Effect of Serum Albumins from Several Mammals on the Photoisomerization of Bilirubin

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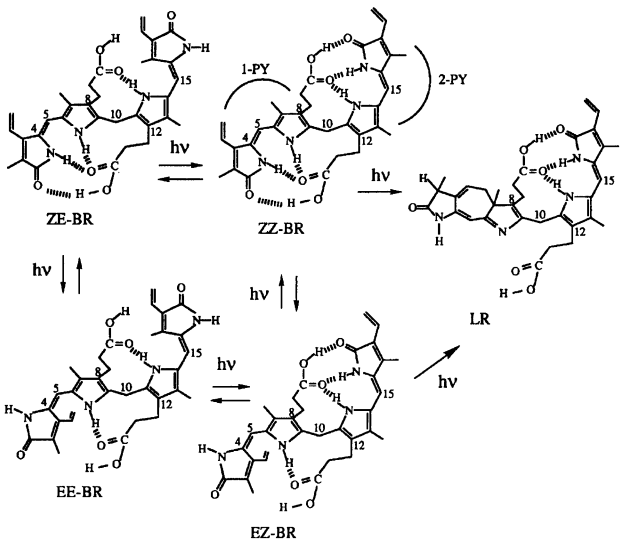
Synopsis. *ZZ*-Bilirubin undergoes photoisomerization to configurational (*ZE* and *EZ* isomers) and structural (LR) isomers in buffered aqueous solution containing serum albumins. The efficiency and the course of the isomerization are very much dependent on the mammals from which serum albumins are taken.

Bilirubin IXα (BR) is continuously produced in mammals such as human through the catabolic process of heme as *ZZ* isomer around the exocyclic double bonds at C4–C5 in the first pyrromethenone moiety (1-PY in Scheme 1) and C15–C16 in the second pyrromethenone moiety (2-PY in Scheme 1).[1—4] *ZZ*-BR is almost insoluble in water at physiological pH and exists in mammals as incorporated into serum albumin (SA); in liver it is converted to a water soluble ester by combining with gluculonic acid and excreted. In the medical treatment for neonate in which the organization of liver is not sufficiently accomplished, photoirradiation is performed to reduce the concentration of *ZZ*-BR in the blood.[1—5] In this case the photoirradiation converts *ZZ*-BR to water soluble BR isomers (*ZE*-, *EZ*-BR and LR in Scheme 1) to be excreted. Without this treatment, *ZZ*-BR is accumulated in the body resulting in serious damage of tissues such as brain.[1]

Many reports of medical treatment of neonatal jaundice appeared in literature for more than last twenty years. Most of them report from qualitative aspect the change of the isomer ratio of BR on irradiation either in vivo[1—5] such as in rat or human body or in vitro[6—15] such as in aqueous buffered solution containing, human (HSA), rat (RATSA) or rabbit serum albumins (RABSA). It is very important, however, to know the quantitative results of the irradiation to reveal and to simulate the photochemistry of bilirubin in human body and the effect of SA on the photoisomerization of BR. Till now the quantum yields of isomerization of *ZZ*-BR are reported only preliminarily for bovin serum albumin (BSA)[14] and HSA solution,[7,11,14,15] to show that HSA leads to more efficient isomerization than BSA. In this respect, we intended to determine the efficiency of photoisomerization of *ZZ*-BR in aqueous buffered solution containing SA from different mammals and now report that only HSA is highly effective for the photoisomerization and those from other mammals examined exhibit nearly equally lower efficiency, which notices the limitation of the use of rats as a model system for human body.



Scheme 1.

Experimental

ZZ-BR supplied from Sigma was purified as previously reported.[16] Essentially fatty acid-free horse serum albumin (HORSA) and BSA were obtained from Sigma as A5280 and A6003, respectively. RATSA and HSA, which are essentially fatty acid- and globulin-free, were obtained from Sigma as A4538 and A8763, respectively. Crystallized and lyophilized RABSA was obtained from Sigma as A0764. These albumins were used without further purification.

A typical procedure for the sample preparation was done as follows. A 10-100 mg of *ZZ*-BR dissolved in 0.1 M NaOH (M=mol dm^{-3}) was added to a 0.05 M potassium phosphate buffer solution (pH 7.4) containing a serum albumin to make the molar ratio of BR and serum albumin as 1. The concentration of BR was made as 1.0×10^{-4} and 1.0×10^{-5} M, for photoisomerization and absorption spectra measurements, respectively.

Absorption spectra of *ZZ*-BR (1.0×10^{-5} M) in various mammalian serum albumin solutions were taken in a 1 cm quarts cuvette on a JASCO 660 spectrophotometer.

The irradiation was performed under argon with 436-nm light from a 400-W high pressure mercury lamp through a solution filter (4.4 g dm^{-3} $CuSO_4\cdot5H_2O$+2.7 M NH_3+75 g dm^{-3} $NaNO_2$ in water). The light intensity was measured by using potassium tris(oxalato)ferrate (III) actinometry.

The isomer concentrations of BR after irradiation were analyzed by a high performance liquid chromatography (HPLC, Waters 600 multisolvent delivery system and 490 programable multiwavelength detector with a 740 data module) with a chemcosorb 5-ODS-H column (4.6×250 mm) eluting with MeOH containg 0.1 M dioctylammonium acetate.[9—11] Their concentrations were determined by

Table 1. Effects of Serum Albumins on the Quantum Yields for Isomerization and the Photostationary State isomer Compositions of BR

The source of serum albumin	Φ_{ZZ-ZE}	Φ_{ZZ-EZ}	Φ_{ZZ-LR}	Composition/%		
				ZZ	*ZE*	*EZ*
Horse (HORSA)	7×10^{-3}	2×10^{-3}	7×10^{-5}	86.2	9.7	4.1
Rat (RATSA)	6×10^{-3}	2×10^{-3}	2×10^{-4}	87.1	7.9	5.0
Bovine (BSA)	7×10^{-3}	3×10^{-3}	1×10^{-4}	85.1	9.3	5.6
Rabbit (RABSA)	5×10^{-3}	1×10^{-2}	2×10^{-3}	83.6	4.3	12.1
Human (HSA) [a]	0.11	<0.02	2×10^{-3}	63.0	35.3	1.7

a) Refs. 14 and 15.

using the sensitivity ratios between the isomers (*ZZ* : *ZE* : *EZ* : LR=1.0 : 0.70 : 0.67 : 0.43) at 450 nm.[15,17]

Results and Discussion

Absorption Spectra. Figure 1 depicts absorption spectra of *ZZ*-BR (1×10^{-5} M) measured in 0.05 M phosphate buffered solution, pH 7.4, containing SA from different mammals.

ZZ-BR exhibits absorption in the region of about 400 to 500 nm in various SA solutions.[18] The maximum wavelength and the shape of the absorption band are varied among the source of SA's. In solution containing HORSA, RATSA, BSA, or HSA, the maximum is found at about 460 nm accompanied by a shoulder at 420 nm. However, in RABSA solution, the wavelength for the absorption maximum and the shoulder for the other SA's solution are replaced each other; thus, the absorption maximum is shifted to 420 nm and a shoulder is found at 460 nm.

ZZ-BR takes two-folded intramolecularly hydrogen bonded conformation in $CHCl_3$.[19] However, in DMSO the intramolecular hydrogen bonds are broken and replaced by intermolecular hydrogen bonds with DMSO.[19] *ZZ*-BR exhibits an absorption maximum at 460 nm in both solvents, but is accompanied by a shoulder at 420 nm only in DMSO; the former absorption maximum is assigned to the intramolecularly hydrogen bonded conformation and the latter to the conformation of no or very weak intramolecular hydrogen bonds.[15,20,21] The similarity between the absorption spectrum in DMSO and those in SA solutions except RABSA indicates that in these SA solutions *ZZ*-BR may take a conformation similar to that in DMSO. However, in RABSA the absorbance is considerably higher at 420 nm than at 460 nm, which indicates that BR takes a more flexible conformation in RABSA than in the other SA solution.

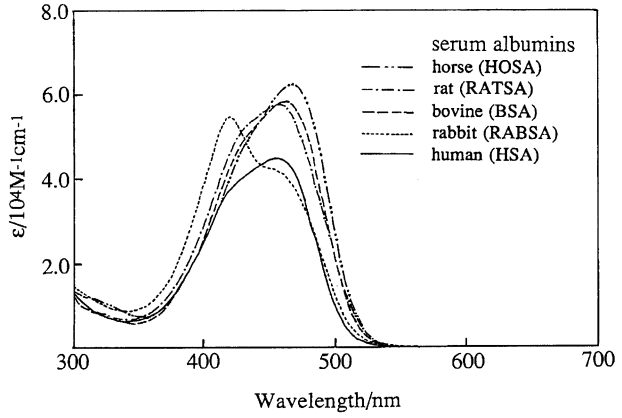


Fig. 1. Absorption spectra of *ZZ*-BR incorporated into serum albumins from different mammals.

Effects of Mammalian SA on Photochemistry of BR. The quantum yields and the photostationary state isomer compositions are varied among the source of SA's as shown in Table 1. The photoirradiation of *ZZ*-BR gives *ZE*, *EZ*, and LR. The HSA solution is the most efficient in the isomerization to *ZE* ($\Phi_{ZZ\rightarrow ZE}$), *EZ* ($\Phi_{ZZ\rightarrow EZ}$), and LR ($\Phi_{ZZ\rightarrow LR}$) with quantum yields of 0.11, <0.02, and 2×10^{-3}, respectively.[14,15]

BSA, HORSA, and RATSA solutions gives *ZE*, *EZ*, and LR with decreasing efficiency in this order like HSA; however, the efficiencies remain as low as $(6-7)\times10^{-3}$, $(2-3)\times10^{-3}$, and $(0.7-2)\times10^{-4}$, respectively. RABSA exhibits different behavior from other SA's; $\Phi_{ZZ\rightarrow EZ}$ is two times higher than $\Phi_{ZZ\rightarrow ZE}$, and both $\Phi_{ZZ\rightarrow EZ}$ and $\Phi_{ZZ\rightarrow LR}$ are nearly ten times larger than those in BSA, HORSA, and RATSA.

The isomer compositions at the photostationary state exhibit essentially the same tendency as their quantum yields. Thus, among SA's HSA shows the highest value of *ZE*-BR and the lowest values of *ZZ*- and *EZ*-BR, and RABSA gives the highest *EZ*-BR value which is still larger than the *ZE*-BR value. HORSA, RATSA, and BSA afford much smaller values for *EZ*- and *ZE*-BR.

Previously, we have shown that the photoisomerization of *ZZ*-BR is influenced by the solvent properties; $\Phi_{ZZ\rightarrow ZE}$, $\Phi_{ZZ\rightarrow EZ}$, and $\Phi_{ZZ\rightarrow LR}$ are higher in DMSO than in $CHCl_3$.[14,15] The quantum yields in BSA, RABSA, or HORSA solution are in similar magnitude to those in DMSO, which indicates that in BSA, RABSA, and HORSA *ZZ*-BR can take a conformation similar to in DMSO as suggested by the absorption spectra.

The observed very efficient isomerization in HSA solution is attributed to its special way of incorporating *ZZ*-BR, since the absorption spectra of *ZZ*-BR are not much different among the SA solutions except RABSA. The amino acid residues in HSA will lead *ZZ*-BR to

take appropriate conformations for the LR and *ZE* formation, while those in the other SA may not. The conformation of *ZZ*-BR incorporated into HSA has been argued in conflicting ways and is not yet clear.[22,23] Thus, the CD spectra indicate that *ZZ*-BR takes intramolecularly hydrogen bonded conformation in HSA, while the binding studies with model compounds of folded and extended conformations suggest that *ZZ*-BR takes an extended conformation where the intramolecular hydrogen bonds are totally broken.

The unique behavior of RABSA solution enhancing *EZ* and LR production suggests that the conformation and the strength of intramolecular hydrogen bonds in this solution should be different from those in BSA, RABSA, and HORSA solutions and suitable for enhancing the efficiency for *EZ* and LR formation without affecting the efficiency of *ZZ*→*ZE* isomerization.

As to the energy diagram concerning to the two dipyrromethenone chromophores, 2-PY is assumed to be lower in energy than 1-PY.[2] This is the reason why the isomerization efficiency of the former double bond is generally higher than the latter. This model can explain most of the reactions of the present work except only those in RABSA solution. The unique behavior in RABSA will reflect the change of the potential energy surface from those for other SA's. Thus, the energy of 1-PY of *ZZ*-BR may be lower than that of 2-PY, or, alternatively the energy barrier for *ZZ*→*EZ* isomerization is decreased without affecting the activation barrier for *ZZ*→*ZE* isomerization. In the latter case, the intramolecular hydrogen bonds between 1-PY and the propionic acid group at C12 might be weaker in RABSA than in other SA solutions.

The present findings clearly demonstrate that SA from different mammals can induce the photochemical reaction of *ZZ*-BR in different manners, probably because of the different environment of BR binding with SA's, and suggest a possibility to use SA as an environment to control photochemical reactions.

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