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Chiral Discrimination in the Transport of Ketoprofen and Ibuprofen Esters through an Aqueous Phase Mediated by Various Serum Albumins

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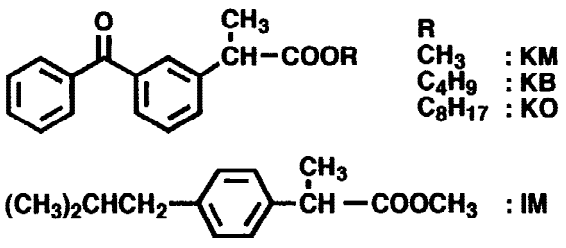
ABSTRACT Serum albumins that act as carriers discriminated between enantiomers of alkyl esters of ketoprofen and ibuprofen in transport in the O/W/O (oil/water/oil) system using a U-shaped cell. The transport rate and the preferred enantiomer of the esters were substantially affected by pH, temperature, and species of albumin. Among five serum albumins studied, bovine serum albumin (BSA) showed the largest rate constant and rat serum albumin (RSA) manifested the highest enantioselectivity. Regarding enantiomer selectivity in transport overall, it is anticipated that the ester uptake step plays an important role for BSA, whereas the ester release is the key step for RSA. *Chirality 11:516–519, 1999.*

KEY WORDS: anti-inflammatory drug esters; induced circular dichroism; bovine serum albumin; rat serum albumin; o/w/o transport; enantiomeric excess

It has been recently reported that serum albumin, one of the transport proteins in blood plasma, can discriminate between enantiomers of antiinflammatory drugs such as ketoprofen in the formation of complexes,[1,2] giving different induced CD (ICD) for the two enantiomers. In the case of ketoprofen methyl ester, however, it is interesting that Cotton effects and intensities of ICD observed for the (R)- and (S)-enantiomer complexes were the same.[1] Thus, we examined chiral discrimination of serum albumins of several species against ketoprofen and ibuprofen alkyl esters practically insoluble in water, by transport experiments through an aqueous phase, because these experiments have been effective for the isolation and purification of isomers.[3] The transport of chiral drugs mediated by serum albumin is a fascinating subject from a biomimetic point of view. So far, investigations have been reported on achiral fatty acid transport using bovine serum albumin (BSA) and human serum albumin.[4] Furthermore, most of the transport studies with a liquid membrane have been W/O/W systems[5] and few have been reported on a water-based liquid membrane (O/W/O system) for the separation of chiral organic molecules.[6,7]

The substrates in the present study were methyl, butyl, and octyl esters of ketoprofen (KM, KB, and KO) and methyl ester of ibuprofen (IM).



MATERIALS AND METHODS

Chemicals and Instruments

Pure enantiomers of ketoprofen and ibuprofen were generous gifts from Nagase & Co., Ltd (Kobe, Japan) and Nissan Chemical Industries, Ltd (Tokyo, Japan). All serum albumins (essentially fatty acid free) were purchased from Sigma (St. Louis, MO). Racemic ketoprofen and ibuprofen were obtained from Wako Pure Chemicals (Osaka, Japan). KM and IM were prepared by esterification of ketoprofen and ibuprofen in methanol in the presence of thionyl chloride, respectively. KB and KO were synthesized by esterification of ketoprofen in n-butanol and n-octanol in the presence of *p*-toluenesulfonic acid, respectively. The solubility of the esters in water were 6.4×10^{-5} mol dm^{-3} for KM, 7.5×10^{-6} mol dm^{-3} for KB, less than 10^{-7} mol dm^3 for KO, and 6.2×10^{-5} mol dm^{-3} for IM.

UV spectra were obtained on a Shimadzu UV-2200A spectrophotometer. CD measurements were carried out with a JASCO J20A spectropolarimeter. The enantiomer ratios of the esters were measured by HPLC using Shimadzu LC-10AT and SPD10A systems equipped with a Chiralcel OB-H column (Daicel) for KM, KB, and KO and a Chiralcel OJ column (Daicel) for IM. The mobile phase was hexane/2-propanol, 9/1 for KM and KB, 20/1 for KO,

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and 40/1 for IM with flow rates of 1, 0.8, 0.5, and 0.15 ml min^{-1} for KM, KB, KO, and IM, respectively. The retention times for the R and S enantiomers were 30.0/38.0, 14.0/21.0, 18.5/27.6, and 27.0/29.0 min for KM, KB, KO, and IM, respectively.

Transport

The cell used in the transport was a U-shaped glass tube with an inside diameter of 1.5 cm. A 7 ml amount of the BSA aqueous solution (3.0×10^{-4} mol dm^{-3}) was placed at the bottom of the cell. n-Octane or n-decane (10 ml) was used as the source and receptor phases, and the substrate concentration in the source phase was 3×10^{-3} mol dm^{-3}. The aqueous phase in the bottom of the cell was maintained in a thermostat and stirred at a constant rate. The transport rate was measured by monitoring the increase in the UV absorbance of the receiving phase with a time interval of 15–30 min. The enantiomer ratio (ER) was determined by HPLC equipped with a chiral column, showing a reproducible value to about ±2%.

ICD of the Complexes of Serum Albumin with the Substrate

All the complex solutions were prepared using pH 8.6 buffer solution. To 5 ml of the BSA (or RSA) aqueous solution (6×10^{-5} mol dm^{-3}) was added 0.1 ml of the substrate in ethanol (6×10^{-3} mol dm^{-3}); the total volume was then made up to 10 ml with the buffer solution to give [BSA (or RSA)]/[substrate] of 1/2. The mixture was agitated gently at 25°C for 30 min, and then the ICD was measured. It was confirmed that no hydrolysis products of the substrates catalyzed by the albumins under these conditions were detected by HPLC.

Release

For the release experiments pH 8.6 buffer solution was used. To 5 ml of the BSA (or RSA) aqueous solution (3.6×10^{-4} mol dm^{-3}) was added 0.1 ml of the substrate in ethanol (6×10^{-3} mol dm^{-3}); the total volume was then made up to 10 ml with the buffer solution. The mixture was incubated at 25°C for 30 min to give a clear solution with [BSA (or RSA)]/[substrate] of 3/1. A 7 ml amount of this solution was placed in a test tube, and 10 ml of decane was floated over the solution. The aqueous layer was stirred at a constant rate, thermostated at 36°C. The substrate transferred from the aqueous to the organic layer was measured by the UV absorbance.

RESULTS AND DISCUSSION

Transport of the Substrates by BSA

The cell used for chiral discrimination in the transport was a U-shaped glass tube[8] as illustrated in Fig. 1. The aqueous solution of BSA that acts as a carrier was placed at the bottom of the cell, with octane or decane used as solvent, a source phase containing the racemic substrates and a receptor phase. The amount transported to the receiving phase increased linearly with time in a zero-order fashion, because of high concentration of substrates initially dissolved in the source phase. Thus, transport rates were determined by the slope of the time course of the transport

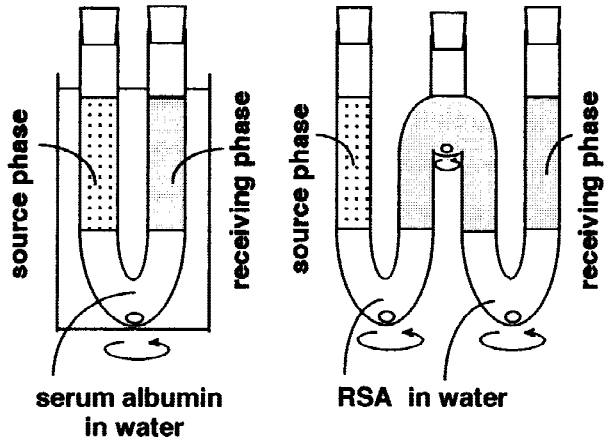

Fig. 1. U- and W-shaped cells with an inside diameter of 1.5 cm.

up to ca. 1% of the initial amount in the source phase. The apparent enantiomeric composition of the substrates transported in the receiving phase remained almost unchanged throughout the time course experiments. The transport rates (pH 8.6, 36°C) per mole of BSA were in the order KM (54.8×10^{-3}) > KB (6.5×10^{-3}) > KO (1.2×10^{-3} mol h^{-1}), reflecting the increasing steric hindrance of alkyl chain. Even taking into account the net transport by BSA, obtained from the apparent transport in the presence of BSA less the control transport without BSA, the ER of the transported esters was small and no conspicuous trend was indicated: (S) ER; KM 2.6%, KB 6.9%, and KO −4.8%. With IM, this value was rather high at 35.0%, but the net transport rate was small (0.66×10^{-3} mol h^{-1}). Thus, as a substrate, we examined mainly KB, which shows relatively high chiral discrimination and net transport rate.

The conditions affecting rate and ER were investigated with respect to pH and temperature. With a rise of pH from 4 to 10, the rate for racemic KB showed an S-shaped change (Fig. 2) which resembles the profile of conformational population changes in the N–B transition of BSA.[9] The ER also showed the inclination of a gradual increase with an increasing pH. This may be ascribable to changes of the uptake site of BSA such as substrate accommodation ability (e.g. size and shape) and hydrogen bonding ability, which were caused by alterations of the sterical structure. As an elevation of temperature activates molecular motion, in this case the transport rate was gradually enhanced, while the discrimination ability of BSA was reduced (Fig. 3). However, it was observed around 40°C that BSA preferred a reverse antipode (vide infra). Irreversible conformational changes begin to occur over ca. 45°C,[10,11] which may reduce the binding ability of BSA to the substrates, resulting in a decrease of both the transport rate and the chiral discrimination.

Transport of KM and IM by Serum Albumins of Several Species

As species differences in structures and properties of serum albumins are considerable as has been previously reported,[12,13] we can anticipate species differences in the

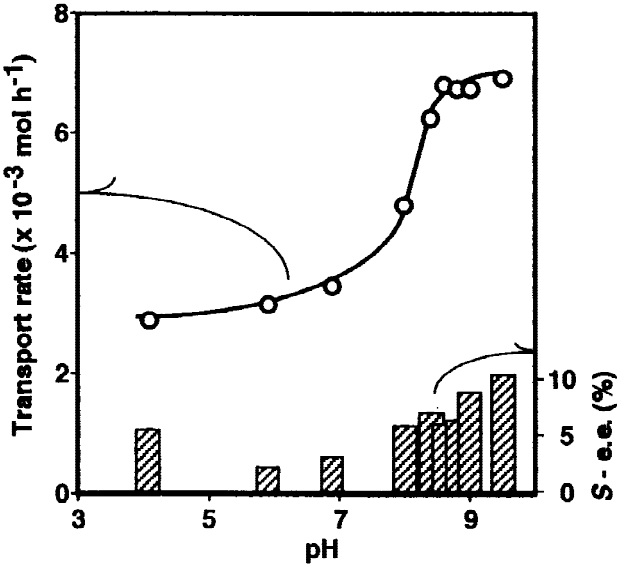

Fig. 2. The pH dependence of the transport rate and the preferential enantiomer of KB mediated by BSA at 36°C. The rates are obtained as per mole of BSA.

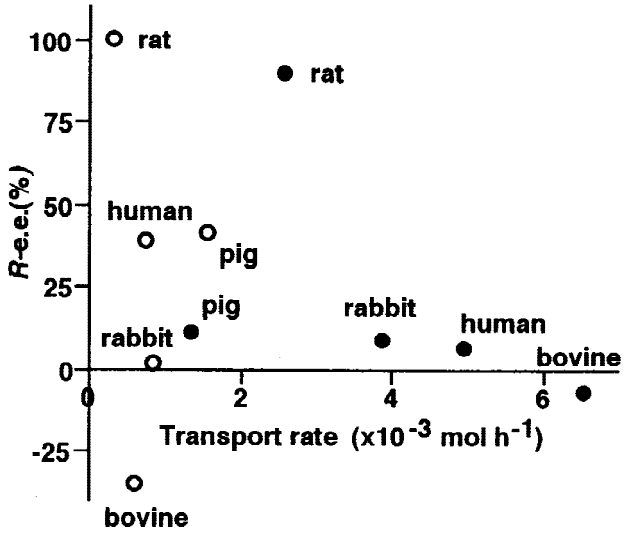

Fig. 4. Species difference of serum albumins in the transport rate and the preferential enantiomer of KB (●) and IM (○) at 36°C and pH 8.6. The rates are obtained as per mole of each serum albumin.

transport of the present substrates. Figure 4 demonstrates the net transport rates and ER for serum albumins from five species using the racemate KB and IM as substrates. The rate was largest for KB-BSA and smallest for IM-rat serum albumin (RSA). All the albumins except for BSA transported the (R)-enantiomer more effectively. Especially, RSA manifested high selectivity toward the (R)-antipode for both KB and IM, though the transport rates were fairly slow.

Taking advantage of this nature of RSA, we examined the effective optical resolution of racemic KB and IM using a W-shaped cell[14] which ensures a two-step chiral discrimination (Fig. 1). When ca. 1% of the substrates initially placed in the source phase was transported, the apparent ERs of the substrates in the receiving phase were 100% for KB (76.3% of the apparent ERs for the U-shaped cell) and 72.3% for IM (50.7%). These results indicate the possibility that further modifications of the equipment and the transport conditions may attain more effective resolution.

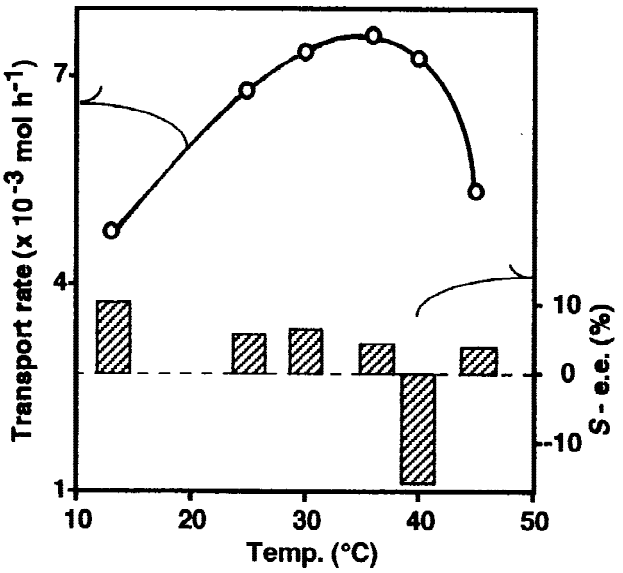

Fig. 3. Temperature dependence of the transport rate and the preferential enantiomer of KB mediated by BSA at pH 8.6. The rates are obtained as per mole of BSA.

Comparison between BSA and RSA at the Uptake and Release Steps

The transport process in this system is considered to be composed of three steps in which the rate and chiral recognition are regulated: the uptake of the substrate from the source phase, the transfer in the aqueous phase, and the release to the receptor phase. The transfer step is not responsible for the selection of antipodes, and the transfer rate was fast enough because of rapid stirring, resulting in the low stationary concentration of the albumin/substrate complex in the aqueous phase ($<10^{-7}$ mol dm^{-3}). Due to the low partition of the substrate in the aqueous phase containing the albumin in comparison with that in the organic phase, the reliable migration amount of the substrate from the organic to the aqueous phase could not be estimated. Thus, we tentatively regarded the intensity of the ICD of the albumin/substrate complex as a measure of the uptake by albumins.

The complex of BSA with the racemate KB exhibited an ICD (broad) maximum around 345 nm with a positive Cotton effect. Similar spectra were obtained for the complexes of BSA with KM and KO (Fig. 5), where the ICD intensities fell in the order of KO > KB > KM. This suggests that these racemic esters may be incorporated into BSA by hydrophobic interactions with the benzoylphenyl group, and the ICD observed are presumably due to the twist of the carbonyl moiety as reported in the literature on the ketoprofen–BSA complex,[1] their intensities being subject also to steric effects by the alkyl chain length of the esters.

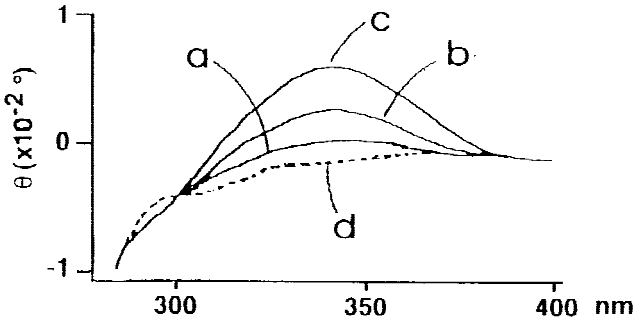

Fig. 5. ICD spectra of the complexes of BSA with the ketoprofen esters: (a) KM-BSA; (b) KB-BSA; (c) KO-BSA; (d) BSA alone.

The ICD of the (R)-KB complex of BSA with the same as that of the (S)-KB complex as long as the shape and the location of the maximum. However, the intensity of the (R)-KB complex was slightly weaker by ca. 16% compared with that of the (S)-KB complex, suggesting the stronger interaction of (S)-KB with BSA than that of (R)-KB. On the other hand, the RSA complex with KB gave a similar ICD to that with BSA. However, no differences were observed for the RSA complexes of racemic, (R)- and (S)-KB concerning their ICD intensities as well as their shapes and maximum wavelength. This implies that RSA does not recognize the enantiomers, at least in the incorporation of KB.

Next, the rate and chiral discrimination at the release step from the aqueous layer to the organic layer were studied by conducting model experiments. An organic layer was floated over an aqueous BSA or RSA solution previously incorporating the same amount of racemic or enantiomeric KB, and the lower aqueous layer was gently stirred. The amount and ER of the substrate which migrated from the aqueous layer to the organic layer were estimated. The migration proceeded in a first-order fashion. In the case of BSA, the rate constants of migration increased in the order of S (0.141) < racemate (0.182) < R (0.365 h^{-1}). The migration of the (R)-enantiomer was 2.6 times faster than that of the (S)-enantiomer. This indicates that the (S)-enantiomer binds more strongly with BSA than the (R)-enantiomer, in agreement with the ICD study. Therefore, the reason why the transport of the racemate KB mediated by BSA brought about the (S) ER may be explained in terms of a larger amount of the (S)-enantiomer incorporated at the uptake step than its antipode. This may explain the reverse preference of BSA for the enantiomer around 40°C as stated above. With a rise of temperature, the discrimination ability of BSA is reduced, causing a decrease of the (S) ER in the uptake step. Eventually, the ratio of (R)-enantiomer surpassed that of its antipode in the overall transport process.

As for the release of the racemate KB from RSA, the migration proceeded also in a first order fashion. The migration rate was 0.02 h^{-1} which was ca. one-ninth that for BSA. The enantiomer ratio (R/S) of KB released to the organic layer was 1.0/0.1, indicating that the (R)-enantiomer was readily released and, thus, that the (S)-enantiomer of KB was more strongly bound to RSA. This is in marked contrast to the result of the ICD study, where no difference was observed between (R)- and (S)-enantiomers. Since RSA preferred the (R)-antipode in the transport, this shows that release is the key step for determining a chirality preference for the substrates in the overall transport process mediated by RSA.

In conclusion, serum albumins effect the transport of the water-insoluble ketoprofen and ibuprofen esters through an aqueous phase. The degree of chiral discrimination in the transport depends upon the species from which the albumins used were derived as carrier. Apparent large differences were noticed in the uptake and release steps by BSA and RSA, which may be attributed to the difference in the uptake sites for the present substrates. The detailed mechanisms and structure alteration in the transport process remain under investigation.

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