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# KINETICS OF THE INHIBITION OF STYRENE OXIDE AND BENZO(*a*)PYRENE-4,5-OXIDE HYDRATION IN RAT LIVER MICROSOMES BY CADMIUM

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## Summary

The kinetics of the inhibition by cadmium of styrene oxide and benzo(*a*)pyrene-4,5-oxide hydration were studied in a microsomal preparation from rat liver. Cadmium inhibited the hydration of styrene oxide in an apparently noncompetitive manner, whereas the inhibition of benzo(*a*)pyrene-4,5-oxide hydration showed a competitive mechanism.

Styrene oxide inhibited the hydration of benzo(*a*)pyrene-4,5-oxide competitively and cyclohexene oxide inhibited the hydration of both styrene oxide and benzo(*a*)pyrene-4,5-oxide competitively.

The present data suggest that cadmium is bound near the active centre of the enzyme. Occupation of this site by cadmium inhibits the enzymic hydration of the polycyclic benzo(*a*)pyrene-4,5-oxide molecule competitively, and that of the smaller monocyclic alkene oxide of styrene noncompetitively. The estimated $K_i$ values were below 10 μmol for hydration of both substrates indicating that cadmium is a very potent inhibitor of epoxide hydration.

The inhibition of epoxide hydration is well established [1—3]. Recently cadmium was found to strongly inhibit drug-metabolizing enzymes including the glutathione S-transferases, the cytochrome P-450-dependent monooxygenases and glucuronide conjugation of 4-methylumbelliferone, as well as, epoxide hydrase [4,5]. In the present report a kinetic study of the inhibition of styrene and benzo(*a*)pyrene-4,5-oxide hydration by cadmium is presented.

As the regulatory effects of the membrane on epoxide hydrase seem limited, as shown by similar activatory and inhibitory properties of the mem-

brane-bound and purified enzyme [6], see also [7,8], hepatic microsomes [9,10] (male Wistar rats 300 g) were used as the enzyme preparation. Hydration of styrene oxide was measured by the method of Oesch et al. [11]. Hydration of benzo(*a*)pyrene-4,5-oxide was measured by the method of Schassmann et al. [12] using the same buffer as with styrene oxide hydration (0.1 mol/l Tris-HCL, pH 9.0, 0.025% Tween 80). Cadmium iodide or sulphate (with analogous results) were added to incubation mixtures as dissolved in water. Styrene oxide and cyclohexene oxide were added in 1 µl of acetonitrile. After extraction of the unreacted substrates with petroleum ether, the water phases were counted in 4 ml of Luma Gel (Lumac Systems AG, Basel, Switzerland). The counting efficiency was 35%.

The inhibition of styrene oxide hydration by cadmium was noncompetitive (Fig. 1). This indicates that cadmium-binding site is separate from the active centre-hydrating styrene oxide. On the other hand, cadmium caused competitive inhibition of the hydration of benzo(*a*)pyrene-4,5-oxide (Fig. 2). This suggests that cadmium prevents the binding of benzo(*a*)pyrene-4,5-oxide to the enzyme molecule. Cyclohexene oxide inhibited the hydration of both styrene oxide and benzo(*a*)pyrene-4,5-oxide competitively (with apparent $K_i$ values of 120 and 85 µmol/l, respectively), and styrene oxide likewise competitively inhibited the enzymic hydration of benzo(*a*)pyrene-4,5-oxide (Figs. 3 and 4). An explanation of these data is that there is a common active centre for both substrates (in the same enzyme molecule). Cadmium is not bound to

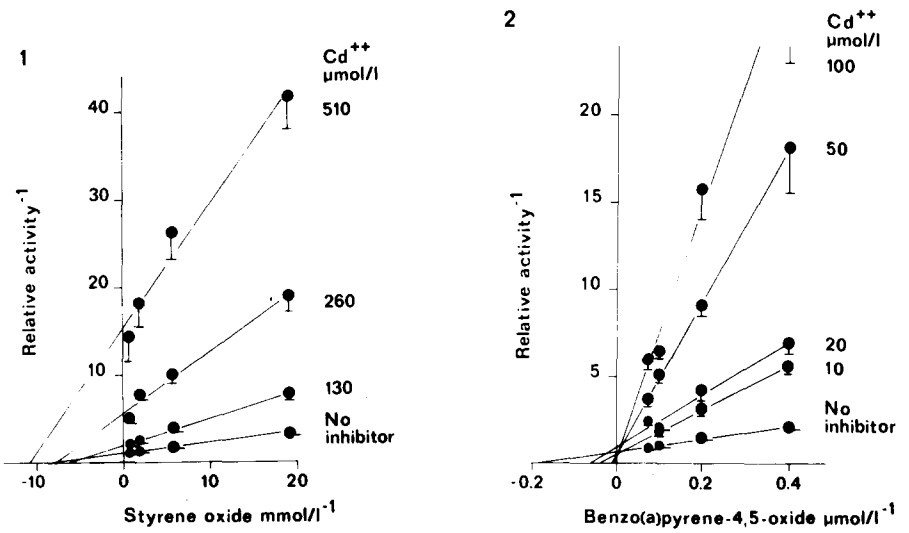


Fig. 1. Inhibition of styrene oxide hydration in rat liver microsomes by cadmium, Lineweaver-Burk plot. Each point represents the mean of five independent (separate enzyme preparations) duplicate measurements. Standard error in one direction is given. The activity is given related to uninhibited activity measured at a styrene oxide concentration of 1.74 nmol/l, which gave an acitvity of 118 ± 12 nmol/min g liver wet weight. The lines were calculated using the least-squares method.

Fig. 2. Inhibition of benzo(*a*)pyrene-4,5-oxide hydration in rat liver microsomes by cadmium, Lineweaver-Burk plot. Each point represents the mean of four to nine independent (separate enzyme preparations) duplicate measurements. Standard error in one direction is given. The point inhibited by 10 µmol/l of cadmium at the 13.5 µmol/l concentration of the substrate was not determined. The activity is given related to uninhibited activity at a benzo(*a*)pyrene-4,5-oxide concentration of 10 µmol/l which gave an activity of 81 ± 7.8 nmol/min g liver wet weight. The lines were calculated using the least-squares method.

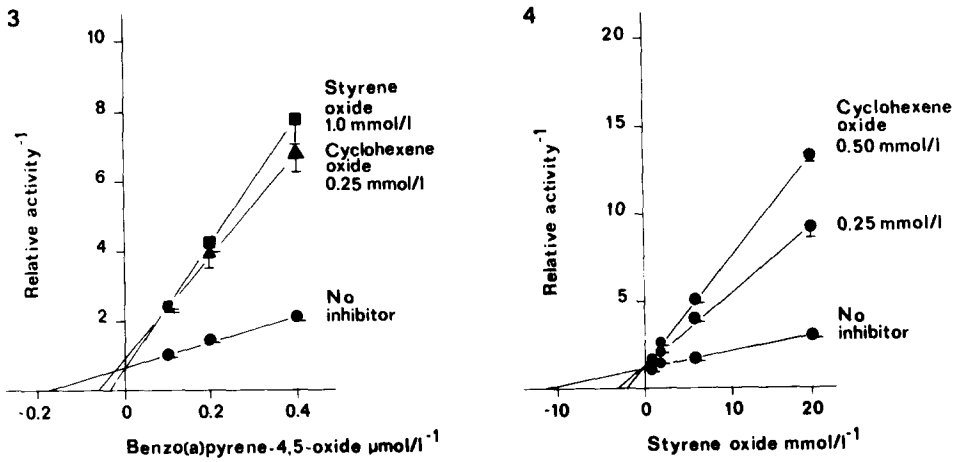


Fig. 3. Inhibition of benzo(*a*)pyrene-4,5-oxide hydration in rat liver microsomes by styrene oxide and cyclohexene oxide in vitro, Lineweaver-Burk plot. Each point represents mean of four independent (separate enzyme preparations) duplicate measurements. Standard error to one direction is given. The activity is given related to uninhibited activity at a benzo(*a*)pyrene-4,5-oxide concentration of 10 μmol/l, which gave an activity of 94.5 ± 15 nmol/min g liver wet weight. The lines were calculated using the least-squares method.

Fig. 4. Inhibition of styrene oxide hydration in rat liver microsomes by cyclohexene oxide in vitro, Lineweaver-Burk plot. Each point represents mean of four independent (separate enzyme preparations) duplicate measurements. Standard error in one direction is given. The activity is given related to uninhibited activity at a styrene oxide concentration of 1.74 nmol/l, which gave an activity of 153 ± 2.5 nmol/min g liver wet weight. The lines were calculated using the least-squares method.

this centre but to a site in close vicinity to it. As bound to this site, cadmium impairs the function of the enzyme on styrene oxide without preventing the binding of this substrate (noncompetitive inhibition). On the contrary, the binding of the larger benzo(*a*)pyrene-4,5-oxide is sterically hindered in binding to the enzyme by cadmium giving rise to competitive inhibition.

Cadmium is bound to microsomal constituents, other than epoxide hydrase, decreasing the actual concentration of the metal in the medium. To estimate the error caused by nonspecific binding by microsomes the apparent $K_i$ for styrene oxide hydration was determined at three enzyme concentrations and extrapolated to zero enzyme concentration using linear regression. (Four independent duplicate measurements were performed with microsome amounts corresponding to 150, 100 and 50 mg liver, wet weight. The styrene oxide and cadmium concentrations used were 52.2 and 260 μmol/l, respectively, $r = 0.87$). This gave a value below 10 μmol/l for cadmium. This is close to the $K_i$ of 5 μmol/l obtained for benzo(*a*)pyrene-4,5-oxide hydration (the data from Fig. 2), where the interfering microsomal amount is very low (microsomes corresponding to 2.5 mg liver, wet weight, were used). The low $K_i$ values estimated indicate that cadmium is a very potent inhibitor of epoxide hydrase. This compares with the apparent $K_i$ of about 100 μmol/l with both of the substrates for cyclohexene oxide, as well known inhibitor of epoxide hydrase. A comparison was also made with trichloropropene oxide, which is regarded as the strongest inhibitor of epoxide hydrase [1–3]. The inhibition of trichloropropene oxide appeared to be strongly dependent on the amount of microsomal preparation present. A 50% inhibition of styrene oxide

hydration was obtained with 20 μmol/l trichloropropene oxide. A similar inhibition of benzo(*a*)pyrene-4,5-oxide was achieved at 2 μmol/l trichloropronene oxide.

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