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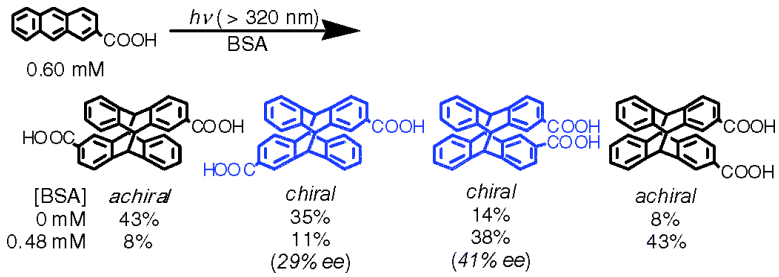


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# Bovine Serum Albumin-Mediated Enantiodifferentiating Photocyclodimerization of 2-Anthracenecarboxylate

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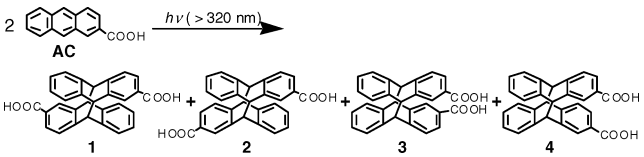
Asymmetric photochemistry provides us with new versatile routes to enantiomerically enriched novel/strained compounds.[1] Recently, much effort has been devoted to supramolecular photochirogenesis, exploiting the chiral environment of natural and synthetic hosts, such as cyclodextrin,[2] modified zeolite,[3] DNA,[4] hydrogen-bonding template,[5] chiral crystal lattice,[6] or host.[7] Supramolecular photochirogenesis with biomolecules is particularly attractive and advantageous in view of their inherently chiral and well-defined 3D structures.

Bovine serum albumin (BSA) binds endogenous as well as exogenous substrates in its hydrophobic pockets.[8] Zandomeneghi et al. demonstrated that racemic 1,1′-binaphthol and ketoprofen are photodecomposed in high enantiomeric excess (ee) in the presence of BSA.[9] This is a clever strategy for effecting the asymmetric photodestruction by selectively exciting the red-shifted band of the BSA-bound substrate enantiomer.

Here we propose a more dynamic supramolecular photochirogenesis using BSA, in which stereogenic centers are *created*, employing 2-anthracenecarboxylate (AC) as a prochiral substrate. Photoirradiation of AC affords four [4 + 4] cyclodimers, *anti*- and *syn-head-to-tail* (HT) (**1** and **2**) and *anti*- and *syn-head-to-head* (HH) dimers (**3** and **4**), of which only **2** and **3** are chiral.[10] In their pioneering work,[11] Tamaki et al. demonstrated that photocyclodimerization of AC in the presence of $\gamma$-cyclodextrin affords optically active cyclodimers, although the ee was not determined at that time. In our recent study, we obtained **2** in up to 41% ee and **3** in very low ee of <5%.[12]




In this study to expand the range of supramolecular photochirogenesis with BSA (from uni- to bimolecular reaction and from photodestruction to photochirogenesis) and to elucidate the factors and mechanisms governing the product ratio and ee in BSA-mediated photochirogenesis, we examined the complexation behavior of AC by BSA, and subsequently performed enantiodifferentiating photocyclodimerization.

The supramolecular interaction of AC with BSA was quantitatively examined by circular dichroism (CD) spectral titration. Gradual additions of AC (0−0.75 mM) to a phosphate buffer solution (pH 7) of BSA (0.075 mM) gave well-structured induced

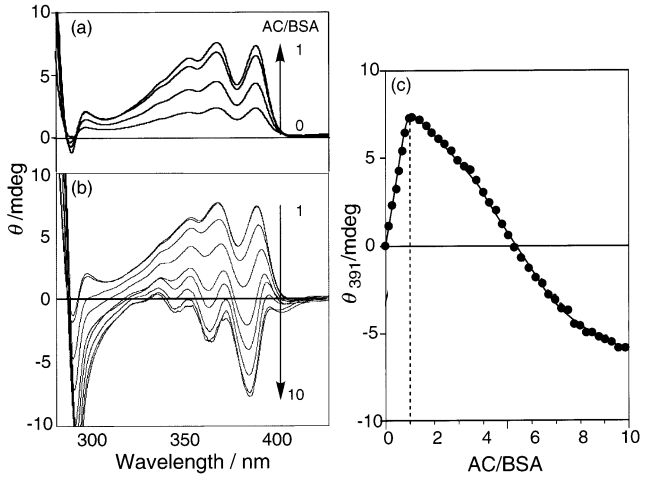



***Figure 1.*** CD spectral change upon addition of AC to a phosphate buffer solution (pH = 7) of BSA (0.08 mM) at 25 °C; (a) [AC] = 0 (baseline), 0.02, 0.04, 0.06, 0.08 mM (from bottom to top); (b) [AC] = 0.08, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, and 0.8 mM (from top to bottom); (c) CD intensity at 391 nm as a function of AC/BSA ratio and curve-fitting analyses.

CD (ICD) bands at 330−400 nm, which exactly correspond to the UV spectral peaks of AC. Interestingly, the CD spectral behavior was a critical function of AC/BSA ratio, with the sign of ICD inverted at high ratios, clearly indicating the operation of multiple binding modes. Positive Cotton effect peaks evolve, and their intensities rapidly grow with increasing AC/BSA ratios from zero to unity (Figure 1a). However, further addition of AC causes a gradual decrease of ICD intensity with increasing AC/BSA ratios, eventually giving negative ICD spectra (Figure 1b). Figure 1c illustrates the ellipticity change at 391 nm versus the AC/BSA ratio. Further detailed Job-plot and curve-fitting analyses of the CD, UV−vis, and fluorescence titration data revealed that there are four independent binding sites for AC in BSA, which bind 1, 3, 2, and 3 AC molecules with binding constants of $5.3 \times 10^7$, $1.3 \times 10^5$, $1.4 \times 10^4$, and $3.0 \times 10^3$ $M^{-1}$, respectively.[13] The exclusive binding of one AC by the first site justifies the monotonic increase of ICD up to AC/BSA = 1. Notably, the subsequent multiple AC bindings cause no appreciable exciton coupling (Figures 1b), indicating that these ACs, even if bound to the same binding site, are not very closely located to each other.

Phosphate buffer solutions (pH 7), containing AC (0.6 mM) and varying amounts of BSA (0−0.6 mM), were irradiated under identical conditions to give the HT (**1** and **2**) and HH dimers (**3** and **4**). The results are listed in Table 1, along with the initial populations of AC prior to irradiation, which are calculated by using the binding constants determined above.

The presence of BSA, particularly at high concentrations, appreciably reduces the photodimerization rate. Thus, the conversion

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***Table 1.*** Enantiodifferentiating Photocyclodimerization of AC in the Presence/Absence of BSA in Aqueous Buffer Solution (pH 7) at 25 °C[a]

| | | % population of AC[b] | | | | | | | % distribution of product (% *ee*)[d] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| [BSA]/mM | AC/BSA | 1st site | 2nd site | 3rd site | 4th site | free | bound/free[c] | % conversion | **1** | **2** | **3** | **4** | HH/HT[e] |
| 0.00 | *f* | 0 | 0 | 0 | 0 | 100 | 0 | 88 | 43 | 35 (*0*) | 14 (*0*) | 8 | 0.28 |
| 0.03 | 20 | 5 | 15 | 8 | 7 | 65 | 0.5 | 73 | 29 | 25 (−*11*) | 26 (*14*) | 20 | 0.85 |
| 0.06 | 10 | 10 | 29 | 15 | 9 | 37 | 1.4 | 64 | 29 | 21 (−*13*) | 29 (*34*) | 21 | 1.0 |
| 0.12 | 5 | 20 | 53 | 15 | 3 | 9 | 7.9 | 55 | 21 | 17 (−*18*) | 35 (*38*) | 27 | 1.6 |
| 0.15 | 4 | 25 | 58 | 11 | 1 | 5 | 14 | 48 | 17 | 15 (−*22*) | 38 (*39*) | 30 | 2.1 |
| 0.48 | 1.3 | 78 | 22 | 0.2 | <0.1 | 0.1 | 220 | 5 | 8 | 11 (−*29*) | 38 (*41*) | 43 | 4.3 |
| 0.60 | 1.0 | 91 | 9 | <0.1 | <0.1 | <0.1 | | < 1 | | | | | |

[a] Irradiated at >320 nm for 1 h under Ar; [AC] = 0.60 mM. [b] Population of AC among the first to fourth sites and the bulk solution, calculated from the binding constants. [c] Molar ratio of AC bound to second, third, and fourth sites against free AC. [d] Enantiomeric excess determined by chiral HPLC; error <3% ee; the positive/negative ee sign corresponds to the excess of the first/second-eluted enantiomer, respectively. [e] [**3** + **4**]/[**1** + **2**]. [f] Not applicable.

of AC decreases moderately from 88% in the absence of BSA to 48% at AC/BSA = 4, then rapidly to 5% at AC/BSA = 1.3, and finally to <1% at AC/BSA = 1. The extremely low conversions (0−5%) at AC/BSA = 1−1.3, where all ACs are populated in the first and second sites, reveal the significant contribution of free AC to the photodimerization. The complete suppression of the photoreaction at AC/BSA = 1 indicates that the single AC tightly bound to the first site is photochemically inactive, probably due to the steric shielding or efficient quenching by electron-donating aromatic amino acid residues such as tryptophan and tyrosine.

The HH/HT ratio shows a rapid increase from 0.28 in the absence of BSA up to 4.3 at AC/BSA = 1.3, accompanying a dramatic inversion of regioselectivity from 78% HT to 81% HH. This change is nicely linked to the rapid decrease of free AC, giving resembling profiles for the HH/HT ratio and the "bound/free" AC ratio (unreactive AC at the first site not taken into account). This clearly indicates that the HH dimers arise from the "intra-site" and "inter-site" reactions at the second, and probably the third and fourth sites, at which the majority of AC (neglecting those at the first site) is populated, particularly at higher [BSA]. Probably, the electrostatic repulsion between negatively charged ACs, discouraging the HH dimerization in the bulk solution, is canceled, and instead attractive interactions are induced between the bound ACs and the cationic amino acid residues of BSA, which accelerates the intra- or inter-site attack, giving the HH dimers.

The ee's of **2** and **3** are also dynamic functions of [BSA], exhibiting completely different profiles (Table 1); the ee of **2** steadily increases with increasing [BSA] to reach 29% at AC/BSA = 1.3, while that of **3** shows a rapid growth to 34% at AC/BSA = 10 with subsequent moderate increases to reach a plateau of 38−41% at AC/BSA = 5−1.3.

The optically active dimers are produced either through the intra- or inter-site reaction of two BSA-bound ACs or by the external attack of free AC to a bound one. The steadily increasing ee profile for **2** indicates that at least one bound AC is involved in its formation. However, the yield of **2**, as a product of conversion and distribution, decreases with lowering $[AC]_{free}$, indicating that the external attack of free AC also participates at least in part in its formation, although a considerable amount of **2** is produced from the intra/inter-site attack. The relative importance of the intra/inter-site attack increases with increasing population of AC at the second site, and with decreasing contribution from the inherently racemic, but unavoidable, photodimerization of free AC. On the other hand, the almost constant ee's of **3** (38−41%) at AC/BSA = 5−1.3 nicely coincide with the predominant population of AC at the second site, while the lower ee's at higher AC/BSA ratios are attributable to the less or nonenantiodifferentiating photodimerization of ACs at the third and fourth sites and in the bulk solution. Hence, the best ee's of 29% and 41% obtained for both **2** and **3** are from the intra/inter-site attack between ACs bound to the second site.

In this first supramolecular photochirogenesis with an achiral substrate, we showed: (1) BSA possesses four discrete binding sites for AC of different affinity, stoichiometry, and chiral environment for photochirogenesis, (2) BSA-mediated photodimerization of AC switches the regioselectivity from HT to HH, and (3) affords optically active **2** and **3** in 22% and 39% ee (48% conversion) and eventually 29% and 41% ee (5% conversion). It is emphasized that the selective excitation of bound substrate, utilizing the bathochromic shift upon BSA complexation, is not a prerequisite for efficient photochirogenesis using biomolecules. This conclusion is highly encouraging in expanding the range of substrates and the scope of supramolecular photochirogenesis with biomolecules.

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**Supporting Information Available:** Experimental procedures and detailed analysis of the binding behavior of AC with BSA (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) For the details of the analyses, see Supporting Information. The existence of multiple binding sites for exogenous substrates is well known (ref 8).

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