Mutagenicity and toxicity of chloroethylene oxide and chloroacetaldehyde

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Summary. Exposure of several *trp*-auxotrophic *Escherichia coli* strains, carrying base-pair substitutions, to chloroethylene oxide or chloroacetaldehyde (two metabolites of vinyl chloride) increased the mutation frequency to tryptophan prototrophy. Strong cytotoxic and mutagenic effects were observed with 2.5 mM chloroethylene oxide, while a higher concentration of chloroacetaldehyde (100 mM) exhibited a mutagenic effect which was 400 times lower.
Key words. Chloroethylene oxide; chloroacetaldehyde; mutagenicity; toxicity, *Escherichia coli.*

Vinyl chloride is a recognized carcinogen in animals and humans. Its biological effects depend on its conversion by microsomal monooxygenases into chloroethylene oxide (CEO)[1,2] which can spontaneously rearrange itself to chloroacetaldehyde (CAA)[3]. The mutagenic activity of vinyl chloride and/or its metabolites has been studied[4-6] by an in vitro technique using the reverse mutation system of *Salmonella typhimurium* developed by Ames et al.[7] in which the genetic indicator reverts to histidine prototrophy. In *S. typhimurium* TA 1535, chloroethylene oxide was found to be approximately 450 times more effective as a mutagen than chloroacetaldehyde when the comparison was based on exposure doses, defined as the time-dependent concentrations of the compounds in the treatment solutions, integrated between the times of onset and termination of treatment[8]. In this paper we report on the mutagenicity and toxicity of chloroethylene oxide and chloroacetaldehyde in several *E. coli* mutants previously obtained by ultra-violet irradiations[9]. The mutants have been extensively characterized by C.Yanofsky; each one has a substitution of a single amino-acid at a particular position in the tryptophan synthetase A protein. Reversion to tryptophan prototrophy induced by the two metabolites of vinyl chloride has been performed in 4 of these mutants: *E. coli* K12 A11, K12 A23, K12 A46, K12 A58.

Material and methods. Chloroethylene oxide was prepared by A. Croisy, INSERM, Institut Curie, Orsay, according to the method of Gross and Freiberg[10]. Chloroacetaldehyde was prepared immediately before use by heating 3-oxa-4-chloro-2-furanone with triethylamine to 200 °C and allowing the chloroacetaldehyde thus gradually formed to distil through a vigreux column, b.p. 87–89 °C[11].

E. coli K12 A mutants were generously provided by Dr C. Yanofsky, Stanford University (California).

Each mutant strain was grown overnight in L broth[12], centrifuged, washed twice with the Vogel-Bonner modified medium (containing 0.2% glucose)[13], then resuspended either in 0.85% saline solution (treatment with chloroethylene oxide) or with the Vogel-Bonner modified medium (treatment with chloroacetaldehyde) to a final population of 2×10^9 cells per ml. In the case of chloroacetaldehyde treatment, the sample was dissolved in water. Owing to the instability of chloroethylene oxide, this compound was dissolved in cold anhydrous acetone (0 °C) and solutions and dilutions of chloroethylene oxide or chloroacetaldehyde were made very quickly. The treatment started by adding 100 µl of the chloroethylene oxide or chloroacetaldehyde solutions to 5 ml aliquots of *Escherichia coli* suspension. Chloroethylene oxide treatment was carried out at 25 °C for 6 min and the treatment was stopped at +4 °C prior to centrifugation. The chloroacetaldehyde treatment was conducted at the same temperature but it lasted longer (60 min). At the end of the treatment, cells were centrifuged and the supernatant immediately removed. Cells were then washed twice with the Vogel-Bonner modified medium, then resuspended in L broth. An aliquot of this suspension (10 µl) was used to measure survivors (B_0 = number of survivors per ml of culture at t = 0, where t = 0 represents the end of treatment) by seeding bacteria on L agar plates (L broth plus 15 g/l agar). The suspension was further incubated for 15 h at 37 °C to allow the growth of survivors, and among them, revertants. At t = 15 h, 10 µl of the culture were used to measure cells (B = number of cells per ml of culture at t = 15 h) as above. This culture was used to look for revertants: cells were centrifuged, washed twice with the Vogel-Bonner medium containing 0.2% of glucose, and resuspended in this medium to a final population of 10^{10} cells ml. 10^9 cells were plated on the Vogel-Bonner agar (Vogel-Bonner medium containing 0.2% of glucose plus 15 g/l agar). Petri dishes were incubated for three days at 37 °C. Revertants were then counted. The number of revertants per ml of culture was determined at t = 15 h (R).

Results and discussion. Each mutant strain was treated with chloroethylene oxide at three concentrations (0.5 mM, 1 mM, 2.5 mM). *E. coli* K12 A23 was treated with chloroacetaldehyde

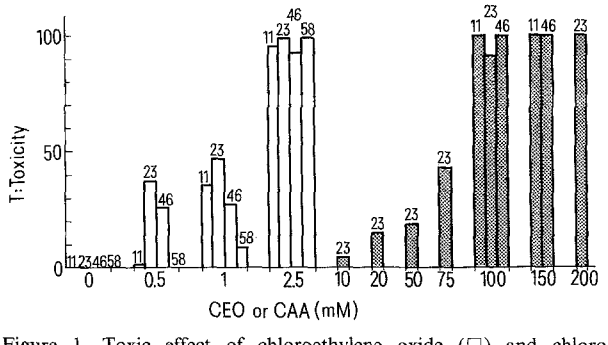

Figure 1. Toxic effect of chloroethylene oxide (□) and chloroacetaldehyde (▨). $T = 100(1-\frac{B_{0(CEO\text{ or }CAA)=c}}{B_{0(CEO\text{ or }CAA)=0}})$. $B_{0(CEO\text{ or }CAA)=c}$: number of survivors per ml of culture just after treatment (at t = 0) for a given concentration c of CEO or CAA.
$B_{0(CEO\text{ or }CAA)=0}$ represents the control: anhydride acetone for CEO and water for CAA.

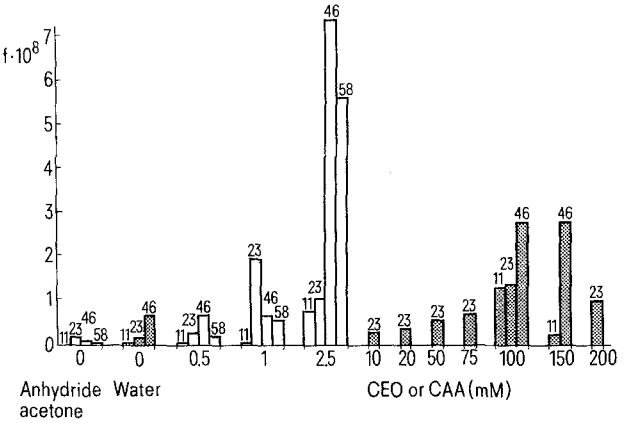

Figure 2. Mutagenic effect of chloroethylene oxide (□) and chloroacetaldehyde (▨). $f = \frac{R}{B}$. f, reversion frequency; B, number of cells per ml of culture at t = 15 h; R, number of revertants per ml of culture at t = 15 h.

at 6 concentrations (10 mM, 20 mM, 50 mM, 75 mM, 100 mM, 200 mM), while two concentrations (100 mM, 150 mM) were tested on *E. coli* K12 A11 and K12 A46.

The toxic effect of the two products is shown in figure 1. For all *E. coli* strains utilized, chloroethylene oxide at 2.5 mM and chloroacetaldehyde at 100 mM showed a strong cytotoxic effect.

The mutagenic effect of the two compounds is shown in figure 2. In the case of chloroethylene oxide, all four strains tested are reverted to tryptophan prototrophy. *E. coli* K12 A46 showed the highest mutagenic response. In the case of chloroacetaldehyde, the three strains tested could also be reverted to tryptophan prototrophy and the highest reversion frequencies are observed at 100 mM.

These results show that chloroethylene oxide is more effective as a mutagen than chloroacetaldehyde in *E. coli* strains, although the durations of treatment are different. *E. coli* strains were treated for only 6 min with chloroethylene oxide because a rearrangement to chloroacetaldehyde occurs at room temperature[10]. Thus, assuming a linear extrapolation with respect to time, a treatment using chloroethylene oxide at 0.25 mM for 60 min could be as effective to obtain a similar mutagenic effect as a treatment using chloroacetaldehyde at 100 mM for the same time. Under such conditions, and with *E. coli*, chloroethylene oxide is approximately 400 times more effective as a mutagen than chloroacetaldehyde. Our results are consistent with those obtained in *Salmonella typhimurium*, especially for chloroethylene oxide[8]. However, in the *S. typhimurium* study, chloroacetaldehyde was more mutagenic than in our experiments.

In conclusion, we have been able to obtain a great number of *E. coli* revertants. Since reversion pathways to prototrophy of Yanofsky *E. coli* strains are known[14, 15], it is of interest to study the type of base pair substitutions or other DNA changes induced by CEO or CAA.

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Genetic differences between inbred strains of mice; a new source of variation in high-sulphur keratins[1]

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Summary. Proteins were extracted by improved techniques from the hair of inbred strains of Peru, C57BL/6 and CBA/Ca mice. The extracts were characterized by amino acid analysis and high resolution polyacrylamide gel electrophoresis, and previously unreported strain differences were observed. Genetic analysis confirmed them.
Key words. Keratins; mouse; electrophoresis; amino acid analysis.

Three major protein fractions can be extracted from mammalian keratins. These are termed high-sulphur (H-S), low-sulphur (L-S) and high tyrosine-glycine (H-T-G-) proteins[2, 3]. The L-S proteins, a high molecular weight fraction, are thought to include the α-helical proteins found in wool filaments extracted from wool fibers[3] and also the tonofilaments of mammalian epidermis and strateum corneum[4]. The matrix in which the filaments are embedded is made up of the lower mol. wt H-S and H-T-G proteins.

There exists substantial heterogeneity in all three types of protein extracted from hair, each class being made up of several components. The H-S proteins, in particular have been reported by many workers as being extremely heterogeneous[5]. The number of variants is not exactly known, but estimates vary from 20 up to 70[3].

The sulphur content of wool proteins has been shown to be related to the sulphur content of the sheep's diet; diets low in sulphur containing amino acids being associated with lower sulphur content of protein extracted from wool[6, 7]. This variation might arise by differential rates of production or complete repression of particular H-S proteins, or by some form of post translational control, but the actual mechanism is not known. No genetic variation in high sulphur keratins has been demonstrated previously[8] although the mouse mutants 'tabby' and 'naked' have been examined for differences in H-S keratins without success[9].

Mouse hair has been examined by Marshall and Gillespie[10, 11] from animals of unspecified genetic background. It contains a relatively large proportion of H-S proteins (30%), which Marshall and Gillespie reported to be extremely heterogeneous in size and charge. Both high and low sulphur keratins from mammalian hair are extremely insoluble, making them difficult to extract and very difficult to separate[3, 12]. The use of agents such as mercaptoethanol or dithioerythreitol during extraction of the proteins is necessary to reduce the disulphide linkages of cysteine. These are prevented from reoxidizing by alkalation of the thiol groups with iodoacetic acid to produce a carboxymethylated protein[12]. Conventionally, harsh conditions have