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Effects of Solvents and Media on the Efficiency and Course of Photoisomerization of Bilirubins

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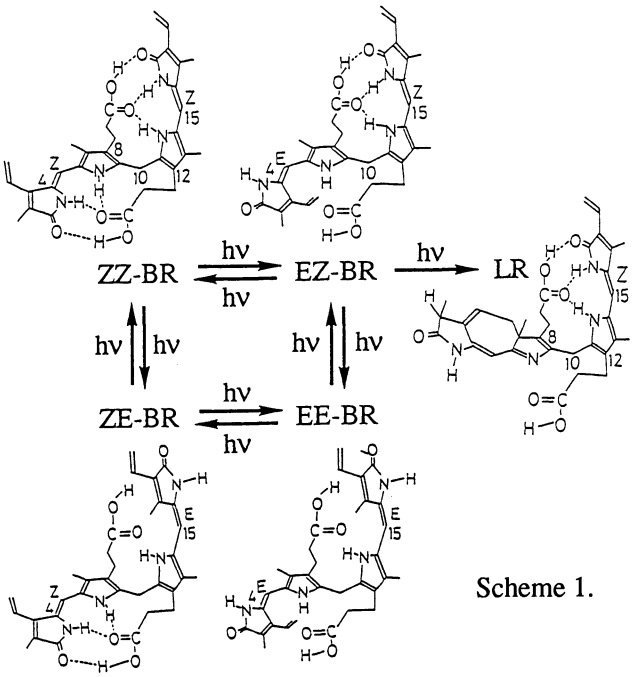
The quantum yields of photoisomerization of bilirubin to various isomers were determined in various solvents. A specific photoisomerization took place only in buffered solutions containing human serum albumin. We discuss the effects of solvents and media surrounding bilirubin on its photoisomerization.

It is well accepted that light affects bilirubin metabolism in jaundiced newborns; 4Z,15Z-bilirubin IXa (ZZ-BR) is converted to more polar products (4E,15Z-, 4Z,15E-, 4E,15E-bilirubin, and lumirubin) and, therefore, they are more easily excreted from mammals than native bilirubin (ZZ-BR).[1,2] However, the role of albumins, which bind to bilirubins in mammals, particularly in the photochemical process is still obscure. Photochemical reactions of ZZ-BR have been reported to proceed *in vitro* through processes shown in Scheme 1.[3] Knowledge of the isomerization efficiency is very important and useful to determine the mechanism of bilirubin metabolism, but it has not been studied in detail except measurements of



Scheme 1.

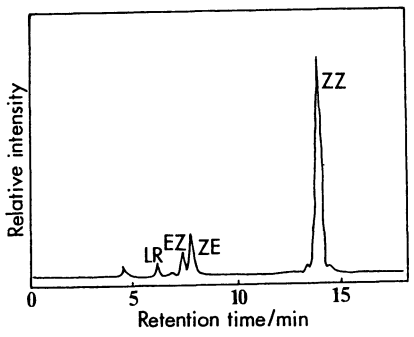


Fig. 1. A typical chromatogram of isomeric bilirubins.

Table 1. Quantum yields for isomerization products from ZZ-BR and photostationary state isomer compositions of bilirubins in various solvents

Solvent	Quantum yield			Isomer ratio		
	$\phi_{ZZ\rightarrow ZE}$	$\phi_{ZZ\rightarrow EZ}$	$\phi_{ZZ\rightarrow LR}$	ZZ	ZE	EZ
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
$CHCl_3$	3×10^{-3}	6×10^{-4}	$<1\times10^{-5}$	77.1	17.3	5.6
$CHCl_3$-Et_3N(1:1)	3×10^{-2}	6×10^{-3}	1×10^{-4}	73.3	20.7	6.0
$CHCl_3$-Oct_3N(1:1)	5×10^{-2}	1×10^{-2}	3×10^{-4}	66.6	25.8	7.5
H_2O (pH 7.4) with BSA	7×10^{-3}	3×10^{-3}	1×10^{-4}	85.1	9.3	5.6
H_2O (pH 7.4) with HSA	0.11	<0.02	2×10^{-3}	63.0	35.3	1.7

the quantum yields for E,Z isomerization (by indirect methods)[4] and LR formation.[5] We now present determination of the quantum yields for bilirubin isomerization and their dramatic dependence on solvents and albumin additives.

The quantum yields for ZE-BR ($\phi_{ZZ\rightarrow ZE}$) and EZ-BR formation ($\phi_{ZZ\rightarrow EZ}$) were determined by irradiating degassed samples of ZZ-BR (5×10^{-5}–2×10^{-3} M) (M=mol dm^{-3}) in THF, DMSO, $CHCl_3$, $CHCl_3$-Et_3N (1:1 by volume), $CHCl_3$-trioctylamine (Oct_3N) (1:1 by volume) and buffered aqueous solutions (potassium phosphate buffer, pH 7.4) containing bovin serum albumin (BSA) or human serum albumin (HSA) with 436-nm light from a 400-W high-pressure mercury lamp through a solution filter (4.4 g/dm^3 $CuSO_4.5H_2O$+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.[6] The isomer concentrations were measured with HPLC (Waters 600 multisolvent delivery system and 490 programmable multiwavelength detector with a 740 data module) with a Chemcosorb 5-ODS-H column (4.5×25 cm) eluting with MeOH containing 0.1 M dioctylammonium acetate.[7] The chromatograms were monitored at 450 nm and the concentrations were determined by using the sensitivity ratios between the isomers (ZZ:ZE:EZ:LR=1:0.70:0.67:0.43).[8] A typical chromatogram is shown in Fig. 1. All the peaks except that ascribed to EE-BR were identified by separating the products according to the reported procedures[9] followed by NMR spectroscopy.

The quantum yields are very much dependent on medium properties to different degrees between the products, as shown in Table 1. The $\phi_{ZZ\rightarrow ZE}$ value is small in $CHCl_3$ and increases in the order of $CHCl_3$, DMSO, THF, BSA solution, $CHCl_3$-Et_3N, $CHCl_3$-Oct_3N, and HSA solution. In $CHCl_3$-Et_3N and $CHCl_3$-Oct_3N $\phi_{ZZ\rightarrow ZE}$ is 10 times larger than that in $CHCl_3$ but is still in the order of 10^{-2}. The $\phi_{ZZ\rightarrow ZE}$ value in the HSA solution is as high as 0.1, which indicates, however, that there are still other deactivation processes not resulting in isomerization.

The photostationary state isomer compositions were measured in various solvents by irradiating ZZ-BR under conditions similar to those employed in the quantum yield measurements, and are shown in Table 1. The ZZ-BR composition is slightly smaller in $CHCl_3$-Et_3N, $CHCl_3$-Oct_3N, and the HSA solution than in the other solvents, but is in a similar range.

It has been accepted that ZZ-BR is incorporated into albumins by hydrogen bonding or salt bridges to their lysine, triptophane, or histidine residues.[10] The higher $\phi_{ZZ \rightarrow ZE}$ values and the lower photostationary ZZ-BR compositions in the HSA solution, $CHCl_3$-Et_3N, and $CHCl_3$-Oct_3N indicate that ZZ-BR can also interact with Et_3N and Oct_3N through the hydrogen bonding or salt bridges. These results suggest that the solvents which break the intramolecular hydrogen bonds of ZZ-BR play an important role in inducing the ZZ-BR isomerization. Thus, the internal conversion in the excited states through the intramolecular hydrogen bonding between the carboxylic groups and pyrrole or lactone rings is diminished in such solvents and the twisting about the double bonds can be facilitated by disruption of the intramolecular hydrogen bonding.

As to the isomerization to LR which is most excretable among the isomers and may play an important role in the metabolism, the quantum yield for its formation (ϕ_{LR}) is remarkably high as 2×10^{-3} in the HSA solution as reported previously,[5] and it is noticeable that this value is 10 times larger than ϕ_{LR}'s in THF, DMSO, $CHCl_3$-Et_3N, and $CHCl_3$-Oct_3N, and even in the BSA solution. In $CHCl_3$ no LR peak was detected in HPLC, which suggests that ϕ_{LR} must be lower than 10^{-5}. The ϕ_{LR} values were calculated by assuming that LR formation occurred through a one-photon process from ZZ-BR. Breaking of the intramolecular hydrogen bonds is supposed to induce ZZ→EZ isomerization. However, in the HSA solution in which the intramolecular hydrogen bonds are broken by the amino acid residues surrounding bilirubin molecules, only a small amount of the EZ isomer was detected compared with ZE-BR and LR formation, suggesting that some special interactions may control the course of isomerization in the HSA solution resulting in the high yields of ZE-BR and LR. However, in the BSA solution, the photochemical behavior of ZZ-BR is very much different from that in the HSA solution, but is similar to those in DMSO and $CHCl_3$-Et_3N. This also indicates that in the presence of HSA some special environment controls the course and the efficiency of isomerization. Thus, the conformation of ZZ-BR incorporated in HSA plays an important role in leading to the different isomerization efficiency from those in the homogeneous and BSA solutions. In other words, the amino acid residues in HSA will contribute to enhancing the LR and ZE formation, while those in BSA will not influence the efficiency.

Two mechanisms have been proposed for the LR formation.[1,3,7] One is a direct

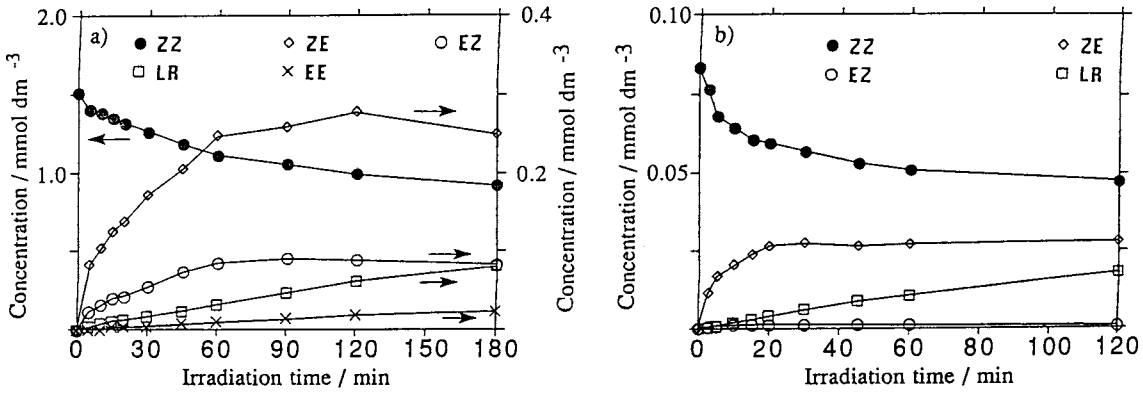


Fig. 2. The time development of the photoproducts starting from ZZ-BR in $CHCl_3$-Et_3N(1:1) (a) and in the buffered solution containing human serum albumin (b).

formation by photolysis of ZZ-BR[1,7] and the other is a consecutive photochemical process by way of EZ-BR[3]. Recently, on the basis of the time development of the product, Onishi et al. reported a possibility of the latter mechanism.[3] However, the present low stationary concentration of EZ-BR and high efficiency in LR formation on photolysis of ZZ-BR in the HSA solution suggest the former mechanism.

In order to get more insight into the mechanism we also studied the time development of the products at very early stages (Fig. 2). In $CHCl_3$-Et_3N ZE-BR and EZ-BR were produced very efficiently. Although LR was formed very slowly and increased with irradiation time, no induction period was observed. In the buffered HSA solution ZE-BR and LR were produced at the early stage, but EZ-BR was given only in a low yield (Table 1). The low stationary state composition of EZ-BR tends us to propose that in this solvent LR may be afforded through the one-photon process from ZZ-BR, although a possibility of the consecutive process involving the secondary EZ→LR conversion with an almost unit efficiency cannot be completely excluded.

It should be noted here that the efficiency and course of the isomerization is very much dependent not only on the properties of solvent but also on the source of albumin. Thus, only in the HSA solution the most excretable bilirubin isomer, LR, is produced very efficiently. This suggests that neonatal jaundice might cause more serious problems to humans than to animals.

In conclusion, HSA exhibited an unusual acceleration of ZZ→ZE and ZZ→LR isomerization and retardation of ZZ→EZ isomerization compared with the other homogeneous solutions, and the important role of protein environment is interpreted as breaking of the intramolecular hydrogen bonding of ZZ-BR and other factors for specific reactions yielding LR.

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