Photoisomerization of Bilirubins and the Role of Intramolecular Hydrogen Bonds

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The quantum yields and the photostationary state isomer ratios in the photoisomerization of bilirubin are strongly governed by the properties of the reaction media. Among the reaction media examined, the quantum yields for $ZZ \rightarrow ZE$ isomerization (ϕ_{ZZ-ZE}) and for cyclization (ϕ_{LR}) were the highest in buffered aqueous solution (potassium phosphate buffer, pH 7.4) containing human serum albumin (HSA) in a 1 : 1 molar ratio to *ZZ*-BR, 0.11 and 2×10^{-3}, respectively, on 436 nm irradiation. The effects of the reaction media surrounding bilirubin are discussed.

(4*Z*,15*Z*)-Bilirubin IXα (*ZZ*-BR) is the final product of hemoglobin metabolism. This compound is composed from two pyrromethenone moieties carrying propionic acid chain. The each propionic group forms strong intramolecular hydrogen bonds with lactam CO/NH and pyrrole NH groups of the adjacent pyrromethenone moiety.[1] These intramolecular hydrogen bonds make *ZZ*-BR take a folded conformation without any free polar groups oriented the outside, therefore, making it lipophilic and stable in the dark. However, on irradiation with visible light, it isomerizes into *E*-isomers (*ZE*-BR, *EZ*-BR, *EE*-BR) and a cyclized product (lumirubin, LR) (Scheme 1).[1—10] These photochemical products have at least one free carboxyl group not bounded by a hydrogen bond, which leads them more hydrophilic than *ZZ*-BR. The conversion of *ZZ*-BR to *ZE*-BR takes place by way of the isomerization of the unsaturated bond in the second pyrromethenone group (2-Py in Scheme 1) by breaking of its hydrogen bonds to the propionic acid group at C-8 in the first pyrromethenone moiety (1-Py in Scheme 1). On the other hand, production of *EZ*-BR and LR is concerned with the change of the first pyrromethenone moiety and breaking of its hydrogen bond to propionic acid residue at C-12. Phototherapy for neonatal jaundice is carried out to isomerize water-insoluble *ZZ*-BR into these water-soluble isomers to be excreted.[1]

In a preliminary paper, we reported that environment surrounding *ZZ*-BR affects the efficiency and course of photoisomerization of *ZZ*-BR.[7] We now present the experimental detail of the photoisomerization and discuss their dramatic dependence on properties of solvent environment.

Experimental

Chemicals and Samples. *ZZ*-BR was supplied from Sigma and was purified as described by McDonagh and Assisi.[11] Human serum albumin (HSA) (fatty acid-free) was obtained from Sigma as A3782 and was used without further purification.

All the solvents were purified by distillation before use. Indole and imidazole were recrystallized from benzene. Dioctylammonium acetate was prepared by neutralization of dioctylamine with acetic acid in benzene.

Photoirradiation Procedures. Except for photochemical experiments, all manipulation were carried out under a photographic safe-light. In all photochemical experiments the solutions were deoxygenated with argon. Most of the irradiation was performed with 436-nm light from a 400-W high-pressure mercury lamp through a solution filter (4.4 g dm^{-3} $CuSO_4 \cdot 5H_2O$ + 2.7 M NH_3 + 75 g dm^{-3} $NaNO_2$ in water, 1 M = 1 mol dm^{-3}). For the irradiation with the mixture of 405 and 436 nm light Toshiba L-39 glass filter was used. The light intensity was measured by using potassium tris(oxalato)ferrate(III) actinometry.

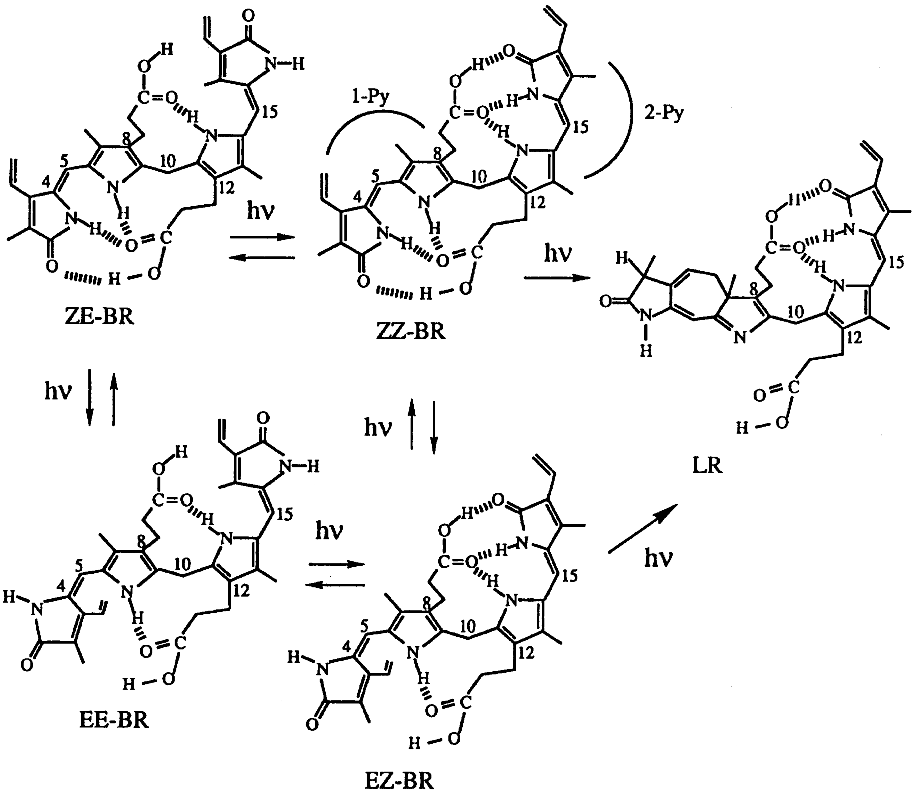
Bilirubin Isomers. Isomers of BR were produced on irradiation of *ZZ*-BR in DMSO in the presence of ethylenediaminetetraacetic acid disodium salt for LR and in chloroform for *ZE*- and *EZ*-BR under N_2 with 436 nm light. Then, photobilirubins (*ZE*-BR, *EZ*-BR, LR) were separated according to the reported procedures and identified by NMR spectroscopy.[3,12,13] The absorption spectra of *EZ*-,*ZE*-BR, and LR were measured by means of a HPLC system using a Shimadzu chromatograph (LC-6A) with a photodiode array detector (Otsuka electronics MCPD-350PC) equipped with a personal computer (NEC PC-9801E) with a chemcosorb 5-ODS-H column (4.6×250 mm) eluting with MeOH containing 0.1 M dioctylammonium acetate.[8—10,12,14]

Absorption Spectra. Absorption spectra of *ZZ*-BR (1.5×10^{-5} M) in various solutions were taken in a 1 cm quartz cuvette on a JASCO 660 spectrophotometer.

Quantum Yields of Isomerization and Photostationary State Isomer Ratios. Photochemical measurements were carried with *ZZ*-BR (5×10^{-5}—2×10^{-3} M) in $CHCl_3$ in the presence or absence of heterocyclic compounds, DMSO, THF, $CHCl_3$–Et_3N (1 : 1 by volume), $CHCl_3$–trioctylamine (Oct_3N) (1 : 1 by volume) and a buffered aqueous solution (potassium phosphate buffer, pH 7.4) containing HSA in a 1 : 1 molar ratio to *ZZ*-BR.

Solution of *ZZ*-BR/serum albumin in 1 : 1 ratio (1.5×10^{-4} M) was prepared by adding 2 ml of a BR solution, made up by dissolving 58 mg of *ZZ*-BR in 25 ml of 0.1 M NaOH, to 50 ml of serum albumin solution (0.51 g/50 ml) in 0.05 M phosphate buffer, pH 7.4. Before HPLC analysis HSA was precipitated by 10 times dilution with ice-cooled mobile phase and was removed by filtration.

The BR isomers were analyzed in triplicate 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 containing 0.1 M dioctylammonium acetate.[8—10,12,14] Their



Scheme 1.

concentrations were determined by using sensitivity ratios among the isomers (*ZZ*: *ZE*: *EZ*: LR=1.0 : 0.70 : 0.67 : 0.43) at 450 nm.[14] A typical chromatogram is shown in Fig. 1.

Under irradiation the concentration of the resulting *ZE*-BR and *EZ*-BR was increased linearly with absorbed light intensity by *ZZ*-BR up to 4% conversion. Calculations by the least-squares method were carried out for these initial reaction period to obtain yields of *ZE*-BR and *EZ*-BR, and the number of photons absorbed by *ZZ*-BR in unit time. The quantum yield for LR formation (Φ_{LR}) was calculated by assuming that LR formation occurred through a one-photon process from *ZZ*-BR.[8] For this calculation the fraction of light absorbed by *ZZ*-BR (ξ_{ZZ}) in solution was calculated from the fraction of *ZZ*-BR in photoequilibrating mixtures determined by HPLC analysis and the absorbance of the solutions at 436 nm before and after irradiation. The photostationary state isomer compositions were determined under conditions similar to the quantum yields measurements.

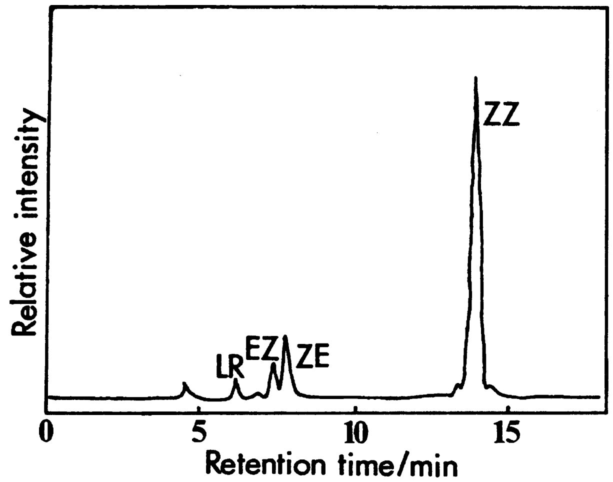


Fig. 1. A typical chromatogram of isomeric bilirubins after irradiation in $CHCl_3$–Et_3N (1 : 1).

Results

ZZ-BR is not soluble in aqueous buffered solution of pH 7.4, but is soluble in the presence of an equimolar amount of serum albumin by their binding. The binding constant between BR and HSA is reported as high as 2×10^8.[15] Thus, in the present aqueous buffered HSA solution, more than 99% bilirubin is estimated as bound to HSA.

Absorption Spectra of *ZZ*-BR. *ZZ*-BR shows absorption around 450 nm in various solvents as depicted in Fig. 2. The maximum wavelength and the shape of the absorption band are dependent on the solvents and added substances. In DMSO, $CHCl_3$–aliphatic amine mixed solvents and the buffered aqueous solution (potassium phosphate buffer, pH 7.4) containing HSA in a 1 : 1 molar ratio to *ZZ*-BR, the absorption exhibits a shoulder at 420 nm, which is absent in $CHCl_3$.

Absorption Spectra of BR Isomers. *ZZ*-isomer is stable in the dark. However, it is difficult to isolate other isomers (*ZE*, *EZ*, *EE*-BR, LR) in high purity because of their low stability. To measure the absorption

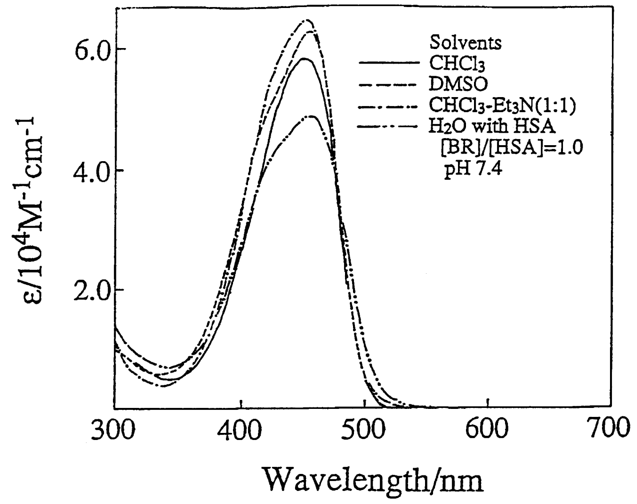


Fig. 2. Absorption spectra of *ZZ*-BR in various solvents and media.

spectra of each photoisomer, *ZZ*-BR was irradiated in $CHCl_3$-Et_3N (1 : 1) and the resulting mixture was subjected to HPLC eluted with MeOH containg 0.1 M dioctylammonium acetate;[14,16] the absorption spectrum of each isomer was measured by means of a photodiode array detector as shown in Fig. 3. The molar extinction coefficients of BR isomers were estimated on the basis of the spectral data of *ZZ*-BR ($\varepsilon = 6\times10^4$ M^{-1} cm^{-1} at 450 nm) and its isomers together with the reported sensitivity ratios[14] between the isomers at 450 nm in the HPLC solvent. The photoproducts (*ZE*-, *EZ*-, *EE*-BR, LR), in which intramolecular hydrogen bonds are partly or totally broken, show absorption maxima at shorter wavelength with smaller absorption coefficients than *ZZ*-BR.

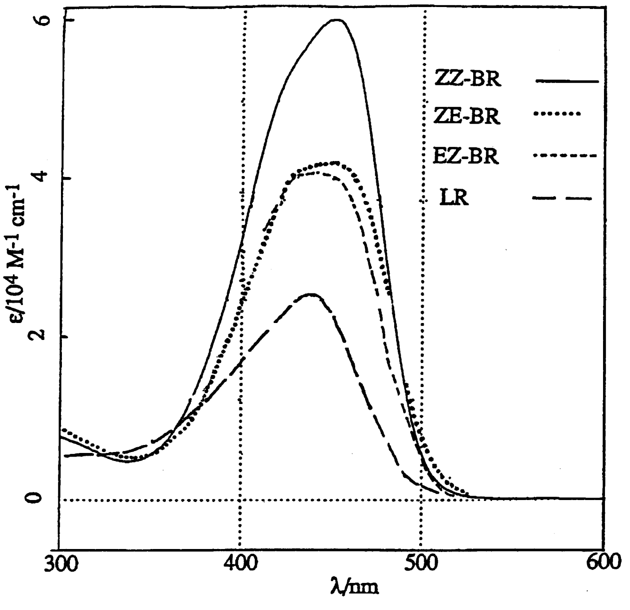


Fig. 3. Absorption spectra of bilirubin isomers.

Quantum Yields of the Isomerization of BR and the Photostationary State Isomer Composition. *ZZ*-BR was irradiated with 436 nm light or mixed light of 405+436 nm in various solvents to investigate the time development of the products (Fig. 4) and to determine the photostationary isomer composition (Table 1).

On 436 nm irradiation the photostationary *ZZ*-BR compositions was varied by the solvents: 77% in $CHCl_3$, 80% in THF, 81% in DMSO, 73% in $CHCl_3$–Et_3N (1 : 1), 67% in $CHCl_3$–Oct_3N (1 : 1) and 63% in the HSA solution. The [*ZZ*]/[*ZE*] is smaller in $CHCl_3$–Et_3N (1 : 1),

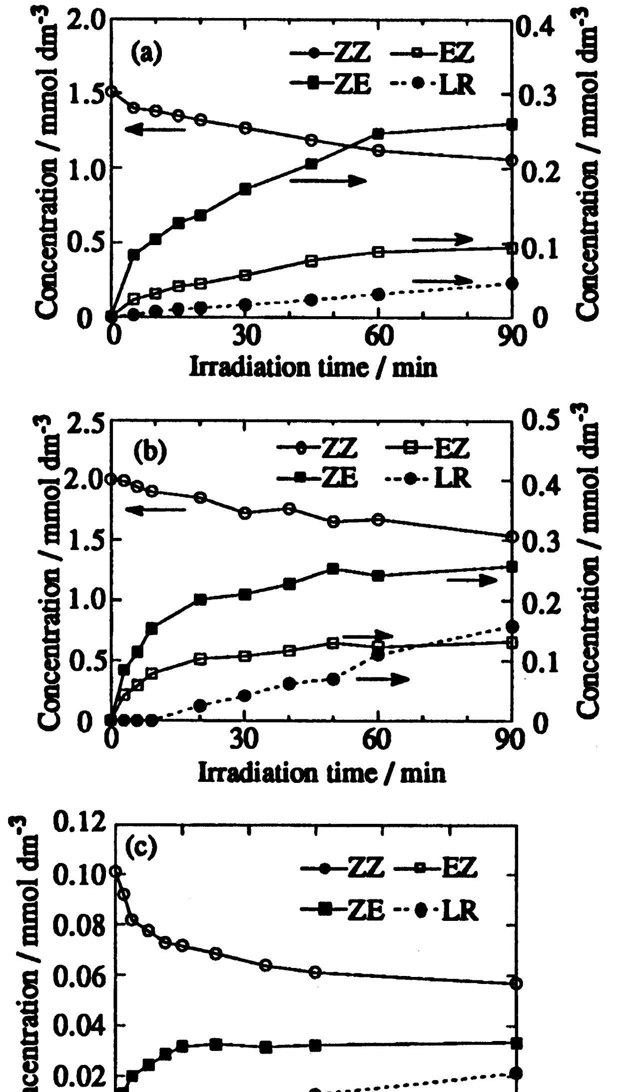


Fig. 4. The time development of the photoproducts starting from *ZZ*-BR on 436 nm irradiation in $CHCl_3$–Et_3N (1 : 1) (a), on 405 and 436 nm irradiation in $CHCl_3$–Et_3N (1 : 1) (b), and on 436 nm irradiation in the buffered solution containing human serum albumin (c).

Table 1. Quantum Yields for Isomerization and Photostationary State Isomer Compositions of BR in Various Solvents

Solvent	Quantum yield			Composition/%		
	Φ_{ZZ-ZE}	Φ_{ZZ-EZ}	$\Phi_{ZZ-\mathrm{LR}}$	ZZ	ZE	EZ
$CHCl_3$	3×10^{-3}	6×10^{-4}	$<1\times10^{-5}$	77.1	17.3	5.6
THF	7×10^{-3}	9×10^{-4}	1×10^{-4}	80.1	15.5	4.4
DMSO	5×10^{-3}	2×10^{-3}	2×10^{-4}	81.1	13.7	5.2
$CHCl_3$–Et_3N (1 : 1)	3×10^{-2}	6×10^{-3}	1×10^{-4}	73.3	20.7	6.0
$CHCl_3$–$(C_8H_{17})_3N$ (1 : 1)	5×10^{-2}	1×10^{-2}	3×10^{-4}	66.6	25.8	7.5
H_2O (pH7.4) with HSA	0.11	<0.02	2×10^{-3}	63.0	35.5	1.7

$CHCl_3$–Oct_3N (1 : 1), and the buffered aqueous HSA solution than in the other solvents.

In organic solvents, the quantum yields for $ZZ\rightarrow ZE$ and $ZZ\rightarrow EZ$ isomerization, $\Phi_{ZZ\rightarrow ZE}$ and $\Phi_{ZZ\rightarrow EZ}$, are small in $CHCl_3$, slightly increased in DMSO and THF, and more increased in $CHCl_3$–Et_3N (1 : 1) and $CHCl_3$–Oct_3N (1 : 1). In $CHCl_3$–Et_3N (1 : 1) or $CHCl_3$–Oct_3N (1 : 1), Φ_{ZZ-ZE} is 10 times larger than in $CHCl_3$ (Table 1). The quantum yield for production of LR, Φ_{LR}, was in the order of 10^{-4} in the solvents examined except in $CHCl_3$, in which Φ_{LR} is lower than 10^{-5}.

Among the reaction media examined, *ZZ*-BR exhibited a specific photochemical behavior in HSA solution. $\Phi_{ZZ\rightarrow ZE}$ and Φ_{LR} are remarkably high as 0.11 and 2×10^{-3}, respectively, though $\Phi_{ZZ\rightarrow EZ}$ remained <0.02.[17] These values are comparable to those recently reported by Agati et al.[10]

In the presence of heterocyclic nitrogen compounds such as indole, imidazole, pyrrole, and pyridine in $CHCl_3$, the photostationary *ZZ*-BR composition is larger than that in pure $CHCl_3$. Φ_{LR} is about 10^{-4} comparable to those in the presence of aliphatic amines; however, the quantum yields of $Z\rightarrow E$ isomerizations remain as low as in $CHCl_3$ and 10 times lower than in the presence of aliphatic amines (Table 2).

Discussion

Effect of Reaction Media. The observed effect of the added alkylamines, Et_3N and Oct_3N, to increase both the $\phi_{ZZ\rightarrow ZE}$ and the photostationary *ZE* composition indicates that these amines interact with *ZZ*-BR to break or weaken the intramolecular hydrogen bonds by making intermolecular hydrogen bonds or salt bridges[2] with propionic acid side chains and (or) pyrrole NH groups in BR. The poor effect of the added heterocyclic bases can be attributed to their weaker property as bases.

As to ϕ_{LR}, the effect of THF, DMSO, Et_3N, and Oct_3N for slightly increasing it compared to $CHCl_3$, and the more pronounced effect of HSA, though remaining in 10^{-3}, can be attributed to the weakening of the hydrogen bonds between the first pyrromethenone moiety and the propionic acid group at C-12. However, the effects of the above proton-accepting solvents, amines and HSA, on the hydrogen bonds between 1-Py and the acid group at C-12 seem to be not so large as on the hydrogen bonds between 2-Py and the acid group at C-8 leading to more efficient $ZZ\rightarrow ZE$ isomerization.

The solvent effects on the absorption spectra also afford an insight into the situation of intramolecular hydrogen bonding in *ZZ*-BR. *ZZ*-BR shows absorption maximum around 450 nm in various solvents (Fig. 2). The observation of a shoulder at ca. 420 nm, in addition to maximum at 450 nm, in DMSO, $CHCl_3$–Et_3N (1 : 1), $CHCl_3$–Oct_3N (1 : 1), and buffered aqueous HSA solution, suggests that *ZZ*-BR can take several conformations and their population is affected by the properties of the solution media. In comparison with the absorption spectra of bilirubin dimethyl ester,[18,19] the absorption at 420 nm can be assigned to conformers in which intramolecular hydrogen bonding is broken or very weak.

In solid, *ZZ*-BR has six intramolecular hydrogen bonds as demonstrated by X-ray crystallography.[20] In chloroform *ZZ*-BR retains its intramolecular hydrogen bonds, while in DMSO the intramolecular hydrogen bonds are broken and replaced by hydrogen bonds to DMSO oxygen as revealled by NMR spectroscopy.[21] Even in the latter case the two pyrromethenone chromophores do not freely rotate and remain at a fixed angle. The above effects of solvents on the efficiency of the isomerization by affecting the strength of intramolecular hydrogen bonds are reasonably supported by these structural findings of *ZZ*-BR.

Therefore, it is obvious that the solvents to break the intramolecular hydrogen bonds of *ZZ*-BR can induce the isomerization of *ZZ*-BR. In other words, in these solvents the internal conversion in the excited states through the intramolecular hydrogen bonds between the carboxyl groups and pyrrole or lactam rings is diminished, and, instead, the twisting around the double bonds can be facilitated by disruption of the intramolecular hydrogen bonds.

Special Effect of HSA. As discussed above breaking of the intramolecular hydrogen bonds induces facile $Z\rightarrow E$ isomerization. The $\phi_{ZZ\rightarrow ZE}$ is the largest in the HSA solution. Therefore, by incorporation of *ZZ*-BR in HSA, the intramolecular hydrogen bonds may be broken by the interaction with amino acid residues of HSA. Production of only a small amount of the *EZ*-BR com-

Table 2. Effects of Additives on the Quantum Yields for Isomerization and Photostationary State Isomer Compositions of BR in $CHCl_3$

Solvent	Quantum yield			Composition/%		
	Φ_{ZZ-ZE}	Φ_{ZZ-EZ}	$\Phi_{ZZ-\mathrm{LR}}$	*ZZ*	*ZE*	*EZ*
$CHCl_3$ with indole (1 M)	6×10^{-3}	1×10^{-4}	9×10^{-6}	82.3	10.9	6.8
$CHCl_3$ with imidazole (1 M)	3×10^{-3}	7×10^{-4}	2×10^{-4}	83.0	13.7	3.3
$CHCl_3$–pyrrole (1 : 1)	3×10^{-3}	7×10^{-4}	5×10^{-5}	90.8	7.2	2.0
$CHCl_3$–pyridine (1 : 1)	7×10^{-3}	1×10^{-3}	8×10^{-5}	83.9	12.3	3.8

pared with *ZE*-BR and LR suggests that some special interactions with HSA may control the course of isomerization of *ZZ*-BR resulting in the high yields of *ZE*-BR and LR.

The conformation of *ZZ*-BR incorporated in HSA has been actively argued.[16,22—26] *ZZ*-BR can take two-folded, intramolecularly hydrogen-bonded, enantiomeric conformations which are in dynamic equilibrium in solution. The CD spectral pattern[22—24] indicates that *ZZ*-BR is incorporated into HSA taking one of the folded structures, probably a right-handed (positive) chiral conformational enantiomer where intramolecular hydrogen bonds of *ZZ*-BR still exist. In contrast to this proposition, solvent effects on the resonance Raman spectra[25c] show that the intramolecular hydrogen bonds of *ZZ*-BR are ruptured when incorporated in HSA. Furthermore, the binding studies[26] using rac-iron(III) *N,N'*-ethylenebis[5-bromo-2-hydroxyphenyl)glycinate] indicates that bilirubin is bound to HSA by taking an extended conformation in which intramolecular hydrogen bondings are totally broken. The present findings that HSA induces efficient and specific isomerization of *ZZ*-BR well accord with the latter proposition.

Mechanism of Photoisomerization of BR. It is generally accepted that the photoisomerization of BR proceeds by Scheme 1 or by its slightly modified scheme with regard to the LR formation:[1,2,4,7—10] LR can be produced directly from *ZZ*-BR in a one-photon process or a consecutive photochemical process by way of *EZ*-BR.

The time development of the products at very early stages affords more insight into the mechanism. As shown in Fig. 4, irradiation of mixed light of 405+436 nm in $CHCl_3$–Et_3N afforded *ZE*- and *EZ*-BR in the early stage. However, LR was formed after an induction period and then increased with the irradiation time. These results indicate that in these conditions LR is a secondary product form *ZZ*-BR. However, 436 nm irradiation gave LR without observable induction period. The wavelength effect on the isomerization efficiency might be one of the reasons for the difference between 405+436 nm and 436 nm irradiation, though difficult to be clearly explained. One could only suspect possible presence of an induction period on 436 nm at earlier than that described in Fig. 4a, during which the products do not attain to sufficient concentration to be accurately determined.

In the buffered HSA solution irradiation of 436 nm produced *ZE*-BR and LR at the early stage, but gave *EZ*-BR only in a low yield (ca. 3% of *ZZ*-BR at the photostationary state). In this solution LR may be afforded through the one-photon process, although one cannot exclude the two-photon process[4a,9] comprising the secondary *EZ*→LR conversion which may proceed with a high efficiency. Actually, Ennever et al. determined the quantum yields of LR formation starting from the pure *EZ* isomer as 0.12 on 450 nm irradiation and suggests the consecutive mechanism.[9] Which of the two mechanisms actually works may be governed by the irradiation wavelength as well as the reaction medium.

We should mention here whether the consecutive mechanism can actually be accepted or not. Usually photochemical reactions proceed as a diabatic process in which the deactivation of the excited state takes place at a conformation different from the product. For example, cis–trans isomerization of aromatic olefins such as stilbene mainly proceed by a diabatic reaction path through deactivation of the perpendicular excited state to the perpendicular ground state finally giving the mixture of cis- and trans- isomers at nearly 1 : 1 ratio.[28] However, the introduction of a large conjugated group such as 2-anthryl group on one of the ethylenic carbon drastically changes the isomerization mechanism from the conventional diabatic mechanism to an adiabatic mechanism in which direct conversion from the cis- to the trans-triplet excited states takes place followed by the deactivation to trans ground state, that is the product.[29] Furthermore, at the excited singlet state even for stilbene an adiabtic conversion from cis ($^1c^*$) to trans ($^1t^*$) isomers takes place though very inefficiently.[30] Moreover, 9-styrylanthracene[31] and 1-styrylpyrene[32] undergoes adiabtaic conversion from $^1c^*$ to $^1t^*$ with a quantum yield as high as 0.5.

Accordingly, if LR is directly produced from photolysis of *ZZ*-BR by a one-photon process, it is necessary that the isomerization of *ZZ*→*EZ* takes place by an adiabatic way followed by the single bond rotation between C_5–C_6.

As regard to the single bond rotation, it has long been believed not to take place within the lifetime of excited singlet state; however, it has recently been shown to take place efficiently in 2-vinylanthracene and its related compounds within the lifetime of their excited singlet state.[33—35] Therefore, the direct production of

LR from *ZZ*-BR on photoexcitation seems not be very unreasonable.

Potential Energy Surface of *ZZ*→*ZE* Isomerization of BR. Until our preliminary report for the effect of solvent on the quantum yields of the isomerization of BR was published,[7)] no precise value for $\Phi_{ZZ\to ZE}$ had been reported. Moreover, the $\Phi_{ZZ\to ZE}$ values were considered to be similar in both $CHCl_3$ and HSA solution.[27)] Our measured values are very much contrasted with these previous assumptions; therefore, it seems worthwhile to discuss here the possible difference in the potential energy surfaces of the isomerization in $CHCl_3$ and HSA solution.

The lifetime (τ_s) of *ZZ*-BR in the excited singlet state ($^1ZZ^*$) was measured to be similar in $CHCl_3$ and HSA solution by absorption and fluorescence spectroscopy,[27,35)] for example, 17 and 19 ps,[27)] respectively, as determined by transient absorption spectroscopy.

The *ZZ*→*ZE* isomerization must take place through the perpendicularly twisted singlet state ($^1Zp^*$) around the double bond of C_{15}=C_{16} as shown in Fig. 5. *ZE*-BR reverts to *ZZ*-BR in the time scale of hours or days depending on the properties of the reaction media.[1)] Therefore, as depicted in Fig. 5, *ZE*-BR is less stable than *ZZ*-BR and the activation barrier for thermal *ZE*→*ZZ* isomerization is assumed to be about 25 kcal mol^{-1}.

The partition ratio to give *ZZ* and *ZE* isomers by the deactivation from $^1Zp^*$ can be assumed to be 1 : 1, therefore, the quantum yield of production of $^1Zp^*$ ($\Phi_{ZZ\to Zp}$) is estimated as 0.006 and 0.22 in $CHCl_3$ and HSA solution, respectively, based on the $\Phi_{ZZ\to ZE}$. The quantum yield of intersystem crossing from the excited singlet state to the triplet state (Φ_{isc}) of *ZZ*-BR as well as the quantum yield of fluorescence emission (Φ_f) at room temperature is reported to be lower than 0.01.[36—38)] Therefore, *ZZ*-BR in the excited singlet state mostly deactivates through a radiationless process (k_d in Fig. 5) before undergoing isomerization to $^1Zp^*$.

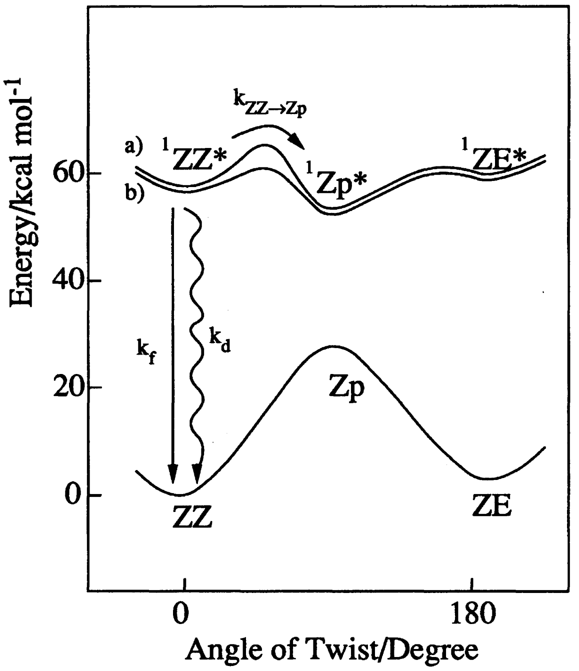


Fig. 5. Potential energy surfaces of *ZZ*→*ZE* isomerization of BR in $CHCl_3$ (a) and HSA solution (b).

By using the values of τ_s and $\Phi_{ZZ\to ZE}$ the rate constant of production of $^1Zp^*$ from $^1ZZ^*$, $k_{ZZ\to Zp}$, is estimated to be 3.5×10^8 and 1.2×10^{10} s^{-1} in $CHCl_3$ and HSA solution, respectively. These rate constants correspond to activation energies (E_a) of 5 (Fig. 5a) and 2.6 kcal mol^{-1} (Fig. 5b) in $CHCl_3$ and HSA solution, respectively, when a frequency factor A for twisting around the double bond is taken as 10^{12} s^{-1} [39)] in Eq. 1.

$$k_{ZZ\to Zp} = A\exp(-E_a/RT). \qquad (1)$$

The rate constant for the radiationless deactivation of $^1ZZ^*$, $k_d(\approx(1-\Phi_f-\Phi_{ZZ\to Zp})/\tau_s)$, is obtained as very similar values, 5.8×10^{10} and 4.1×10^{10} s^{-1}, in $CHCl_3$ and HSA solution, respectively. Thus, the remarkable effect of HSA on the efficiency of the photoisomerization of BR arises not from the change of k_d value but from the increase of $k_{ZZ\to Zp}$ value.

From the spectroscopic data it was reported that in the ground state *ZZ*-BR forms intramolecular hydrogen bonds and the activation barrier of the conformational inversion associated with this interaction is estimated as high as 17.9 kcal mol^{-1};[40)] the barrier in the excited singlet state is decreased to 5.5 kcal mol^{-1} in HSA solutions.[41)]

The present values, which are slightly lower than those previously estimated, clearly indicate that the photoexcitation of *ZZ*-BR weaken the intramolecular hydrogen bonds more than 10 kcal mol^{-1} to result in the facile isomerization around the double bond.

Conclusion

The present findings indicate that the strength of the intramolecular hydrogen bonds in *ZZ*-BR governs the efficiency of its isomerization: The more effectively the hydrogen bonds are weakened, the more efficiently the isomerization takes place. Thus, the solvents which break the hydrogen bonds of *ZZ*-BR play an important role in inducing its isomerization.

However, even in the presence of Et_3N or Oct_3N, the quantum yields of the isomerization of *ZZ*-BR is still less than 0.1 indicating that the excited *ZZ*-BR undergoes the radiationless deactivation much more predominantly preceding to its isomerization.

In HSA solution *ZZ*-BR undergoes specific photochemical reactions, which is attributed to the incorporation of *ZZ*-BR into HSA through formation of hydrogen bonds or salt bridges with its amino acid residue taking a conformation very much different from that in organic solvents such as an extended conformation as previously proposed based on Raman spectroscopy and model compounds studies.

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