

Genotoxicity of Acrylamide and Glycidamide

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Background: Acrylamide, a known rodent carcinogen, is found in the human diet. However, the mechanism by which acrylamide exerts its carcinogenic effects remains unclear. **Methods:** Normal human bronchial epithelial cells and Big Blue mouse embryonic fibroblasts that carry a λ phage cII transgene were treated *in vitro* with acrylamide, its primary epoxide metabolite glycidamide, or water (control) and then subjected to terminal transferase-dependent polymerase chain reaction to map the formation of DNA adducts within the human gene encoding p53 (TP53) and the cII transgene. The frequency and spectrum of glycidamide-induced mutations in cII were examined by using a λ phage-based mutation detection system and DNA sequence analysis, respectively. All statistical tests were two-sided. **Results:** Acrylamide and glycidamide formed DNA adducts at similar specific locations within TP53 and cII, and DNA adduct formation was more pronounced after glycidamide treatment than after acrylamide treatment at all doses tested. Acrylamide-DNA adduct formation was saturable, whereas the formation of most glycidamide-DNA adducts was dose-dependent. Glycidamide treatment dose-dependently increased the frequency of cII mutations relative to control treatment ($P < .001$). Glycidamide was more mutagenic than acrylamide at any given dose. The spectrum of glycidamide-induced cII mutations was statistically significantly different from the spectrum of spontaneously occurring mutations in the control-treated cells ($P = .038$). Compared with spontaneous mutations in control cells, cells treated with glycidamide or acrylamide had more A→G transitions and G→C transversions and glycidamide-treated cells had more G→T transversions ($P < .001$). **Conclusion:** The mutagenicity of acrylamide in human and mouse cells is based on the capacity of its epoxide metabolite glycidamide to form DNA adducts. [J Natl Cancer Inst 2004;96:1023–9]

The recent discovery of acrylamide, a proven rodent carcinogen, in a variety of food products consumed by humans has raised public health concerns (1–5). Acrylamide finds its way into the human diet when amino acids and sugars present in food are heated at high temperature, i.e., during food processing (e.g., frying) (6,7). Substantial quantities of acrylamide are found in commonly consumed foods (e.g., breakfast cereals, french fries, and potato chips) and beverages (e.g., coffee) (1–4).

It has long been known that administration of acrylamide to laboratory animals results in the formation of tumors in various organs. For example, in carcinogenicity experiments, acrylamide administered via different routes increased the incidence of lung adenomas and initiated skin tumorigenesis in mice, and induced scrotal mesotheliomas, thyroid adenomas, mammary gland tumors, uterine adenocarcinomas, clitoral gland adenomas, and oral papillomas in rats [reviewed in (8)]. However, the mechanism by which acrylamide exerts these effects remains elusive (8). Indeed, organ-specific tumorigenicity of acrylamide is not always directly related to the extent to which acrylamide binds

DNA in the affected tissues, casting doubt on a possible genotoxic mechanism involved in the tumorigenicity of acrylamide (8). We recently investigated the biologic and biochemical fates of acrylamide administered *in vitro* to embryonic fibroblasts derived from mice that carry a λ phage cII transgene (9). We found that acrylamide interacts with DNA in the treated cells, giving rise to promutagenic acrylamide-DNA adducts. Because the induction of mutations in cancer-related genes such as TP53, the gene that encodes the tumor suppressor p53, may initiate the multistage process leading to tumorigenesis, we proposed that the DNA adduct- and mutation-inducing properties of acrylamide might be responsible for its tumorigenicity. Our observation that the sites of DNA adduct formation and induced mutations in the cII gene did not always coincide led us to believe that only a subset of DNA adducts induced by acrylamide are involved in its mutagenicity. Moreover, the non-dose dependency of DNA adducts and the higher induction of mutations at lower concentrations of acrylamide were indicative of a saturable process that generated the promutagenic DNA adducts (9).

Previous studies have documented the mutagenicity of glycidamide, an epoxy derivative of acrylamide and the primary metabolite of acrylamide, in Salmonella assays that either contained or lacked a microsomal activating system (10,11). In addition, results of kinetics studies have shown that epoxidation of acrylamide, which follows a Michaelis-Menten process, is saturated when the concentration of acrylamide exceeds the Michaelis constant (K_m) for its metabolizing enzyme, namely cytochrome P-450 2E1 (CYP2E1) (8,10,11). Furthermore, pretreatment with 1-aminobenzo-triazole, an inhibitor of CYP2E1, diminished or substantially reduced the frequency of acrylamide-induced dominant lethal mutations in spermatids of male mice (12).

In this study, we examined the mechanistic role of glycidamide in acrylamide-induced mutagenesis in Big Blue mouse embryonic fibroblasts. This transgenic system has an easily recoverable target gene that can be used for simultaneous detection of DNA adducts and mutations at the nucleotide level. The system is amenable to various experimental conditions and is cost-effective for DNA sequence analysis and adduct mapping because the cII transgene is only 294 base-pairs (bp) long (13). We also examined the formation of DNA adducts induced by acrylamide and glycidamide in TP53 in normal human bronchial epithelial cells, a target cell type for acrylamide-induced tumorigenesis (8,10,11).

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MATERIALS AND METHODS

Cell Culture and Chemical Treatment

Acrylamide (Boehringer Mannheim, Indianapolis, IN) and glycidamide (LKT Laboratories, St. Paul, MN) were each dissolved in double-distilled water and sterilized by passage through 0.2- μ m filters. Early-passage embryonic fibroblasts were prepared from 13.5-day-old embryos of Big Blue mice (Stratagene, La Jolla, CA) and grown in Dulbecco's modified Eagle Medium (Irvine Scientific, Santa Ana, CA) supplemented with 10% fetal bovine serum. Normal human bronchial epithelial cells (Cambrex, Walkersville, MD) were grown in Small Airway Cell Basal Medium supplemented with 10% fetal bovine serum (Cambrex). After the cultures reached \approx 50% confluence, the media were removed and replaced with the respective serum-free media for several hours before cells were treated with various doses of acrylamide, glycidamide, or control solvent for 4 hours. The cells were detached with trypsin, harvested immediately after the 4-hour treatment or at various time points (up to 72 hours in the absence of test compound), and processed for determination of cell viability and quantification of DNA adducts. Cell viability was determined with the trypan blue dye exclusion assay. For mutation assays, mouse embryonic fibroblasts were treated for 4 hours and then grown in complete medium for an additional 8 days, after which they were analyzed for the frequency and spectrum of mutations in the cII transgene. Each experimental condition was performed in triplicate, and each experiment was conducted two or three times.

Genomic DNA Isolation

Genomic DNA was isolated using a standard phenol and chloroform extraction and ethanol precipitation protocol (14). The DNA was dissolved in 1 \times TE (10 mM Tris-HCl, 1 mM EDTA [pH 7.5]) and kept at -80 °C until further analysis.

Terminal Transferase-Dependent Polymerase Chain Reaction To Map DNA Adducts

Terminal transferase-dependent polymerase chain reaction (TD-PCR) is a polymerase arrest-based assay that involves an initial gene-specific primer extension step, followed by PCR amplification and labeling of the extended product and separation of the labeled products by gel electrophoresis (15). During the initial primer extension, all DNA lesions that impede progression of the polymerase produce prematurely terminated single-stranded DNA fragments. These fragments were then labeled after undergoing exponential amplification by PCR and were visualized by autoradiography after electrophoresis on a sequencing gel (15). To increase the sensitivity of the assay for detecting N7-dG-glycidamide, the major DNA adduct formed by acrylamide and glycidamide (8) in TP53, we digested genomic DNA isolated from normal human bronchial epithelial cells with human *N*-methylpurine-DNA glycosylase and a mild piperidine treatment prior to TD-PCR (16). Briefly, the pretreated genomic DNA (1 μ g) was used as a template, and single-stranded products were made by repeated primer extensions. The extension protocol consisted of a custom-made biotinylated primer [information on primers and primer design is available in (17,18)] in a mixture of Vent (exo-) DNA polymerase (New England Biolabs, Beverly, MA) with a thermocycler setting of 2 minutes at 95 °C, 2 minutes at $T_m + 5$ °C, 3 minutes at 72 °C, and nine

cycles of 45 seconds at 95 °C, 2 minutes at $T_m + 5$ °C, and 3 minutes at 72 °C. The resulting product was mixed with streptavidin-coupled magnetic beads (Dynal Biotech ASA, Oslo, Norway), and the mixture was gently rotated for 45 minutes at room temperature to allow primer extension products to bind. The magnetic bead-bound DNA was incubated with 0.15 *M* NaOH at 37 °C for 10 minutes. The beads were thoroughly washed with 1 \times TE (pH 7.5) in a magnetic particle concentrator (Dynal ASA), and the bead-bound single-stranded DNA was resuspended in 0.1 \times TE (pH 7.5) and subjected to homopolymeric ribotailing and adapter ligation, as previously described (15). The ligation product was washed in the magnetic particle concentrator with 1 \times TE (pH 8.0), resuspended in 0.1 \times TE (pH 8.0), and PCR-amplified by using a second specific primer, the linker primer, and Expand Long Polymerase (Roche, Indianapolis, IN), as previously described (15). The thermocycler setting was as follows: 2 minutes at 95 °C; 2 minutes at $T_m - 1$ °C; 3 minutes at 72 °C; 18 cycles of 45 seconds at 95 °C, 2 minutes at $T_m - 1$ °C, and 3 minutes at 72 °C; followed by 45 seconds at 95 °C; 2 minutes at $T_m - 1$ °C; and 10 minutes at 72 °C (T_m = melting temperature). The PCR products were then labeled by subjecting them to an extension reaction with a fluorescence infrared dye-labeled primer (IRD-700; LI-COR, Lincoln, NE) (18) in a thermocycler at the following setting: 2 minutes at 95 °C, 2 minutes at $T_m + 5$ °C, 3 minutes at 72 °C, 45 seconds at 95 °C, 2 minutes at $T_m + 5$ °C, 3 minutes at 72 °C, 45 seconds at 95 °C, 2 minutes at $T_m + 5$ °C, and 10 minutes at 72 °C. The labeled reaction products were subjected to electrophoresis on a 5% polyacrylamide-urea gel in an IR² Long Ranger 4200 system (LI-COR) with simultaneous detection. The locations of DNA adduct formation and/or DNA strand breaks were identified as the sites at which progression of the DNA polymerase was arrested, resulting in an intense dark band (dependent on the lesion frequency) in the sequencing gel.

cII Mutation Frequency Analysis

The cII mutation frequency was examined by using the λ Select-cII Mutation Detection System for Big Blue Rodents (Stratagene). The assay system is based on the ability of the λ phage to multiply either lytically or lysogenically in *Escherichia coli* host cells (13). The commitment of the λ phage to lysis or lysogeny upon infection of the host is dependent on a chain of events, of which transcription of the cII gene is a determining factor (19). The cII protein activates the transcription of the genes encoding λ cI repressor and λ integrase, both of which obligate the phage to undergo lysogenization (19). Only the λ phages bearing a mutant version of cII can enter the lytic pathway and, as a result, form visible plaques on an *E. coli* lawn (13). The λ LIZ vector harbors a cI857 temperature-sensitive (ts) mutation that makes the cI_{ts} protein labile at temperatures higher than 32 °C (20). Thus, all λ phages, regardless of their cII mutation status, multiply lytically in host *E. coli* incubated at temperatures exceeding 32 °C (i.e., nonselective conditions) (13).

Briefly, we recovered the λ LIZ shuttle vectors from the Big Blue mouse embryonic fibroblast genomic DNA (\approx 5 μ g) and packaged them into viable phage particles using Transpack packaging extract (Stratagene), according to the manufacturer's instructions. The phage particles were then pre-adsorbed to G1250 *E. coli*, and the bacteria were plated on TB1 agar plates.

The plates were incubated for 48 hours at 24 °C (selective conditions) or overnight at 37 °C (nonselective conditions). The cII mutation frequency was expressed as the ratio of the number of plaques formed on plates incubated under selective conditions to the number of plaques formed on plates incubated under nonselective conditions. We screened a minimum of 3×10^5 rescued phages for each experimental condition, as recommended by the manufacturer (Stratagene). For quality assurance, control phage solutions containing a mixture of cII⁺ and cII⁻ λ phages (Stratagene), each with known mutation frequencies, were assayed in all runs.

cII Mutational Spectrum Analysis

Plaques containing putative mutants of λ cII were verified after being replated on a second TB1 agar plate and incubated under selective conditions. The verified plaques were PCR-amplified using λ select-cII sequencing primers (Stratagene), according to the manufacturer's recommended protocol. The PCR products were purified by using a QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) and sequenced by using a Big Dye terminator cycle sequencing kit on an ABI-377 DNA Sequencer (ABI Prism; PE Applied BioSystems, Foster City, CA).

Statistical Analysis

Results are expressed as mean values with 95% confidence intervals. cII mutation frequencies were determined by repeated measurements in various groups treated with increasing concentrations of glycidamide and were analyzed by one-way analysis of variance. We compared the entire mutational spectra and the specific types of mutations in the treated versus control groups by using the hypergeometric test of Adams and Skopek (21) and the chi-square test, respectively. All statistical tests were two-sided. *P* values less than .05 were considered statistically significant. The statistical software packages we used included Statview SM⁺ Graphics (Abacus Concepts, Amsterdam, The Netherlands) and DNA Mutation Analysis Application (21) (available at: <ftp://anonymous@sunsite.unc.edu/pub/academic/biology/>).

RESULTS

Glycidamide Cytotoxicity

We first examined whether glycidamide was cytotoxic to Big Blue mouse embryonic fibroblasts. The cells were exposed to increasing concentrations of glycidamide (50 nM, 500 nM, 5 μM, 50 μM, 500 μM, 5 mM, and 10 mM) for 4 hours, and cell viability was determined by trypan blue dye exclusion over a period of 24 hours (Fig. 1). The cytotoxic effects appeared only when the cells were treated with concentrations of glycidamide in the millimolar range and were time- and dose-dependent. We observed similar patterns of cytotoxicity for glycidamide as well as for acrylamide in normal human bronchial epithelial cells (data not shown).

Mapping of DNA Adducts

We used our modified TD-PCR technique to map polymerase-blocking DNA adducts induced in the cII transgene of Big Blue mouse embryonic fibroblasts and in TP53 of human

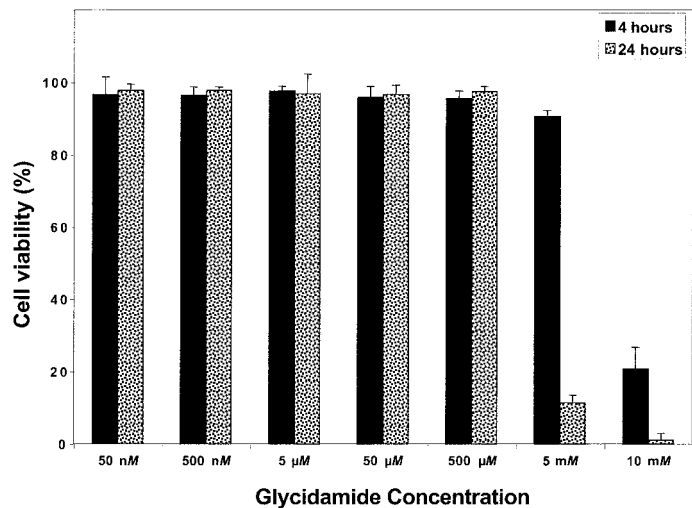


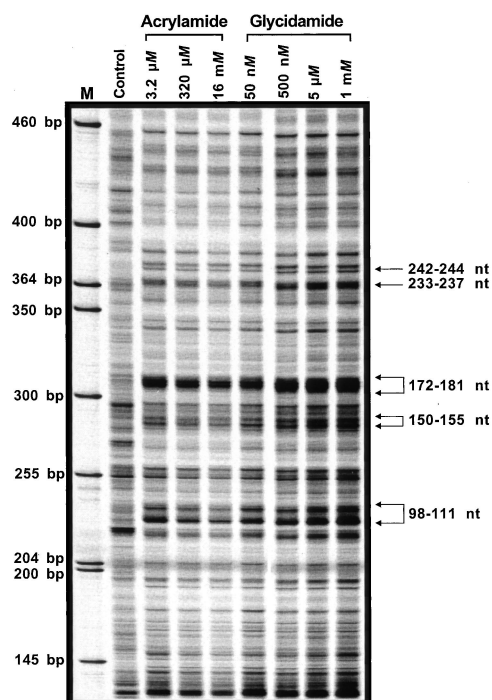
Fig. 1. Cytotoxic effects of glycidamide on Big Blue mouse embryonic fibroblasts. Confluent cell cultures were treated with increasing concentrations of glycidamide for 4 hours, and cell viability was determined by the trypan blue dye exclusion assay immediately after treatment or after a further 24 hours of incubation in the absence of glycidamide. Viability is expressed as a percentage of the total number of cells. Results are expressed as means of two independent experiments, with each experiment run in triplicate. **Error bars** = 95% confidence intervals.

bronchial epithelial cells exposed to various doses of glycidamide or acrylamide (Fig. 2, A and B). At all tested doses of glycidamide or acrylamide, the induced DNA adducts were more easily detectable in the cII transgene than in TP53, probably because the transgene is present in 40 copies in the mouse genome, whereas TP53 is present in two copies in the human genome (Fig. 2, A and B). Both acrylamide and glycidamide treatments resulted in the formation of DNA adducts at specific locations within the cII transgene and within TP53. Formation of most of the acrylamide–DNA adducts was saturable (at a micromolar dose range), whereas all glycidamide–DNA adducts in the cII transgene and in TP53 were, to some extent, dose-dependent (Fig. 2, A and B). Some of the induced DNA adducts observed immediately after the 4-hour treatments were not observed at 24–48 hours after treatments, presumably because they were repaired. However, there were acrylamide– and glycidamide–DNA adducts that persisted for 72 hours after treatment at specific nucleotide positions within the cII transgene and at specific codons within TP53 (data not shown). Although the distribution of acrylamide– and glycidamide–DNA adducts was similar in both the cII transgene and TP53, the amount of DNA adduction was more pronounced after treatment with glycidamide than after treatment with acrylamide at all doses tested (Fig. 2, A and B).

Analysis of Induced cII Mutations

We previously reported that treatment with acrylamide statistically significantly increases the frequency of mutations in the cII transgene in Big Blue mouse embryonic fibroblasts compared with control treatment and gives rise to specific types of mutations (i.e., an excess of A→G transitions and G→C transversions) (9). We examined the mutagenic potency and specificity of glycidamide in the same transgenic mouse cells. As shown in Fig. 3, Big Blue mouse embryonic fibroblasts treated with glycidamide displayed a statistically significant and

A



B

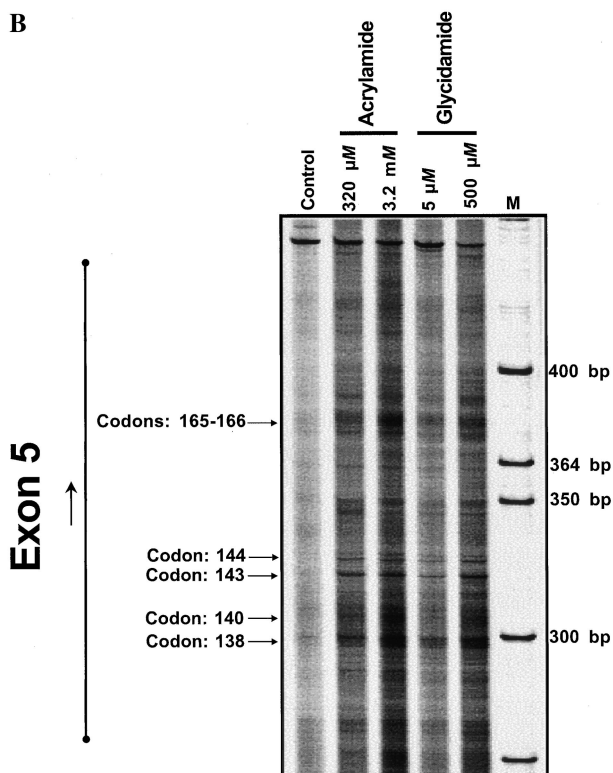


Fig. 2. Mapping of DNA adducts in acrylamide- and glycidamide-treated cells. Adduct formation within the cII transgene in Big Blue mouse embryonic fibroblasts (A) and TP53 in normal human bronchial epithelial cells (B) treated with increasing concentrations of acrylamide or glycidamide or control solvent (double-distilled water). Genomic DNA was extracted and subjected to terminal transferase-dependent polymerase chain reaction. Results are shown for selected doses. bp = base pair; M = sizing standard; nt = nucleotide position. **Arrows** indicate the position of adducts with respect to nucleotides (A) or codons (B).

dose-dependent increase in the frequency of cII mutations compared with control-treated fibroblasts ($P < .001$; analysis of variance). In addition, glycidamide was more mutagenic than acrylamide at all doses tested; for example, the highest mutation frequency induced by glycidamide was 4.1-fold over background (at 5 mM glycidamide) (Fig. 3), whereas the highest mutation frequency induced by acrylamide was twofold over background (at 320 μ M acrylamide) (9).

To examine the specificity of the glycidamide-induced cII gene mutations, we sequenced the DNA isolated from 134

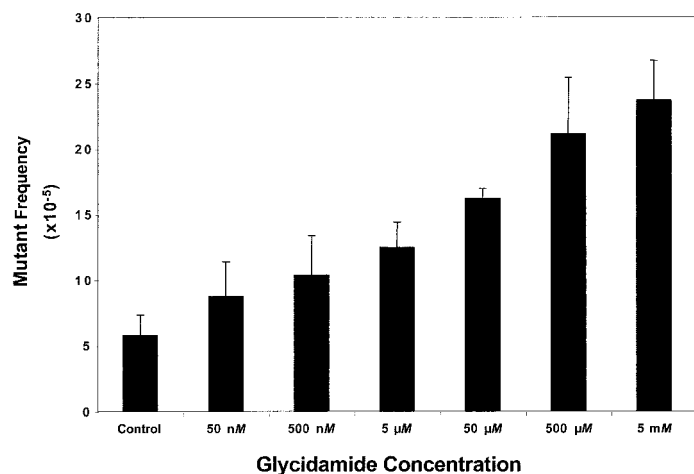


Fig. 3. Mutation frequency of the cII transgene in Big Blue mouse embryonic fibroblasts treated with increasing concentrations of glycidamide or control solvent (double-distilled water). Mutations were quantified 8 days after treatment with the use of a λ Select-cII Mutation Detection System for Big Blue rodents (Stratagene), a phage-based assay that permits detection of mutations within the transgene on the basis of plaque formation. Results are expressed as means of two independent experiments, with each experiment run in triplicate. **Error bars** = 95% confidence intervals.

cII-containing plaques derived from Big Blue mouse embryonic fibroblasts treated with 500 μ M glycidamide. Eight plaques (6.0%) had no mutation in the cII transgene. Presumably, these wild-type cII-containing plaques were recovered under selective conditions because they contain a mutation within the cI fragment of the λ construct (22–24). The remaining 126 cII-containing plaques harbored single-base substitutions as well as rare instances of single-base insertions and deletions in the cII transgene (Fig. 4, A). The spectrum of glycidamide-induced mutations consisted of several “jackpot” mutations at specific nucleotide positions in the cII gene. These jackpot mutations are common phenomena in transgenic model systems and have been consistently reported by us (9,18,25) and others (26,27). Jackpot mutations are thought to occur during early development of the transgenic rodents and to undergo clonal expansion such that many cells from various tissues harbor the same type of mutation. Alternatively, they might represent actual hotspots of spontaneous mutagenesis (22–24). Regardless of the origin of jackpot mutations, it is methodologically appropriate to exclude them from the comparative mutational spectra analyses. The spontaneous mutational spectrum for the control-treated cells included four jackpot mutations: a G insertion/deletion at nucleotide positions 179–184; a G→A transition at nucleotide position 196; a G→C transversion at nucleotide position 211; and a T→G transversion at nucleotide position 221 (Fig. 4, B). After these jackpot mutations were excluded from the analysis, the spectrum of mutations induced by glycidamide was statistically significantly different from the spontaneous spectrum of mutations observed in control cells ($P = .038$; Adams and Skopek test).

Because the cII transgene is almost certainly not transcribed after being integrated into the genome (22,23), it is unlikely that strand-biased mutagenesis, a phenomenon caused by transcription-coupled DNA repair in mammalian endogenous genes

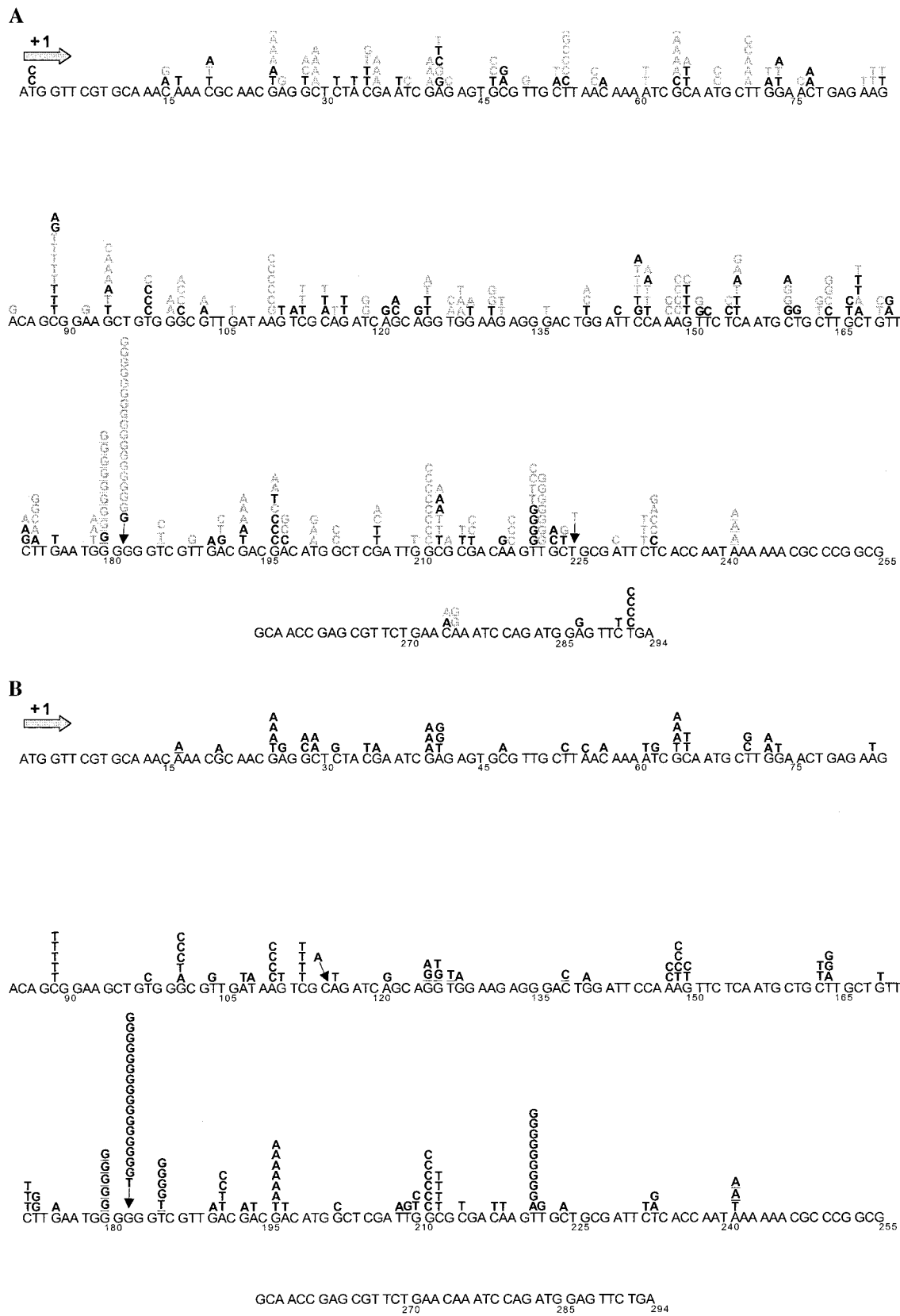


Fig. 4. Comparative mutational spectra of the cII transgene in Big Blue mouse embryonic fibroblasts. Fibroblasts were treated with 500 μ M glycidamide (**bold black typeface**) or 320 μ M acrylamide (**bold gray typeface**) (A) or control solvent (double-distilled water) (B). Mutations were quantified 8 days after treatment with the use of a λ Select-cII Mutation Detection System for Big Blue Rodents (Stratagene), a phage-based assay that permits detection of mutations

within the transgene on the basis of plaque formation. Verified mutant plaques were subsequently subjected to DNA sequence analysis. The total number of plaques sequenced was 134 for glycidamide-treated cells and 173 for control-treated cells. Substituted bases are in **bold**. Deleted bases are underlined. Inserted bases are shown with an **arrow**. Numbers below the bases are the nucleotide positions. The data for acrylamide were taken from (9).

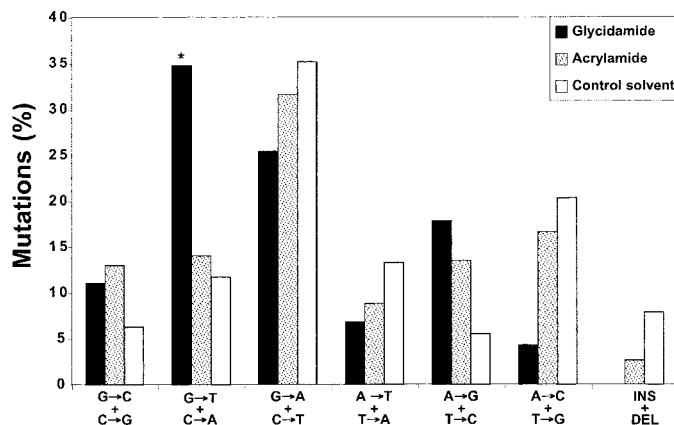


Fig. 5. Detailed mutational spectra of the cII transgene in Big Blue mouse embryonic fibroblasts treated with 500 μ M glycidamide, 320 μ M acrylamide, or control solvent (double-distilled water). Mutations were quantified 8 days after treatments with the λ Select-cII Mutation Detection System for Big Blue Rodents (Stratagene), a phage-based assay that permits detection of mutations within the transgene on the basis of plaque formation. Verified mutant plaques were subsequently subjected to DNA sequence analysis. For comparison, the strand mirror counterparts of all transitions (e.g., G→A and C→T) and transversions (e.g., G→T and C→A) were combined. All jackpot mutations found in control-treated cells (i.e., G insertion/deletion at nucleotide positions 179–184, G→A transition at nucleotide position 196, G→C transversion at nucleotide position 211, and T→G transversion at nucleotide positions 221) were excluded from the analyses. Total numbers of sequenced plaques were 134, 232, and 173 from glycidamide-treated, acrylamide-treated, and control samples, respectively. INS = insertion; DEL = deletion. The data for acrylamide were taken from (9). * $P < .001$ (χ^2 test) for glycidamide treatment versus control.

(28,29), occurs in the Big Blue transgenic system. Accordingly, we combined the strand mirror counterparts of all transitions (e.g., G→A + C→T) and transversions (i.e., G→T + C→A and G→C + C→G) and compared the specific types of mutations among the different treatment groups. Relative to control, the spectrum of mutations induced by glycidamide featured an excess of A→G transitions and G→C transversions, which are characteristic of acrylamide-induced mutagenesis (9), as well as an excess of G→T transversions (approximately 35% of all induced mutations; $P < .001$, χ^2 test) (Fig. 5). Many of the glycidamide-induced mutations that clustered at specific locations within the cII transgene coincided with sites of glycidamide- and/or acrylamide-DNA adduct formation (Fig. 2, A and B, and Fig. 4, A).

DISCUSSION

To date, epidemiologic studies have not convincingly addressed the possible link between acrylamide exposure and human cancer (30–34). Given the ubiquity of acrylamide in the human diet and in the environment (i.e., in various work settings or in the ambient air [for example, as a constituent of environmental tobacco smoke]) and the variable amounts of this compound generated during food processing, the profile of human exposure to acrylamide remains complex (8,10,11). The potential contribution of acrylamide to human cancer risk is ideally examined in dietary intervention studies, in which human subjects consume a diet supplemented with foods rich in acrylamide and are monitored for the relevant biomarkers of exposure and effects before and after the intervention. An informative but less definitive approach is to investigate the biologic consequences

of acrylamide exposure in strictly controlled *in vitro* test systems or in animal models. In this study, we examined the *in vitro* effects of acrylamide and its epoxide metabolite glycidamide on DNA damage as a function of mutagenicity, both in transgenic mouse embryonic fibroblasts and in normal human bronchial epithelial cells, a known target cell type for acrylamide-induced tumorigenesis (8,10,11).

Treatment of the cells with increasing concentrations of acrylamide or glycidamide resulted in the formation of DNA adducts at specific sites within the cII transgene and within human TP53. Glycidamide was more mutagenic than acrylamide at all doses tested. The saturation of acrylamide-DNA adduct formation we observed suggests that the conversion of acrylamide to DNA-reactive derivatives is limiting. This observation is in accord with the kinetics data, which show that CYP2E1 is the rate-limiting factor for metabolic activation of acrylamide to glycidamide (8,10,11). We also found that glycidamide-DNA adduct formation was, for the most part, dose-dependent. In agreement with these findings, we found that acrylamide and glycidamide treatment produced similar patterns of DNA adducts, both in the cII transgene and in TP53 (Fig. 2, A and B).

We previously showed that, compared with control treatment, acrylamide is weakly but statistically significantly ($P < .001$) mutagenic in the cII transgene in mouse embryonic fibroblasts, producing a distinct mutational signature, i.e., an excess of A→G transitions and G→C transversions (9). In this study, we showed that glycidamide gives rise to similar types of mutations as are specifically induced by acrylamide in the cII transgene. In addition, we demonstrated that glycidamide produces the hallmark G→T transversion mutations in this transgenic system (approximately 35% of all induced mutations; $P < .001$; χ^2 test, relative to control) (Fig. 5). One explanation for the distinctive excess of G→T transversions induced by glycidamide as compared with acrylamide is that acrylamide can generate a variety of DNA adducts via different pathways. For example, acrylamide can react with DNA directly by the Michael addition reaction (8,10,11), yielding DNA adducts that are structurally distinct from glycidamide-DNA adducts generated via the epoxidation pathway (8,10,11). The TD-PCR assay we used to detect DNA adducts cannot distinguish between DNA adducts generated by different pathways. Nonetheless, the different frequency of G→T transversions induced by glycidamide and acrylamide may reflect differences in the pathways via which these two compounds form DNA adducts.

Altogether, the overall mutational spectra of glycidamide and acrylamide are consistent with the known structural identities and mutagenic potencies of DNA adducts induced by both agents (8,10,11). N7-dG-glycidamide is the predominant DNA adduct induced by both acrylamide and glycidamide, whereas N1-dA-glycidamide and N3-dA-glycidamide are the minor adducts induced by acrylamide and glycidamide, respectively (35,36). Both N7-dG-glycidamide and N3-dA-glycidamide are promutagenic because they can undergo spontaneous depurination (8,35,36). The abasic sites that are produced by depurination of N7-dG-glycidamide are likely to promote incorporation of deoxyadenosine during DNA replication, leading to G→T transversions. N1-dA-glycidamide is also highly promutagenic because of its impaired base-pairing potential (8,35,36). The involvement of glycidamide-DNA adducts in acrylamide-induced mutagenesis is reaffirmed by our observation that many of the glycidamide-induced mutations, which were clustered at specific

locations within the cII transgene, coincided with sites of acrylamide–DNA adduct formation.

In summary, we have shown that glycidamide is largely responsible for the mutagenicity of acrylamide. The involvement of glycidamide in genotoxicity of acrylamide was shown by the pronounced formation of DNA adducts in the TP53 and cII genes, the higher induction of cII mutations, and the intensified signature of induced cII mutations in cells treated with glycidamide relative to acrylamide. At first glance, the micromolar doses of acrylamide that effectively induced promutagenic DNA adducts in the genes coding for cII and p53 might seem too high to be achieved by human dietary exposure alone. However, the ever-presence of human exposure to acrylamide on a daily basis makes these estimates somehow more tangible if not achievable. From a public health standpoint, these findings reiterate the need for reconsidering the presence of acrylamide in the human diet and the environment.

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NOTES

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