Kinetic behaviour of microsomal styrene monooxygenase and styrene epoxide hydratase in different animal species[1]

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Summary. The apparent K_m and V_{max} values of styrene epoxide forming monooxygenase and styrene epoxide hydratase have been evaluated in the liver microsomes of male rats, mice, guinea-pigs and rabbits. Epoxide hydratase gave much higher and more uniform K_m values than the monooxygenase in the species considered.

Styrene, vinyl chloride and other olefins with higher molecular weights are commonly used in plasticizers or sodium alkyl sulphate detergents. It has been suggested in recent years that most of these agents may be carcinogenic in laboratory animals, through the formation of highly reactive metabolic intermediates. One of the major metabolic pathways of these compounds is now assumed to be oxidation to the corresponding epoxides by microsomal mixed function oxidase enzymes, followed by conversion of the products to glycols by hepatic microsomal epoxide[3,4].

In the present paper we report the kinetic parameters of 2 key enzymes which form and hydrate epoxides, i.e. the epoxide monooxygenase and hydratase. Styrene and styrene epoxide were used as substrates. These compounds are particularly useful, since they represent 2 consecutive substrates in vivo also. The knowledge of their specific activities and apparent K_m values may partly help in predicting the ratio between the formation and detoxification of unstable styrene epoxide.

The experiments were carried out in liver microsomes from 4 commonly used animal species, i.e. rat, mouse, guinea-pig and rabbit. The differences in kinetic parameters from one species to another might help in choosing the most sensitive one, in order to develop new and more specific experimental approaches to study the chemical induction of cancer.

Materials and methods. Adult male animals of the following species were used in all experiments: Sprague Dawley rats (150–180 g); New Zealand rabbits (2–5 kg); albino Swiss mice (20–25 g) and Dunkin Hartley guinea-pigs (300–350 g). Animals were allowed food and water ad libitum, and were killed by decapitation. Microsomes were prepared according to the method of Kato and Takayanaghi[5]. The isolation medium contained 50 mM phosphate buffer pH 7.4, 150 mM KCl and 5 mM $MgCl_2$. After the last centrifugation, the microsomes were resuspended in the same medium and used the day they were prepared. Protein concentrations were determined by the method of Lowry et al.[6] with crystalline bovine serum albumin as standard.

Enzyme assay. The activities of styrene monooxygenase and epoxide hydratase were determined, using styrene and styrene epoxide as substrates, as previously described[7]. Enzyme activities were assayed under conditions in which the reaction was linear with respect to the incubation time and protein concentration. To assay the activity of both enzymes, 1–2 mg/ml microsomal protein was used in a final volume of 5 ml of incubation medium consisting of the medium used for microsome preparation, microsomes and substrates. Preparations were incubated at 37 °C for 5 min.

The reaction for assay of styrene monooxygenase was started by the addition of various amounts of a 250 mM acetone solution of styrene, in order to have 6 final styrene concentrations ranging from 0.1 to 5.0 mM. The reaction for assay of styrene epoxide hydratase was started by adding various amounts of a 42 mM acetone

Table 1. Apparent K_m and V_{max} values of styrene epoxide forming monooxygenase in the liver of different animal species

Species	K_m(mM)	V_{max} (nmoles/min/mg protein)
Rat	0.23 ± 0.04	2.71 ± 0.17
Mouse	0.04 ± 0.01	3.81 ± 0.12
Guinea-pig	0.18 ± 0.03	5.41 ± 0.07
Rabbit	0.08 ± 0.02	4.18 ± 0.08

Each value is the average ± SE of 3 analysis of pools of 5 mouse livers or individual livers for the other species.

Table 2. Apparent K_m and V_{max} values of styrene epoxide hydratase in the liver of different animal species

Species	K_m (mM)	V_{max} (nmoles/min/mg protein)
Rat	0.93 ± 0.05	10.5 ± 0.57
Mouse	0.73 ± 0.07	5.52 ± 0.23
Guinea-pig	0.73 ± 0.03	8.97 ± 1.66
Rabbit	0.51 ± 0.04	9.98 ± 0.13

Each value is the average ± SE of 3 analysis of pools of 5 mouse livers or individual livers for the other species.

Table 3. Ratios of apparent K_m and V_{max} values of styrene epoxide hydratase to styrene monooxygenase in different animal species

Species	K_m hyd/K_m monoox.	V_{max} hyd/V_{max} monoox.
Rat	4.0	3.9
Mouse	18.2	1.4
Guinea-pig	4.0	1.6
Rabbit	6.4	2.4

1 Acknowledgments. This work has been partially supported by the Fondazione Valenti (Milano).

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solution of styrene oxide in order to have 6 final styrene oxide concentrations ranging from 0.1 to 3.0 mM. Blank values due to non-enzymatic opening of the epoxide were subtracted from the 2 highest concentrations. Enzyme activities are expressed in nmoles/min/mg of protein.
Statistics. At least 3 animals were used for each species. For the mouse, 3 different pools of 5 livers each were used. Kinetic parameters were calculated according to the Woolf plot.
Results and discussion. Table 1 reports the apparent K_m and V_{max} values for styrene epoxide forming monooxygenase, and table 2 those for the epoxide hydratase, in the 4 animal species considered. Table 3 shows the apparent K_m and V_{max} ratios of the epoxide hydratase to the monooxygenase in the different species. In the mouse and rabbit, K_m values for the epoxide hydratase are 18.2 and 6.4 times those of the monooxygenase, while the specific activity of the hydratase for the rabbit is only twice that of the monooxygenase and is almost the same in the mouse.
This broad variability of K_m might imply that at the styrene concentrations (0.1–5 mM) we used in vitro, which presumably reflect those in vivo, the affinity of styrene epoxide for the hydratase is the rate limiting step for the overall metabolic transformation of styrene. This means that it is the speed of hydration of styrene epoxide and not of its formation that determine the rate at which styrene is detoxified.
The effect was less pronounced in the rat and guinea-pig. Table 1 indicates the broad inter-species variability in the K_m of styrene monooxygenase, whereas homogeneous values were found for the epoxide hydratase. These findings are in agreement with data that show there are multiple forms of P-450 dependent monooxygenases with different affinities[8,9].
The greater affinity of styrene monooxygenase, compared to the hydratase, and its considerable inter-species variability seems an important factor in establishing which animal species is most suitable as a model for studies on carcinogenesis. The species with higher rates of epoxide formation (mouse and rabbit) and slower epoxide hydration should be preferred for research into toxicity, such as liver necrosis or tumours in target organs due to accumulation of highly reactive epoxidic metabolic intermediates of foreign compounds.

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Coupling of fructose-1,6-P_2 to aminated agarose by Schiff base reduction. Affinity chromatography of yeast aldolase[1]

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Summary. Fructose-1,6-P_2 was immobilized by sodium borohydride reduction of the Schiff base formed with aminated agarose (AH-Sepharose 4B ®). The coupling occurs with high yield (25 μmoles immobilized fructose-1,6-P_2 per ml packed gel) at neutral pH and room temperature. Schiff base reduction thus provides a convenient and mild coupling procedure for sugar phosphates preserving their labile phospho ester bonds. As exemplified by a new isolation procedure for fructose-1,6-P_2 aldolase from yeast, sugar phosphates insolubilized in this manner may be used for affinity chromatography of the corresponding enzymes, provided that contaminating unspecific phosphatases are removed in a preceding fractionation step.

Sugar phosphates might afford suitable ligands for affinity chromatography of a large number of enzymes. However, the majority of sugar phosphates are labile to hydrolysis by, e.g., alkaline or acid pH, at temperatures above room temperature[4,5], the conditions required for the presently available coupling procedures for carbohydrates[6,7]. This report presents a coupling procedure which is operative at room temperature in the neutral pH range and thus does not affect labile phospho ester bonds. Its practicability was tested by isolating aldolase from yeast by affinity chromatography with insolubilized fructose-1,6-P_2.
Materials. Baker's yeast was purchased from Presshefefabrik Hindelbank; DEAE-Cellulose (DE 52) from Whatman; AH-Sepharose 4B (cross-linked agarose in bead form) from Pharmacia Fine Chemicals; sodium borohydride, ethylenediamine tetraacetic acid, 2-mercaptoethanol and 4-nitrophenyl phosphate from Fluka; enzyme grade ammonium sulfate from Schwarz/Mann; phenylmethylsulfonyl fluoride from Sigma. Fructose-1,6-P_2 tetracyclohexylammonium salt (for aldolase activity assay), fructose-1,6-P_2 trisodium salt, NADH, glucosephosphate isomerase (EC 5.3.1.9), glucose-6-phosphate dehydrogenase (EC 1.1.1.49), and glycerol-3-phosphate dehydrogenase/triosephosphate isomerase (EC 1.1.1.8/EC 5.3.1.1) were from Boehringer. Alkaline phosphatase (EC 3.1.3.1) was prepared from E. coli[8].
Methods. Aldolase activity was determined by the coupled assay with glycerol-3-phosphate dehydrogenase omitting 2-mercaptoethanol[9]. In the first steps of the preparation, protein concentrations were determined by the biuret method calibrated with bovine serum albumin; concen-

1 This work was supported by the Swiss National Science Foundation, grant No. 3.620-0.75. A preliminary account has been presented at the 10th Int. Congress of Biochemistry, Hamburg, 1976, Abstracts, p. 194.
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3 Acknowledgment. The excellent technical assistance of Alice Gasser is gratefully acknowledged.
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