HHS Public Access

Author manuscript

Chem Res Toxicol. Author manuscript; available in PMC 2015 June 03.

Published in final edited form as:

Chem Res Toxicol. 2009 September ; 22(9): 1499–1508. doi:10.1021/tx900147g.

MOLECULAR MECHANISMS OF 4-HYDROXY-2-NONENAL AND ACROLEIN TOXICITY: NUCLEOPHILIC TARGETS AND ADDUCT FORMATION

Richard M. LoPachin[†,*], **Terrence Gavin**[§], **Dennis R. Petersen**[¥], and **David S. Barber**[*f*]

[†]Department of Anesthesiology, Albert Einstein College of Medicine, Montefiore Medical Center, 111 E. 210th st., Bronx, NY 10467

[§]Department of Chemistry, Iona College, New Rochelle, NY 10804

[¥]Department of Pharmaceutical Sciences, School of Pharmacy, University of Colorado, Denver, CO 80262

[*f*]Center for Environmental and Human Toxicology, University of Florida, Gainesville, Fl 32611-0885

Abstract

Acrolein and 4-hydroxy-2-nonenal (HNE) are by-products of lipid peroxidation and are thought to play central roles in various traumatic injuries and disease states that involve cellular oxidative stress; e.g., spinal cord trauma, diabetes, Alzheimer's disease. In this Commentary, we will discuss the chemical attributes of acrolein and HNE that determine their toxicities. Specifically, these aldehydes are classified as type-2 alkenes and are characterized by an α,β-unsaturated carbonyl structure. This structure is a conjugated system that contains mobile *pi* electrons. The carbonyl oxygen atom is electronegative and can promote the withdrawal of mobile electron density from the β carbon atom causing regional electron deficiency. Based on this type of electron polarizability, both acrolein and HNE are considered to be soft electrophiles that preferentially form 1,4-Michael type adducts with soft nucleophiles. Proteomic, quantum mechanical and kinetic data will be presented indicating that cysteine sulfhydryl groups are the primary soft nucleophilic targets of acrolein and HNE. This is in contrast to nitrogen groups on harder biological nucleophiles such as lysine or histidine residues. The toxicological outcome of adduct formation is not only dependent upon residue selectivity, but also the importance of the targeted amino acid in protein function or structure. In attempting to discern the toxicological significance of a given adduct, we will consider the normal roles of cysteine, lysine and histidine residues in proteins and the relative merits of corresponding adducts in the manifestations of diseases or toxic states. Understanding the molecular actions of acrolein and HNE could provide insight into many pathogenic conditions that involve initial cellular oxidative stress and could, thereby, offer new efficacious avenues of pharmacological defense.

[*]Richard M. LoPachin, Ph.D. Montefiore Medical Center Moses Research Tower – 7 111 E. 210th St. Bronx, NY 10467 (718) 920-5054 (telephone) (718) 515-4903 (fax) lopachin@aecom.yu.edu.

Keywords

α,β-unsaturated carbonyl; Alzheimer's disease; acrolein; 4-hydroxy-2-nonenal; oxidative stress

INTRODUCTION

There is now general agreement that the pathogeneses of many diseases, xenobiotic intoxications and traumatic injuries (e.g., Alzheimer's disease, spinal cord injury, alcoholism) are characterized by a common pathophysiological cascade involving cellular oxidative stress and membrane lipid peroxidation (1-5). The peroxidative destruction of membrane lipids has direct toxic effects on the structural integrity of cellular membranes. In addition, the fragmentation of polyunsaturated fatty acids during lipid peroxidation generates highly electrophilic α,β-unsaturated carbonyl derivatives including acrolein, 4-hydroxy-2-nonenal (HNE) and 4-oxononenal (ONE; 6-8). These lipid by-products are capable of modifying nucleophilic sidechains on amino acid residues (Cys, His, Arg, Lys) primarily through 1,4-Michael-type conjugate reactions (e.g., see 1, 9-12-16). That the generation of these electrophilic aldehydes and subsequent adduction of protein nucleophiles might have pathophysiological significance is evidenced by the elevated tissue levels of HNE, acrolein and their respective protein adducts in disease processes that involve cellular oxidative damage (e.g., see 17-21; reviewed in 14, 22). Furthermore, the formation of adducts by these reactive aldehydes has been linked to numerous cytotoxic consequences including the disruption of cell signaling, inhibition of enzyme activity and mitochondrial dysfunction (e.g., see 23-25; reviewed in 26). Finally, substantial research indicates that protein adduction, as opposed to the depletion of glutathione and other cellular reducing equivalents, is the primary mechanism of acrolein and HNE toxicity (reviewed in 16, 27-30).

Thus, a large database suggests that the liberation of acrolein, HNE and other α,β-unsaturated aldehyde derivatives during membrane peroxidation could mediate, at least in part, many disease processes that involve cellular oxidative stress. However, despite the obvious pathogenic relevance of these aldehydes, a detailed understanding of their cytotoxicities is missing. Clearly, the corresponding molecular mechanism is a multi-step process that is initiated by the covalent interaction of an electrophilic aldehyde, such as acrolein, and a target nucleophile, such as a cysteine sulfhydryl group. The rate of this reaction is dependent upon the electrophilic reactivity of the aldehyde and the corresponding receptivity of the nucleophile. The consequences of adduct formation at the protein level (e.g., enzyme inhibition or altered tertiary structure) can then lead to defective cellular processes (e.g., reduced energy metabolism, loss of cytoskeletal structure) and eventual cytotoxicity. However, it is not known whether such toxicity involves adduction of multiple amino acids or the selective targeting of a specific residue. The mechanistic relationship between amino acid adduct formation and subsequent cellular damage also has not been established. Finally, it is not clear whether these toxicants act via a common molecular mechanism or whether different mechanisms are involved on a cell- and/or disease-specific basis. The goal of this commentary is to address these data gaps. To accomplish this, we will discuss how electrophilicity determines the relative abilities of acrolein and HNE to form

adducts with different biological nucleophiles. We will also discuss how the role of a given amino acid residue in protein function determines the toxicological relevance of subsequent adduct formation. Based on our interpretation, we propose a unified mechanistic hypothesis of aldehyde toxicity; i.e., acrolein and HNE, like other conjugated α,β-unsaturated carbonyl derivatives, produce cytotoxicity by forming Michael-type adducts with highly nucleophilic sulfhydryl thiolate groups on cysteine residues of functionally critical proteins. Because acrolein and other structurally related chemicals (e.g., acrylamide, acrylonitrile, methylvinyl ketone) are significant environmental pollutants, we will also present the possibility that exogenous exposure to these toxicants can accelerate the onset and development of diseases mediated by endogenous aldehyde generation.

NUCLEOPHILIC TARGETS AND ADDUCT CHEMISTRY OF CONJUGATED α,β-UNSATURATED CARBONYL DERIVATIVES

Physiochemical principles governing electrophile-nucleophile interactions

Acrolein and HNE are α,β-unsaturated carbonyl derivatives and, as such, they are classified as type-2 alkenes (Fig. 1; see also 31). Most chemicals in this class are composed of an alkene (a carbon to carbon double bond) linked to an electron withdrawing group, which in this case is a carbonyl group (carbon to oxygen double bond). The resulting structure (an α,β-unsaturated carbonyl) is a conjugated system that contains mobile outer shell *pi* electrons. Normally an alkene functional group is electron rich. However, the combination of polarizable mobile electrons and the electron withdrawing capacity of the carbonyl group, creates an area of electron deficiency at the alkene β carbon atom of the α,β-unsaturated carbonyl derivatives (see Fig. 2 for detailed explanation). Therefore, acrolein, HNE and other type-2 alkenes are electrophiles (electron deficient species) that form adducts with nucleophiles (electron rich species). Although notable exceptions exist, many toxicants are, in fact, electrophiles that covalently interact with biological nucleophilic targets (32, 33).

Electrophiles, however, do not simply react with nucleophiles, rather such interactions occur along a continuum of relative reactivity. As explained in the following discussion, there is a significant degree of selectivity in electrophile-nucleophile interactions, which is predicted by the Hard and Soft, Acids and Bases (HSAB) theory of Pearson (reviewed in 26, 33, 34). According to this theory, electrophiles and nucleophiles are classified as either relatively “hard” or “soft”, based on inherent electronic characteristics. Hard electrophilic toxicants (e.g., chloroethylene oxide, dimethylnitrosamine) have high positive charge densities (carbocation) at their electrophilic centers and, consequently, these chemicals are characterized by low electron polarizability. In contrast, soft electrophiles (e.g., quinones) have low charge density and their electrons are highly polarizable. The α,β-unsaturated carbonyl structures of acrolein and HNE are, therefore, considered soft electrophiles based on the mobility of their corresponding *pi* electrons. Similarly, the softness of a nucleophile is determined by the polarizability of corresponding valence electrons. Sulfur has a large atomic radius with highly polarizable valence electrons and is the softest nucleophile in biological systems. In contrast, harder biological nucleophiles such as nitrogen and oxygen have small atomic radii and are highly electronegative with low electron polarizability. Based on the HSAB model, soft electrophiles preferentially form adducts with nucleophiles

of comparable softness, whereas hard electrophiles form adducts with hard nucleophiles (reviewed in 26, 32, 34, 35). This model therefore predicts that the preferred nucleophilic targets of acrolein, HNE and other type-2 alkenes are sulfur atoms, as opposed to harder nucleophiles such as nitrogen or oxygen. Consistent with the selectivity principle of this theory, the reaction rate between a soft electrophile and a hard nucleophile is expected to be relatively low (36, 37).

Quantum mechanical descriptors of electrophile-nucleophile interactions

HSAB principles are grounded in the Frontier Molecular Orbital (FMO) theory which, in its most simplistic form, describes covalent bond formation as the overlap occurring exclusively between the respective outermost (frontier) orbitals of the reacting molecules. The frontier molecular orbital of the nucleophile consists of the highest energy orbital holding electrons, known by the acronym HOMO (Highest Occupied Molecular Orbital). In contrast, the frontier molecular orbital for the electrophile is the lowest energy orbital that is vacant or LUMO (Lowest Unoccupied Molecular Orbital). Covalent bonding, such as adduct formation, occurs when the nucleophile donates high-energy HOMO electrons into the empty LUMO of the electrophile. Hence, the propensity of an electrophile and a nucleophile to form adducts should be predictable if knowledge of the relevant frontier orbital energies is available. Values for these FMO energies have been derived from computer-based quantum mechanical calculations and are the basis of algorithms used to compute several previously mentioned HSAB parameters; e.g., hardness ($\eta = [E_{LUMO} - E_{HOMO}]/2$). These quantitative parameters have been demonstrated to be reliable descriptors of electrophile-nucleophile interactions (33, 34, 38-40).

Softness (σ), defined as the inverse of hardness or $\sigma = 1/\eta$, measures the ease with which electron redistribution takes place during covalent bonding. Therefore, with respect to electrophilic species, the softer the electrophile (i.e., higher σ value), the more readily it will form adducts by accepting electrons from a nucleophile. The electrophilic index ($\omega = \mu^2/2\eta$) is an important higher order parameter that combines softness ($1/\eta$) with chemical potential ($\mu = [E_{LUMO} + E_{HOMO}]/2$) and, it is believed, represents a more sensitive measure of electrophilic reactivity. In previous studies, these quantum mechanical parameters were calculated for a series of structurally related type-2 alkenes (41-43) to determine how softness and electrophilicity were related to the induction of nerve terminal (synaptosomal) toxicity. The data presented in Table 1 show that, among the type-2 alkenes tested, NEM is the strongest electrophile; i.e., both σ and ω are numerically larger (more positive) than those of other class members. Based on their respective quantum mechanical parameters, acrylamide (ACR) and methyl acrylate (MA) are substantially weaker electrophiles, whereas acrolein, methylvinyl ketone (MVK) and HNE have intermediate electrophilic reactivity. In this type-2 alkene series, relative differences in electrophilicity were determined by the substituent functional groups and their contribution to the electron density of the α,β-unsaturated carbonyl structure; e.g., the amide group of acrylamide contributes electron density to the conjugated system, which increases the respective E_{LUMO} and decreases softness. The non-conjugated analogs, allyl alcohol and propanal, are not Michael acceptors and, therefore, have substantially lower values of softness and electrophilicity (Table 1). The respective quantum mechanical values indicate the following rank order of type-2 alkene

electrophilicity: NEM>>acrolein>HNE>MVK>>MA≥ACR. In general, this spectrum of electrophilicity was closely correlated ($r^2 \geq 0.95$) to the rank order of the corresponding rate constants (k_2) and in vitro neurotoxic potencies (IC_{50}; 41-43). For HNE, however, steric hindrance imposed by the alkane tail (43) slowed the corresponding adduct reaction (59). Thus, although the respective values of σ and ω reflect electrophilicity equivalent to that of acrolein (Table 1), the rank order of the respective k_{RS}- and IC_{50} values indicate reactivity less than that of MVK (Table 4). Such disagreement between direct chemical measurements and calculated parameters should be expected, since the algorithms for σ and ω do not consider steric factors. These data nonetheless demonstrate that the type-2 alkenes are electrophiles of varying softness and that the degree of softness determines their relative abilities to impair function and cause toxicity. Compared to other type-2 alkenes, acrolein and HNE are moderately reactive soft electrophiles and their corresponding toxicity is commensurate with their electrophilicity.

According to the selectivity principle of the HSAB theory, soft electrophiles should preferentially react with soft nucleophiles. Although several nucleophilic amino acids are present in biological systems, sulfhydryl groups on cysteine residues are the softest (discussed above). Alternatively, the imidazole sidechain of histidine and the ε-amino group of lysine contain nucleophilic nitrogens that are also potential sites for acrolein or HNE adduction. Indeed, there is abundant in vitro evidence that these toxicants can form adducts with lysine and histidine residues; e.g., 44-52. However, substantial proteomic data indicate that cysteine sulfhydryl groups are the preferential targets for acrolein, HNE and other type-2 alkenes (12, 23, 41, 45, 50, 51, 53-57; also see early studies 58, 59). This cysteine preference is consistent with the fact that the sidechain amino nitrogen groups of lysine and histidine are harder nucleophiles and, therefore, have inherently lower reactivity for soft electrophiles such as acrolein and HNE. These electronic and structural restrictions are reflected in the corresponding second order rate constants (mean $k_2 \pm$ SD $M^{-1}s^{-1}$), which demonstrate that Cys (1.33±0.083) is approximately one-thousand fold more reactive toward HNE than His ($2.14 \pm 0.312 \times 10^{-3}$) or Lys ($1.33 \pm 0.050 \times 10^{-3}$; 12, 45; see also 57, 60). The relatively high toxicant-to-protein molar ratios (50:1) and long incubation times (≥ 24 hrs) necessary to produce Lys and His adducts during in vitro acrolein/HNE experiments (e.g., see 9, 10, 47, 49, 61-63) are a reflection of the correspondingly slow rate of adduct formation.

To assess quantitatively the relative nucleophilicity of Cys, Lys and His residues, the respective frontier molecular orbital energies (E_{HOMO}, E_{LUMO}) can be used to calculate nucleophilic softness (σ) and chemical potential (μ). The latter parameter represents the ability of a nucleophilic species to transfer electron density to the electrophile. Calculated values of μ are independent of pH (i.e., $\mu = E_{LUMO}+E_{HOMO}/2$) and, therefore, reflect the inherent electronic nature of the structural moiety upon which the computations are based. At physiological pH (7.4), the sulfhydryl sidechain of cysteine is protonated and, therefore, exists primarily as the neutral (0) thiol. Also at this pH, the imidazole secondary amine of histidine is mostly deprotonated (0) based on a corresponding pKa of 6.0 and the primary ε-amino group amine of lysine (pKa = 10.5) is protonated (+1). As reflected in the μ and σ values (Table 2), the respective nucleophilicities of these residues are surprisingly low; i.e., the corresponding HOMO energies are relatively low and the μ values are negative.

However, at physiological pH, a small but significant fraction (~10%) of the cysteine sulfhydryl groups is deprotonated and exists in the anionic (-1) thiolate state. In fact, the thiolate fraction is more prevalent than predicted due to the existence of low pKa cysteine sulfhydryl groups within highly specialized amino acid sequences known as catalytic triads (see below). As the quantum mechanical descriptors indicate, thiolates are much softer nucleopilies than lysine, histidine or the corresponding thiols; i.e., the E_{HOMO} energy is more positive and the corresponding σ and μ values are larger. This analysis indicates that thiolates are the preferred nucleophilic targets of acrolein, HNE and other type-2 alkenes. Furthermore, the likelihood that a given nucleophile will form an adduct with a type-2 alkene can be predicted by calculating the nucleophilicity index [$\omega^- = \eta_A(\mu_A - \mu_B)^2/2(\eta_A - \eta_B)^2$]. This recently developed higher order parameter considers the hardness (η) and chemical potential (μ) of both the electrophilic (type-2 alkene) and nucleophilic (cysteine, histidine or lysine) reactants (64). As suggested by the respective ω^- values (Table 3), acrolein, HNE and the type-2 alkenes preferentially form adducts with cysteine thiolate sites as opposed to histidine, lysine or thiol residues, which have significantly lower ω^- values.

If, as the calculated nucleophilic descriptors suggest, the thiolate is the preferred target for acrolein and HNE, this should be reflected in a correspondingly faster rate of adduction. Thus, rates of sulfhydryl adduct formation with type-2 alkenes are known to be dependent upon the pH of the solution. For example, in a recent study, L-cysteine (pKa = 8.15) was shown to react with acrolein fifteen times faster when the pH was increased from 7.4 to 8.8. Corroborative studies showed that this pH-dependent increase in rate occurred for the reactions of L-cysteine with all type-2 alkenes evaluated (42,43). The observed changes in second-order rate constant (k_2) obviously reflected the increase in thiolate concentration at the higher pH value and thus, confirms the notion that the operable reactive species is indeed the anionic thiolate. Moreover, it has been convincingly demonstrated that experimentally determined rate constants like k_2 can be used to derive an anionic rate constant (k_{RS}-) as a quantifiable measure of inherent nucleophilic strength (58). Using the expression log (k_{RS}- - k_2) = log k_2 + pK_a – pH (where k_{RS}- represents the anionic rate constant), a representative series of thiolate rate constant were calculated. For each α,β-unsaturated carbonyl derivative, the corresponding thiolate rate constants (k_{RS}^-) were highly correlated to μ(r^2 = 0.96; Table 2) and ω^- (r^2 = 0.91; Table 3). Furthermore, the fact that ω^- and k_{RS}^- were closely correlated to the neurotoxic potencies (IC_{50}'s; Table 4) provides evidence that thiolate targeting by acrolein and HNE has toxicological relevance (42, 43).

The adduct chemistry of acrolein and HNE

It is clear that acrolein and HNE are relatively soft electrophiles that target the soft nucleophilic thiolate-state of cysteine residues. The soft-soft interaction between these type-2 alkenes and their sulfhydryl target occurs via a 1,4-Michael-type conjugate reaction; i.e., nucleophilic attack at the β-carbon of the α,β-unsaturated carbonyl structure with subsequent addition across the carbon-carbon double bond. The resulting intermediate product, a saturated aldehyde, then undergoes an intramolecular reaction with the hydroxyl group to form a cyclic hemiacetal, which is the predominant adduct form (reviewed in 1, 16). That acrolein, HNE and other type-2 alkenes preferentially form stable 1,4-adducts with cysteine sulfhydryl groups has been demonstrated by isolation of corresponding protein

adducts and subsequent quantitation using mass spectrometry and other proteomic approaches (12, 23, 41, 45, 50, 51, 53-57; also see early studies by 58, 59).

As an alternative to 1,4-Michael addition, the carbonyl carbon atom of acrolein and HNE could form adducts with primary amines (e.g., Lys) via a 1,2-addition. Nadkarni and Sayre (61) have provided indirect evidence that HNE forms such adducts with primary amines and that subsequent Schiff base formation is prevalent in solvent-isolated (buried) hydrophobic protein microenvironments. However, the corresponding kinetics are inherently slow and the Schiff base product is reversible (10, 11, 16). That reactions with carbonyl groups (Schiff base formation) on acrolein and other type-2 alkenes are not neurotoxicologically relevant is suggested by recent in vitro studies, which showed that graded exposure of striatal synaptosomes to propanal (an aldehyde) did not affect function (41). Alternatively, because α,β-unsaturated carbonyls are bifunctional (i.e., electrophilic reactivity at the β-carbon and carbonyl carbon atoms), HNE, acrolein and other chemicals in this class could cause toxicity by crosslinking proteins rather than by forming monoadducts (1, 62, 65). However, the results of recent immunoblot analyses and studies with pharmacological crosslink blockers (41) did not support a mechanistic role for protein crosslinks in the production of alkene toxicity.

HISTIDINE, LYSINE AND CYSTEINE RESIDUES: RESPECTIVE ROLES IN PROTEIN STRUCTURE AND FUNCTION

The chemical and proteomic data presented thus far indicate that the soft-soft interactions between, for example, acrolein and cysteine thiolates is a kinetically favored reaction that occurs rapidly. In contrast, soft-hard interactions between, for example, acrolein and lysine residues, are unfavored and, therefore, significantly slower reactions. It is important to realize that, among potential amino acid targets, differences in nucleophilic reactivity and, therefore, selectivity are not necessarily related to the relative degree of toxicological relevance. That is, it cannot be assumed that rapid adduct formation with a given residue has inherent toxic significance. Rather, it is the role of the residue in protein structure or function and the resulting disruptive consequences of adduction that determine the relevance of an amino acid adduct. In the following subsections, we provide a brief overview of the respective roles that histidine, lysine and cysteine play in determining protein activity. For more comprehensive discussions, the reader is referred to several relevant reviews; i.e., 29, 71, 74, 79. In a subsequent section, we will discuss how amino acid function might be related to the toxicological consequences of adduct formation.

Histidine is a basic amino acid that plays an important role in cellular buffering. At physiological pH, the imidazole sidechain (pK_a = 6.04) is mostly deprotonated (0) and, therefore, free histidine has limited buffering capacity. Nonetheless, in combination with other amino acids (e.g., alanine), the pK_a of the imidazole can increase, which makes histidine residues in proteins and peptides a dominant buffering system in many cells. When paired with an acidic amino acid (e.g., aspartic acid), histidine residues can participate in the protonation/deprotonation of central nucleophiles in catalytic triads (e.g., see 66). As will be discussed, these acid-base motifs determine the nucleophilic state of, for example, cysteine sulfhydryl groups, which in turn regulates the activity of critical proteins. Histidine residues

can also play more direct roles in modulating protein activity. For example, several functionally critical histidine residues are present within the active site of creatine kinase (CK). In a transition state of this enzyme, His 66 forms a salt bridge with the carboxyl group of Asp 326 and thereby locks two flexible loops (residues 60-70 and 323-332) over the active site. Latching of these loops brings together two hydrophobic residues (Ile 69 and Val 325), which presumably creates a binding pocket for the N-methyl group of creatine and imparts specificity for this substrate (67). His 191 and His 296 are also located in the active site of CK and are involved in orienting the adenosine rings. Specifically, His 296 and the six-membered adenine ring undergo a stacking interaction, whereas His 191 forms a hydrogen bond with the 2'-hydroxyl group of the ribose ring (67). Mutation of these active site histidine residues substantially decreased enzyme activity (68).

The ε-amino group of lysine is protonated (+1; pKa = 10.5) at neutral pH and can participate in the electrostatic interactions of amino acids (e.g., lysine-glutamate) that mediate protein-protein associations. Similar to histidine, lysine residues are often the basic amino acid component of many catalytic triads (69). The primary amine sidechain of lysine is also subject to substantial posttranslational modifications (PTMs); e.g., acetylation, methylation, sumoylation and ubiquitylation, (70-73). These reactions are not favored thermodynamically and are, therefore, mediated by specific enzymes (e.g., lysine acetyltransferases and deacetylases). Lysine PTMs can influence protein function by changes in charge density or by reversible interactions with crucial lysine residues of, for example, structural proteins (e.g., Lys 40 of α-tubulin) or enzymes (e.g., Lys 609 of acetyl-CoA synthase). Such modifications alter the activities of many protein classes; e.g., histones, cytoskeletal proteins, energy metabolism, mitochondrial enzymes and plasma membrane associated receptors (72, 74, 75) and, similar to protein phosphorylation, lysine modulation might represent a signaling program that orchestrates cell processes (e.g., see 70, 72, 73, 76).

Cysteine is a polar, uncharged amino acid with a pKa of 8.3 and, therefore, at physiological pH the sidechain sulfhydryl group is protonated (0). The biological importance of cysteine stems from the unique ability of this amino acid to undergo reversible oxidation/reduction. Based on this ability, cysteine residues can act as redox-sensors that detect and transduce changes in cellular redox status caused by the generation of toxic reactive oxygen species (ROS). ROS oxidation of these cysteines produces protein conformational changes that stimulate antioxidant pathways through subsequent induction of gene expression; e.g., activation of the Nfr2-Keap1 pathway by ROS and electrophiles (77-79; reviewed in 80). In addition to the management of toxic ROS, reactive cysteines are now recognized as acceptors for redox signaling systems; e.g., nitric oxide (NO), hydrogen peroxide (H_2O_2). The redox status of these cysteines regulates the function of proteins that are involved in numerous critical cell processes (reviewed in 29, 81-85). For example, Cys264 is a reactive cysteine residue located within the nucleotide-binding consensus sequence of *N*-ethylmaleimide sensitive factor (NSF). The ATPase activity of this enzyme, which is critically involved in membrane fusion reactions (e.g., exocytosis), is regulated by reversible S-nitrosylation of Cys264 through NO-signaling. As alluded to above, not all cysteine residues are sensitive to ROS or redox signaling. Indeed, the nucleophilicity and, therefore, reactivity of most biological cysteine thiols (RSH) with, for example, NO is too low to be

relevant (Table 2). However, as discussed above, the nucleophilicity of sulfhydryl groups is markedly increased in the anionic thiolate state. Since the pKa of cysteine is 8.3, sulfhydryl thiolates can exist only in protein microenvironments where the pKa is lowered. Such conditions exist in catalytic triads where proton shuttling between flanking or proximal (≤ 6Å) basic amino acid residues (histidine, arginine, lysine) and their acidic counterparts (aspartate, glutamate) can deprotonate the sulfhydryl group and thereby lower the corresponding pKa by several units (e.g., see 66, 69). The highly nucleophilic thiolate groups of catalytic triads (and diads) therefore represent "receptors" for electrophilic transmitters such as NO and H_2O_2 (29, 85-87). Cysteine-based catalytic triads are found within the active sites of many proteins (e.g., NSF - Cys264; glyceraldehyde-3-phosphate dehydrogenase - Cys525; vacuolar-ATPase - Cys254) and play a critical role in modulating corresponding function. Through these diverse cysteine effectors, redox signaling can influence almost all aspects of cell physiology.

The Toxicological Significance of Amino Acid Adduct Formation

Clearly, cysteine sulfhydryl thiolate groups are critically involved in the majority of cellular processes; i.e., they are essential for enzyme catalytic activity, metal chelation, they function as ROS sensors and they are acceptors for redox signaling pathways. Lysine and histidine residues also are physiologically important; lysine residues undergo posttranslational modifications, histidine residues function as cellular buffers and both amino acids participate as the basic components of catalytic triads. Given the apparent significance of lysine, histidine and, especially, cysteine in protein structure and function, it would seem that adduction of these residues by acrolein or HNE could have toxicological implications. Nonetheless, little direct information is available regarding the toxicological consequences of corresponding adduct formation. Proteomic research has identified HNE or acrolein adducts at specific amino acid residues following in vivo or in vitro exposure of proteins; e.g., Cys47 of glutathione S-transferase P1-1; His178 in the phosphorylation lip of Erk-2; Cys29 in the A chain of cathepsin B (11, 25, 53, 55, 57, 88-90). However, the toxicological significance of adduct formation was inferred from the presumed role of the targeted amino acid in corresponding protein function. Additional research has shown that incubation of purified proteins (e.g., glyceraldehyde-3-phosphate dehydrogenase) with acrolein, HNE or other type-2 alkenes disrupts function and produces lysine, histidine or cysteine adducts (23, 41, 46, 48, 50, 54, 89, 91). In these studies, it was not determined whether adduct formation was causally related to protein dysfunction.

A few studies, however, have specifically addressed the toxicological significance of adduct formation by HNE and acrolein. Stewart et al. (92) used an in vitro model of tubulin polymerization to show that lysine adducts formed by HNE and 4-oxononenal (ONE) had limited functional effects. In contrast, these investigators found that adduction of cysteine residues on the α- and β-tubulin subunits (Cys347α, Cys376α and Cys303β) inhibited polymerization. In another study, Carbone et al. (93) reported that in vitro HNE exposure (10 and 100μM) impaired the chaperone function of Hsp72 and that this inhibition was associated with adduct formation at Cys267 in the ATPase domain of this protein. The relevance of this adduct was suggested by experiments showing that DnaK, a bacterial Hsp70 variant that lacks Cys267, was resistant to HNE inactivation and that

malondialdehyde, a nonconjugated aldehyde analog, did not affect Hsp72 activity. Research by Eliuk et al. (94) showed that HNE, at a pathophysiologically relevant concentrations (10-30 μM), selectively formed Michael adducts with Cys 283 in the active site of recombinant human brain creatine kinase (CK; 10μM). Adduction of this cysteine residue was associated with 30-40% inhibition of enzyme activity. At higher HNE concentrations (100-300 μM), active site histidine residues (His 66, His 191 and His 296; see preceding section) were also adducted and enzyme activity was progressively inhibited. Together, these studies provide preliminary evidence that adduction of certain protein cysteine residues by α,β-unsaturated carbonyl derivatives has potential toxicological significance.

From the preceding overview, it is clear that data gaps exist and, therefore, more research is needed to establish the toxicological relevance of lysine, histidine and cysteine adducts in acrolein and HNE toxicity. Nonetheless, the rank order of amino acid reactivity (Cys>>His>Lys) and the relative importance of these residues in cell physiology provide insight into the corresponding molecular mechanisms. Thus, as discussed above, lysine and histidine are relatively poor nucleophiles and are, therefore, unlikely to be immediate targets for soft electrophiles. This also applies to the transient ionization states of these residues that occur in catalytic triads and other microenvironments where the respective pK_a's are modified (compare corresponding data in Table 2). However, the relatively high thiolate reactivity of acrolein and HNE and the well-known critical roles of this sulfhydryl in cell physiology argue that the initial aspects of toxicity are mediated by cysteine adduction. The slower rate of lysine or histidine adduction might be relevant to toxicities involving high concentrations or subchronic exposure durations. Accordingly, at lower cellular toxicant concentrations (nM to very low μM range; 95) that might develop during the early stages of a disease processes or following chronic environmental acrolein exposure, cysteine adduction will predominate due to the higher nucleophilic reactivity of this residue (see 23, 44, 53, 55). Although Lys is slightly more abundant in proteins than either Cys or His residues (7%, 3% and 2%, respectively; 96), this difference is not sufficient to alter the residue selectivity of the type-2 alkenes through changes in mass action kinetics (23, 53-55, 63). Results from extensive research on type-2 alkene toxicity (e.g., see 1, 11-13, 41-43, 55, 56, 92, 106), suggests that the acute effects of acrolein and HNE are mediated by a common mechanism involving adduction of sulfhydryl thiolates in the catalytic triads of proteins that regulate critical cellular processes. Because many of the thiolates in these catalytic centers function as NO acceptors, the irreversible formation of HNE/acrolein adducts at these sulfhydryl groups might disrupt NO signaling (reviewed in 14, 29, 30, 99). As cellular toxicant concentrations rise (low μM range) or as the exposure duration increases, the available cysteine thiolate pool will diminish and adduct formation will shift to residues with lower reactivity; i.e., lysine or histidine. This scenario could account for the detection of Lys and His adducts, as well Cys adducts, in tissues of patients with chronic diseases that presumably involve oxidative generation of acrolein/HNE; e.g., Alzheimer's disease, atherosclerosis, diabetes (see 17, 51, 52, 100, 101). The toxicity associated with lysine adduction is likely mediated by disruption of posttranslation modifications; e.g., phosphorylation, acetylation, methylation (see above). With respect to long-term (chronic) exposure conditions, the cell-types or –regions most susceptible to HNE or acrolein toxicity are those characterized by slower protein turnover rates. In these conditions, adducts are

removed slowly, which favors the accumulation of dysfunctional proteins and the development of cumulative toxicity (reviewed in 14, 30, 102).

ROLE OF HNE AND ACROLEIN IN DISEASE PROCESSES

Cellular oxidative stress and associated peroxidation of membrane polyunsaturated fatty acids are implicated in the pathogenesis of many diseases including alcoholic liver damage, diabetes, reperfusion injury (e.g., stroke or myocardial infarction), atherosclerosis and Alzheimer's disease (reviewed in 2-4, 103-105). There is considerable evidence that major aspects of these disease processes are mediated by oxidative stress and the subsequent liberation of acrolein, HNE and other toxic aldehydes during membrane peroxidation (reviewed in 1, 14, 30, 106-108). Whereas some studies have focused on glutathione (GSH) depletion as the critical event (e.g., see 109-112), a substantial database suggests that the in vivo toxicity of acrolein and other type-2 alkenes primarily involves protein adduct formation (e.g., see 56, 113-117; reviewed in 28). Based on a weight of evidence approach, we propose the following hypothesis: acrolein, HNE and other lipid by-products (e.g., ONE, HHE) are soft electrophiles that form irreversible 1,4-Michael-type adducts with soft nucleophilic sulfhydryl thiolate groups of cysteine. These anionic residues are the central nucleophilic components of catalytic triads in the active sites of many critical proteins. Reversible redox modulation of these catalytic sulfhydryl groups by, for example, the endogenous NO or H_2O_2 pathways regulates protein activity. Therefore, irreversible adduction of these regulatory thiolate groups by acrolein or HNE will disrupt redox control of protein function and, thereby, produce cytotoxicity (reviewed in 14, 29, 30, 99). Although lysine and histidine residues are also targets for type-2 alkene chemicals, these residues are relatively hard nucleophiles with significantly slower adduction kinetics. As a result, the toxic consequences of lysine or histidine adduction are more likely to develop during high-dose intoxication or during the late stages of chronic diseases when adduction of the cysteine thiolate pool has saturated.

The proposed pathophysiological scenario has significant implications for disease states and traumatic tissue injuries that involve initial oxidative stress and subsequent liberation of toxic aldehydes in specific cell-types. Thus, for example, a large body of evidence indicates that the pathobiology of Alzheimer's disease involves oxidative damage and subsequent generation of toxic aldehydes in nerve cells of the hippocampus and cerebral cortex. It has been hypothesized that acrolein and HNE selectively target regional nerve terminals and that the ensuing disruption of synaptic function promotes problems with declarative memory and cognition (reviewed in 14, 30). Certainly, pathokinetic differences such as the affected cell-type, the rate of acrolein/HNE generation and resulting cellular levels will distinguish each disease or injury state. Nonetheless, adduction of NO-targeted sulfhydryl thiolate-sites on proteins is a rational, common mechanism for pathogenic conditions that involve cellular oxidative stress. As indicated above, acrolein is a member of the type-2 alkene chemical family that includes acrylamide, methyl acrylate and acrylonitrile. These chemicals have extensive industrial utility and are pervasive environmental pollutants (ambient, occupational, dietary). Therefore, the proposed mechanism is applicable to acquired toxicities that develop as a result of environmental or occupational exposures to acrolein and other α,β-unsaturated aldehyde derivatives (14). Since the type-2 alkenes presumably

operate via a common mechanism, it is also possible that environmental exposure to these toxicants (e.g., acrolein, acrylonitrile, MVK) will accelerate the onset and development of chronic conditions such as diabetes or Alzheimer's disease that involve liberation of endogenous toxic aldehydes (e.g., acrolein, HNE, ONE). Finally, our proposed mechanism of electrophile toxicity suggests that nucleophilic scavengers such as *N*-acetylcysteine (NAC) might be cytoprotective and, consequently, have possible therapeutic value. However, it should be recognized that nucleophiles would also scavenge the electrophilic mediators of endogenous redox pathways (e.g., NO, H_2O_2) and would, therefore, constitute a significant toxic threat (see 42).

ACKNOWLEDGEMENT

Research presented in this manuscript was supported by NIH grants to R.M.L (NIEHS RO1 ES03830-21; ES07912-10) and to D.R.P. (NIAAA AAR37-09300-13).

REFERENCES

1. Esterbauer H, Schaur RJ, Zollner J. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Rad. Biol. Med. 1991; 11:81–128. [PubMed: 1937131]
2. Halliwell B. Oxidative stress and neurodegeneration: where are we now? J. Neurochem. 2006; 97:1634–1658. [PubMed: 16805774]
3. Lefer DJ, Granger N. Oxidative stress and cardiac disease. Am. J. Med. 2000; 109:316–323.
4. Lieber CS. Alcoholic liver injury: pathogenesis and therapy in 2001. Pathol. Biol. 2001; 49:738–752. [PubMed: 11762137]
5. Povlishock JT, Kontos HA. The role of oxygen radicals in the pathobiology of traumatic brain injury. Hum Cell. 1992; 5:345–353. [PubMed: 1304799]
6. Catala A. Lipid peroxidation of membrane phospholipids generates hydroxy-alkenals and oxidized phospholipids active in physiological and/or pathological conditions. Chem. Phys, Lipids. 2009; 157:1–11. [PubMed: 18977338]
7. Grimsrud PA, Xie H, Griffin TJ, Bernlohr DA. Oxidative stress and covalent modification of protein with bioactive aldehydes. J. Biol. Chem. 2008; 283:21837–21841. [PubMed: 18445586]
8. Schneider C, Porter NA, Brash AR. Routes to 4-hydroxynonenal: fundamental issues in the mechanisms of lipid peroxidation. J. Biol. Chem. 2008; 283:15539–15543. [PubMed: 18285327]
9. Amarnath V, Valentine WM, Montine TJ, Patterson WH, Amarnath K, Bassett CN, Graham DG. Reactions of 4-hydroxy-2(*E*)-nonenal and related aldehydes with proteins studied by carbon-13 nuclear magnetic resonance spectroscopy. Chem. Res. Toxicol. 1998; 11:317–328. [PubMed: 9548802]
10. Bruenner BA, Jones AD, German JB. Direct characterization of protein adducts of the lipid peroxidation product 4-hydroxy-2-nonenal using electrospray mass spectrometry. Chem. Res. Toxicol. 1995; 8:552–559. [PubMed: 7548735]
11. Crabb JW, O'Neil J, Miyag M, West K, Hoff HF. Hydroxynonenal inactivates cathepsin B by forming Michael adducts with active site residues. Protein Sci. 2002; 11:831–840. [PubMed: 11910026]
12. Doorn JA, Petersen DR. Covalent adduction of nucleophilic amino acids by 4-hydroxynonenal and 4-oxononenal. Chem. Bio. Interact. 2003; 143-144:93–100. [PubMed: 12604193]
13. Witz G. Biological interactions of α,β-unsaturated aldehydes. Free Rad. Biol. Med. 1989; 7:333–349. [PubMed: 2673948]
14. LoPachin RM, Barber DS, Gavin T. Molecular mechanisms of the conjugated α,β-unsaturated carbonyl derivatives: relevance to neurotoxicity and neurodegenerative diseases. Tox. Sci. 2008; 104:235–249.
15. Cai J, Bhatnagar A, Pierce WM. Protein modification by acrolein: formation and stability of cysteine adducts. Chem. Res. Toxicol. 2009; 22:708–716. [PubMed: 19231900]

16. Petersen DR, Doorn JA. Reactions of 4-hydroxynonenal with proteins and cellular targets. Free Rad. Biol. Med. 2004; 37:937–945. [PubMed: 15336309]
17. Calingasan NY, Uchida K, Gibson GE. Protein-bound acrolein: a novel marker of oxidative stress in Alzheimer's disease. J. Neurochem. 1999; 72:751–756. [PubMed: 9930749]
18. Castellani RJ, Perry G, Siedlak SL, Nunomura A, Shimohama S, Zhang J, Montine T, Sayre LM, Smith MA. Hydroxynonenal adducts indicate a role for lipid peroxidation in neocortical and brainstem Lewy bodies in humans. Neurosci Letters. 2002; 319:25–28.
19. Grune T, Siems WG, Schneider W. Accumulation of aldehyde peroxidation products during postanoxic reoxygenation of isolated rat hepatocytes. Free Radical Biol. Med. 1993; 15:125–132. [PubMed: 8375689]
20. Ohsawa I, Nishimaki K, Murakami Y, Suzuki Y, Ishikawa M, Ohta S. Age-dependent neurodegeneration accompanying memory loss in transgenic mice defective in mitochondrial aldehyde dehydrogenase 2 activity. J. Neurosci. 2008; 28:6239–6249. [PubMed: 18550766]
21. Uchida K. Current status of acrolein as a lipid peroxidation product. TCM. 1999; 9:109–113. [PubMed: 10639724]
22. Uchida K. 4-Hydroxy-2-nonenal: a product and mediator of oxidative stress. J. Prog. Lip. Res. 2003; 42:318–343.
23. Ishii T, Tatsuda E, Kumazawa S, Nakayama T, Uchida K. Molecular basis of enzyme inactivation by an endogenous electrophile 4-hydroxy-2-nonenal: identification of modification sites in glyceraldehyde-3-phosphate dehydrogenase. Biochemistry. 2003; 42:3473–3480.
24. Picklo MJ, Montine TJ. Acrolein inhibits respiration in isolated brain mitochondria. Biochem. Biophys. Acta. 2001; 1535:145–152. [PubMed: 11342003]
25. Sampey BP, Carbone DL, Doorn JA, Drechsel DA, Petersen DR. 4-Hydroxy-2-nonenal adduction of extracellular signal-regulated kinase (Erk) and the inhibition of hepatocyte Erk-Est-like protein-1-activating protein-1 signal transduction. Mole. Pharmacol. 2007; 71:871–883.
26. LoPachin RM, DeCaprio AP. Protein adduct formation as a molecular mechanism in neurotoxicity. Tox Sci. 2005; 86:214–225.
27. Friedman M. Chemistry, biochemistry and safety of acrylamide. A review. J. Agric. Food Chem. 2003; 51:4504–4526. [PubMed: 14705871]
28. Kehrer JP, Biswal SS. The molecular effects of acrolein. Toxicol Sci. 2000; 57:6–15. [PubMed: 10966506]
29. LoPachin RM, Barber DS. Synaptic cysteine sulfhydryl groups as targets of electrophilic neurotoxicants. Tox. Sci. 2006; 94:240–255.
30. LoPachin RM, Gavin T, Barber DS. Type-2 alkenes mediate synaptotoxicity in neurodegenerative diseases. NeuroToxicology. 2008; 29:871–882. [PubMed: 18582500]
31. Kemp, DS.; Vellaccio, F. Carbonyl condensation reactions. Organic Chemistry Worth Publishers; New York: 1980. p. 839-874.Chapt. 24
32. Hinson JA, Roberts DW. Role of covalent and noncovalent interactions in cell toxicity: effects on proteins. Ann. Rev. Pharmacol. Toxicol. 1992; 32:471–510. [PubMed: 1605575]
33. Schultz TW, Carlson RE, Cronin MTD, Hermens JLM, Johnson R, O'Brien PJ, Roberts DW, Siraki A, Wallace KB, Veith GD. A conceptual framework for predicting the toxicity of reactive chemicals: modeling soft electrophilicity. SAR QSAR Eviron. Res. 2006; 17:413–428.
34. Pearson RG. Hard and soft acids and bases – the evolution of a chemical concept. Coord. Chem. Rev. 1990; 100:403–425.
35. Coles B. Effects of modifying structure on electrophilic reactions with biological nucleophiles. Drug Met. Rev. 1984-85; 15:1307–1334.
36. Chattaraj PK. Chemical reactivity and selectivity: local HSAB principal versus frontier orbital theory. J. Phys. Chem. A. 2001; 105:511–513.
37. Pearson RG. Maximum chemical and physical hardness. J. Chem. Edu. 1999; 76:267–275.
38. Cronin MTD, Manga N, Seward JR, Sinks GD, Schultz TW. Parametrization of electrophilicity for the prediction of the toxicity of aromatic compounds. Chem. Res. Toxicol. 2001; 14:1498–1505. [PubMed: 11712907]

39. Geiss KT, Frazier JM. QSAR modeling of oxidative stress in vitro following hepatocyte exposures to halogenated methanes. Toxicol. In Vitro. 2001; 15:557–563. [PubMed: 11566591]
40. Maynard AT, Huang M, Rice WG, Covell DG. Reactivity of the HIV-1 nucleocapsid protein p7 zinc finger domains from the perspective of density-functional theory. Proc. Natl. Acad. Sci. 1998; 95:11578–11583. [PubMed: 9751708]
41. LoPachin RM, Barber DS, Geohagen BC, Gavin T, He D, Das S. Structure-toxicity analysis of Type-2 alkenes: in vitro neurotoxicity. Tox. Sci. 2007a; 95:136–146.
42. LoPachin RM, Gavin T, Geohagen BC, Das S. Neurotoxic mechanisms of electrophilic type-2 alkenes: soft-soft interactions described by quantum mechanical parameters. Tox. Sci. 2007b; 98:561–570.
43. LoPachin RM, Gavin T, Geohagen BC, Das S. Synaptosomal toxicity and nucleophilic targets of 4-hydroxy-2-nonenal. Tox. Sci. 2009; 107:171–181.
44. Aldini G, Gamberoni L, Orioli M, Beretta G, Regazzoni L, Facino RM, Carini M. Mass spectrometric characterization of covalent modification of human serum albumin by 4-hydroxy-*trans*-2-nonenal. J. Mass Spec. 2006; 41:1149–1161.
45. Doorn JA, Petersen DR. Covalent modification of amino acid nucleophiles by the lipid peroxidation products 4-hydroxynonenal and 4-oxo-2-nonenal. Chem. Res. Toxicol. 2002; 15:1445–1450. [PubMed: 12437335]
46. Friguet B, Stadtman ER, Szweda LI. Modification of glucose-6-phophate dehydrogenase by 4-hydroxy-2-nonenal. J. Biol. Chem. 1994; 269:21639–21643. [PubMed: 8063806]
47. Liu Z, Minkler PE, Sayre LM. Mass spectroscopic characterization of protein modification by 4-hydroxy-2-(*E*)-nonenal. Chem. Res. Toxicol. 2003; 16:901–911. [PubMed: 12870893]
48. Szweda LI, Uchida K, Tsai L, Stadtman ER. Inactivation of glucose-6-6phophate dehydrogenase by 4-hydroxy-2-nonenal. J. Biol. Chem. 1993; 268:3342–3347. [PubMed: 8429010]
49. Uchida K, Stadtman ER. Modification of histidine residues in proteins by reaction with 4-hydroxynonenal. Proc. Natl. Acad. Sci. 1992; 89:4544–4548. [PubMed: 1584790]
50. Uchida K, Stadtman ER. Covalent attachment of 4-hydroxynonenal to glyceraldehyde-3-phosphate dehydrogenase. J. Biol. Chem. 1993; 268:6388–6393. [PubMed: 8454610]
51. Uchida K, Kanematsu M, Morimitsu Y, Noriko T, Noguchis N, Niki E. Acrolein is product of lipid peroxidation reaction. J. Biol. Chem. 1998; 273:16058–16066. [PubMed: 9632657]
52. Uchida K, Kanematsu M, Sakai K, Matsuda T, Hattori N, Mizuno Y, Suzuki D, Miyata T, Noguchi N, Niki E. Protein-bound acrolein: Potential markers for oxidative stress. Proc. Natl. Acad. Sci. 1998; 95:4882–4887. [PubMed: 9560197]
53. Aldini G, Dalle-Donne I, Vitoli G, Facino RM, Carini M. Covalent modification of actin by 4-hydroxy-*trans*-2-nonenal (HNE): LC-ESI-MS/MS evidence for Cys374 Michael adduction. J. Mass Spec. 2005; 40:946–954.
54. Barber DS, LoPachin RM. Proteomic analysis of acrylamide-protein adduct formation in rat brain synaptosomes. Toxicol. Appl. Pharmacol. 2004; 201:120–136. [PubMed: 15541752]
55. Dalle-Donne I, Vistoli G, Gamberoni L, Giustarini D, Colmbo R, Facino RM, Rossi R, Milzani A, Aldini G. Actin Cys374 as a nucleophilic target of α,β-unsaturated aldehydes. Free Rad. Biol. Med. 2007; 42:583–598. [PubMed: 17291982]
56. LoPachin RM, Barber DS, He D, Das S. Acrylamide inhibits dopamine uptake in rat striatal synaptic vesicles. Tox Sci. 2006; 89:224–234.
57. Van Iersel MLPS, Ploemen J-PHTM, LoBello M, Federici G, van Bladeren PJ. Interactions of α,β-unsaturated aldehydes and ketones with human glutathione *S*-transferase P1-1. Chem-Biol. Inter. 1997; 108:67–78.
58. Friedman M, Cavins JF, Wall JS. Relative nucleophilic reactivities of amino groups and mercaptide ions in addition reactions with α,β-unsaturated compounds. J. Am. Chem. Soc. 1965; 87:3672–3682.
59. Friedman M, Wall JS. Additive linear free-energy relationships in reaction kinetics of amino groups with α,β-unsaturated compounds. J. Org. Chem. 1966; 31:2888–2894.
60. Lin D, Lee J, Liu Q, Perry G, Smith MA, Sayre LM. 4-Oxo-2-nonenal is both more neurotoxic and more protein reactive than 4-hydroxy-2-nonenal. Chem. Res. Toxicol. 2005; 18:1219–1231. [PubMed: 16097795]

61. Annangudi SP, Deng Y, Gu X, Zhang W, Crabb JW, Salomon RG. Low-density lipoprotein has an enormous capacity to bind (*E*)-4-hydroxynon-2-enal (HNE): detection and characterization of lysyl and histidyl adducts containing multiple molecules of HNE. Chem Res. Toxicol. 2008; 21:1384–1395. [PubMed: 18570390]
62. Nadkarni D, Sayre LM. Structural definition of early lysine and histidine adduction chemistry of 4-hydroxynonenal. Chem. Res. Toxicol. 1995; 8:284–291. [PubMed: 7766813]
63. Xu G, Liu Y, Kansal MM, Sayre LM. Rapid cross-linking of proteins by 4–ketoaldehydes and 4-hydroxy-2-alkenals does not arise from the lysine-derived monoalkylpyrroles. Chem. Res. Toxicol. 1999; 12:855–861. [PubMed: 10490508]
64. Jaramillo P, Periz P, Contreras R, Tiznada W, Fuentealba P. Definition of a nucleophilicity scale. J. Phys. Chem. A. 2006; 110:8181–8187. [PubMed: 16805506]
65. Kurtz AJ, Llyod RS. 1,N^2-deoxyguanosine adducts of acrolein, crotonaldehyde, and *trans*-4-hydroxynonenal cross –link to peptides via Schiff base linkage. J. Biol. Chem. 2003; 278:5970–5976. [PubMed: 12502710]
66. Britto PJ, Knipling L, Wolff J. The local electrostatic environment determines cysteine reactivity of tubulin. J. Biol. Chem. 2002; 277:29018–29027. [PubMed: 12023292]
67. McLeish MJ, Kenyon GL. Relating structure to mechanism in creatine kinase. Crit. Rev. Biochem. Mol. Biol. 2005; 40:1–20. [PubMed: 15804623]
68. Chen LH, Borders CL Jr. Vasquez JR, Kenyon GL. Rabbit muscle creatine kinase: consequences of the mutagenesis of conserved histidine residues. Biochemistry. 1996; 35:7895–7902. [PubMed: 8672491]
69. Sfakianos MK, Wilson L, Sakalian M, Falany CN, Barnes S. Conserved residues in the putative catalytic triad of human bile acid coenzyme A:amino acid N-acetly transferase. J. Biol. Chem. 2002; 277:47270–47275. [PubMed: 12239217]
70. Huang J, Berger SL. The emerging field of dynamic lysine methylation of non-histone proteins. Curr. Op. Cell Biol. 2008; 18:152–158.
71. Yang W, Sheng H, Homi HM, Warner DS, Paschen W. Cerebral ischemia/stroke and small ubiquitin-like modifier (SUMO) conjugation – a new target for therapeutic intervention? J. Neurochem. 2008; 106:989–999. [PubMed: 18410505]
72. Yang X-J, Gregoire S. Metabolism, cytoskeleton and cellular signaling in the grip of protein N^{ε}- and O-acetylation. Mole. Cell. 2007; 31:449–461.
73. Yang X-J, Seto E. Lysine acetylation: codified crosstalk with other posttranslational modifications. Mole. Cell. 2008; 31:449–461.
74. Hammond JW, Cai D, Verhey KJ. Tubulin modifications and their cellular functions. Curr. Op. Cell Biol. 2008; 20:71–76. [PubMed: 18226514]
75. Kim SC, Sprung R, Chen Y, Xu Y, Ball H, Pei J, Tzuling C, Kho Y, Xiao H, Grishin NV, White M, Yang X-J, Zhao Y. Substrate and functional diversity of lysine acetylation revealed by a proteomics survey. Mole. Cell. 2006; 23:607–618.
76. Kouzarides T. Acetylation: a regulatory modification to rival phosphorylation? EMBO J. 2000; 19:1176–1179. [PubMed: 10716917]
77. Dinkova-Kostova AT, Massiah MA, Bozak RE, Hicks RJ, Talalay P. Potency of Michael reaction acceptors as inducers of enzymes that protect against carcinogenesis depends on their reactivity with sulfhydryl groups. Proc. Natl. Acad. Sci. 2001; 98:3404–3409. [PubMed: 11248091]
78. Satoh T, Okamoto S-I, Cui J, Watanabe Y, Furuta K, Suzuki M, Tohyama K, Lipton SA. Activation of the keap1/nrf2 pathway for neuroprotection by electrophilic phase II inducers. Proc. Natl. Acad. Sci. 2006; 103:768–773. [PubMed: 16407140]
79. Wakabayashi N, Dinkova-Kostova AT, Holtzman WD, Kang M-I, Kobayaski A, Yamamoto M, Kensler TW, Talalay P. Protection against electrophile and oxidant stress by induction of the phase 2 response: fate of cysteines of the Keap1 sensor modified by inducers. Proc. Natl. Acad. Sci. 2004; 101:2040–2045. [PubMed: 14764894]
80. Dinkova-Kostova AT, Holtzclaw WD, Kensler TW. The role of Keap1 in cellular protective responses. Chem. Res. Toxicol. 2005; 18:1779–1791. [PubMed: 16359168]

81. Forman HJ, Fukuto JM, Torres M. Redox signaling: thiol chemistry defines which reactive oxygen and nitrogen species can act as second messengers. Am. J. Physiol Cell Physiol. 2004; 287:C246–C256. [PubMed: 15238356]
82. Forman HJ, Fukuto JM, Miller T, Zhang H, Rinna A, Levy S. The chemistry of cell signaling by reactive oxygen and nitrogen species and 4-hydroxynonenal. Arch. Biochem. Biophys. 2008; 477:183–195. [PubMed: 18602883]
83. Hess DT, Matsumoto A, Kim S-O, Marshal HE, Stamler JS. Protein S-nitrosylation: purview and parameters. Nat Rev. 2005; 6:150–166.
84. Mannick JB, Schonhoff CM. Nitrosylation: the next phosphorylation. Arch. Biochem. Biophys. 2002; 408:1–6. [PubMed: 12485597]
85. Stamler JS, Lamas S, Fang FC. Nitrosylation: the prototypic redox-based signaling mechanism. Cell. 2001; 106:675–683. [PubMed: 11572774]
86. Hess DT, Matsumoto A, Nudelman R, Stamler JS. S-nitrosylation: spectrum and specificity. Nat Cell Biol. 2001; 3:E46–E49. [PubMed: 11175760]
87. Stamler JS, Toone EJ, Lipton SA, Sucher NJ. (S)NO signals: translocation, regulation and a consensus motif. Neuron. 1997; 18:691–696. [PubMed: 9182795]
88. Carbone DL, Doorn JA, Petersen DR. 4-Hydroxynonenal regulates 26S proteasomal degradation of alcohol dehydrogenase. Free Rad. Biol. Med. 2004; 37:1430–1439. [PubMed: 15454282]
89. Carbone DL, Doorn JA, Kiebler Z, Ickes BR, Petersen DR. Modification of heat shock protein 90 by 4-Hydroxynonenal in rat model of chronic alcoholic liver disease. J. Pharmacol. Exp. Ther. 2005; 315:8–15. [PubMed: 15951401]
90. Codreanu SG, Zhang B, Sobecki SM, Billheimer DD, Liebler DC. Global analysis of protein damage by the lipid electrophile 4-hydroxy-2-nonenal. Mole. Cell. Prot. 2009 In press.
91. Uchida K, Toyokuni S, Nishikawa K, Kawashaki S, Oda H, Hiai H, Stadtman ER. Michael addition-type-4-hydroxy-2-nonenal adducts in modified low-density lipoproteins: markers for atherosclerosis. Biochem. 1994; 33:12487–12494. [PubMed: 7918471]
92. Stewart BJ, Doorn JA, Petersen DR. Residue-specific adduction of tubulin by 4-hydroxynonenal and 4-oxononenal causes cross-linking and inhibits polymerization. Chem. Res. Toxicol. 2007; 20:1111–1119. [PubMed: 17630713]
93. Carbone DL, Doorn JA, Kiebler Z, Sampey BP, Petersen DR. Inhibition of Hsp72-mediated protein refolding by 4-Hydroxynonenal. Chem. Res. Toxicol. 2004; 17:1459–1467. [PubMed: 15540944]
94. Eliuk SM, Renfrow MB, Shonsey EM, Barnes S, Kim H. Active site modifications of the brain isoform of creatine kinase by 4-hydroxy-2-nonenal correlate with reduced enzyme activity: mapping of modified sites by Fourier transform-ion cyclotron resonance mass spectrometry. Chem. Res. Toxicol. 2007; 20:1260–1268. [PubMed: 17696488]
95. Poli G, Schaur RJ. 4-Hydroxynonenal in the pathomechanisms of oxidative stress. IUBMB life. 2000; 50:315–321. [PubMed: 11327326]
96. Klapper MH. Biochem. Biophys. Res. Commun. 1977; 78:1018–1024. [PubMed: 911323]
97. Barber DS, Stevens S, LoPachin RM. Proteomic analysis of rat striatal synaptosomes during acrylamide intoxication at a low dose-rate. Toxicol. Sci. 2007; 100:156–167. [PubMed: 17698512]
98. LoPachin RM, Schwarcz AI, Gaughan CL, Mansukhani S, Das S. In vivo and in vitro effects of acrylamide on synaptosomal neurotransmitter uptake and release. NeuroToxicology. 2004; 25:349–363. [PubMed: 15019298]
99. LoPachin RM, Gavin T. Acrylamide-induced nerve terminal damage: relevance to neurotoxic and neurodegenerative mechanisms. J. Agric. Food Chem. 2008; 56:5994–6003. [PubMed: 18624437]
100. Montine KS, Kim PJ, Olson SJ, Markesbery WR, Montine TJ. 4-hydroxy-2-nonenal pyrrole adducts in human neurodegenerative disease. J. Neuropathol. Exp. Neurol. 1997; 56:866–871. [PubMed: 9258256]
101. Siems W, Grune T. Intracellular metabolism of 4-hydroxynonenal. Mol. Aspects Med. 2003; 24:167–175. [PubMed: 12892994]
102. LoPachin RM, Ross JF, Lehning EJ. Nerve terminals as the primary site of acrylamide action: A hypothesis. NeuroToxicology. 2002; 23:43–60. [PubMed: 12164547]

103. Jenner P. Oxidative stress in Parkinson's disease. Ann. Neurol. 2003; 53:S26–S38. [PubMed: 12666096]
104. Montine TJ, Neely MD, Quinn JF, Beal FM, Markesbery WR, Roberts LJ, Morrow J. Lipid peroxidation in aging brain and Alzheimer's disease. Free Rad. Biol. Med. 2002; 33:620–626. [PubMed: 12208348]
105. Weight SC, Bell PRJ, Nicholson ML. Renal ischaemia-reperfusion injury. Br. J. Surg. 1996; 83:162–170. [PubMed: 8689154]
106. Beauchamp RO, Andjelkovich DA, Kligerman AD, Morgan KT, Heck H. d'A. A critical review of the literature on acrolein toxicity. Crit. Rev. Toxicol. 1985; 14:309–380. [PubMed: 3902372]
107. Izard C, Libermann C. Acorlein. Mut. Res. 1978; 47:115–138. [PubMed: 415230]
108. Zarkovic K. 4-Hdroxynonenal and neurodegenerative diseases. Mol. Asp. Med. 2003; 24:293–303.
109. Adams JD, Klaidman LK. Acrolein-induced oxygen radical formation. Free Rad. Biol. Med. 1993; 15:187–193. [PubMed: 8397144]
110. Kinter M, Roberts RJ. Glutathione consumption and glutathione peroxidase inactivation in fibroblast cell lines by 4-hydroxy-2-nonenal. Free Rad. Biol. Med. 1996; 21:457–462. [PubMed: 8886795]
111. Luo J, Shi R. Acrolein induces oxidative stress in brain mitochondria. Neurochem. Internl. 2005; 46:243–252.
112. Yousefipour Z, Ranganna K, Newaz MA, Milton SG. Mechanism of acrolein-induced vascular toxicity. J. Physiol. Pharmacol. 2005; 56:337–353. [PubMed: 16204758]
113. Biswal S, Acquaah-Mensah G, Datta K, Wu X, Kehrer JP. Inhibition of cell proliferation and AP-1 activity by acrolein in human A549 lung adenocarcinoma cells due to thiol imbalance and covalent modifications. Chem. Res. Toxicol. 2002; 15:180–186. [PubMed: 11849044]
114. Biswal S, Maxwell T, Rangaswamy T, Kehrer JP. Modulation of benzo[a]pyrene-induced p53 DNA activity by acrolein. Carcinogenesis. 2003; 24:1401–1406. [PubMed: 12807757]
115. Grafström RC, Dypbukt JM, Willey JC, Sundqvist K, Edman C, Atzori L, Harris CC. Pathological effects of acrolein in cultured human bronchial epithelial cells. Cancer Res. 1988; 48:1717–1721. [PubMed: 3349453]
116. Ku RH, Billings RE. The role of mitochondrial glutathione and cellular protein sulfhydryls in formaldehyde toxicity in glutathione-depleted rat hepatocytes. Arch Biochem Biophys. 1986; 247:183–189. [PubMed: 3707139]
117. Patel JM, Block ER. Acrolein-induced injury to cultured pulmonary artery endothelial cells. Tox Appl Pharmacol. 1993; 122:46–53.

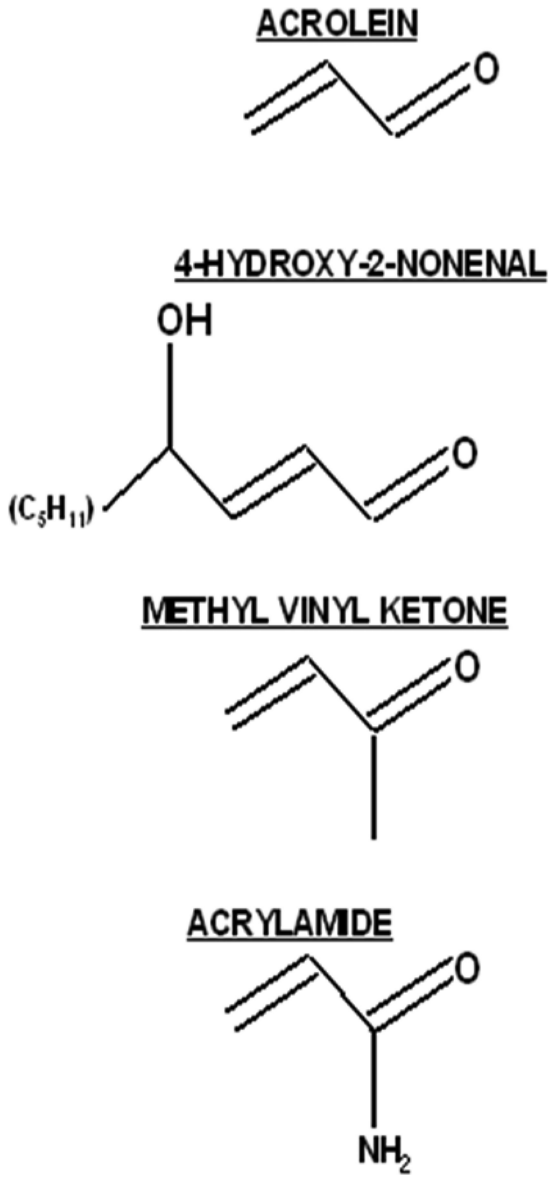

Figure 1.

This figure presents line structures for acrolein, HNE and several structurally related α,β-unsaturated carbonyl derivatives of the type-2 alkene chemical class.

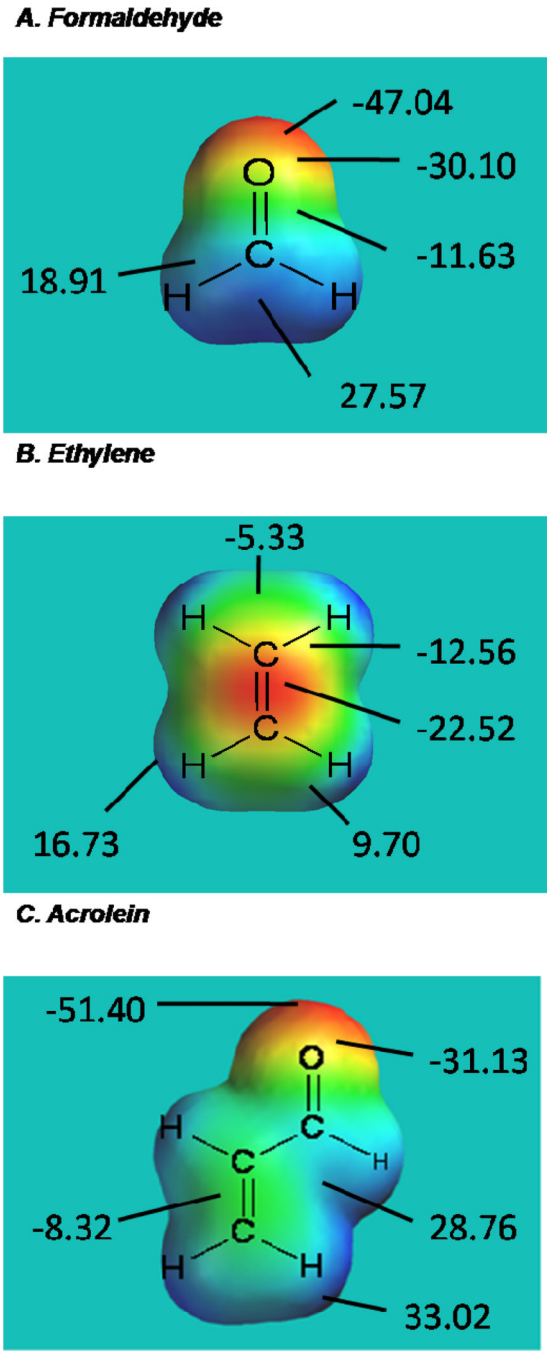

Figure 2.

The concept of soft electrophilicity is illustrated in this figure, which shows the color-coded electrostatic potential maps for formaldehyde (2A), ethylene (2B) and acrolein (2C; calculated using Spartan ’04, Wavefunction Inc., Irvine, CA). The line structures for each chemical are inserted within the corresponding potential figure. The color gradient for each map illustrates how charge is distributed across the molecule and, therefore, indicates the relative degree to which the corresponding atoms attract oppositely charged atoms. Accordingly, red signifies the most negative electrostatic potential and is used for regions

that attract positively charged molecules most strongly. Blue denotes areas with the most positive electrostatic potential and is used for regions that attract negatively charged molecules most strongly. The orange-yellow-green spectrum indicates intermediate (from negative to positive, respectively) electrostatic potential. For each chemical, a numerical example of local electron density or distribution (expressed as kJ/mol) is provided for each color. If we first consider the relatively simple polar covalent bond of formaldehyde (2A), it is clear that the highly electronegative oxygen atom (Pauling electronegativity = 3.44) draws electron density as indicated by the localized red-colored zone (-47.04) from the less electronegative carbon (2.55) and hydrogen (2.20) atoms. Here, the resulting electron deficiency of the carbon-hydrogen bonds is reflected in the green-blue gradient; i.e., respective electron density from -11.63 to 27.57. We next consider the carbon-carbon double bond of ethylene (2B). A red-yellow gradient (from –22.52 to –12.56, respectively) is centered over the double bond, which indicates the covalent sharing of electron density between two atoms (carbon) of equal electronegativity. Acrolein (2C) combines the carbonyl of formaldehyde and the carbon-carbon double bond of ethylene, which is considered to be a conjugated system. As the corresponding red color-coding indicates, the electronegative carbonyl oxygen atom has withdrawn electron density (-51.40) from the normally electron rich carbon-carbon double bond (see the ethylene double bond; 2B). As a result, the β-carbon atom becomes an electron deficient or electrophilic center (green = -8.32). Such electron delocalization is possible because the π orbitals of the conjugated α,β-unsaturated carbonyl structure overlap. Consequently, the respective π electrons are mobile or polarizable and can, therefore, relocate to the electronegative oxygen atom. The quantum mechanical parameter softness (σ), is an index of π electron mobility and, based on their respective σ values (Table 1), acrolein and HNE are relatively soft electrophiles that will rapidly form adducts with sulfhydryl groups.

Table 1

Calculated Quantum Mechanical Parameters for α,β-Unsaturated Carbonyl Derivatives and Non-Conjugated Analogs

Conjugated alkenes	E_{LUMO} (ev)	σ (ev^{-})	ω (ev)
NEM	−2.36	0.406	4.73
Acrolein	−1.70	0.379	3.57
HNE	−1.53	0.381	3.29
MVK	−1.33	0.372	3.00
MA	−1.01	0.315	2.76
ACR	−0.69	0.329	2.30
Non-conjugated analogs			
Propanal	−0.33	0.307	1.98
Allyl alcohol	+0.51	0.269	1.39

The Lowest Unoccupied Molecular Orbital (LUMO) energy (E_{LUMO}) and Highest Occupied Molecular Orbital (HOMO) energy (E_{HOMO}), were calculated using Spartan04 (version 1.0.3) software (Wavefunction Inc., Irvine CA). Global (whole molecule) hardness (η) was calculated as $\eta = (E_{LUMO}-E_{HOMO})/2$ and softness (σ) was calculated as the inverse of hardness or $\sigma = 1/\eta$. The electrophilicity index (ω) was calculated as $\omega = \mu^2/2\eta$, where μ is chemical potential of the electrophile and was calculated as $\mu = (E_{LUMO}+E_{HOMO})/2$ (For details see 42). Abbreviations: NEM = *N*-ethylmaleimide, HNE = 4-hydroxy-2-nonenal, MVK = methyl vinyl ketone, MA = methyl acrylate and ACR = acrylamide.

Table 2

Calculated Quantum Mechanical Parameters for Nucleophilic Amino Acids

Amino Acid Residue	E_{HOMO} (ev)	μ (ev)	σ (ev)
[a]Cysteine thiol (0)	−5.87	−2.87	0.330
Histidine (0)	−5.75	−2.75	0.331
Lysine (+1)	−10.39	−6.69	0.270
[b]Cysteine thiolate (−1)	−0.35	2.21	0.391
Histidine (+1)	−10.03	−7.31	0.368
Lysine (0)	−5.59	−2.60	0.334

[a]For each nucleophile, quantum mechanical parameters were calculated based on the predominant ionization-state (in parentheses) at pH 7.4.

[b]For each residue, quantum mechanical parameters were calculated based on the predominant ionization-state (in parentheses) in a catalytic triad with cysteine as the central nucleophile. E_{LUMO} (not shown) and E_{HOMO} values were used to calculate the chemical potential (μ) of the nucleophile and corresponding softness (σ). Global (whole molecule) softness (σ) was calculated as the inverse of hardness or $\sigma = 1/\eta$. The chemical potential (μ) was calculated as $(E_{LUMO}+E_{HOMO})/2$ (see 42 for details).

Table 3

Calculated Nucleophilic Indices (ω^-) for Type-2 Alkene Reactions With Possible Nucleophilic Targets

Electrophile[a]	ω^- Cys (−1)	ω^- Cys (0)	ω^- His (0)	ω^- Lys (+1)
NEM	2.51	0.194	0.250	0.277
Acrolein	2.03	0.103	0.123	0.253
HNE	1.93	0.083	0.102	0.287
MVK	1.83	0.064	0.081	0.319
MA	1.59	0.069	0.063	0.332
ACR	1.50	0.036	0.048	0.346

The nucleophilicity index (ω^-) was calculated as $\omega^- = \eta_A (\mu_A - \mu_B)^2/2(\eta_A - \eta_B)^2$, where $\eta = (E_{LUMO}-E_{HOMO})/2$, $\mu = (E_{LUMO}+E_{HOMO})/2$, A = reacting nucleophile and B = reacting electrophile (see 42 for details). For each nucleophile, the respective ionization-state is presented in parentheses. The nucleophilicity index is a higher order parameter that considers the respective hardness and chemical potential of the electrophilic (type-2 alkene) and nucleophilic (cysteine, histidine or lysine) reactants and is, therefore, a measure of the likelihood of subsequent adduct formation. As suggested by the respective ω-values, the type-2 alkenes preferentially form adducts with cysteine thiolate sites as opposed to histidine, lysine or thiol residues. Abbreviations: NEM = *N*-ethylmaleimide, HNE = 4-hydroxy-2-nonenal, MVK = methyl vinyl ketone, MA = methyl acrylate and ACR = acrylamide.

Table 4

Type-2 Alkene Reactivity: Comparisons of Nucleophilic Indices (ω^-), Thiolate Rate Constants (k_{RS}-) and Neurotoxic Potencies (IC_{50}'s).

Electrophile	ω^-Cys (−1)	log k_2	log k_{RS}-	log IC_{50}
NEM	2.51	6.536	7.912	−4.33
Acrolein	2.03	2.596	3.417	−4.28
HNE	1.93	0.938	1.759	−3.40
MVK	1.83	2.048	2.953	−3.48
MA	1.59	−0.936	1.011	−0.34
ACR	1.50	−1.804	0.767	−0.36

Second order rate constants (k_2) were determined for type-2 alkene reactions with L-cysteine at pH 7.4 (n=4-6 experiments). The k_2 values at pH 7.4 were corrected for the corresponding cysteine thiolate concentration (k_{RS}-) according to the algorithm: log (k_{RS}- -k_2) = log k_2 + pKa – pH. Inhibition of membrane ^{3}H-dopamine transport was determined in rat striatal synaptosomes exposed in vitro to graded concentrations of each type-2 alkene. The concentration-response data for transport were fitted by nonlinear regression analysis and the respective IC_{50}'s were calculated by the Cheng-Prusoff equation (see 41-43 for methodological details).