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VITAMIN K EPOXIDE REDUCTASE ACTIVITY IN THE METABOLISM OF EPOXIDES

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Abstract—The importance of vitamine K epoxide reductase for the metabolism of a range of structurally diverse epoxides has been investigated. Vitamin K_1 epoxide is reduced by rat liver microsomes at a rate of 0.47 nmoles/g liver/min. The rate of menadione oxide reduction is not significantly higher than the non-enzymatic reduction rate. No measurable reduction of benzo[*a*]pyrene 4,5-oxide, benzo[*a*]pyrene 7,8-oxide, phenanthrene 9,10-oxide, styrene 7,8-oxide, and dieldrin has been detected, nor could trichothecene T-2 toxin inhibit reduction of vitamin K_1 epoxide. Thus, vitamin K epoxide reductase is very specific for vitamin K_1 epoxide. Taking into account the range of structurally diverse epoxides investigated and the high specific activities of microsomal epoxide hydrolase and cytosolic glutathione transferase for these epoxides it may be concluded that vitamin K epoxide reductase, in all likelihood, generally does not significantly contribute to the control of epoxides metabolically formed from xenobiotics.

Reactive metabolites are responsible for the harmful effects of many foreign compounds. Aromatic and olefinic compounds can be metabolized to epoxides, which are generally electrophilically reactive and therefore bind to cellular macromolecules such as DNA, RNA and proteins. Epoxides are produced during oxidation of foreign and endogenous compounds by the microsomal monooxygenase system [1–5]. These epoxides may then be further metabolized either by epoxide hydrolase [2] or by conjugation with glutathione [6]. In 1976, Kato demonstrated the involvement of cytochrome P-450 in the reduction of benzo[*a*]pyrene 4,5-oxide to benzo[*a*]pyrene [7], showing that this is another possible pathway for deactivation. At this epoxide reduction was already inhibited by low concentrations of oxygen, the physiological significance of the reductive reaction remains unclear [8]. However, there exists an enzyme system, which reconverts vitamin K_1 epoxide to the corresponding olefin, vitamin K_1, in the presence of oxygen [9]. Therefore, the possibility arises that this enzyme may reduce and hereby inactivate foreign and endogenous epoxides under physiological conditions. In order to gain an insight into the substrate specificity of vitamin K epoxide reductase and to probe the potential importance of this enzyme in the control of toxic epoxides we have examined the metabolism of a range of structurally diverse epoxides by vitamin K epoxide reductase, in presence of ambient air as oxygen source: benzo[*a*]pyrene 4,5-oxide, benzo[*a*]pyrene 7,8-oxide, phenanthrene 9,10-oxide, sytrene 7,8-oxide, dieldrin, trichotecene T-2 toxin and menadione oxide.

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§ Abbreviations: DTT, dithiothreitol; TCPO, 1,1,1-trichloropropene 2,3-oxide.

MATERIALS AND METHODS

[Phytyl-1,2-^{3}H]vitamin K_1 was a gift from Hoffman–La Roche (Basel, Switzerland). [^{3}H]vitamin K_1 epoxide (2 Ci/mole) was prepared as described by Tishler *et al.* [10]. [^{3}H]benzo[*a*]pyrene 4,5-oxide (2.5 Ci/mole) and [^{3}H]styrene 7,8-oxide (0.5 Ci/mole) were synthesized as described previously [11, 12]. [^{3}H]Phenanthrene 9,10-oxide (0.9 Ci/mole) and [^{3}H]benzo[*a*]pyrene 7,8-oxide (1 Ci/mole) were gifts from Dr. P. Sims (Institute of Cancer Research, London). Trichothecene T-2 toxin was isolated as previously described [13]. Livers from untreated male Sprague–Dawley rats (200 g, Wiga, Sulzfeld, FRG) and Wistar rats (200 g, Han, Hannover, FRG) were used for preparation of microsomes as described by Sadowski *et al.* [14]. The vitamin K epoxide reductase activity in non-solubilized microsomes was determined under aerobic conditions by the method of Whitlon *et al.* [15] in presence of DTT as sole reducing agent. The microsomal and cytosolic epoxide hydrolase activity was measured as previously described ([16], U. Vogel-Bindel and F. Oesch, unpublished results). Dieldrin and aldrin were determined according to the method of Wolff [17].

For the determination of epoxide reductase activity with the other substrates, the conditions for determination of vitamin K epoxide reductase were modified as follows: the incubation mixture contained microsomes from 0.5 g liver, 2 mM DTT,§ 2 mM TCPO, in a final volume of 1 ml and the reaction was started with 40 nmoles epoxide. Incubations were carried out at 37° for 5 min under aerobic conditions, followed by two extractions with 2 ml of ethyl acetate. The ethyl acetate phases were combined and the solvent evaporated to dryness under a stream of nitrogen. The residue was redissolved in

Table 1. Vitamin K epoxide reductase activity in rat strains and effect of TCPO

Rat strain	Inhibitor	Vitamin K epoxide reductase activity* nmoles/g Leber/min	t-Test
Sprague–Dawley	None	0.25 ± 0.03	
Wistar	None	0.47 ± 0.03	
Wistar	1 mM TCPO	0.43 ± 0.08	No significant difference†

* The results are given as means ± S.E.M. from three incubations.
† $P > 0.05$, compared with incubation in absence of TCPO.

50 μl of methanol. 20 μl were analyzed by HPLC using a Zorbax ODS column (Du Pont). For the separation of oxidized and reduced compounds, different solvent gradients of methanol and water were used. 1 ml fractions were collected, and the radioactivity was determined, after the addition of 3 ml Unisolve I (Roth), in a Tricarb 300 C liquid scintillation counter (Packard). The reduced compounds were identified by cochromatography with authentic standards. Benzo[*a*]pyrene was detected with fluorometric methods. Fluorescence experiments were performed with an Aminco Spectrofluorometer (excitation wavelength: 294 nm, emission wavelength: 404 nm).

RESULTS

Vitamin K epoxide reductase activity in different rat strains and effect of TCPO

Vitamin K epoxide reductase activity was measured in male Sprague–Dawley and Wistar rats (Table 1). As microsomes from Wistar rats displayed a higher specific activity, microsomes from this strain were used in further experiments.

TCPO is a potent inhibitor of the microsomal epoxide hydrolase in all species investigated, including the rat [18, 19]. However, TCPO did not significantly change vitamin K epoxide reductase activity with vitamin K epoxide as substrate (Table 1). Therefore, to inhibit substrate removal by epoxide hydrolase, TCPO was included in experiments for measuring vitamin K epoxide reductase activity with the compounds mentioned below, since several of them are excellent substrates of microsomal epoxide hydrolase [1, 2, 16].

Benzo[a]pyrene 4,5-oxide, benzo[a]pyrene 7,8-oxide, phenanthrene 9,10-oxide, styrene 7,8-oxide, dieldrin, trichothecene T-2 toxin, menadione oxide as substrates of Vitamin K epoxide reductase activity

Substrates were incubated with microsomes in the presence of DTT which was included as a reducing agent for epoxide reduction by vitamin K epoxide reductase [15] and 1 mM TCPO to reduce substrate depletion due to hydrolysis by epoxide hydrolase. The reaction products were analyzed by HPLC. Quantitation was performed by measuring radioactivity and fluorescence. No reduction of benzo[*a*]pyrene 4,5-oxide, benzo[*a*]pyrene 7,8-oxide, phenanthrene 9,10-oxide, styrene 7,8-oxide or dieldrin was observed (Table 2). Since these compounds are rapidly metabolized by other detoxifying enzymes

Table 2. Comparison of vitamin K epoxide reductase activity with the activity of other detoxifying enzymes

Substance	Vitamin K epoxide reductase activity nmoles/g liver/min	Activity of other epoxide metabolizing enzymes nmoles/g liver/min		
Benzo[*a*]pyrene 4,5-oxide	<0.007	EH*	95	
		GST†	300	(22)
Benzo[*a*]pyrene 7,8-oxide	<0.007	EH	69	(21)
Phenanthrene 9,10-oxide	<0.04	EH	560	(21)
		GST	6000	(22)
Styrene 7,8-oxide	<0.04	EH	160	(21)
		GST	5000	(22)
Dieldrin	<0.06			
Menadione oxide	<0.05 (non-enzymatic 1 nmole/min)			
Vitamin K_1 epoxide	0.47			

* EH, microsomal epoxide hydrolase.
† GST, cytosolic glutathione transferase.

Table 3. The lack of an inhibitory effect of trichothecene T-2 toxin on vitamin K epoxide reductase activity*

Reductase activity	Vitamin K epoxide inhibitor nmoles/g liver/min	*t*-Test
None	0.47 ± 0.03†	
Solvent only (40 μl acetonitrile)	0.27 ± 0.04†	
0.1 mM T-2 toxin (in 40 μl acetonitrile)	0.27 ± 0.01†	No significant difference§
0.2 mM T-2 toxin (in 40 μl acetonitrile)	0.28‡	No significant difference§

* Concentration of trichothecene T-2 toxin 0.1 and 0.2 mM, concentration of substrate vitamin K_1 epoxide 0.04 mM.
† The results are given as means ± S.E.M. from two incubations.
‡ Material was sufficient for one incubation only.
§ $P > 0.05$, compared with solvent control.

(Table 2), it is clear that a reductase activity, which is smaller than the detection limit indicated in Table 2, cannot have any significance for the metabolism of these substances.

We also studied whether menadione oxide, a structural analogue of vitamin K_1 epoxide was reduced by vitamin K epoxide reductase. As menadione oxide is also reduced non-enzymatically, enzymatic activity would have to be higher than for vitamin K_1 oxide in order to detect it. Indeed, no significant enzymatic metabolism of this compound could be measured (Table 2). A potential enzymic reduction of trichothecene T-2 toxin by vitamin K epoxide reductase could not be determined directly, since a standard of the reduced compound was not available. Therefore, we determined whether trichothecene T-2 toxin inhibited the reduction of vitamin K_1 epoxide by vitamin K epoxide reductase. Table 3 shows that up to 200 μM of trichothecene T-2 toxin exerted no effect on vitamin K epoxide reductase (concentration of the substrate vitamin K epoxide: 40 μM).

Effect of vitamin K_1 epoxide on microsomal and cytosolic epoxide hydrolase

Vitamin K_1 epoxide, which was, amongst the tested compounds the only substrate for vitamin K_1 epoxide reductase, was investigated for a potential interaction with microsomal or cytosolic epoxide hydrolases. No effect of vitamin K_1 epoxide on microsomal or cytosolic epoxide hydrolase was observed, even at concentrations of vitamin K_1 epoxide which were considerably higher than those of the substrates i.e. benzo[*a*]pyrene 4,5-oxide and *trans*-stilbene oxide (Fig. 1a, b). As no inhibition of microsomal or cytosolic epoxide hydrolase was observable, in presence of vitamin K_1 epoxide, this compound is probably not a substrate of these enzymes.

DISCUSSION

Among the various epoxides tested, activity of vitamin K epoxide reductase has been shown to be restricted to vitamin K_1 epoxide. Thus, this enzyme appears to be very specific for vitamin K epoxide. If any metabolism occurred with any of the other epoxides, the rate was below the detection limit of the assay used. TCPO, an inhibitor of microsomal epoxide hydrolase, has been included in the epoxide reductase assays to reduce substrate depletion. Vitamin K epoxide reductase activity (using vitamin K epoxide as substrate) was not significantly inhibited in presence of 1 mM TCPO. Likewise, the activities for other potential substrates with higher K_ms than

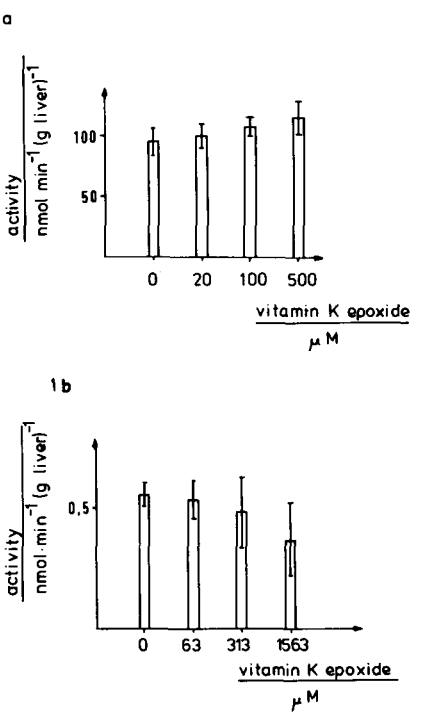

Fig. 1. The inhibitory effect of vitamin K_1 epoxide on microsomal (a) and cytosolic (b) epoxide hydrolase. The substrate for microsomal epoxide hydrolase was benzo[*a*] pyrene 4,5-oxide (40 μM), for cytosolic epoxide hydrolase *trans*-stilbene oxide (62.5 μM). The results are given as means ± S.D. from three incubations.

vitamin K epoxide (K_m = 8 μM [20]) would also not be seriously affected. This conclusion was reached based on theoretical calculations for competitive, non-competitive and uncompetitive inhibition (not shown). Therefore, taking into account the high specific activities of epoxide hydrolase and glutathione transferase with these epoxides, we suggest that vitamin K epoxide reductase activity is of no importance for the metabolism of these compounds. Conversely, the data presented also show, that vitamin K_1 epoxide does not inhibit the microsomal or cytosolic epoxide hydrolase even at concentrations of vitamin K_1 epoxide which were considerably larger than those of the epoxide hydrolase substrates used. It is therefore unlikely that vitamin K_1 epoxide could interfere with this important step of epoxide metabolism and that vitamin K_1 epoxide serves as a substrate for microsomal or cytosolic epoxide hydrolase. The data also indicate that neither microsomal nor cytosolic epoxide hydrolase have any influence on the vitamin K redox cycle.

For the investigation of vitamin K_1 epoxide reductase activity, epoxides of different size, structure and polarity were used in this study. Since none of them was measurably reduced by vitamin K epoxide reductase, it is probable, that also other xenobiotic epoxides are not metabolized by this enzyme. Thus it is also unlikely that the vitamin K cycle could be blocked by epoxides metabolically produced from foreign compounds.

We conclude, that vitamin K epoxide reductase activity is very specific for the conversion of vitamin K_1 epoxide and that the enzyme plays no role in the control of any of the reactive epoxides metabolically produced from foreign compounds which were investigated in the present study. Since these cover a broad array of diverse structures, it is quite likely that this enzyme plays no important role in the control of xenobiotic epoxides.

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REFERENCES

1. F. Oesch, *Xenobiotica* **3**, 305 (1973).
2. D. M. Jerina and J. W. Daly, *Science* **185**, 573 (1974).
3. P. Sims and P. L. Grover, *Adv. Cancer Res.* **20**, 165 (1974).
4. D. W. Nebert, J. R. Robinson, A. Niwa, K. Kumaki and A. P. Poland, *J. Cell Physiol.* **85**, 393 (1975).
5. F. J. Wiebel, J. K. Selkirk, H. V. Gelboin, D. A. Haugen, T. A. Van der Hoeven and M. J. Coon, *Proc. natn Acad. Sci. U.S.A.* **72**, 3917 (1979).
6. D. M. Jerina, in *Glutathione: Metabolism and Function* (Eds. I. M. Arias and W. B. Jakoby), p. 267. Raven Press, New York (1976).
7. R. Kato, K. Iwasaki, T. Shiraga and N. Naguchi, *Biochem. biophys. Res. Commun.* **70**, 681 (1976).
8. M. Sugiura, Y. Yamazoe, T. Kamataki and R. Kato, *Cancer Res.* **40**, 2910 (1980).
9. J. T. Matschiner, A. Zimmermann and R. G. Bell, *Thromb. Diath. Haemorrh., Suppl.* **57**, 45 (1974).
10. M. Tishler, L. F. Filser and N. L. Wendler, *J. Am. chem. Soc.* **62**, 2866 (1940).
11. P. M. Dansette and D. M. Jerina, *J. Am. chem. Soc.* **96**, 1224 (1974).
12. F. Oesche, D. M. Jerina and J. W. Daly, *Biochim. biophys. Acta.* **227**, 685 (1971).
13. R. Schmidt, E. Ziegenhagen and K. Dose, *J. Chromatogr.* **212**, 370 (1981).
14. J. A. Sadowski, H. K. Schnoes and J. W. Suttie, *Biochemistry* **16**, 3856 (1977).
15. D. S. Whitlon, J. A. Sadowski and J. W. Suttie, *Biochemistry* **17**, 1371 (1978).
16. H. Schmassmann, H. R. Glatt and F. Oesch, *Analyt. Biochem.* **74**, 94 (1976).
17. T. Wolff, in *Biochemistry, Biophysics and Regulation of Cytochrome* P-450 (Eds. J. Å. Gustafsson, F. Karlstedt-Duke, A. Moder and F. Raster), p. 49. Elsevier, London (1980).
18. F. Oesch, N. Kaubisch, D. M. Jerina and J. W. Daly, *Biochemistry* **10**, 4858 (1971).
19. F. Oesch, *Biochem. J.* **139**, 77 (1974).
20. P. C. Preusch and J. W. Suttie, *J. biol. Chem.* **258**, 714 (1983).
21. P. Bentley and F. Oesch, in *Primary Liver Tumours* (Eds. H. Remmer, H. M. Bolt and P. Bannasch), p. 239. MTP Press, Lancaster (1978).
22. J. R. Bend, Zvi Ben-Zvi, J. Van Anda, P. M. Dansette and D. M. Jerina, in *Carcinogenesis*, Vol. 1, *Polynuclear Aromatic Hydrocarbons: Chemistry, Metabolism, and Carcinogenesis* (Eds. R. J. Freudenthal and P. W. Jones), p. 63. Raven Press, New York (1976).