Kinetics of the photochemical interconversion among geometric photoisomers of bilirubin

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Kinetic study of the photochemical interconversion of three geometric photoisomers of bilirubin, namely peaks 1, 2 and 3, under anaerobic conditions was performed by using high-pressure liquid chromatography. Rapid and definite interconversion among these three peaks and the parent pigment occurred on irradiation by blue light.

Phototherapy is now an established procedure in treatment of neonatal jaundice (Cohen & Ostrow, 1980). Excretion of the major photoderivative and unconjugated bilirubin in the bile together account for 70% of the increased bilirubin turnover during phototherapy in the Gunn rat (Stoll *et al.*, 1979). A previous paper reported that exact correspondence was observed between the two main photoproducts, unknown pigment and photobilirubin IX α from biological fluids and the comparable main peaks 2 and 3 formed *in vitro* (Onishi *et al.*, 1980a). Each fraction of these peaks 1, 2 and 3 was separated by h.p.l.c. from bilirubin photoproducts produced by the action of light on a bilirubin–albumin solution. Peak 2 corresponds to photobilirubins (IIA) and (IIB) and peak 3 to photobilirubin (IB) described by Stoll *et al.* (1979). In the present paper we have studied the kinetics of the photochemical interconversion among these three peaks.

Materials and methods

Separation of each peak

Isolation of peaks 1, 2 and 3 was achieved by irradiation of an equimolar solution (850 μ M) of human serum albumin and bilirubin in 0.02 M-phosphate buffer, pH 7.4, followed by h.p.l.c. separation after sample preparation and collection of each peak (Onishi *et al.*, 1980a). Thermal reversion of each peak after elution was inhibited by cooling the collecting test tubes to -70°C . All manipulations except photochemical experiments were carried out in subdued light.

Photochemical experiments

Photochemical experiments under anaerobic con-

ditions were carried out in stoppered 10 ml Pyrex tubes, containing a solution of one of peaks 1, 2 and 3 separated by h.p.l.c. (Onishi *et al.*, 1980a,b). The tubes were placed 3 cm above the canopy of four fluorescent lamps (20 W Matsushita fluorescent blue–white fluorescent tubes). In addition to each peak of photoproducts, these solutions consisted of the h.p.l.c. eluent by which each peak was separated. Light intensity, determined by a PR III phototherapy radiometer (Air Shields), at the surface of the solution was $11.8 \mu\text{W}\cdot\text{cm}^{-2}\cdot\text{nm}^{-1}$. Chromatographic operation, preparation of a calibration curve of peak area, sample preparation and numbering of each peak separated by h.p.l.c. were carried out as described previously (Onishi *et al.*, 1980a,b).

Results and discussion

Analysis by h.p.l.c. of photochemical conversion of peak 1 (Fig. 1) showed that, within 1 min of irradiation, the peak decreased abruptly and peak 2 appeared at the same time and exceeded peak 1 in amount. Subsequently, the slopes of disappearance curves of these two peaks were parallel. Within 1 min, peak 5, which is identical with lipid-soluble (ZZ)-bilirubin IX α (the parent pigment), appeared and then increased gradually. The change in peak 3, which is identical with (EZ)- and (ZE)-photobilirubin IX α , was the same as that described for peak 5, although its change was slight. A summary of these changes plotted against time on a semi-logarithmic scale is shown in Fig. 2. A good correspondence between the disappearance of peak 1 and the sum of the appearance of peaks 2, 3 and 5 was observed.

Peak 2 (Fig. 3) also decreased abruptly and peak 1, which is a more polar substance than peak 2, appeared precipitously. After 1 min of irradiation, the slopes of disappearance curves of these two peaks were also parallel. Within 3 min peak 5

Abbreviation used: h.p.l.c., high-pressure liquid chromatography.

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Fig. 1. *H.p.l.c.* of solutions obtained at various intervals during anaerobic illumination of peak 1 in the eluent (a) shows the chromatographic scan for the control non-irradiated solution; (b) and (c) show the scans after exposure to light for 1 min and 30 min respectively.

Fig. 3. *H.p.l.c.* of solutions obtained at various intervals during anaerobic illumination of peak 2 in the eluent (a) shows the chromatographic scan for a control non-irradiated solution; (b) and (c) show the scans after exposure to light for 10 min and 30 min respectively.

Fig. 2. Plot of the changes in peaks 1, 2, 3 and 5 with time on a semi-logarithmic scale

appeared and then peak 3 appeared at 10–30 min. These peaks increased with time. These changes plotted against time on a semi-logarithmic scale are shown in Fig. 4. Analogous to the case for peak 1, a good correspondence of the disappearance of peak 2 with the appearance of peaks 1, 3 and 5 was observed.

On the contrary, on irradiation, peak 3 reverted rapidly and exclusively to peak 5. The extent of reversion was 70.8% at 15 s and 80.0% at 30 s. When compared with other peaks, the rate of reversion was very rapid and the manner was very simple and different from the other peaks, namely one 'step' to the parent pigment.

The relation between these peaks and the hitherto described photoproducts is as follows. Peak 1 does not correspond to any photobilirubin described by

Fig. 4. Plot of the changes of peaks 1, 2, 3 and 5 with time on a semi-logarithmic scale

Stoll *et al.* (1979). When the irradiated solution, after the addition of chloroform, is washed with water to remove all dimethyl sulphoxide in the preparation of photobilirubins (IIA) and (IIB) (Stoll *et al.*, 1979), peak 1, the most polar substance among these three peaks, is lost with the aqueous phase. Peak 2, the same substance as the unknown pigment obtained from bile (Onishi *et al.*, 1980a), corresponds probably to the (*EE*)-isomer of photobilirubin described by McDonagh *et al.* (1979) and by Lightner *et al.* (1979a,b) and to the mixture of photobilirubins (IIA) and (IIB) described by Stoll *et al.* (1979), because the retention times of both these photobilirubins fractionated by t.l.c. agree with that of the unknown pigment, which appeared in the

h.p.l.c. method for photobilirubin (Onishi *et al.*, 1980a). In addition, since a part of the fractionated photobilirubin (IIA) by t.l.c. was photochemically converted into (*ZZ*)-bilirubin XIII α , photobilirubin (IIA) contains a XIII α isomer corresponding to the unknown pigment, which is a IX α isomer. Because peak 3 can be divided into two peaks by the recycling method of h.p.l.c. and because both photobilirubins (IA) and (IB) isolated by t.l.c. as described by Stoll *et al.* (1979) are spontaneously converted into (*ZZ*)-bilirubin IX α , it was considered that peak 3 [the same substance as photobilirubin IX α obtained from neonatal hyperbilirubinaemic serum (Onishi *et al.* 1980a)] corresponds to the mixture of the (*ZE*)- and (*EZ*)-isomers described by McDonagh *et al.* (1979) and by Lightner *et al.* (1979a,b) and to the mixture of photobilirubins (IA) and (IB) described by Stoll *et al.* (1979).

From these observations we conclude that, in photochemical interconversion, there is a mutual relationship between peaks 1, 2, 3 and 5 (parent pigment).

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