Conversion of Racemic 2-Hydroxymethyl[5]thiaheterohelicene into a Single Enantiomer on the Uptake by Bovine Serum Albumin

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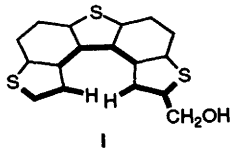
Racemic 2-hydroxymethylthieno[3,2-e:4,5-e']di[1]benzothiophene with a labile helical structure was converted into a (*P*) enantiomer upon uptake by bovine serum albumin, in which the existence of two substrate-binding sites with a distinct ability to recognize chirality was revealed.

Bovine serum albumin (BSA) has recently found wide use as a resolving agent for racemic substrates in both homogeneous and heterogeneous phases,[1] owing to its ability to recognize chirality of enantiomers of a racemic mixture. We describe herein another new function of BSA concerning chirality recognition, converting a racemic substrate into a single enantiomer during the formation of a complex.† Such behaviour of BSA differs explicitly from an ordinary optical resolution of racemates, in which the ratio between enantiomers remains unchanged. The helical shape of 2-hydroxymethylthieno[3,2-e:4,5-e']di[1]benzothiophene (2-hydroxymethyl[5]thiaheterohelicene, **I**) investigated in this study arises from steric repulsion between the terminal hydrogen atoms in the molecule.[4] Inversion of the helix, however, occurs easily in solution, causing so rapid a racemization at room temperature, that **I** cannot be resolved into each enantiomer[5] nor provide a CD absorption spectrum.

When a mixture of an ethanolic solution of **I** (1.0×10^{-4} g, 3.06×10^{-7} mol, 0.1 ml) and an aqueous solution of BSA (2.02×10^{-2} g, 3.06×10^{-7} mol, 5 ml) was diluted to 10 ml with water and incubated by stirring mildly for *ca.* 30 min at 25 ± 1 °C, a clear solution was obtained. The solution exhibited intense CD absorptions which are assigned to the (*P*) enantiomer of **I**, by comparison with the UV absorptions of **I** and the CD absorptions of (*P*)-[7]thiaheterohelicene ((*P*)-[7]TH),‡ a higher homologue of **I**,[6] (Fig. 1). **I** is practically insoluble in water and slightly soluble (<1.7×10^{-7} mol dm^{-3}) in 1% ethanol–water.

However, in the presence of two equivalents of BSA (1.53×10^{-5} mol dm^{-3}) **I** can be dissolved in 1% ethanol–water. When a mixture containing BAS and **I** (1 : 5) was stirred for 2 days, uptake of **I** did not exceed 2 mole equivalents. When the solution of the I-BSA complex was extracted with an equal volume of diethyl ether at room temperature, **I** was quantitatively recovered in the ethereal phase and gave no CD absorptions. Addition of **I** to the extracted aqueous solution of BSA gave the same CD absorption peaks at the same wavelengths. These facts suggest that the uptake of **I** by BSA is completely reversible and the interaction between both components seems to be too weak to withstand extraction by ether, but is strong enough to prevent **I** undergoing inversion.

The intensity of the CD absorptions of the I-BSA solution increased linearly with an increase of the molar ratio **I**:BSA up to 2 : 1, the slope of the linear plots, however, differed in the ranges of 0–1 : 1 and 1–2 : 1 (Fig. 2). This difference implies that BSA possesses two uptake sites (1 and 2) with different abilities to discriminate between the chiralities of the enantiomers of **I**, in agreement with the existence of the two substrate-binding domains in human serum albumin (HSA) as determined by X-ray crystallography.[7] Furthermore, when BSA was dissolved in an aqueous solution of **I**:BSA (2 : 1) leading to a 1 : 1 mixture the apparent molecular ellipticity of each CD absorption band was enhanced by *ca.* 16%. It was



I

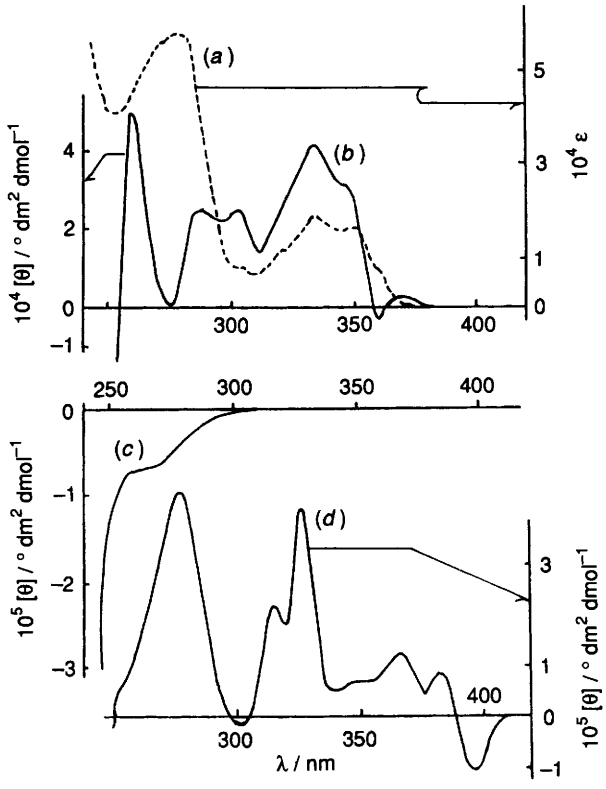


Fig. 1 UV (*a*) and CD (*b*) spectra of an aqueous **I**–BSA solution, CD spectrum of aqueous BSA solution (*c*) and of (*P*)-[7]TH in $CHCl_3$ (*d*); [**I**] = [BSA] = 1.68×10^{-5} mol dm^{-3} (*a*), [**I**] = [BSA] = 3.06×10^{-5} mol dm^{-3} (*b*), [BSA] = 1.68×10^{-5} mol dm^{-3} (*c*) and [(*P*)-[7]TH] = 8.34×10^{-5} mol dm^{-3} (*d*)

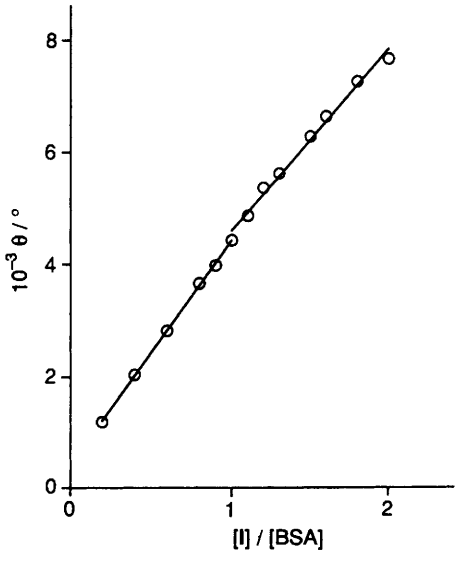


Fig. 2 Dependence of the observed ellipticities (θ) on the molar ratio of **I**:BSA {[BSA] = 3.06×10^{-5} mol dm^{-3}}. Values of θ were obtained from θ (peak at 334 nm) − θ (trough at 311 nm).

ascertained that further addition of BSA to the solution containing only BSA resulted in almost no change in the longer wavelength region measured for the I-BSA complex. In a 2:1 solution of I–BSA, both sites 1 (provisionally with a higher ability for chiral discrimination) and 2 (with a relatively lower ability) are anticipated to be coordinated by **I**. Further addition of BSA then compels **I** at site 2 to migrate to unoccupied site 1 raising the CD intensities in accord with the difference of abilities in discriminating chirality. We can deduce that site 1, with a higher discrimination ability, also possesses a higher affinity towards the substrate than site 2 and hence uptake of **I** occurs predominantly at site 1 up to a **I** BSA ratio of 1:1, with subsequent uptake at site 2.

The temperature-dependence of the CD absorptions was examined in oder to evaluate the lability of each component. Upon increasing the temperature, the CD absorptions were significantly reduced for solutions of I–BSA at 1:1 and 1:2 ratios (Fig. 3), in contrast to only a small temperature dependence for the CD absorptions of (*P*)-[7]TH in CH_2ClCH_2Cl. This observation can be explained by the configurational fixation of **I** being weakened by increased thermal motions of BSA with increase in temperature, resulting in the displacement of the equilibrium BSA–(*P*)-**I** ⇌ BSA–(*M*)-**I** to the right. Therefore, while the alteration in CD-absorptional intensities of the **I**:BSA (1:1) solution reaches a plateau below *ca.* 10 °C, a decrease in the apparent ellipticity above 10 °C is assumed to arise from the generation of BSA–(*M*)-**I**. Ellipticity for species at site 1, $[\theta]_{site\ 1}$, obtained for I-BSA (1:1) solutions can lead to an estimation of $[\theta]_{site\ 2}$ through the relation $[\theta]_{site\ 2} = [\theta]_{site\ (1+2)} - [\theta]_{site\ 1}$, where $[\theta]_{site\ (1+2)}$ is the apparent ellipticity measured for I-BSA(1:2) solutions at the same BSA concentration. In this manner the population of each diastereomeric complex can be evaluated for the two sites, respectively, to provide equilibrium constants at each temperature. The equilibrium constants thus obtained were plotted according to the van't Hoff equation, and good straight lines were obtained to give ΔH 70.3 and 28.4 kJ mol^{-1} for sites 1 and 2, respectively, for the bonding interactions between the *M* and *P* forms of **I**. These ΔH values imply differing binding strengths for the BSA–(*P*)–**I** and BSA–(*M*)–**I** species, and thus explain the difference in ability to recognize the chirality of **I** at sites 1 and 2. The larger ΔH value at site 1 is in reasonable accordance with the observed behaviour (Fig. 2).

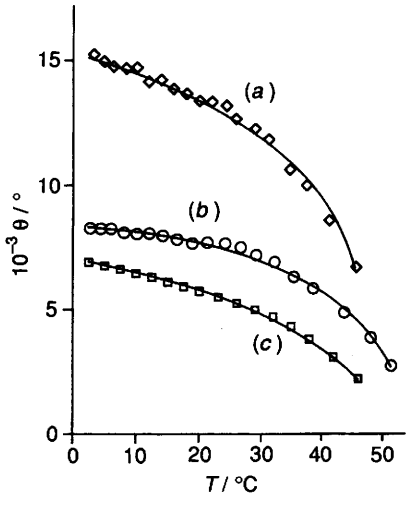


Fig. 3 Temperature dependence of the observed ellipticities (θ) with [BSA] = 3.06 × 10^{-5} mol dm^{-3}: (*a*) θ_a for **I**:BSA = 2:1, (*b*) θ_b for **I**:BSA = 1:1, (*c*) calculated curve from ($\theta_a - \theta_b$) at the respective temperatures. Values of θ were obtained from θ (peak at 334 nm) − θ (trough at 311 nm).

Similar experiments were performed using chicken egg albumin (CEA) and HSA. CEA showed no ability for the uptake of **I**, whereas HSA showed both uptake and chiral recognition towards **I**, although the latter was not as effective as found for BSA. The results described herein indicate that **I** may act as an effective probe for measuring the extent of chiral recognition of several proteins and biochemical polymers.

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Footnotes

† Recently, the following studies on albumin concerning chirality conversion have been reported: the chirality of racemic 1,1′-bi-2-naphthol complexed to BSA was partially biased upon UV irradiation[2] and the sign of the CD absorption of an aqueous bilirubin–HSA solution was inverted in the presence of a drop of chloroform.[3]

‡ IUPAC name: Bisthieno[3′,2′:4,5]benzo[1,2-b:4,3-b′]di[1]benzothiophene.

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