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Highly Enantiomeric Supramolecular [4 + 4] Photocyclodimerization of 2-Anthracenecarboxylate Mediated by Human Serum Albumin

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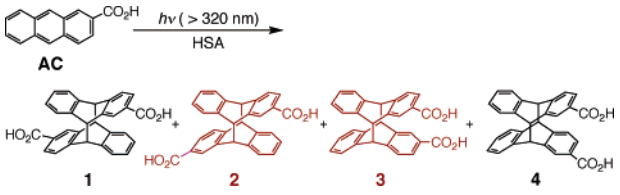
A supramolecular approach to chiral photochemistry has become more popular,[1] and a variety of natural and synthetic chiral hosts have hitherto been employed, including cyclodextrins,[2] modified zeolites,[3] DNA,[4] hydrogen-bonding templates,[5] and nanoporous materials.[6] Nevertheless, photochirogenesis with biomolecules is still one of the most challenging topics. This is due to the ambivalent nature of biomolecules that possess inherently chiral, finely defined 3D structures on the one hand but behave as multicomponent, multisite binders to exogenous guests on the other.

We have recently found that bovine serum albumin (BSA) efficiently binds multiple 2-anthracenecarboxylate (AC) molecules, providing good photochirogenic environments for photocyclodimerization to give *syn* head-to-tail (HT) dimer **2** in 29% ee and *anti* head-to-head (HH) dimer **3** in 41% ee.[7] The performance of BSA as a photochirogenic host is advantageous compared to cyclodextrin (CDx) because the ee's for both products are of the same magnitude. For CDx's, the ee of one product is enhanced (40−60%) while the ee for the other product is low (<15%),[8−10] revealing an apparent "tradeoff". Despite the moderate ee's obtained with BSA,[7] the use of proteins in supramolecular photochirogenesis is promising in view of the concomitant enhancement of the ee of *both* chiral products. In the present study, we employed human serum albumin (HSA), which differs from BSA in 26 amino acid residues[11] and exhibits significant differences in drug binding, photophysical, and/or photochemical behavior.[11−15] In addition, in contrast to BSA, the X-ray crystallographic structures are known for HSA and its complexes with various drugs.[16,17] We show that with HSA an excellent ee (80−90%) is achieved for both **2** and **3**.



We first examined the binding behavior of AC to HSA by using circular dichroism (CD) spectroscopy. Addition of AC (0−1.2 mM) to a phosphate buffer solution (pH 7.0) of HSA (0.06 mM) induced Cotton effect peaks in the AC absorption region (300−450 nm), indicating complexation of AC to HSA binding sites. The changes in intensity and shape of the CD spectra depend on the AC/HSA ratios, indicating the presence of multiple binding sites (Figure 1a,b). The ellipticities at 330, 390, and 420 nm were plotted against the AC/HSA ratio (Figure 1c). The profiles at the three wavelengths differ dramatically, reflecting the nature of the chiral environment

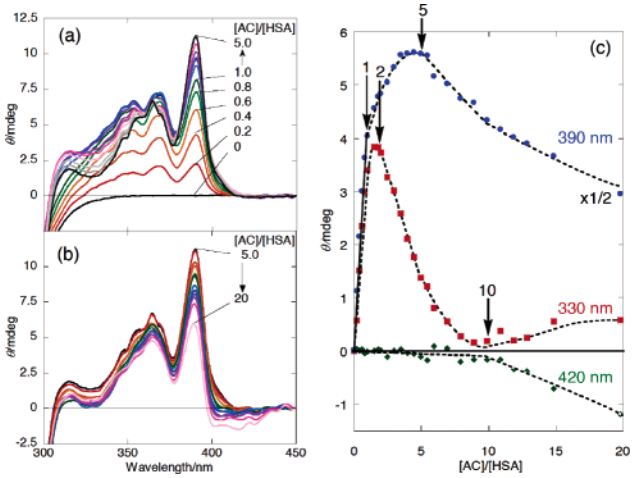


Figure 1. CD spectral changes upon addition of AC to a buffer solution of HSA (0.06 mM) at 25 °C: (a) [AC] = 0−0.3 mM; (b) [AC] = 0.3−1.2 mM; (c) ellipticities at 330, 390, and 420 nm versus AC/HSA ratio.

and chemical species involved, which enabled us to estimate the number and stoichiometry of the AC binding sites. Four inflection/turning points are readily identified at AC/HSA ratios of 1, 2, 5, and 10, showing that the stoichiometries of sites 1−4 (in the order of decreasing affinity) are 1, 1, 3, and 5. Weak binding occurs at higher AC/HSA ratios (site 5) as indicated by the CD signals at 330 and 420 nm. The striking difference observed upon CD spectral titration with HSA and BSA (Supporting Information) may rationalize the significantly different photochirogenic behavior described below.

Fluorescence spectral titration of AC (0.28 μM) with HSA (0−1.42 μM) and the subsequent nonlinear least-squares analysis, assuming the 1:1 stoichiometry, allowed us to determine a binding constant of the first site of 3.0×10^8 M^{-1} (Supporting Information), which is appreciably larger than the equivalent value for BSA (5.3×10^7 M^{-1}).[7] To elucidate the nature of each binding site, fluorescence experiments were performed by adding HSA (0−1.2 mM) to a buffer solution of AC (0.6 mM) at 25 °C (Supporting Information). Upon addition of HSA of up to 0.06 mM (AC/HSA = 10), the broad, structureless fluorescence of AC was reduced in intensity without any appreciable change in shape, but further addition of HSA (AC/HSA = 2) caused changes in shape with a continued intensity decrease to give a sharp fluorescence spectrum with vibrational fine structure. This sharp spectrum is characteristic of AC emission in nonpolar solvents and was observed for AC bound to site 1 of BSA.[7] At yet higher HSA concentrations of 0.3−1.2 mM (AC/HSA = 2−0.5), the fluorescence intensities increased while the shape of the spectra remained sharp. These results suggest that the first and second sites are highly hydrophobic, while the

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Table 1. Photocyclodimerization of AC in the Absence/Presence of HSA in Aqueous Buffer Solution at pH 7[a]

			relative yield/%[c]				% ee[d]		
AC/HSA	temp/°C	conv/%[b]	**1**	**2**	**3**	**4**	**2**	**3**	HT/HH[e]
∞[f]	25	88	43	36	14	7	0	0	3.7
25	25	72	42	38	12	8	50	14	4.0
15	25	67	42	39	11	8	58	22	4.3
10	25	59	49	36	7	8	61	33	5.7
8	25	55	43	41	8	8	59	28	5.3
5	25	33	44	42	9	5	59	42	6.1
3	5	13	42	45	8	5	82	90	6.7
	25	20	42	42	11	6	79	88	4.9
	50	*g*	44	37	11	8	67	89	4.3
2	25	<5							
1	25	<5							

[a] Irradiated at $\lambda > 320$ nm for 1 h under Ar; [AC] = 0.60 mM. [b] Consumed AC; error <5%. [c] Product distribution; error <2%. [d] Enantiomeric excess; error <5%; the positive/negative sign indicates an excess of the first/second-eluted enantiomer, respectively. [e] HT/HH = ([**1**] + [**2**])/([**3**] + [**4**]). [f] No HSA added. [g] Not determined.

third one is less hydrophobic, and the fourth and fifth sites are hydrophilic and spectrally indistinguishable from water.

Photoirradiation of AC (0.6 mM) was carried out at $\lambda > 320$ nm in the presence of HSA (0−0.6 mM) at 25 °C. In a selected case, product studies were done at 5 and 50 °C (Table 1).

The conversion of AC decreased from 88% in the absence of HSA to 20% at AC/HSA = 3 and finally to <5% at AC/HSA = 1 and 2, indicating that progressive complexation with HSA decelerates external and/or intrasite attack of AC due to the steric hindrance and lower mobility within the sites. The lack of conversion at AC/HSA ratios of 1 and 2 shows that the first and second sites, each binding a single AC molecule, are nonproductive due to the limited access from the bulk solution.

The HT/HH ratio was high at 4−6 throughout the AC/HSA ratios studied, which is completely different from the BSA-mediated case,[7] where the HT/HH ratio decreases from 3.7 (without BSA) to 0.23 (at AC/BSA = 1.3). Such contrasting behavior suggests that the relative orientations of the ACs bound to HSA are substantially different from those in BSA.

The ee's of **2** and **3** significantly increased with increasing HSA concentration to reach the maximum values of 79 and 88% at AC/HSA = 3, both of which are unprecedentedly high for an enantiodifferentiating supramolecular photochirogenesis. The origin for the strikingly higher ee with HSA when compared to that of BSA is related to differences in the AC binding motifs, where opposite induced CD signals were observed for AC/albumin ratios above 3 (Supporting Information). Interestingly, the ee profiles differ for **2** and **3**, indicating that each HSA site produces **2** and **3** in significantly different ee's. Despite the lack of quantitative equilibrium constant values, one can readily understand from Table 1 that the third binding site provides an excellent photochirogenic environment for both **2** and **3**, while the fourth and fifth sites are less effective. In particular, the sudden drop of ee at AC/HSA = 5 suggests the formation of antipodal **2** and **3** at the fourth site. It is somewhat unusual that the ee of **2** stays constant at 58−61% over the AC/HSA range of 5−15 and is only slightly decreased to 50% even at AC/HSA = 25. In contrast, the ee of **3** rapidly fades out from 42 to 14% in the same AC/HSA range. This contrasting behavior for **2** and **3** may be explained by assuming that the fifth site gives **2** in a better ee than the fourth site, and hence the contribution of racemic **2** produced in the bulk water is cancelled to some extent even at AC/HSA = 25, whereas the ee of **3** produced in the fifth site is lower than that formed from the fourth site and is not able to cancel the contribution of racemic **3** from the bulk water.

The temperature effect on the product ratio and ee was examined for the first time for the biomolecular photochirogenesis. CD spectral inspection of HSA at 5−50 °C did not show any sign of serious conformational changes or denaturation (Supporting Information). Upon irradiation of AC (0.6 mM) with HSA (0.2 mM) at 5−50 °C, the sterically less-hindered HT dimers were favored at lower temperatures to give the highest HT/HH ratio of 6.7 at 5 °C (Table 1). The temperature effect on ee was modest for **2** and negligible for **3**; the highest ee's of 82 and 90% were achieved at 5 °C for both **2** and **3**.

We have shown that the supramolecular photochirogenesis with biomolecules, particularly, HSA, is a mechanistically interesting and synthetically promising strategy to photochemically transfer the microenvironmental chirality to a prochiral substrate through supramolecular interactions. Further work to elucidate the binding and enantiodifferentiation mechanisms through point mutation of HSA and to expand the scope of supramolecular photochirogenesis is in progress.

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Supporting Information Available: CD and Job plots, determination of the binding constant, fluorescence spectra of AC with HSA, CD spectra of HSA at 5−70 °C, and CD spectra comparing AC binding to HSA and BSA. This material is available free of charge via the Internet at http://pubs.acs.org.

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