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COMMENTARY

DNA adducts of chemical carcinogens

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Introduction

During the 30 years or so following the identification of the first pure chemical carcinogen (1), no common factors or pathways in the mechanism of action of carcinogens from different chemical classes were evident. For this reason perhaps, each class of carcinogen, e.g. the polycyclic aromatic hydrocarbons, the aromatic amines and the nitrosamines, was often reviewed and discussed separately. The discovery by the Millers and their colleagues of the role of metabolism in carcinogen activation (2) revealed a commonality amongst many carcinogens, i.e. a chemical reactivity towards cellular macromolecules, such as DNA (3,4). Today, DNA adduct formation is recognized as a common property of most potent carcinogens and the formation of such adducts is the basis of several current strategies in molecular epidemiology and biomonitoring. Despite this common aspect of mechanism for many chemical carcinogens, the complexities of metabolic activation and of the chemistry and stereochemistry of adduct formation have tended to keep some degree of compartmentalization in research and in literature reviews of DNA adduct formation by different classes of chemical compounds (5).

In this article, an attempt is made to summarize carcinogen adduct formation in a fashion that emphasizes features that are common to different chemical classes. This perspective is achieved by classifying chemical carcinogens on the basis of the chemistry of DNA adduct formation in aqueous solution rather than on chemical structure. Adopting this approach, the major division of chemical carcinogens into those intrinsically reactive towards DNA, such as alkylating agents, and those requiring metabolic activation, such as nitrosamines for example, disappears. While mechanisms of metabolic activation remain important concerns, alkylation of DNA is the chemical mechanism through which both types of agent, exemplified above, form adducts with DNA. Indeed, examination of the literature on adduct formation (reviewed in 5), indicates that the majority of known carcinogens react with DNA through one of only three general types of chemical reaction. These involve the transfer to DNA of either: (i) an alkyl residue; (ii) an arylamine residue; or (iii) an aralkyl residue.

Metabolic activation

For intrinsically reactive carcinogens, division into the three categories above can be substantiated by an inspection of carcinogen structures. For other carcinogens, examination of the known metabolic activation reactions, along with the type of reactive species generated is necessary to evaluate the type of residue transferred to DNA (see Figure 1). Review of this information indicates that oxidations at carbon–carbon double bonds yield alkylating or arylalkylating agents, depending on the structural context of the bond, i.e. aliphatic or aromatic. Similarly, the surrounding structure determines whether an alkylating or aralkylating agent results from oxidation at a saturated carbon atom. In this latter reaction, the oxidation itself may be sufficient to generate a reactive entity, e.g. oxidation of an α-carbon atom in a nitrosamine. Alternatively, a further esterification step could be required to make the hydroxyl group into a better leaving group. Oxidations of aromatic amines or amides or reductions of aromatic nitro compounds both yield agents that transfer an arylamine residue to DNA (referred to here as arylaminating agents), either directly or after esterification. Finally, conjugations, as already described for various hydroxy compounds, lead to aralkylating or arylaminating agents and conjugation between glutathione and dihaloalkanes can lead to alkylating agents.

There are, of course, a few carcinogens that do not fit into the three categories for reaction with DNA, e.g. acylating agents, α,β-unsaturated aldehydes and agents such as chloroethylene oxide. However, these few exceptions do not invalidate any insights that might emerge from examination of the

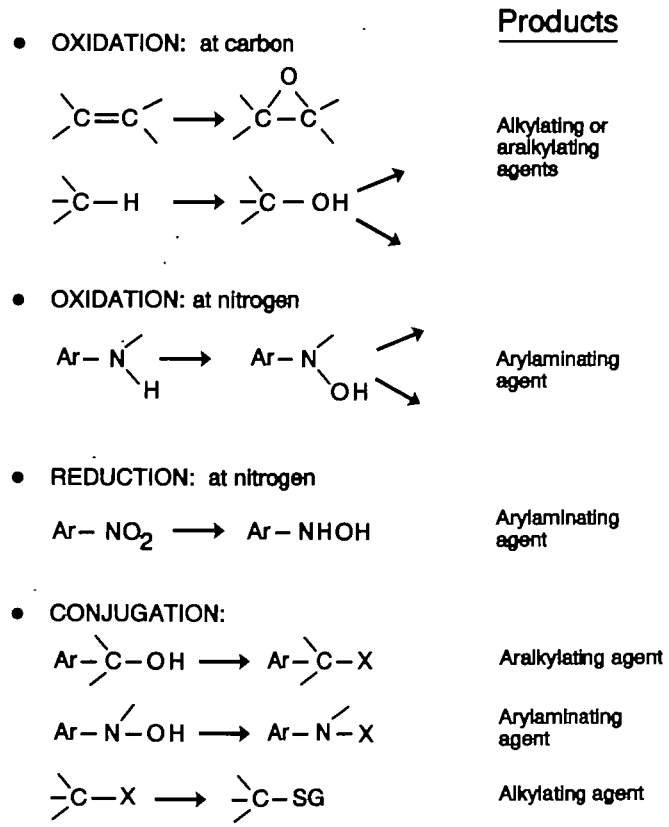

Fig. 1. Summary of the types of metabolic reactions that have been associated with the generation of reactive ultimate carcinogens.

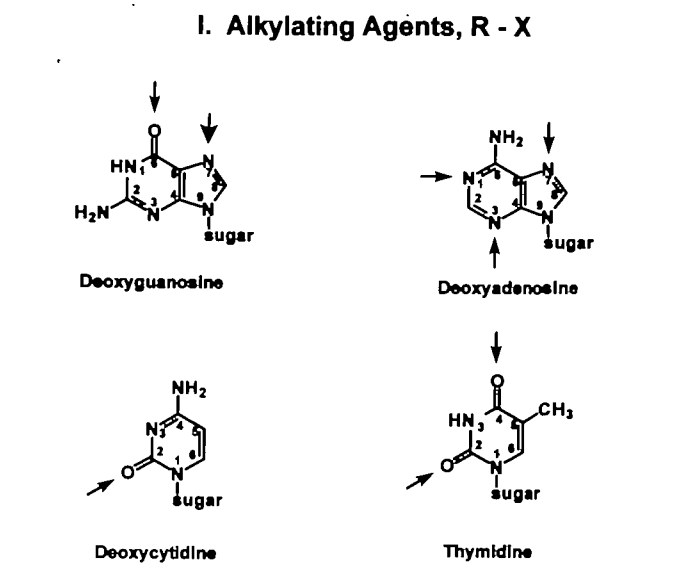

Fig. 2. Summary of the sites on DNA bases modified by alkylating agents.

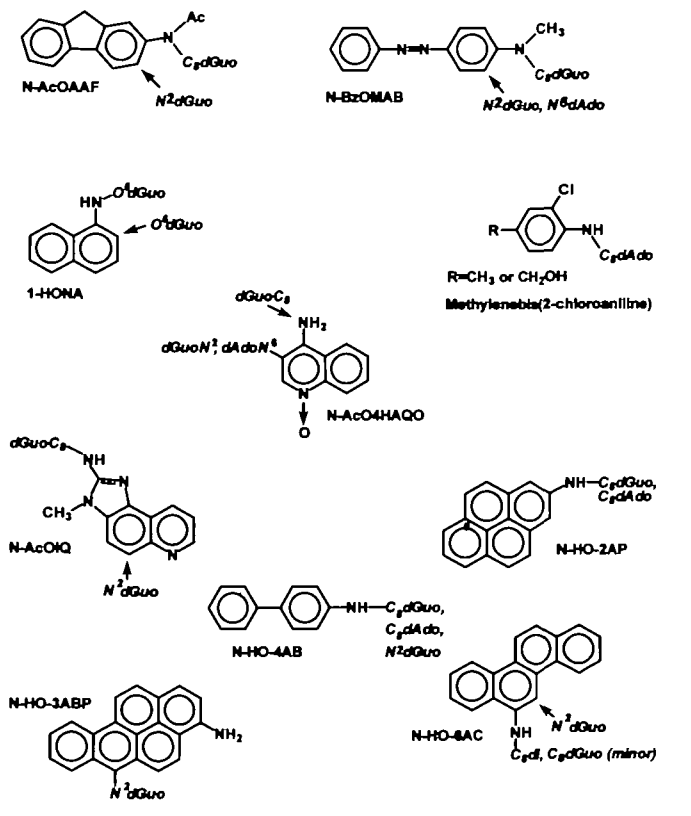

Fig. 3. Reaction products resulting from transfer of arylamine residues to DNA. The major product formed is indicated by a bond to a specific site on deoxyguanosine (dGuo), deoxyadenosine (dAdo) or deoxyinosine (dI). Secondary products are separated from the primary product by a comma if the same site on the carcinogen is involved or indicated by an arrow pointing to an alternative site. N-AcOAAF, *N*-acetoxy-2-acetylaminofluorene; N-BzOMAB, *N*-benzoyloxymethylaminoazobenzene; 1-HONA, 1-naphthylhydroxylamine; N-AcO4HAQO, *N*-acetoxy-4-hydroxylaminoquinoline-*N*-oxide; N-AcOIQ, *N*-acetoxy-2-amino-3-methylimidazo[4,5-*f*]quinoline; N-HO-4AB, *N*-hydroxy-4-aminobiphenyl; N-HO-2AP, *N*-hydroxy-2-aminopyrene; N-HO-3ABP, *N*-hydroxy-3-aminobenzo[*a*]pyrene; N-HO-6AC, *N*-hydroxy-6-aminochrysene. Information was taken from articles by Kadlubar, by Turesky and by Beland and Marques in (5).

great majority of carcinogens in the context of their DNA adduct formation chemistry.

Alkylation

Carcinogens that transfer alkyl residues (these may contain quite complex structures including an aromatic system not conjugated with the site of substitution) to DNA include the nitrosamines, aliphatic epoxides, aflatoxins, lactones, nitrosoureas, mustards, haloalkanes, alkyl triazenes and sultones. The sites of substitution of DNA bases by alkyl residues are numerous and are illustrated in Figure 2 by the presence of arrows that have been placed at all sites that account for at least 1% of total alkylation in reactions of DNA with methyl methanesulfonate, ethyl methanesulfonate, *N*-methyl-*N*-nitrosourea or *N*-ethyl-*N*-nitrosourea (6). Most ring nitrogen atoms and exocyclic oxygen atoms are targets for alkylation, with the 7-position of guanine (large arrow in Figure 2) frequently being the site that is modified most extensively. Notably, the exocyclic amino groups are not effectively targeted by the alkylating agents.

Individual alkylating agents distribute themselves over the target sites summarized in Figure 2 in different fashions of course, and this is a major determinant of the subsequent biological effect. This latter was noted initially by Loveless (7), who attributed mutagenic properties of alkylating agents to their capacities for alkylation at the exocyclic oxygen of guanine residues. More recently, it has become clear that alkylation at the exocyclic oxygens of thymine residues is also important in mutagenesis (8). In general, it seems that alkylating agents that are not particularly ionic in nature are localized more on the ring nitrogen atoms, whereas those that have greater ionic character show greater preferences for reaction at the oxygen atoms in DNA (6,9).

Another complexity of alkylation chemistry is that the ring nitrogen-substituted products are all unstable in some fashion and thus biological effects can be influenced by chemical transformations secondary to the initial DNA alkylation. Examples of such instability are the fairly facile depurination and imidazole ring-opening of 7-substituted deoxyguanosine residues and the ready depurination of 3- and 7-alkyldeoxyadenosines (10).

Arylamination

The most heterogeneous group of carcinogens are those that transfer an arylamine residue to DNA. This group includes, for example, the aromatic amines and amides, the aminoazo dyes, the nitroaromatics and the heterocyclic aromatic amines found in trace amounts in cooked fish and meats. The structures of adducts formed from some of these compounds are summarized in Figure 3. In this figure, the major adduct formed is displayed and the structures of other adducts formed are indicated by an arrow linking the sites of substitution on the DNA nucleoside with that on the reactive carcinogen. Although the site of substitution on the nucleoside in the major product varies substantially, it seems initially that the sites of substitution on deoxyribonucleosides (Figure 4) form a pattern that is very different from that displayed by the alkylating agents (Figure 2). Thus, the C-8 atoms and the amino groups of the purine nucleosides, particularly of deoxyguanosine, are the major targets for the arylaminating agents. These sites are unaffected by alkylating agents.

Important recent studies (11) have suggested that the aromatic amine adducts formed at C-8 of deoxyguanosine arise from a 7-substituted deoxyguanosine precursor, indicating that

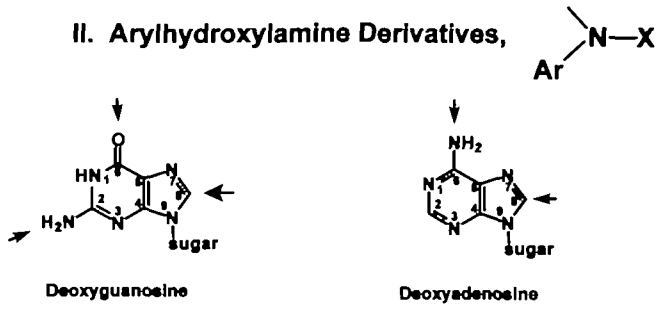

Fig. 4. Sites of substitution of DNA bases by arylaminating agents.

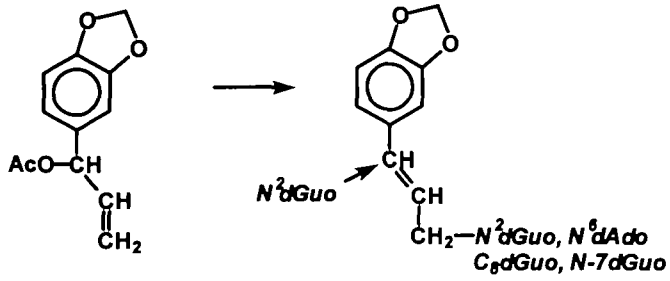

Fig. 5. DNA adduct formation by 1′-acetoxysafrole, taken from Wiseman *et al.* (13).

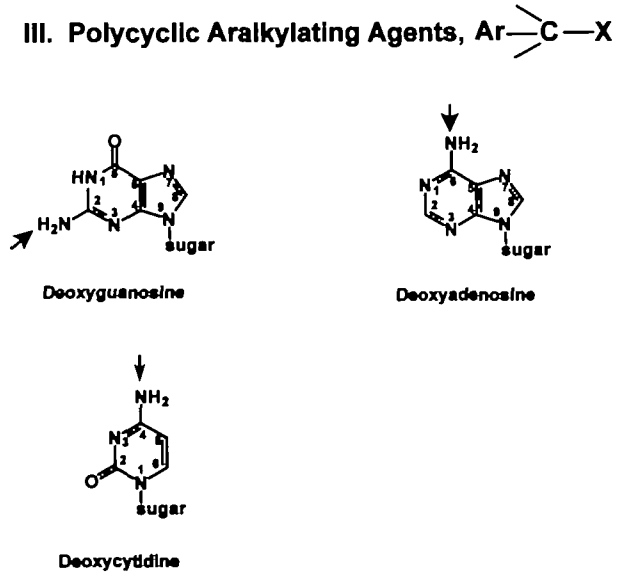

Fig. 6. Sites of substitution of DNA bases by polycyclic aralkylating agents.

a reactivity towards the 7-position of deoxyguanosine residues may be common to both the alkylating and arylaminating agents. In these recent studies, an adduct in which the nitrogen of an aromatic amine was bound to the N-7 position of an 8-methylguanine derivative was characterized.

The products of arylamination of DNA are more stable than alkylation products, but some C-8 substituted deoxyguanosine adducts undergo 8,9-purine ring-opening fairly readily (12).

Aralkylation

Carcinogens that transfer an aralkyl group to DNA include the pyrrolizidine alkaloids, alkenyl benzenes, the large group of polycyclic aromatic hydrocarbons and those nitroaromatics

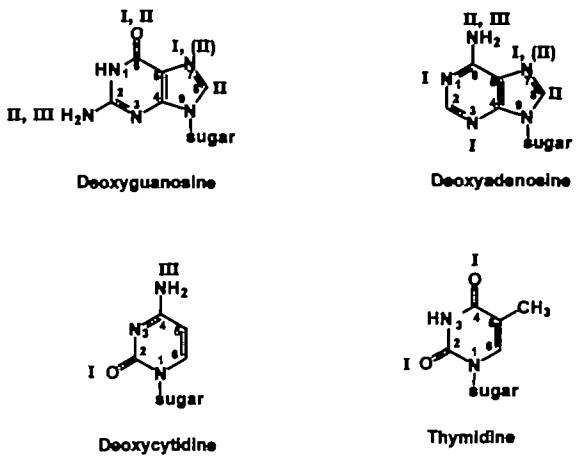

Fig. 7. Sites of substitution of DNA bases by genotoxic carcinogens. Sites modified by alkylating agents are marked by the numeral I, those modified by arylaminating agents by a II and those modified by polycyclic aralkylating agents by a III. Since it has been suggested that C-8 substituted arylamino adducts may have arisen from N-7 substituted precursors (11), the arylaminating agents are listed parenthetically at the 7-position of the purines.

that are activated through the dihydrodiol epoxide mechanism. The alkenyl benzenes react with DNA in a fashion that is not dissimilar to the reactions of the arylaminating agents. Thus, 1′-acetoxysafrole reacts with DNA through the formation of adducts at the amino group of deoxyguanosine residues and product is also formed at the C-8 position. As indicated in Figure 5, most product formation with the amino group of deoxyguanosine arises at a carbon conjugated to the reactive center, rather than at that center itself, thus showing a strong resemblance to the arylaminating agents described above (13).

The polycyclic aralkylating agents, typified by the dihydrodiol epoxides of the polycyclic aromatic hydrocarbons, exhibit a chemistry of DNA adduct formation that seems distinct from that of the agents summarized so far. The reactions of the hydrocarbon dihydrodiol epoxides with DNA result primarily in modification of the exocyclic amino groups of deoxyadenosine and deoxyguanosine residues [minor products at N-7 of guanine (14) and the amino group of deoxycytidine (15) have been characterized in isolated cases], as shown in Figure 6. With a few exceptions (16), these adducts are relatively stable and, as for the other classes of reaction discussed here, the distribution of hydrocarbon residues over the target sites indicated in Figure 6 varies substantially with the structure of the hydrocarbon. Dihydrodiol epoxides derived from planar hydrocarbons react predominantly at the amino group of deoxyguanosine residues in DNA. However, dihydrodiol epoxides derived from hydrocarbons that are substantially distorted from planarity, by the presence of a fjord region or by a substituent methyl group in the bay region, react extensively at the amino groups of both deoxyguanosine and deoxyadenosine (17). (There have been reports of hydrocarbon reactions with DNA through one-electron oxidation products (18), but the relevance of these reactions to carcinogenesis is not yet clear.)

Conclusions

The sites of reaction of each of the three categories of agent discussed above are summarized together in Figure 7. In this figure, the possibility that the C-8 substituted products of the arylaminating agents might arise from precursors at the 7-

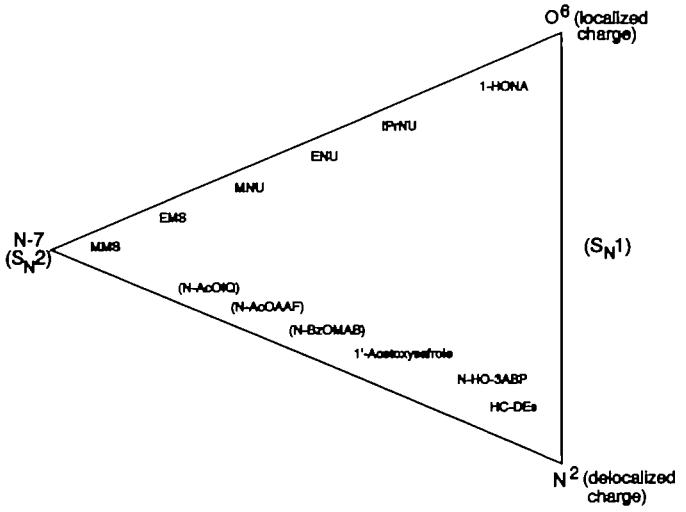

Fig. 8. Diagram summarizing the sites of reaction of various carcinogens on guanine residues in DNA. Reaction through an S_N2 mechanism is believed to result largely in 7-substituted guanine adducts, whereas more ionized intermediates give rise to O^6-substitution if charge is not readily delocalized and N^2 substitution if it is delocalized. MMS, methyl methanesulfonate; EMS, ethyl methanesulfonate; MNU, methylnitrosourea; ENU, ethylnitrosourea, iPrNU, isopropylnitrosourea; HC-DEs, polycyclic hydrocarbon dihydrodiol epoxides; 1-HONA, N-HO-3ABP, N-AcIOQ, N-AcOAAF and N-BzOMAB are defined in the legend to Figure 3.

position of purines has been indicated parenthetically. The main point that arises from this comparison is that sites of alkylation and arylamination might overlap, that sites of arylamination and polycyclic aralkylation do overlap (i.e. both agents modify amino groups), but that sites of alkylation and of polycyclic aralkylation do not overlap. Thus, if there is some overall continuum of mechanism governing these DNA adduct forming reactions, then the polycyclic aralkylating agents and the alkylating agents may be separated from one another in this continuum by the arylaminating agents, along with the monocyclic aralkylating agents.

Previously, based on extensive model studies of the benzylation of guanosine (9,19,20), the formation of different alkylation and polycyclic aralkylation products was rationalized by proposing that the reactivity of alkylating and aralkylating agents should be viewed as areas on a two-dimensional reactivity surface that described the ionic character of the reactive agent in one dimension, i.e. its S_N2 or S_N1 character, and the ability of the developing ion to localize or delocalize charge (21) in a second dimension. Figure 8 reproduces a similar analysis wherein each corner of a triangle represents reactivity toward one of the three major sites of reaction on guanine residues in DNA, i.e. the N-7, O^6, and N^2 sites, and the closer an agent is placed to a given corner, the more predominant is that site of reaction. Agents are known that react fairly exclusively with each of these sites, i.e. the simple alkylating agents, such as methyl methane sulfonate, yield principally N-7 substituted products (6), 1-naphthylhydroxylamine is reported to react exclusively at the O^6 position (22) and the dihydrodiol epoxides of the polycyclic aromatic hydrocarbons (17) and 3-benzo[*a*]pyrenylhydroxylamine (23) react exclusively at the N^2 position. In the reactivity diagram, these chemicals are positioned, therefore, close to the appropriate corners of the diagram.

Other reactive chemicals show a broader specificity in their reactions with guanine residues in DNA. For example, the acetoxy derivatives of IQ and of acetylaminofluorene react primarily at the C-8 of guanine, but also form some N^2-substituted adducts. If these agents react through an N-7 precursor, they could be placed between the N-7 and N^2 corners of the diagram. Similarly, the alkylating agents that modify both the N-7 and O^6 sites in guanine residues are placed between the N-7 and O^6 corners of the diagram. Any agent that reacts with one or more of these three sites on guanine residues could be placed on this diagram such that its preference for reaction at a given site is roughly inversely proportional to the distance from the corner representing that site.

It might be reasonable to assume that some mechanistic chemical rules govern the location of the various reactive carcinogen derivatives in this diagram. For the specific cases of the alkylating and aralkylating agents, we have previously suggested that the horizontal dimension of this diagram might measure the ionic character of the reactive species and that the vertical dimension might measure the ability to delocalize charge in any partially or fully ionized species (as indicated in parentheses in Figure 8) (21,24). The realization that the arylaminating agents might initially attack the N-7 position of guanine in forming C-8 adducts has allowed these agents to be provisionally included in this diagram and it is conceivable then that the majority of DNA adduct forming reactions with exogenous carcinogens may all be controlled by the same general chemical considerations. An important point that has been made before (9) and reiterated recently (25) is that the products of these DNA adduct forming reactions are not readily accounted for by any one-dimensional measure of chemical reactivity.

The adducts formed with deoxyadenosine residues in DNA could presumably be placed on a similar reactivity diagram, although only sites analogous to the N-7 and N^2 sites in guanine residues are available in adenine residues (i.e. N-1 and N^6). There is not much information available at present, however, on the factors that determine the relative extents of reactions of carcinogens with deoxyguanosine versus deoxyadenosine residues in DNA and this is a topic that deserves further investigation.

In summary, although the chemistry of carcinogen–DNA adduct formation is complex, most carcinogens either alkylate, arylaminate or aralkylate DNA. The sites of alkylation and polycyclic aralkylation on DNA do not overlap, but monocyclic aralkylating agents (and possibly arylaminating agents) attack some sites that are targets for polycyclic aralkylating agents and some that are targets for simple alkylating agents. These observations suggest that carcinogen–DNA adduct formation follows some coherent pattern and, therefore, should become increasingly predictable as understanding of the basic chemistry continues to improve.

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