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ENANTIOSELECTIVITY IN GLUTATHIONE CONJUGATION OF 1,2-EPOXY-1,2,3,4-TETRAHYDRONAPHTHALENE BY HEPATIC GLUTATHIONE *S*-TRANSFERASE

Tadashi Watabe, Akira Hiratsuka, and Toshiki Tsurumori

Laboratory of Drug Metabolism & Toxicology, Department of Hygienic Chemistry, Tokyo College of Pharmacy, Hachioji-shi, Tokyo 192-03, Japan

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SUMMARY. Enantiomers of 1,2-epoxy-1,2,3,4-tetrahydronaphthalene (ETN) were conjugated with glutathione (GSH) specifically at their benzylic oxiran carbons, with a marked difference in rate [(1*R*,2*S*)-(+)- < (1*S*,2*R*)-(-)-ETNs] as well as in affinity for GSH *S*-transferase [*K*m: (1*S*,2*R*)-(-)- < (1*R*,2*S*)-(+)-ETNs], in rat liver cytosol to yield two diastereomeric *S*-(2-hydroxy-1,2,3,4-tetrahydronaphth-1-yl)glutathiones which were separable by reverse partition hplc. Enzymatic GSH conjugation of racemic ETN occurred preferentially with the (1*S*,2*R*)-(-)-component as a result of its retarding effect on the conjugation of the (1*R*,2*S*)-(+)-counterpart, one half of which remained in enantiomerically pure form in the incubation medium when the (1*S*,2*R*)-(-)-component had been completely conjugated.

The study on metabolic inactivation of epoxides by hepatic soluble glutathione (GSH) *S*-transferase has been started with racemic 1,2-epoxy-1,2,3,4-tetrahydronaphthalene (ETN) by Boyland and his coworkers in relation to the mechanism of urinary excretion of α-hydroxy-tetrahydronaphthyl-mercapturic acid in rats given dihydronaphthalene (1,2). They isolated a GSH conjugate of racemic ETN from a hepatic incubation mixture and suggested that the conjugation occurred specifically at the benzylic carbon of ETN (2). Direct evidence for the regiospecific conjugation of GSH at the benzylic carbon of racemic ETN has very recently been obtained by the assignment of the ^{13}C nmr spectrum of the conjugate isolated by hplc (3).

Regiospecific conjugation of GSH at the benzylic carbon of the oxiran has been also demonstrated with epoxides of *trans*-β-methylstyrene (3) and other dihydrobenzarenes, such as 3,4-dihydrophenanthrene (3), 7,8-dihydrobenzo[*a*]-pyrene (4), and 9,10-dihydrobenzo[*a*]pyrenes (3). Epoxides consisting of both non-benzylic carbons, however, undergo specific introduction of the sulfhydryl group of GSH into their less hindered oxiran carbons as had been demonstrated

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with cholesterol 5,6α-epoxide (5) and 7-glycidoxycoumarin (6). Phenyloxiran, the simplest epoxide with benzylic and non-benzylic carbons in its oxiran ring, is conjugated at both of them in the higher ratio of the former to the latter (7,8). There is a marked difference in rate of the enzymatic GSH conjugation between *R*- and *S*-phenyloxirans, the former of which showed a higher rate than the latter (8). K-Region epoxides of polynuclear arenes and aza-arenes have been demonstrated to be conjugated with GSH at their *R*-carbons with extremely high regioselectivity by rat liver cytosolic GSH *S*-transferases C_2 (9) and AC (10).

During the course of further investigation of the GSH conjugate formed from racemic ETN, the authors found that it could be separated into two components by reverse partition hplc carried out on an octadecylsilicone (ODS) column. The present paper deals with a unique profile of the enzymatic conjugation reaction of the enatiomers in racemic ETN by rat liver cytosol.

MATERIALS AND METHODS

Materials

Racemic (1), (1*R*,2*S*)-(+)- and (1*S*,2*R*)-(-)-ETNs (11) were synthesized as previously reported. Enantiomeric homogeniety of these epoxides was confirmed by determining their $[\alpha]_D^{20}$ values after derivatization from diastereomeric *l*-menthoxyacetates of *trans*-2-bromo-1-hydroxy-1,2,3,4-tetrahydronaphthalene (11), which were separated at retention times of 9.7 and 10.6 min by hplc on a Nucleosil 50-5 column (10 mm x 25 cm, 5 μ in particle size) eluted with 0.5% THF in *n*-hexane (7.0 ml/min).

GSH, free from its oxidized form, was obtained from Yamanouchi Pharmaceutical Co. Ltd., Tokyo.

Incubation of ETNs and isolation of their GSH conjugates

ETNs were incubated with the dialyzed soluble supernatant fraction from male Wistar rat (100-120 g body weight) liver in the presence of GSH, and their GSH conjugates were isolated on an Amberlite XAD-2 column as previously reported (12).

Chromatography

Hplc was carried out on a Constametric Model II G high pressure liquid chromatograph equipped with a Shimadzu Model SPD-1 stop and flow spectrophotometer. Tlc was carried out on Merck cellulose F_{254} plates in *n*-butanol-pyridine-water (2:1:1). The chromatograms were visualized as uv-absorbing spots with a uv lamp (254 nm) or as coloring spots with ninhydrin.

Spectroscopy

^{13}C nmr and uv spectra were recorded as previously reported (3) with the conjugates isolated by hplc.

Circular dichroism (CD)

CD curves of the GSH conjugates were recorded on a JASCO Model J-500 C spectropolarimeter after being isolated by hplc. CD at 273 nm of ETN was determined after it was extracted from the incubation mixture with *n*-pentane, condensed and isolated by hplc on a Spherisorb silica column (4 mm x 30 cm, 5 μ in particle size) eluted with 0.5% isopropyl alcohol in *n*-hexane (2.0 ml/min); retention time of ETN was 5.0 min.

RESULTS AND DISCUSSION

Racemic ETN was incubated with rat liver cytosol containing GSH until the epoxide was completely consumed, whose concentration was checked by absorptiometry at 254 nm after extracting the mixture with *n*-hexane. A uv-absorbing and ninhydrin-positive product, which showed a single spot at Rf 0.45 in the thin-layer chromatogram, was isolated from the incubation mixture by using an Amberlite XAD-2 column. The product showed the same ^{13}C nmr spectrum as that of the previously isolated ETN-GSH conjugate, which had been assigned as *S*-(2-hydroxy-1,2,3,4-tetrahydronaphth-1-yl)glutathione (3), and was resolved into two peaks with the same peak area at retention times of 15.4 min (conjugate I) and 16.3 min (conjugate II) in the reverse partition hpl-chromatogram (Fig. 1). The conjugates separately eluted from the hplc column showed uv-absorbing,

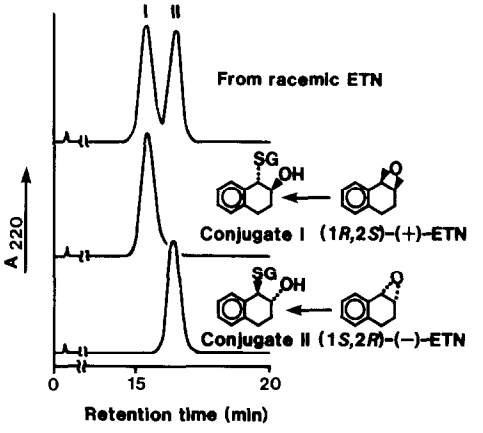

Fig. 1. Separation by Hplc of Diastereomeric GSH Conjugates Formed from Racemic and Enantiomeric ETNs in Rat Liver Cytosol. ETNs (0.3 mM each) were incubated at 37°C with rat liver cytosol (3.5 mg protein/ml) in 0.1 M phosphate buffer, pH 7.4, containing acetone (2.5%, v/v), until the substrates were completely consumed; 80, 60, and 20 min for racemic, (1*R*,2*S*)-(+)- and (1*S*,2*R*)-(-)-ETNs, respectively. Each ETN was dissolved in the acetone and added to the incubation mixture. The conjugates were quantitatively isolated by using Amberlite XAD-2 columns. Hplc conditions —— column: Nucleosil ODS 7C18 (4 mm x 30 cm, 7 μ in particle size); solvent: methanol-water-acetic acid (30:70:0.5, v/v); flow rate:1 ml/min at 20°C.

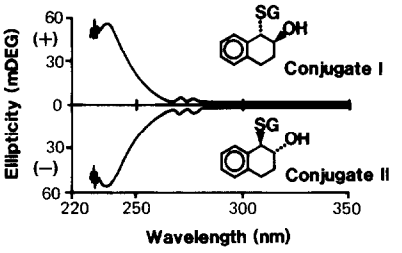

Fig. 2. Circular Dichroism Curves of GSH Conjugates Formed from Racemic and Enantiomeric ETNs. The conjugates were isolated from the incubation mixtures as described in Fig. 1 and dissolved in water for recording their CD at 0.77 mM and 20°C after removing the solvent at 80°C in a stream of nitrogen.

ninhydrin-positive, single spots with the same Rf value in the tl-chromatogram obtained under the same conditions as mentioned above.

Circular dichroism (CD) curves of conjugates I and II had positive and negative ellipticities with the same amplitude, respectively, in the wavelength region of 230-350 nm (Fig. 2). The same molar concentration of GSH, however, showed no appreciable CD curve under the same conditions. CD of the conjugates, therefore, indicates that they are antipodes each other in absolute configuration of C_1 and C_2 of the tetrahydronaphthalene moiety.

Incubation of enantiomerically pure (1*R*,2*S*)-(+)- and (1*S*,2*R*)-(-)-ETNs with rat liver cytosol in the presence of GSH under the same conditions as used for racemic ETN specifically yielded conjugates I and II, respectively (Fig. 1), which showed the same Rf value and CD curves as those from the racemate.

There was a marked difference in rate of formation of conjugates I and II from racemic ETN by hepatic cytosol. A time course study on the enzymatic conjugation of the racemic epoxide by hplc indicated that the rate of formation of conjugate II from the (1*S*,2*R*)-(-)-component was 4.7 times larger than that of conjugate I from the (1*R*,2*S*)-(+)-counterpart at 5 min of the reaction and also that the (1*S*,2*R*)-(-)-component was completely converted into conjugate II at 20 min while only 45% of the (1*R*,2*S*)-(+)-counterpart was consumed for the formation of conjugate I at the time (Fig.3). The remaining epoxide isolated from the incubation mixture at 25 min, which corresponded to one quarter of the racemic substrate used, had an almost completely enantiomeric purity : + 4.6 m° (0.11 mM in EtOH at 20°C). At the initial stage of the enzymatic

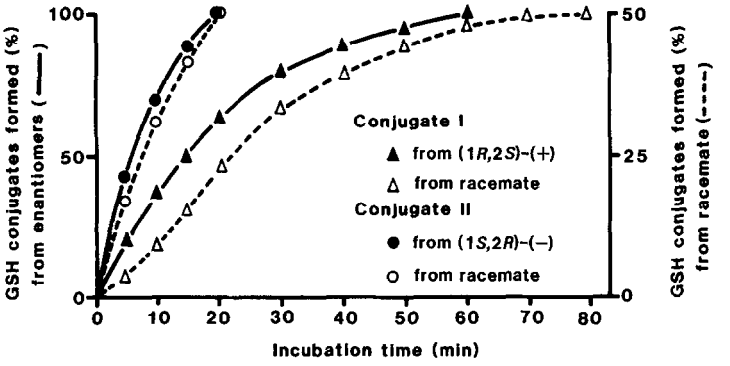

Fig. 3. Time Courses of Diastereomeric GSH Conjugates Formed from Enantiomeric and Racemic ETNs in Hepatic Cytosol. ETNs were incubated under the same conditions, and the conjugates formed were isolated and determined by hplc in the same manner as described in Fig. 1.

conjugation reaction of racemic ETN, the rate for the conjugate I formation was significantly retarded. However, it increased rapidly according to the decrease in concentration of the (1*S*,2*R*)-(-)-component from 50 to 25%. It took the (1*R*,2*S*)-(+)-counterpart about 80 min to disappear completely from the incubation mixture for the formation of the corresponding amount of conjugate I.

A marked difference also existed between the rates of formation of conjugates I and II from enantiomerically pure (1*R*,2*S*)-(+)- and (1*S*,2*R*)-(-)-ETNs by hepatic cytosolic GSH *S*-transferase. However, the difference was much smaller than the case of the aforementioned GSH conjugation of racemic ETN; conjugate II formation from the (1*S*,2*R*)-(-)-epoxide was twice as fast as conjugate I formation from the (1*R*,2*S*)-(+)-enantiomer so far as estimated from the zero-order kinetics region of the enzymatic reactions (Fig. 3). There was only little difference in rate between the enzymatic formation of conjugate II from enantiomerically pure (1*S*,2*R*)-(-)-ETN and from the same enantiomer in racemic ETN while a marked difference was there in the conjugate I formation from both (1*R*,2*S*)-(+)-ETNs. The difference in the conjugates I formation would be attributable to the inhibitory effect of the (1*S*,2*R*)-(-)-component on the enzymatic conjugation of the (1*R*,2*S*)-(+)-counterpart in the racemic substrate because kinetic data obtained by the double reciprocal plot method indicated that enantiomerically pure (1*S*,2*R*)-(-)-ETN had a higher affinity for GSH *S*-transferase than did its enantiomer; apparent *K*m values were 377 and 265 μM,

and *V*max values 94.3 nmol conjugate I and 179 nmol conjugate II formed/mg protein/min for (1*R*,2*S*)-(+)- and (1*S*,2*R*)-(-)-ETNs, respectively.

Racemic and enantiomeric ETNs (0.3 mM each) were considerably stable at pH 7.4 in the presence of the same concentration of GSH as used for the enzymatic reactions; they were non-enzymatically conjugated with little difference in rate only at 1.83 nmol/min. However, the rate of the non-enzymatic conjugation increased according to the increase in pH of the aqueous medium up to 10, and both racemic and enantiomeric ETNs were completely conjugated with GSH within 30 min at room temperature. At any pH higher than 7.4, non-enzymatic GSH conjugation of the enantiomeric ETNs also occurred regiospecifically at their benzylic carbon to specifically yield conjugates I and II from (1*R*,2*S*)-(+)- and (1*S*,2*R*)-(-)-ETNs, respectively, without any appreciable difference in rate. It might be predictable to exist a stereochemical rule in the enzymatic GSH conjugation reaction of non-bay region olefinic epoxides of dihydrobenzoarenes since a very recent study has demonstrated that the 7*S*-carbon of enantiomeric 9,10-dihydrobenzo[*a*]pyrene 7,8-epoxides is also preferentially conjugated with GSH at a higher rate with smaller *K*m (4).

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