Solvent effects on the photoisomerization of bilirubin

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Studies of the photolysis of bilirubin IX-α by absorbance difference (AD) spectroscopy show that in solvents which do not interfere with the intramolecular hydrogen bonding, e.g., $CHCl_3$, *EZ/ZE* isomers and lumirubins are formed, with the former being predominant. The addition of ethanol to $CHCl_3$, either before or during photolysis, greatly changes the AD spectra, possibly because of a reaction of photoproducts with the ethanol or solvatochromism of the *EZ/ZE* isomers. Solvents which interfere with the intramolecular hydrogen bonding of bilirubin, e.g., DMSO, increase the importance of the photoreaction that forms lumirubins. The kinetics of product formation indicate that the lumirubins result from a photoreaction of *EZ/ZE* isomers. The addition of aqueous buffer to DMSO lowers the extent of photoisomerization and leads to AD spectra that resemble those obtained for the photolysis in $CHCl_3$. The addition of human serum albumin (HSA), using stopped flow techniques, to aqueous buffer solution of bilirubin immediately after photolysis gives evidence that the HSA traps the *EZ/ZE* isomers and prevents their reversion. However, in the presence of HSA the predominant pathway for photoreaction of bilirubin to *EZ/ZE* isomers is by reaction of the bound bilirubin. The AD spectra are similar to those obtained for solvents that interfere with hydrogen bonding.

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Les études de spectroscopie par absorbance différentielle (AD) ont démontré que la photolyse de la bilirubine-IXα poursuivie en milieux qui ne perturbent pas les liaisons hydrogènes, tel que le chloroforme, produit principalement les isomères *EZ/ZE*, ainsi que des lumirubines. L'addition d'éthanol, par exemple au chloroforme, soit avant ou après la photolyse transforme les spectres AD, peut-être à la suite d'une réaction des photoproduits avec l'éthanol ou en raison du solvatochromisme des isomères *EZ/ZE*. Par contre, la formation des lumirubines par photoréaction est favorisée dans des solvants tel que le sulphoxyde diméthylique, c'est-à-dire dans des solvants qui perturbent les liaisons hydrogènes. Ainsi, les résultats d'études de cinétique démontrent que les lumirubines sont formées par la photoréaction des isomères *EZ/ZE*. L'addition de solutions tampons aqueuses au sulphoxyde diméthylique diminue le degré de photoisomérisation et les spectres AD obtenus ressemblent à ceux obtenus après la photolyse dans le chloroforme. L'addition de la sérum-albumine humaine (SAH), immédiatement après la photolyse, à des solutions tampons aqueuses de bilirubine en utilisant des techniques d'additions simultanées, indique que la SAH capte les isomères *EZ/ZE* et empêche leur inversion. Cependant, la photoréduction de la bilirubine en isomères *EZ/ZE* en présence de la SAH se poursuit principalement par réaction des bilirubines liées. Les spectres AD obtenus sont semblables à ceux obtenus dans des milieux qui perturbent les liaisons hydrogènes.

Introduction

During the past fifteen years the photochemistry of bilirubin has been studied extensively (1–4), particularly photoisomerization. It is related to phototherapy, used for the treatment of neonatal jaundice (5). Originally it was thought that the major mechanism leading to a lowering in the plasma bilirubin level during phototherapy involves photooxidation of bilirubin (6) but it has subsequently been shown that photoisomerization is a much faster reaction and hence precedes photooxidation (7–9).

Initial evidence suggested that the photoisomerization of bilirubin leads to only one photoproduct, first named "photobilirubin" (8). Further work showed that there are in fact a number of photoproducts, including both structural and geometric isomers (10–13). Furthermore, the photoproduct formed, and in what proportion, seems to be a function of many parameters, including the nature of the solvent and the presence or absence of oxygen or of proteins such as albumin.

The conformation of the bilirubin molecule in solution is highly solvent dependent. In aqueous solution bilirubin is folded into a configuration that has six intramolecular hydrogen bonds (14). This folded, "ridge tile" structure results in the molecule being highly hydrophobic and thus sparingly soluble in aqueous solution. The reported solubility of bilirubin in aqueous solution at physiological pH ranges from 0.007 to 100 μ*M*, with the lower value being favoured according to Brodersen (15). The bilirubin molecule assumes this tight, intramolecularly hydrogen bonded stucture in water and in chloroform (16) but in solvents which interfere with hydrogen bonding, e.g., dimethyl sulfoxide (DMSO), the molecule "opens up" as some of the hydrogen bonds are weakened (10, 17).

Certain conclusions from studies of the photolysis of bilirubin in organic solvents have been extended to phototherapy conditions. Since the ultimate goal of phototherapy is to lower the bilirubin levels in the infant's blood plasma, it is imperative that effect of solvent on the mechanisms of the photoisomerization and photodestruction of bilirubin be understood. Thus, we have studied the photoreaction in various organic and aqueous media, with and without HSA and BSA, and offer some explanation for the photoisomerization results with respect to the environment of the bilirubin.

Experimental

Solutions of bilirubin were prepared daily by dissolving the powder (from bovine gallstones, Sigma) in the appropriate solvent. The purity of the bilirubin was checked using HPLC (18). Chloroform (Spectrograde, Anachemia), triethylamine (Reagent grade, Anachemia) and dimethylsulfoxide (HPLC grade, Anachemia) were used as received, except in experiments for which the ethanol stabilizer in the chloroform was removed immediately prior to use by first distilling over P_2O_5 and then passing the distillate through a column of basic I alumina (Sigma).

Aqueous solutions were made by first dissolving the bilirubin in 0.10 *M* NaOH and adjusting the volume of KH_2PO_4/NaOH buffer to achieve a final pH of 7.8 ± 0.1. Prior to use the solvents were purged with dried nitrogen.

Aliquots of the aqueous solution were added to HSA/buffer solutions to obtain the desired ratio of [HSA]/[BR]. Human serum (Cutter Laboratories) and bovine serum (Sigma, Fraction V) albumin were

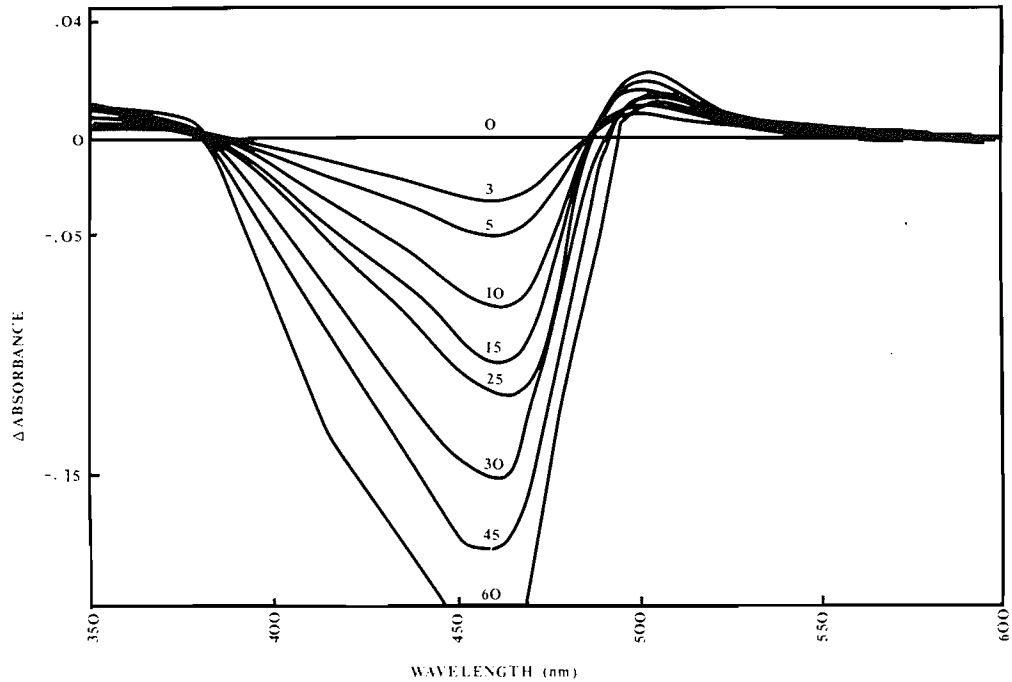

FIG. 1. Absorbance difference spectra for a solution of bilirubin in pure $CHCl_3$ after various times of irradiation. (The numbers refer to the irradiation times, in minutes.)

used as received. For all solutions the final concentration of bilirubin was 1.71×10^{-5} *M*. Studies were done at room temperature, except for those with the pure buffer solutions, which were kept in an ice bath prior to use.

The solutions (< 1 mL), contained in 1.00 cm path length cells, were irradiated directly in the cell holder of the Hewlett Packard Model 8451A Diode Array Spectrophotometer by placing the light source (Cole-Parmer Fiber Optic Illuminator Model 9745-00, fitted with a blue filter with a bandpass of 410–470 nm) over the cell, i.e., by shining the light down through the cell opening (intensity = 0.5 $\mu W/cm^2$). Since the course of bilirubin photochemistry has been shown to depend upon the excitation wavelength (19), the same light source was used throughout this study. The spectrophotometer was capable of recording a spectrum within 1 s *while* the irradiation light source was still on.

The HPLC apparatus and methods, described previously by McCarthy *et al.* (18), used reverse phase methods. The chromatograph consisted of a Waters Associates Model 590 pump, a Rheodyne 7125 injection valve and a Schoeffel Spectraflow (Kratos Analytical Instruments, Ramsay, NJ) Model SF770 variable wavelength detector. The column was a 15.0 × 0.46 cm blank packed in-house with 5 μm Spherisorb (Chromatographic Specialties Company, Town of Mount Royal, Quebec) with a guard column consisting of a 3.5 × 0.46 cm blank packed with a 30–38 μm octyldecyl silica (Whatman Inc., Clinton, NJ). The mobile phase contained 50% phosphate buffer (pH = 7.4), 25% acetonitrile, and 25% dimthylsulfoxide, and 1.5 g/L of tetrabutylammonium chloride (Sigma) as an ion-pairing agent. Prior to sample injection the sample was dissolved in the appropriate amount of phosphate buffer/acetonitrile/dimethylsulfoxide so that the solvent phase matched the mobile phase. All analyses were done at room temperature using a flow rate of 1.0 mL/min with the detector wavelength set at 455 nm.

The stopped flow experiments, made with a Model 1A Stopped-Flow Apparatus (Cantech Scientific Ltd., Winnipeg, Canada), monitored the changes in absorbance, at a fixed wavelength, with a TDI photometer and stored the data with a TDI 1024C transient recorder. The data were transferred to an IBM PC that performed the desired calculations. For these experiments, an aqueous solution of bilirubin was placed into one syringe and of HSA ([HSA]/[BR] = 10) in the second syringe. The syringe containing the bilirubin could be irradiated, using the light source described above, prior to mixing. The two syringes emptied into the observation cell, where the data points (208 points) were collected over a 20 s time span, beginning within 5 ms.

Results and discussion

(A) Photolysis in pure chloroform

When a solution of bilirubin in pure $CHCl_3$ purged with N_2 is irradiated a characteristic absorbance difference (AD) spectrum is obtained (Fig. 1), as reported previously (8, 20). During the first 25 min of irradiation two isosbestic points are obtained. The isosbestic point at 490 nm is not maintained at irradiation times >30 min, but that at 380 nm remains, suggesting two separate photoproducts. The production of *EZ/ZE* isomers of bilirubin leads to the isosbestic point at 490 nm (9). The second isosbestic point results from the formation of a second photoproduct, previously identified as "lumirubins" (12), also referred to as "cyclobilirubin" (9), "unknown pigment" (11), and "pigment 430" (21), that has an absorbance maximum around 430 nm (10). Lumirubin is a configurational isomer of bilirubin formed by an *endo*-vinyl cyclization on one end ring of the molecule (11, 13, 22).

Based on structural arguments obtained by use of molecular models, it seems likely that cyclization to form lumirubins occurs *after* a rotation about the C4—C5 bond of the bilirubin, i.e., by a reaction of *EZ/ZE* isomers. This has been the subject of considerable dispute (11, 22), and is considered more fully later. Although an induction period for the formation of lumirubins would be expected for such a mechanism, it is not detected, probably because the necessary buildup in the concentration of *EZ/ZE* isomers is fairly rapid compared to the frequency at which spectra were taken.

(B) Photolysis in chloroform/ethanol

The photolysis behaviour of bilirubin in $CHCl_3$ containing 1% ethanol stabilizer is similar to that in pure $CHCl_3$ with the exception that, at a given time of irradiation, the resulting gain

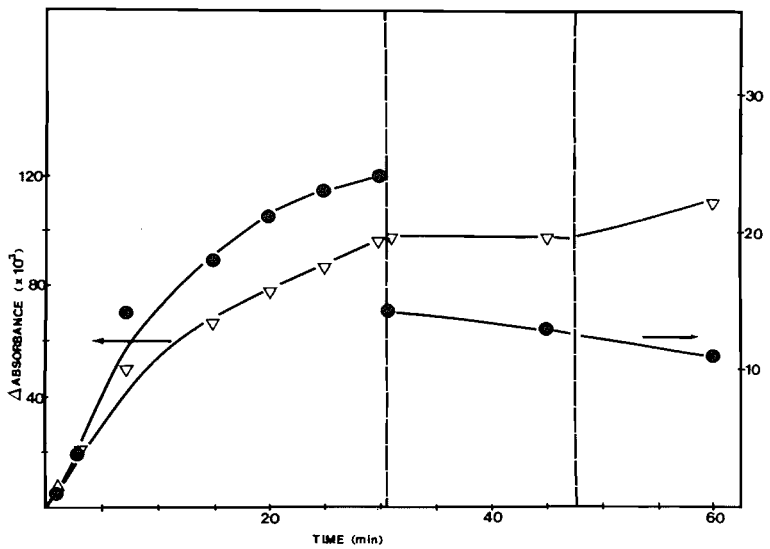

FIG. 2. The absolute values of the absorbance difference of the gain peak (500 nm) and the loss peak (460 nm) for a photolyzed solution of bilirubin in pure $CHCl_3$ as a function of irradiation time. At t = 31 min, irradiation was discontinued and ethanol was added. At t = 47 min, photolysis was continued: ●, gain peak; ▽, loss peak.

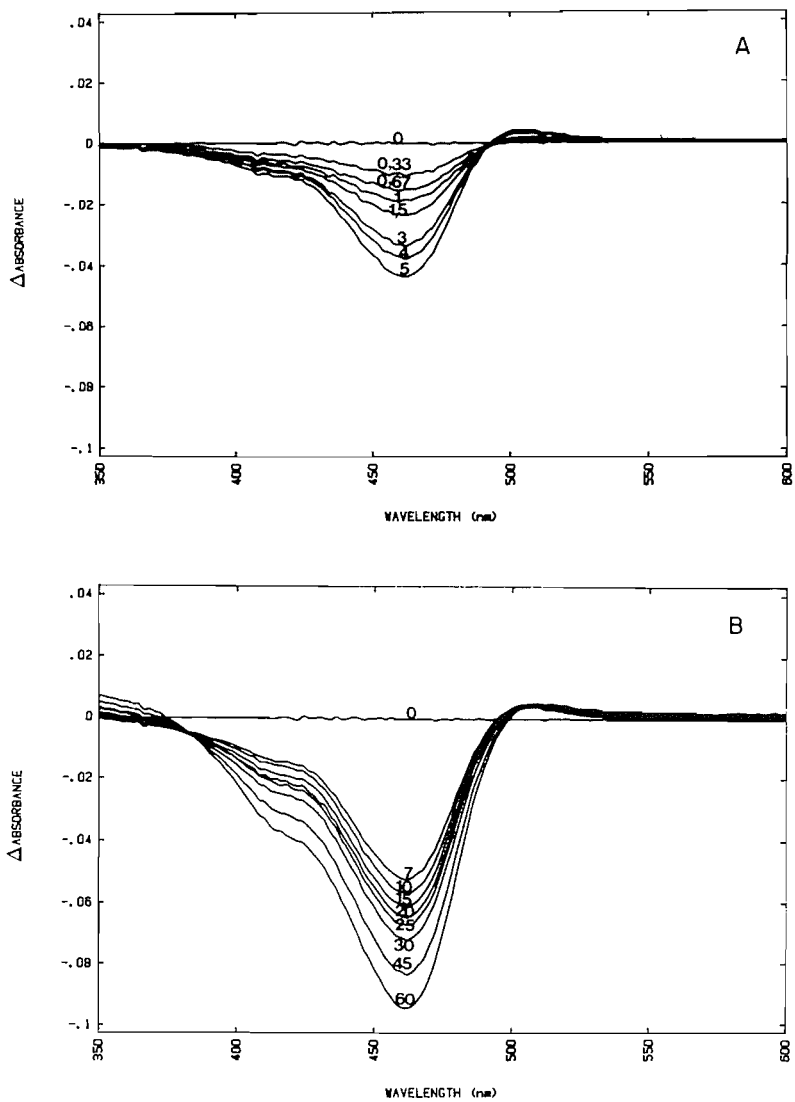

FIG. 3. A/B. Absorbance difference spectra for a solution of bilirubin in DMSO for various irradiation times, in minutes. (The numbers refer to the irradiation times, in minutes.)

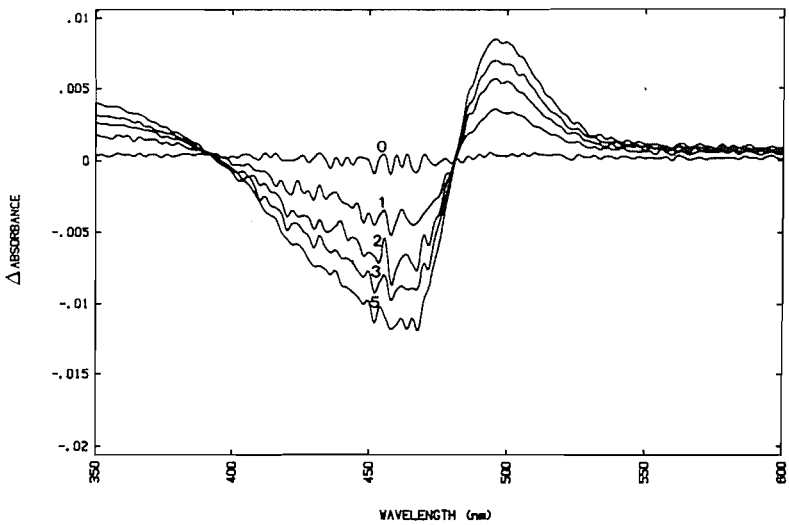

FIG. 4. Absorbance difference spectra for a solution of bilirubin in DMSO/buffer (90:10) for various irradiation times. (The numbers refer to the irradiation times, in minutes.)

peaks are less than one half the size of those using pure $CHCl_3$. However, within experimental uncertainty, the loss peaks remain unchanged. It should be noted that even 1% stabilizer in the solvent corresponds to a molar ethanol concentration that is much greater than the bilirubin concentration.

The following experiment was made to elucidate the role of ethanol: bilirubin in pure $CHCl_3$ purged with N_2 was irradiated for 30 min after which 10 μL of ethanol (1% of the total volume of the solution being irradiated) was added. The cell was gently shaken, the irradiating light was turned off, and additional spectra were taken. Possible side effects due to the addition of ethanol were accounted for by simultaneously adding an equal amount of ethanol to the reference solution. Upon addition of ethanol the height of the gain peak, due to the *EZ/ZE* isomers, immediately decreased substantially while the bilirubin loss peak remained essentially unchanged (Fig. 2). Concomitantly the isosbestic points disappeared. When irradiation of this solution was resumed the gain peak showed a further decrease while the loss peak continued to increase, and the isosbestic point at 380 nm was restored. These observations are consistent with the continued formation of lumirubins, since there is still a loss of bilirubin and an isosbestic point. Meanwhile the peak at 500 nm, corresponding to the *EZ/ZE* isomers, is decreasing slightly such that a new photostationary state is established. Interestingly, the absorbance difference at 500 nm reaches the same final value, 0.011, as in the $CHCl_3$/ethanol system discussed above.

The explanation of these results must account for a rapid decrease in the gain peak due to added ethanol that is not accompanied by any change in the size of the loss peak. A possible explanation is a reaction of ethanol with the *EZ/ZE* isomers. Photochemical addition of alcohols to the *exo*-vinyl group of bilirubin has been reported previously by Garbagnati and Manitto (23). However, the data are not consistent with Manitto's overall mechanism which requires the reaction to be light induced and irreversible. Since the *EZ/ZE* isomers are not entirely consumed, despite a large molar excess of ethanol, the reaction must be an equilibrium process. Furthermore, since the reaction occurs after the light is turned off, it is a reaction with the photoproduct. A reversible reaction of photoproducts with ethanol is consistent with a decrease in the concentration of *EZ/ZE* isomers in $CHCl_3$/EtOH solutions relative to that in pure $CHCl_3$ as well as the immediate decrease in its concentration when ethanol is added after the irradiation.

Alternatively, the changes in the height of the gain peak upon addition of ethanol may indicate solvatochromism of the photoisomers, similar to that exhibited by bilirubin dimethyl ester (24). It is well known that bilirubin dimethyl ester in dilute solution exhibits solvatochromism which has been attributed to the presence of two co-existing conformers with different orientations of the A/B and C/D pyrromethenone moieties with respect to each other (24). It seems likely that the *EZ/ZE* photoisomers can also co-exist in two orientations. Since these photoisomers have less intramolecular H-bonding than bilirubin they are more polar and should have characteristics similar to the bilirubin dimethyl ester so that the relative proportions are likely to be solvent dependent. Thus, while little solvatochromism is expected for bilirubin IX-α in weakly H-bonding solvents, it may well be significant in the case of the *EZ/ZE* photoisomers.

(C) Photolysis in dimethyl sulfoxide

A more dramatic effect on the photoisomerization due to environment can be seen when DMSO is used as solvent rather than $CHCl_3$ (Fig. 3). Similar results were obtained for both DMSO and $CHCl_3/Et_3N$. Comparison with Fig. 1 indicates a difference in shape on the short wavelength side of the loss peak. Specifically, the shoulder at ca. 430 nm seen previously for long irradiation times with $CHCl_3$ is now more distinct such that the loss peak is double humped. Furthermore, the two isosbestic points do not appear simultaneously. The isosbestic point at ca. 490 nm occurs only at short irradiation times (<7 min), while the isosbestic point at 380 nm develops only after longer irradiation times (>7 min).

These data are consistent with the two-step process presented above. The spectra for short times of irradiation indicate that production of *EZ/ZE* isomers predominates (one isosbestic point). An induction period is now evident which could not be seen when bilirubin was irradiated in $CHCl_3$. Since an initial buildup of *EZ/ZE* isomers is required before lumirubins are formed, it is now apparent that the latter results by a reaction of the former. As the time of irradiation is increased, the isosbestic

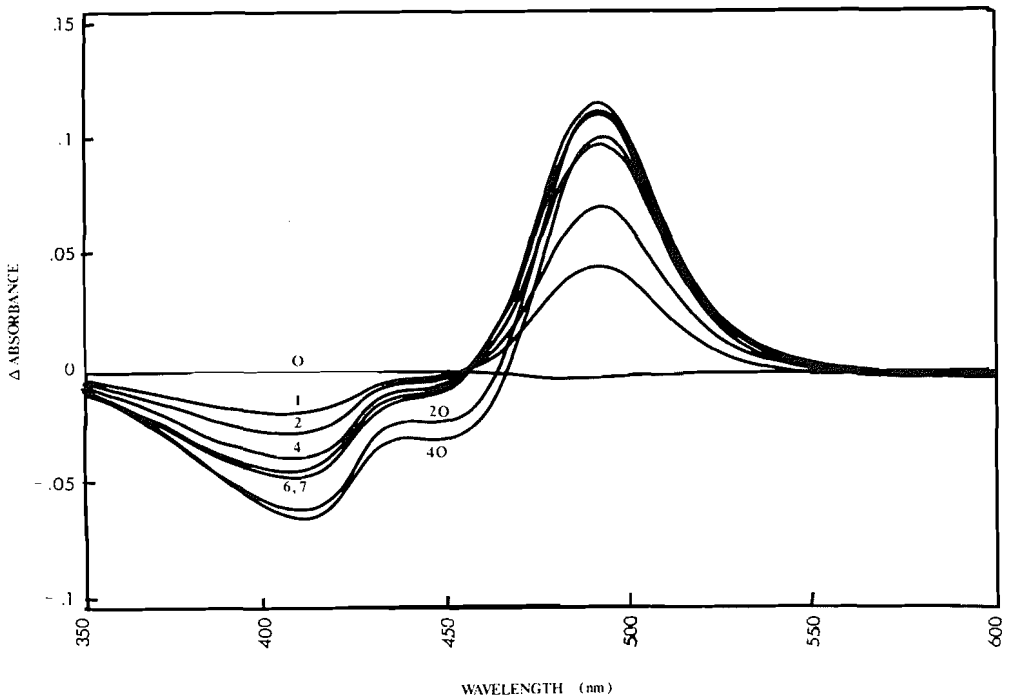

FIG. 5. Absorbance difference spectra for an aqueous buffer solution of bilirubin and human serum albumin for various irradiation times. (The numbers refer to the irradiation times, in minutes.)

point is no longer maintained, indicating that *EZ/ZE* isomers have reached an equilibrium concentration, but another isosbestic point appears at shorter wavelength, indicating that the lumirubins continue to be formed. The increased distortion of the loss peak at 430 nm for DMSO as compared to that with $CHCl_3$ is probably due to an increased concentration of this photoproduct relative to that of *EZ/ZE* isomers. Ostrow and co-workers have shown that in DMSO lumirubins can be produced in sufficient quantities to be isolated (10).

The development of a double-humped loss peak on irradiation of aqueous solutions of bilirubin containing human serum albumin (HSA) has also been attributed, by Davies and Keohane (25), to the presence of a second synthesis peak. To verify that the double-humped loss peaks in the AD spectra result from a superimposed gain peak, experiments were made using HPLC methods described previously by McCarthy *et al.* (18). Chromatograms of the DMSO solution of bilirubin after irradiation confirmed the presence of more than one photoproduct. In fact, McDonagh *et al.* (22), have also reported that irradiation of bilirubin in a $CHCl_3/Et_3N$ system results in a second photoproduct that absorbs at 434 nm. This is consistent with the hypothesis that lumirubins can be formed in solvents which interfere strongly with the intramolecular hydrogen bonding of the bilirubin.

Thus, the mechanism for the formation of photoproducts in DMSO is similar to that in $CHCl_3$ but appears to differ only in the relative quantum yields for the formation of the various photoproducts. It seems that solvents like DMSO and Et_3N permit the *EZ/ZE* isomers to attain certain orientations which favour this internal cyclization. Similar behaviour is seen during photolysis of bilirubin in aqueous solutions containing HSA. As will be shown later, the photoisomers of bilirubin are detected in aqueous solution only when HSA is added. The formation of lumirubins from *EZ/ZE* isomers is consistent with the mechanism proposed previously by Onishi *et al.* (11), rather than that of McDonagh *et al.* (22), who propose that the cyclization process takes place directly from the bilirubin, and not from *EZ/ZE* isomers.

(D) Photolysis in dimethyl sulfoxide/water

To obtain a better understanding of the effect of changes in the polarity a solution of bilirubin in DMSO/buffer (90:10) was irradiated. The AD spectra obtained during the first 5 min of irradiation are analogous to those for the $CHCl_3$ system in that there is only one loss peak with two isosbestic points (Fig. 4). As for photolysis in $CHCl_3$, with increasing time the isosbestic point at 490 nm disappears. Simultaneously, a shoulder begins to form on the loss peak, indicating that the production of *EZ/ZE* isomers has reached a photostationary state but lumirubins are still being formed.

Thus, the relative importance of the different pathways for bilirubin photoisomerization, photodegradation, and photooxidation is highly dependent upon the environment of the bilirubin molecule. The AD spectra resulting from irradiation of bilirubin in DMSO are considerably different from those for bilirubin in $CHCl_3$. Yet, if 10% aqueous phase is added to the DMSO, the spectra become very similar. The addition of water to DMSO causes the bilirubin molecule to adopt a tightly closed, hydrophobic structure, similar to that in $CHCl_3$, and not the more open structure thought to be present in DMSO. The resulting photoprocess in DMSO/buffer becomes more similar to that in the $CHCl_3$/EtOH system rather than that in pure DMSO. In fact, not only are the shapes of the AD spectra very similar, but the absorbance difference is 0.011 in the $CHCl_3$/EtOH system and 0.010 in the $DMSO/H_2O$.

(E) Photolysis of aqueous solutions containing albumin

The dramatic changes resulting from the addition of water to DMSO suggested that photolysis of bilirubin in aqueous medium should differ considerably from photolysis in organic solvents. Indeed, as reported previously (26), attempts to identify

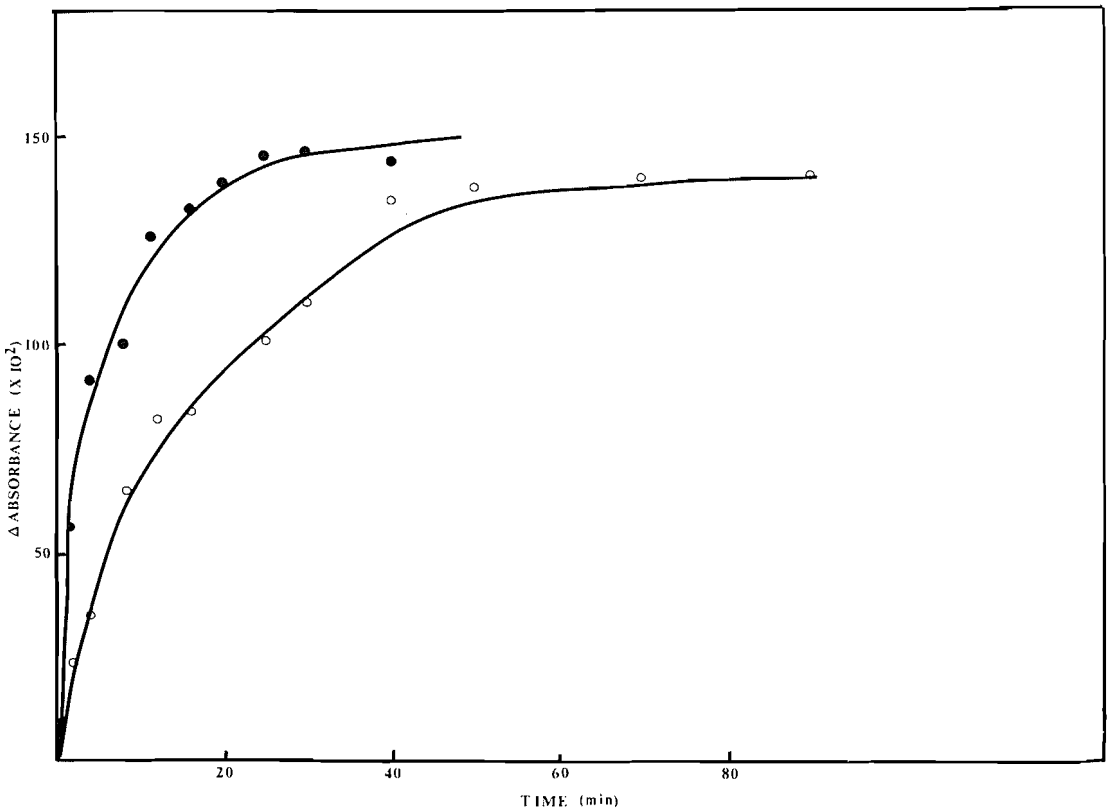

FIG. 6. The absolute values of the absorbance difference of the gain peak (490 nm) for a photolyzed aqueous buffer solution of bilirubin and human serum albumin at different light intensities: ●, high intensity; ○, low intensity.

photoproducts after irradiation of bilirubin in aqueous phosphate buffer were unsuccessful. However, photoproduct formation is readily apparent after the irradiation of the aqueous bilirubin solution containing a small amount of HSA.

When a solution of bilirubin containing HSA with a molar albumin concentration seven times that of the bilirubin is irradiated, the resulting AD spectra (Fig. 5) show three peaks: two loss peaks at 408 and 448 nm, and one large gain peak at 492 nm. In contrast, replacement of HSA with BSA results in one gain peak at ca. 508 nm and a corresponding loss peak at ca. 460 nm (20). These AD spectra show characteristics similar to those for irradiated DMSO solutions of bilirubin. That is, at short irradiation times there is only one isosbestic point, at 460 nm, indicating that initially there is only one product being formed, *EZ/ZE* isomers. As the time of irradiation is increased, this isosbestic point disappears, indicating a disruption in the equilibrium, BR ⇆ *EZ/ZE* isomers, while a second isosbestic point (360 nm) appears, indicating that a second product is now being formed. Thus, the mechanism for photoproduct formation in HSA appears to be the same as in DMSO.

When the height of the gain peak (492 nm) is plotted as a function of the time of irradiation in the presence of HSA a sharp increase is seen initially, followed by the appearance of a photostationary state (Fig. 6). Similar behaviour is seen, but with an increase in initial slope by a factor of 3, so that the photostationary state is reached more rapidly when the intensity of the irradiating light is increased approximately threefold. Thus, a direct relationship is seen between the intensity of the irradiating light and the initial rate of formation of photoproduct. The occurrence of a photostationary state indicates that the formation of *EZ/ZE* isomers from bilirubin is reversible. Furthermore, since the AD spectrum of irradiated bilirubin remains unchanged when it is left in the dark, the reverse process must also be light induced, as proposed by McDonagh and Palma (22).

As mentioned above, it has been suggested previously that the double loss peak is in fact due to the development of a synthesis peak (25). Further evidence is obtained from a plot of peak height after irradiating for 7.0 min, as a function of log {[HSA]/[BR]}, at a fixed bilirubin concentration of $1.71 \times 10^{-5} M$ (Fig. 7). The loss peak at 408 nm behaves identically with the gain peak at 492 nm, while the loss peak at 448 nm behaves differently. (The values have been corrected for the absorbance by HSA at the illuminating wavelength.) The magnitude of the loss peak at 448 nm is, within experimental error, independent of [HSA]/[BR] whereas the other two peaks go through a maximum at a ratio of ca. 1.5/1.0 and then decrease to a plateau. The limit appears to be related to the binding of the bilirubin and/or its photoproducts to the HSA. As the HSA concentration increases, more photoproduct is produced until a [HSA]/[BR] of 1.5 is reached. At higher concentrations of HSA, the amount of photoproduct decreases, perhaps because the binding of bilirubin decreases due to protein aggregation. Such a decrease in binding affinity at high concentrations of HSA has been reported previously (27). When both HSA and bilirubin concentrations are decreased by a factor of 10 a plateau without a "hump" occurs.

The apparent requirement of albumin in aqueous solution of bilirubin to bring about the production of photobilirubin suggests that: (*a*) photoisomerization requires that the albumin bind the bilirubin to facilitate the isomerization, or (*b*) unbound bilirubin can undergo photoisomerization but rapid reversion occurs in the absence of albumin. If the latter were true it should be possible to "trap" photoproducts formed by the

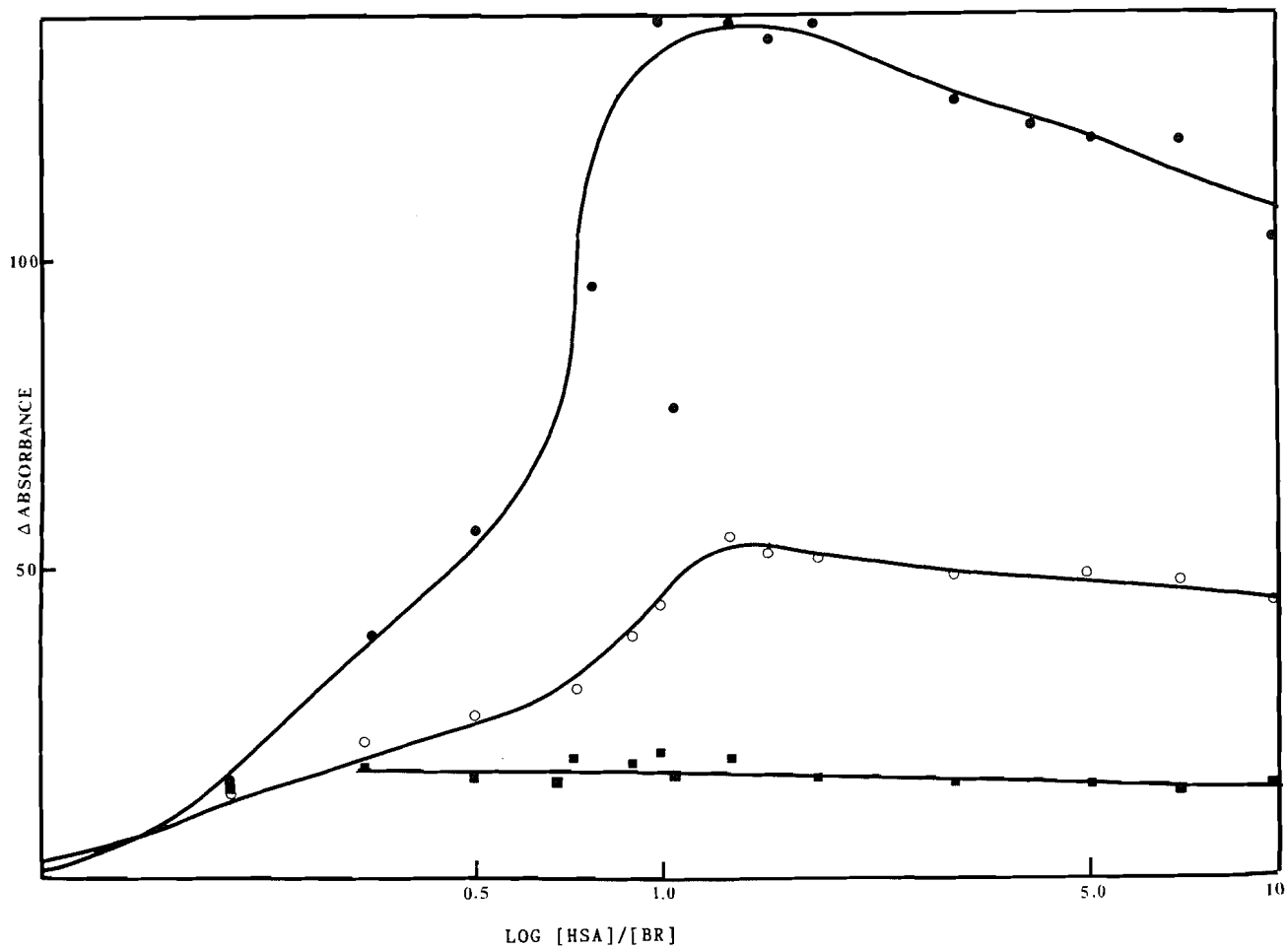

FIG. 7. The absolute values of the absorbance difference, multiplied by 100, of the gain peak (490 nm) and the two loss peaks (448 nm and 408 nm) for a photolyzed aqueous buffer solution of bilirubin and human serum albumin as a function of the log [HSA]/[BR], at 7 min of irradiation: ●, 492 nm; ○, 408 nm; ■, 448 nm.

irradiation of an aqueous solution of bilirubin by adding albumin to bind the photoproducts immediately after irradiation. This was done using the method of stopped flow analysis. The bilirubin buffer solution was irradiated for a given period of time, mixed with a solution of HSA (total mixing time of 20 ms) and the absorbance at the desired wavelength was recorded immediately. A small amount of photoproduct was formed, as evidenced by an increase in absorbance at 490 nm, that increases with time of irradiation (Fig. 8). As expected a concomitant decrease in absorbance was noted at 410 nm while, within experimental error, there was no change in absorbance at 460 nm, which corresponds to an isosbestic point in the AD spectrum of an irradiated solution of bilirubin/HSA. In contrast, no photoproduct formation was detected in an analogous experiment when an irradiated aqueous solution of bilirubin was mixed with buffer, indicating that the presence of HSA is indeed necessary to protect the photoproduct. By comparison, there was approximately a threefold increase in photoproduct formation when the irradiated solution contained both HSA and bilirubin. Thus, it would appear that it is possible for bilirubin to isomerize to form the *EZ*/*ZE* isomers in aqueous solution, but that under these conditions the reversion is too fast to permit their detection. However, when albumin is added to the solution immediately following the irradiation, the *EZ*/*ZE* isomers are bound to the albumin and do not revert to bilirubin.

The fact that more photoproduct is formed when the albumin is present during the irradiation than when it is added just after the irradiation follows from Fig. 7 which shows an increase in photoproduct formation with increasing [HSA]/[BR]. Below the [HSA]/[BR] ratio of 1/1 there is relatively little photoproduct formed. Since the first molecule of bilirubin binds strongly to HSA, with a binding constant of 10^6–10^7 (28), the fraction of free bilirubin is very small at the ratio of [HSA]/[BR] of 1/1, suggesting that it is the irradiation of bound bilirubin that gives rise to the increased formation of photoproducts. Lee and Gillispie (29) have shown that the bilirubin molecule is buried deeply within the HSA. Consequently the HSA can provide a protective environment where the photoproduct is more readily formed. It has been suggested (28) that the HSA molecule maintains the bilirubin in an environment that changes the energies of the transition states leading to the formation of photoproduct. The idea that bilirubin is protected by albumin is also discussed by Rubaltelli and Jori (30).

Summary

It is apparent that the photolysis of bilirubin is a very complex process, involving various photoisomerizations, photodestruction, and photooxidation. Although photoisomerization reactions predominate at short irradiation times under anaerobic conditions, the relative yields of isomers is highly depen-

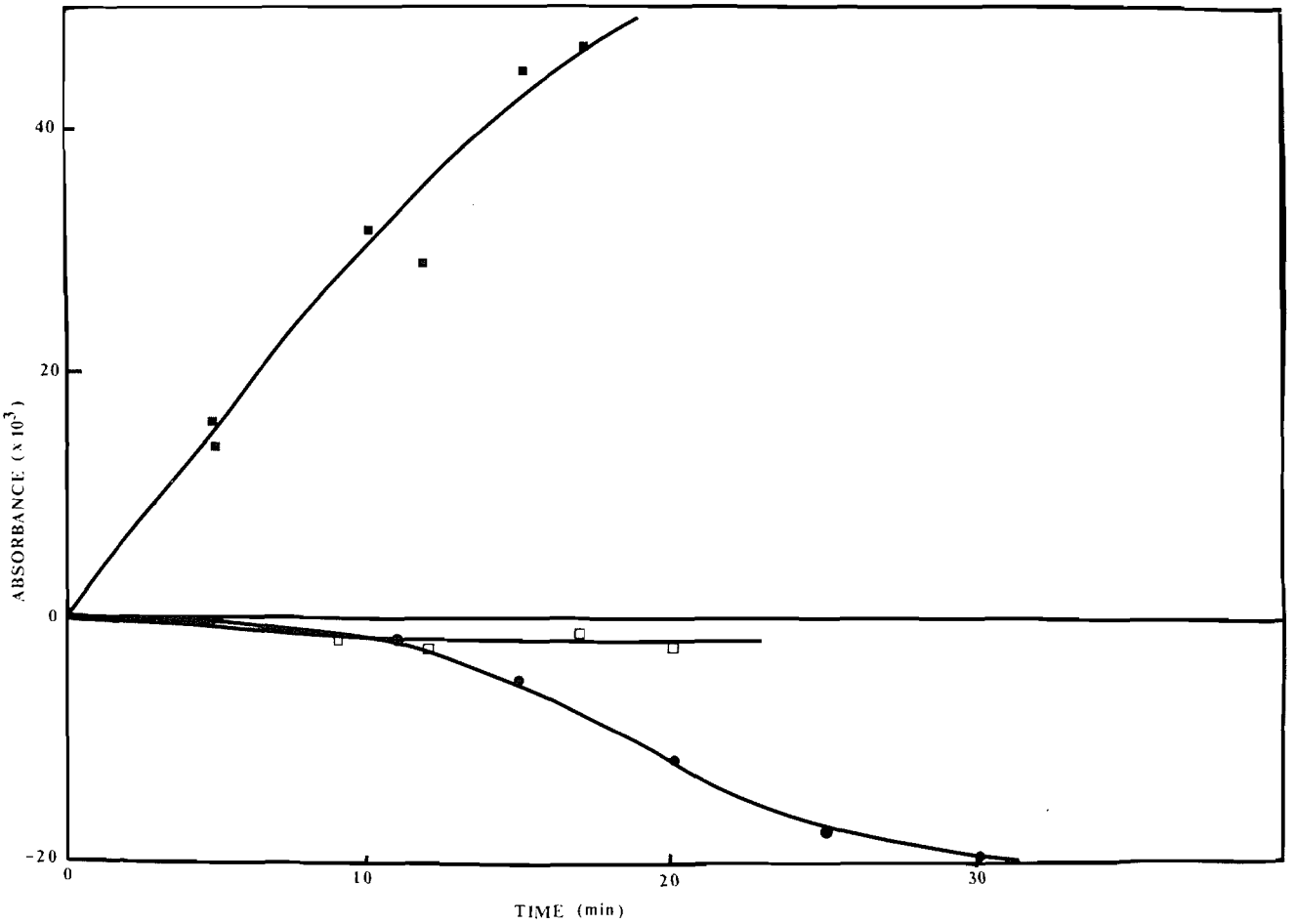

FIG. 8. The absolute values of the absorbance difference at three separate wavelengths (490 nm, 460 nm, and 410 nm) as a function of the irradiation time for a photolyzed aqueous solution of bilirubin after mixing with human serum albumin: ■, 490 nm; □, 460 nm; ●, 410 nm.

dent on the environment of the bilirubin at the time of irradiation. In solvents which interfere weakly with the intramolecular hydrogen bonds of bilirubin, solvents like water or ethanol, formation of lumirubins from *EZ/ZE* isomers is less than in stronger interfering solvents like DMSO and Et_3N. Although small amounts of photoisomers can be "trapped" in irradiated aqueous buffer solutions of bilirubin, substantially greater amounts are formed in the presence of HSA. Indeed, photoisomer production in the presence of HSA is similar to that in DMSO solutions.

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