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Inactivation of a Diol Epoxide by Dihydrodiol Dehydrogenase but Not by Two Epoxide Hydrolases

Abstract. *The mutagenicity of* r-8,t-9-*dihydroxy*-t-*10,11-oxy-8,9,10,11-tetrahydrobenz*[a]*anthracene (BA-8,9-diol 10,11-oxide) toward* Salmonella typhimurium *TA 100 is not decreased by the presence of large amounts of highly purified microsomal or cytosolic epoxide hydrolase. However, highly purified dihydrodiol dehydrogenase inactivates this diol epoxide, which is a major DNA-binding metabolite of benz*[a]*anthracene. The K-region epoxide, benz*[a]*anthracene 5,6-oxide (BA 5,6-oxide) is efficiently inactivated by microsomal epoxide hydrolase, is much less readily inactivated by cytosolic epoxide hydrolase, and is not inactivated by dihydrodiol dehydrogenase. This inactivation of a diol epoxide by dihydrodiol dehydrogenase points to a new significance of this enzyme and a new level of control for diol epoxides.*

Metabolic inactivation is one factor determining whether reactive intermediates formed from a polycyclic hydrocarbon will modify tissue macromolecules. In some cases, such modifications lead to mutation or to initiation of tumors. For example, K-region epoxides of polycyclic aromatic hydrocarbons are potent mutagens in microorganisms (*1, 2*) but are much less active in mammalian systems (*3*) and appear to contribute little to the total DNA binding of metabolically produced active intermediates in mammalian cells (*4*). These observations are consistent with the rapid detoxication of these epoxides by microsomal epoxide hydrolase (*5*) and by cytosolic glutathione *S*-transferases (*6, 7*), enzymes that are present in all the mammalian tissues investigated (*8*).

Much less is known of the enzymic inactivation of vicinal diol epoxides, the metabolites of polycyclic hydrocarbons that seem to be responsible for most of the carcinogenic and mutagenic effects induced by polycyclic hydrocarbons (*3, 4, 9, 10*). Mutagenicity and DNA-binding experiments with *trans*-7,8-dihydro-7,8-dihydroxybenzo[*a*]pyrene indicate that some inactivation is caused by the presence of glutathione (*7, 11, 12*) but not by microsomal epoxide hydrolase (*12–14*). Consistent with the latter observation is the inability of microsomal epoxide hydrolase to inactivate the *anti* isomer of the corresponding "bay-region" diol epoxide and the very weak effect on the activity of the *syn* isomer (*14*). These negative findings may result from the short half-life of the diol epoxide in an aqueous environment, although this may not necessarily be the same in a biological membrane. Low stability is not, however, a property of all vicinal diol epoxides. *r*-8,*t*-9-Dihydroxy-*t*-10,11-oxy-8,9,10,11-tetrahydrobenz[*a*]anthracene (BA-8,9-diol 10,11-oxide), which is the *anti* isomer of a non-bay-region diol epoxide, has a half-life of many hours (*15*) and is therefore useful for metabolic studies. In addition to serving as a model for less stable diol epoxides, its own metabolism is of interest, since it is mutagenic (*16, 17*) and is often the major DNA-binding species formed from benz[*a*]anthracene in vivo and in vitro (*10*). Using BA-8,9-diol 10,11-oxide, we show here that a diol epoxide can be metabolically inactivated. Inactivation did not occur with either microsomal or cytosolic epoxide hydrolase but was obtained with dihydrodiol dehydrogenase. If one assumes that the activities of the investigated enzymes in vivo are comparable to those that are present in our experiments in vitro, inactivation of the diol epoxide by dihydrodiol dehydrogenase would be slower than the rate of inactivation of the K-region oxide by microsomal epoxide hydrolase, but might still be sufficiently rapid to substantially affect diol epoxide concentrations in mammalian systems.

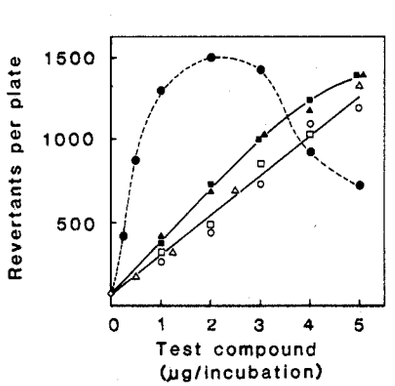

Fig. 1. Dose-dependent mutagenicity, in the absence of pure enzymes, of BA 5,6-oxide in phosphate-buffered KCl (●) and of BA-8,9-diol 10,11-oxide in phosphate-buffered KCl (○, □, and △; experiments performed on different days) or in glycine buffer with (■) or without (▲) NADP. Values are means of duplicate determinations, which differed by less than 10 percent.

To study their role in metabolic inactivation, we purified the three enzymes and used bacterial mutagenicity as an indication of their effects on the mutagenicity of BA-8,9-diol 10,11-oxide and, for comparison, benz[*a*]anthracene 5,6-oxide (BA 5,6-oxide), the K-region epoxide. The synthesis of the epoxides used here has been described (*18*). Microsomal epoxide hydrolase was purified from rat liver to apparent homogeneity (*19*). This enzyme converts a variety of alkene and arene oxides to dihydrodiols (*5, 20*). Cytosolic epoxide hydrolase was purified sufficiently (*21*) from rabbit liver to yield a preparation that contained less than 20 percent impurities. Although to date only a few animal species have been investigated, pertinent differences between cytosolic and microsomal epoxide hydrolase have been observed in all situations that have been studied. The cytosolic enzyme differs from the microsomal epoxide hydrolase immunologically (*22*) and in substrate specificity in that the cytosolic enzyme is relatively inactive toward K-region arene oxides (*23*), but is efficient in the hydrolysis of other epoxides—for example, trisubstituted oxiranes (*24*)—that are not or are only poor substrates for the microsomal enzyme (*25*). Dihydrodiol dehydrogenase was purified to apparent homogeneity from rat liver cytosol (*26*). The enzyme converts dihydrodiols such as *trans*-1,2-dihydro-1,2-dihydroxybenzene to catechols using nicotinamide adenine dinucleotide phosphate (NADP) as a cosubstrate. With carcinogenic polycyclic hydrocarbons such as benzo[*a*]pyrene and dimethylbenz[*a*]anthracene it reduces the mutagenicity in the Ames test (*27*). However, the mechanisms and metabolites involved in these effects are unknown.

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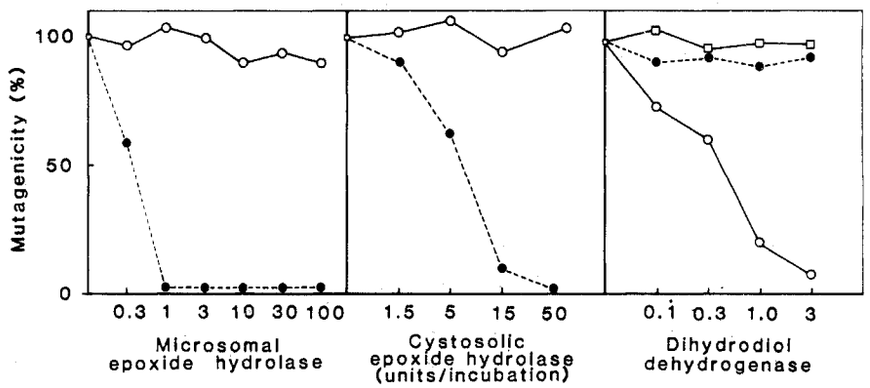

Fig. 2. Effect of purified enzymes on the mutagenicity of BA 5,6-oxide (●) and BA-8,9-diol 10,11-oxide (○, in the presence of NADP; □, in the absence of NADP). The number of mutants above solvent control induced by 1 μg of BA 5,6-oxide or 3 μg of BA-8,9-diol 10,11-oxide in the presence of various amounts of purified enzymes is expressed as the percentage of the corresponding value without enzyme. The absolute values of colonies in the absence of enzyme were 92 to 150 for solvent controls, 920 to 1060 for BA 5,6-oxide, and 560 to 860 (depending on the incubation conditions; see Fig. 1) for BA-8,9-diol 10,11-oxide. Triplicate incubations were performed. The coefficients of variation in the numbers of colonies were less than 10 percent. Enzyme units are described in the papers which describe their purification (*19, 21, 26*). One gram of liver from an adult Sprague-Dawley rat contains approximately 300 units of microsomal epoxide hydrolase and 2.5 units of dihydrodiol dehydrogenase, whereas 1 g of liver from an adult male rabbit contains about 250 units of cytosolic epoxide hydrolase.

After test compounds were incubated with the purified enzymes under favorable conditions for enzyme activity, the incubation mixture was tested for mutagenicity according to a modification of the method of Ames *et al.* (*28*). The experimental protocol was as follows. The enzyme under investigation was placed in an assay tube in 500 μl of an appropriate buffer. Control tubes contained buffer alone. The buffer consisted of 150 m*M* KCl and 10 m*M* sodium phosphate (*p*H 7.4) in the experiments with epoxide hydrolases. In the experiments with dihydrodiol dehydrogenase, 50 m*M* glycine buffer (*p*H 9.0) with or without 2.7 m*M* NADP was used. After the tube was prewarmed for 1 minute at 37°C, the test compound, dissolved in 20 μl of acetone-triethylamine (1000:1), was added. After a 20-minute incubation at 37°C, 1.7×10^8 bacteria in 100 μl of nutrient broth were added and the incubation was continued for another 20 minutes. Top agar, which contained 0.55 percent agar, 0.55 percent NaCl, 50 μ*M* biotin, 50 μ*M* histidine, and 25 m*M* sodium phosphate buffer (*p*H 7.4) and which was kept at 45°C, was then added and the mixture was poured onto minimal agar plates. Revertant colonies were counted after incubation for 3 days at 37°C. During the first incubation time (20 minutes), no decrease in mutagenic activity was found with either epoxide in the absence of enzyme (data not shown). To study the effects of the purified enzymes we used the test compounds at concentrations that are in the increasing portion of the concentration-mutagenicity curve (Fig. 1). The data of Fig. 2 show that microsomal epoxide hydrolase, as expected from its substrate specificity (*5*), readily inactivated BA 5,6-oxide. Even a 100-fold excess over that required for complete inactivation of the K-region oxide was without significant effect on the mutagenicity of BA-8,9-diol 10,11-oxide. Cytosolic epoxide hydrolase also inactivated BA 5,6-oxide, but relatively large amounts of enzyme were required. As with microsomal epoxide hydrolase, cytosolic epoxide hydrolase did not inactivate the diol epoxide. However, the diol epoxide was inactivated by dihydrodiol dehydrogenase and this inactivation required the presence of NADP. The inability of either NADP (Fig. 1) or of dihydrodiol dehydrogenase alone (Fig. 2) to inactivate the diol epoxide, and the lack of inactivation of BA 5,6-oxide by dihydrodiol dehydrogenase either with or without NADP, indicate that inactivation is not the result of nonspecific binding of the diol epoxide to protein or to NADP but rather a consequence of enzymic activity.

The chemical nature of the products formed by dihydrodiol dehydrogenase from BA-8,9-diol 10,11-oxide is unknown; the enzyme may convert the diol epoxide to a ketol epoxide. Several routes are conceivable by which a ketol epoxide could subsequently isomerize nonenzymically to a benz[*a*]anthracene derivative possessing three hydroxy groups on an aromatic 8,9,10,11-benzo ring. Enediol epoxides, tautomers of the ketol epoxides, offer a direct analogy to simple non-K-region arene oxides that isomerize readily to phenols. Both non-K-region arene oxides (*1, 29*) and phenols (*29, 30*) generally are intrinsically poor mutagens.

Whereas a low concentration of microsomal epoxide hydrolase was sufficient for the inactivation of BA 5,6-oxide, much greater concentrations of dihydrodiol dehydrogenase were necessary for inactivation of the diol epoxide. From the data of Fig. 2 it can be calculated that a 50 percent inactivation of 1 μg of BA 5,6-oxide was achieved with either 0.4 unit of purified rat microsomal epoxide hydrolase, equivalent to 1.3 mg of liver, or with 7 units of purified rabbit cytosolic epoxide hydrolase, equivalent to 28 mg of liver. These are relatively small quantities of enzyme. Dihydrodiol dehydrogenase equivalent to 200 mg of liver was required for 50 percent inactivation of 3 μg of BA-8,9-diol 10,11-oxide, whereas microsomal epoxide hydrolase equivalent to 330 mg of rat liver and cytosolic epoxide hydrolase equivalent to 200 mg of rabbit liver did not inactivate this mutagen. Hence, with regard to the concentrations of the enzymes present in vivo, the K-region epoxide is inactivated noticeably more efficiently than the diol epoxide. This may be one of the reasons for the much stronger biological activities in mammalian systems of diol epoxides than of K-region epoxides. An effective but moderate rate of inactivation of diol epoxides, furthermore, suggests that differences in enzyme activity (*31*) among species, organs, and physiological states are a likely cause for differences in susceptibility to the effects of polycyclic aromatic hydrocarbons. Finally, dihydrodiol dehydrogenase very likely is more important for the inactivation of diol epoxides than either microsomal or cytosolic epoxide hydrolase.

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31. Dihydrodiol dehydrogenase was also found in various extrahepatic organs such as lung, kidney, testes, adrenals, and brain. The specific activity of the cytosolic fraction of these organs was 2.4 to 21 percent of that of liver cytosol (K. Vogel and F. Oesch, unpublished results).

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Convergent and Alternative Designs in the Digital Adhesive Pads of Scincid Lizards

Abstract. Prasinohaema virens, *an arboreal scincid lizard, differs from its closest relatives in that it exhibits subdigital adhesive setae resembling those of anoles in shape and those of geckos in some aspects of size. The other scincid species in this genus as well as those in a presumed ancestral genus exhibit pad scales with surface folds and ruffles but no setae; at least one of these species uses an adhesive grip similar to that of anoles and geckos. Thus, there appear to be two strikingly different epidermal specializations for adhesive grip within this small radiation.*

Two primarily arboreal groups of lizards, the family Gekkonidae and the anoline section in the family Iguanidae, have subdigital pads covered with microscopic hairs or setae. Setae enable the animals to climb by adhering to surfaces that will not accept the claw, such as smooth walls, leaf surfaces, and glass. Geckos and anoles each constitute large, diverse radiations whose success is at least in part based on the adaptive value of the adhesive pad (*1, 2*). Several arboreal lizards in the family Scincidae have adhesive pads that are grossly similar to those of anoles and geckos, and previous descriptions of sectioned and whole digits suggest the presence of setae (*3, 4*). Scanning electron microscopy of these forms (*5*) reveals that one species, *Prasinohaema virens*, exhibits remarkable convergence in setal morphology to *Anolis* and some geckos, whereas the reputed closest relatives of *P. virens* exhibit a wholly different pad fine structure.

The setae of *Prasinohaema virens* and *Anolis* are similar in shape (Fig. 1, A, B, D, and E). There is a triangular tip attached at the apex to a tall unbranched stalk; but the *P. virens* setae are significantly larger and less densely packed than those of any anoline examined to date (*5*) (Table 1). The seta stalk diameter, the density of stalks, and the total tip area per stalk are more like those of some geckos, although the basic shape or design of the seta is distinct from that of most geckos (Table 1 and Fig. 1, A and B and F to H) (*1, 6*). The adhesive setae of some Coleoptera approach those of *P. virens* in size and shape (*7*).

Prasinohaema virens and the other scincid lizards that we examined are a terminal twig of the most evolutionarily derived of the four subfamilies of the

Table 1. Comparison of setal morphology.

Item	*Prasinohaema virens*	*Anolis cuvieri*	*Gekko gecko* (*6*) (*Gekko vittatus*)
Setal shape	Single stalk ending in a triangular tip	Single stalk ending in a triangular tip	Multibranched stalk ending in 100 to 100(small triangular tips
Stalk dimensions			
Diameter (μm)	2.0 ± 0.2	0.51 ± 0.04	5 (2.1 ± 0.3)
Height (μm)	26.0 ± 2.0	22.4 ± 0.5	110 (33 to 42)
Stalk density (stalks per square micrometer)	0.03	1	0.005 (0.01)
Tip dimensions			
Length (μm)	6.2 ± 0.4	0.624 ± 0.045	0.2 (0.2)
Width (μm)	6.3 ± 0.4	0.729 ± 0.045	0.2 (0.2)
Estimated area (μm^2)	19.5 ± 1.7	0.229 ± 0.025	0.02
Estimated total tip area per stalk (μm^2	19.5 ± 1.7	0.229 ± 0.025	2 to 20
Ratio of estimated total tip area to cross-sectional area of stalk	6.2	1.1	0.1 to 1.0

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