Interaction of Bilirubin with Human Serum Albumin and Cationic Detergents

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Bilirubin shows a red-shift in the visual spectra on binding to albumin. In bilirubin-cationic detergent systems a similar spectral change was observed. These comparisons allow one to deduce that the environment of the binding site in albumin is most likely apolar than when bilirubin is solubilized by the cationic detergents.

In biological systems, once the unconjugated bilirubin is released into the plasma, it is transported rather firmly bound to albumin [1–3]. The binding equilibria of bilirubin to albumin and tissues are of great interest in order to be able to understand the development of hyperbilirubinemia [3]. It is well established that while the first mole of bilirubin is tightly bound to albumin, the subsequent moles of bilirubin show a less affinity [4, 5]. Free bilirubin at pH about 7 exhibits in the visual spectra an absorption maximum at approx. 440 nm [5–7]. Its spectra show a red-shift after binding to human serum albumin, at a bilirubin : albumin molar ratio of 1 : 1, where the maximum shifts to around 457 nm, depending on the ionic strength, pH and method of preparation [8, 9]. Recent studies by various investigators have indicated that a variety of non-covalent and hydrophobic interactions may contribute to the stability of the human- or bovine serum albumin-bilirubin complex [6, 10]. It was therefore considered of interest to compare the spectral changes of bilirubin on binding to human serum albumin with spectral changes of bilirubin caused by a. cationic detergents and b. addition of ethanol.

Material and Methods

Human Serum Albumin (HSA) was used as supplied by Behringwerke A.G., Germany (electrophoretic purity ~100%). Cetyl trimethylammonium bromide (CTAB) was of ~99% purity (Schuchardt, Germany). Cetyl pyridinium chloride (CPC) was of ~99% purity (Schuchardt, Germany). Bilirubin (purity 96%) was used as supplied by B.D.H., U.K. All solutions were made in phosphate buffer of pH = 7.4 (0.009 M KH_2PO_4 + 0.03 M Na_2HPO_4, containing 9.0 g/l NaCl, ionic strength = 0.25). All reagents were of analytical grade.

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Bilirubin solution and spectral measurements: 5 mg bilirubin was dissolved in 1.0 ml of 0.1 N NaOH, and diluted with distilled water to 5 ml. This solution was stored in the dark at about 4 °C and used within 4 hours. Bilirubin solution in buffer was prepared from this by diluting 26 times with buffer. For spectral measurements, 400 µl of this bilirubin buffer solution was diluted with 2000 µl of buffer in cells with path length 1.0 cm (bilirubin concentration $6.4\,10^{-3}$ mg/ml = 10.9 µmoles). The difference spectra were measured as follows: 400 µl bilirubin buffer solution + 1520 µl of buffer solution were placed in the reference and the sample beams. 480 µl solution of HSA (7.5 mg/ml) or detergent (CTAB or CPC) (20 mg/ml) was added to the sample cell, while an equivalent volume of buffer was added to the reference cell. After mixing the spectra were recorded manually between 550 – 350 nm. The spectra were measured on a Cary 16 spectrophotometer. It was observed that bilirubin was precipitated from buffer solutions on addition of cationic detergents (CTAB, CPC) when the detergent concentration was under the critical micelle concentration (cmc). However, when the detergent concentration was much over cmc no precipitation was observed. The detergent concentration (10^{-2} M) used was much higher than the cmc. Spectra were measured where molar ratios of bilirubin : HSA, bilirubin : CTAB and bilirubin : CPC were 1 : 2, 1 : 1000 and 1 : 1073 respectively. Difference spectra of bilirubin in 10 – 15 – 20% ethanol in buffer were measured by using bilirubin in the reference cell.

Results and Discussion

As described above, bilirubin was precipitated on addition of cationic detergents (CTAB and CPC)

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under cmc. Since no precipitation was observed when the detergent concentration was over cmc, all experiments were performed at detergent concentration much over cmc. In Fig. 1 the difference spectra of bilirubin + HSA (molar ratio 1 : 2) are given,

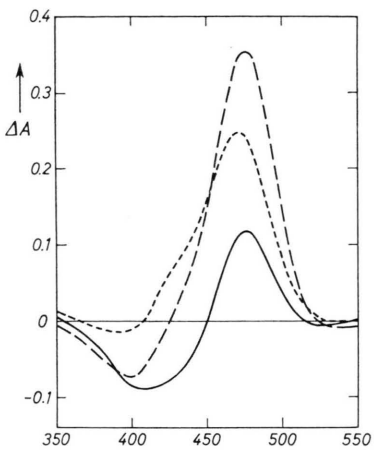

Fig. 1. Differenc spectra of bilirubin + human serum albumin (———), bilirubin + cetylpyridinium chloride (- - - -) and bilirubin + cetyltrimethylammonium bromide (— — —) : reference bilirubin solution. pH 7.4.

bilirubin + CTAB (molar ratio 1 : 1000) and bilirubin + CPC (molar ratio 1 : 1073) against bilirubin. In Fig. 2 the spectra of bilirubin, bilirubin + HSA, bilirubin + CPC and bilirubin + CTAB are

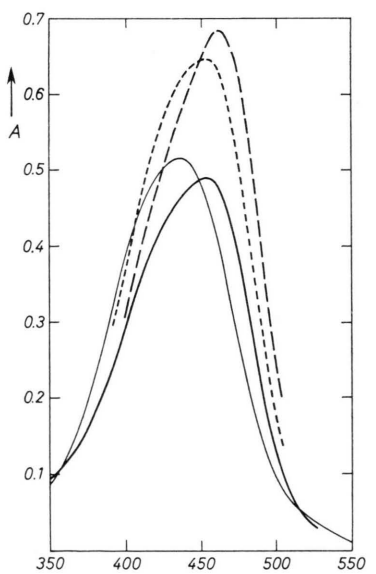

Fig. 2. Spectra of bilirubin (———), bilirubin + human serum albumin (———), bilirubin + cetylpyridinium chloride (- - - -) and bilirubin + cetyltrimethylammonium bromide (— — —). pH 7.4.

given. The spectra of bilirubin show a red-shift after binding to albumin in agreement with the investigations of other workers [5, 6]. It is seen that the bilirubin spectra change in the bilirubin-detergent (CTAB, CPC) systems is analogous to the bilirubin-albumin system (Fig. 1). Although the difference spectra exhibit similar changes, but the degrees are different. The absorption change at 477 nm in the case of bilirubin + HSA system is much lower than in the bilirubin-detergent systems. These spectral changes suggest that the environment of bilirubin is predominantly apolar and increases in the following order HSA-CPC-CTAB. Further, the difference spectra in CPC and CTAB systems would have been expected to be similar if the bilirubin molecule was mainly in the apolar region of the micelles in the solubilized state, since the alkyl chain length is equal in these detergents. However, as the spectra differ significantly, this clearly indicates that the bilirubin is at the micelle-water interface. Similar conclusions were arrived from solubilization studies of water insoluble dyes by detergents [11]. The bilirubin difference spectra in aqueous solutions with various concentrations of ethanol (Fig. 3) also agree with

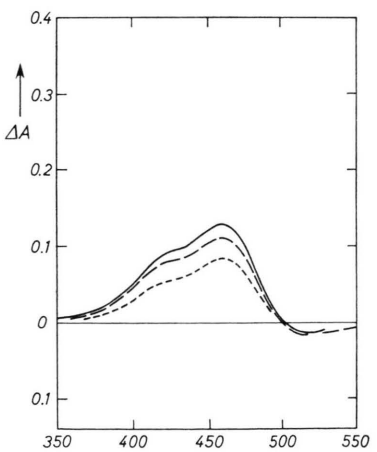

Fig. 3. Difference spectra of bilirubin in ethanol solutions: 10% (- - - -), 15% (— — —) and 20% (———) ethanol. pH 7.4. Reference bilirubin solution without ethanol.

the above conclusions. These spectra indicate that as the polarity of the solvent decreases (with increasing concentration of ethanol) the difference absorption increases at 460 nm. Since the difference spectra allow to differentiate the environment of bilirubin, these results indicate that the binding site of bilirubin in HSA is somewhat less apolar than at the micelle-water interface.

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