OPTICAL ACTIVITY OF HUMAN SERUM IN THE VISIBLE REGION COMPARED WITH THAT OF THE COMPLEX BILIRUBIN–SERUM ALBUMIN

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1. Introduction

On the basis of recent investigations on the optical activity of the complex formed between bilirubin and serum albumin in aqueous solution* [1–4], it was anticipated that blood serum would also show optical activity in the visible region. It is known that human adult blood normally contains between about 0.2 to 1.4 mg/100 ml of total bilirubin and 4.2 to 5.4 g/100 ml of serum albumin [5, 6]. Bilirubin has been shown to be bound to albumin in blood serum [7, 5]. The present results indicate that the optical activity in the visible region of human serum is indeed due to a large extent to that of the bilirubin bound to serum albumin.

2. Materials and methods

HSA** (crystalline, Control No. 4619) and bilirubin were obtained from Nutritional Biochemicals and were used without further purification (fatty acid content: 2.4 moles per mole protein, determined as quoted by [8]).

Sample of normal adult serum: non-esterified fatty acid content, 319 μequiv. per ℓ (Method: [9]).

CD measurements were carried out on a Cary Model 60 recording spectropolarimeter with a Model 6002 accessory. For experimental details and preparation of the complex bilirubin–HSA, see [2]. The optical density of the serum did not exceed 0.8 (near 460 nm) and that of the complex, 1.35, in the spectral range investigated.

3. Results and discussion

Fig. 1 shows a typical CD spectrum in the region 330 to 560 nm of human undiluted blood serum of about pH 8, taken freshly from a normal individual. These results are compared on a molar bilirubin basis with those obtained previously [2] for a complex of bilirubin with HSA at pH 7.3 (similar results were obtained for the complex at pH 10). The extrema of the three longer wavelengths bands are at 508, 462 and 410 nm, respectively, for the serum as compared to 510–520, 458 and 407 nm, respectively, for the complex. Despite fair agreement of the band positions in both cases, the ellipticity magnitudes differ. Part of this discrepancy can be attributed to the large errors inherent in the analytical methods for determining bilirubin (see [5]). We have observed large variations even in the molar ellipticity values at the band extrema among sera of different individuals and also in a given individual at different times. It is possible that, besides analytical errors, other factors such as variations in fatty acid content or in other molecules bound to the

* Referred to below as "complex".

** *Abbreviations:*
CD : circular dichroism;
HSA: human serum albumin;
RSA: rabbit serum albumin.

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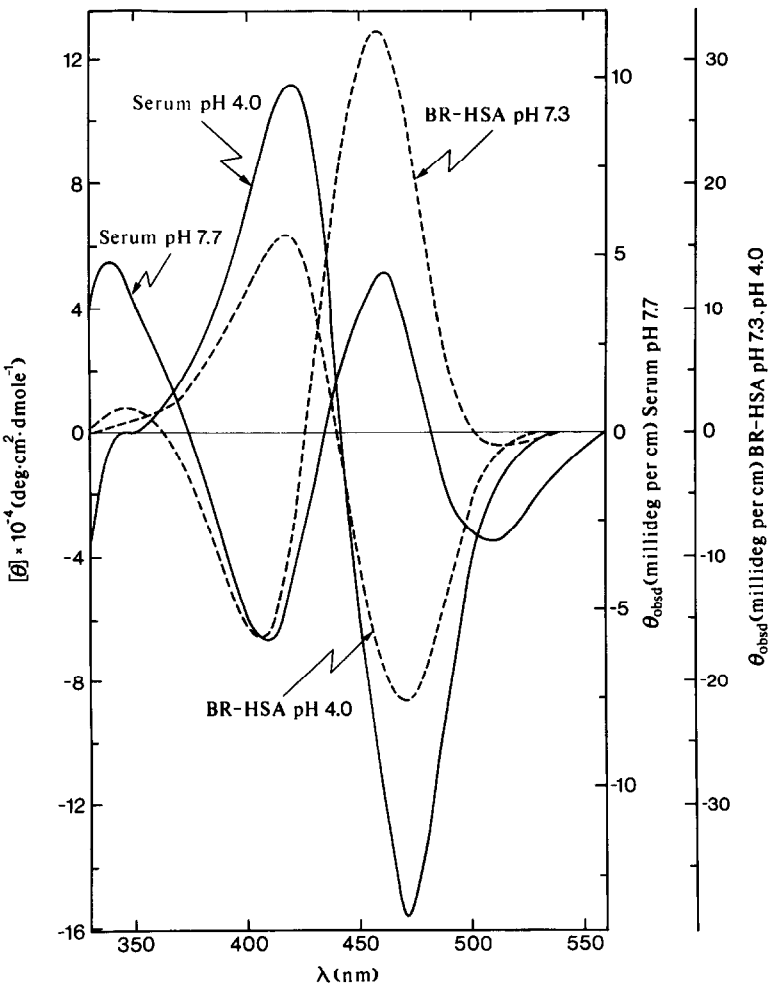

Fig. 1. CD Spectra of a sample of normal human adult serum (female) and of a complex bilirubin–HSA in aqueous solution at different pH values. Temp., 27.0 ± 0.5°C; 1.0 cm cells. The reference solvent was water in all cases except for the complex at pH 4.0, where the protein was used. The CD spectra of the complex were measured within 1 hr from its preparation and the spectra of the serum within several hours from the time of blood drawing. Ellipticity values are corrected by a factor of 1.07 [2]. The molar ellipticity $[\theta]$ is based on (indirect) bilirubin. Serum: Total bilirubin: 9.6×10^{-6} M (Method: [11]). Indirect bilirubin: 8.8×10^{-6} M [11]. Albumin content: 7.1×10^{-4} M (Method: [12]). At pH 4.0, all concentrations should be multiplied by a factor of 0.89 due to pH adjustment. Complex: Bilirubin, 2.5×10^{-5} M; HSA, 12.5×10^{-5} M; NaCl, 0.1 M.

serum albumin, as well as subtle differences in the albumin component are responsible for these quantitative variations observed. It should be noted that significant differences in CD spectra have also been recorded for complexes of bilirubin with differently treated HSA [2]. The present choice of a bilirubin–HSA complex for comparison with the serum (fig. 1) is, therefore, arbitrary to some degree. Similar considerations may apply to the shorter wavelength band (340–350 nm), where the band of the complex is considerably smaller than that of the serum. Certain contributions of hemoglobin Soret Cotton effects, due to some hemolysis during blood drawing and separation of the serum, may also affect the results. Also, the presence of other proteins and substances in the serum may interfere with the binding of bilirubin to

the albumin. It may be noted that the visible-range CD spectra of the serum were considerably more stable with time than the corresponding spectra of the complexes [2].

Further support for the correlation between a large part of the optical activity of the serum and the bilirubin–serum albumin complex is derived from CD data obtained by us for rabbit serum. This serum usually contains relatively low concentrations of bilirubin. Indeed, the two main longer wavelength bands observed for the complex bilirubin–RSA in the range 400–500 nm at pH 7.4 [10] cannot be recognized under the conditions used. Because of extensive hemolysis, typical CD bands of hemoglobin are present.

Additional evidence for a major contribution of the bilirubin–serum albumin complex to the observed dichroic bands in the serum appears from measurements at pH 4. Despite the formation of some turbidity in the serum and a time-dependent decrease in the CD bands of the complex at this pH, which require caution in quantitative evaluation of the optical activity, the similarity between the CD spectra of the serum and those of the complex is again demonstrated: in both cases, the two bands near 470 and 420 nm (472 and 420 nm in the serum), respectively, show an inversion in their sign and the smaller band near 500 nm is absent in both cases (fig. 1).

In view of the observed similarities in the CD spectra between human adult serum and bilirubin–HSA complexes, essentially the same mechanism for generation of a large part of the rotatory power should be operative in both systems: at least in the range 400–500 nm, exciton coupling between electric transition dipole moments of the dipyrromethene chromophores of bound bilirubin was considered to contribute to a considerable extent to the optical activity observed [2]. The inversion in the sign of the dichroic bands observed at lower pH was attributed to a change with pH in the dissymmetric orientation of the transition moments, leading to a change in the sign of the vector product [2, 10]. At the large molar excess of serum albumin over bilirubin normally present, these dipole–dipole interactions are likely to occur within the two halves of a single bound bilirubin molecule. Other contributions to the optical activity should also be considered (see [2]). A more detailed account of the present work and further studies will be published elsewhere.

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