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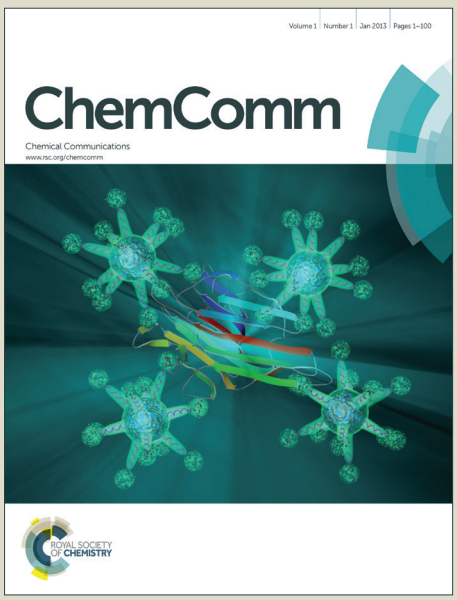





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# Mammalian serum albumins as a chiral mediator library for bio-supramolecular photochirogenesis: Optimizing enantiodifferentiating photocyclodimerization of 2-anthracencecarboxylate

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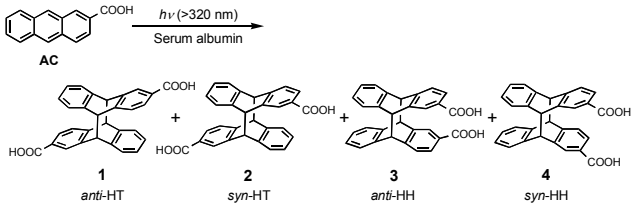
**A simple strategy for choosing optimal bio-supramolecular mediators from the mammalian serum albumin library is proposed for bimolecular photochirogenic reaction. Thus, the enantiodifferentiating photocyclodimerization of 2-anthracencecarboxylate (AC) was optimized in chemical and optical yields, when mediated by porcine and canine serum albumins, both of which bound two AC molecules in the first productive site to give the (*P*)-enantiomer of *syn-head-to-tail*-cyclodimer in 69% yield and 89% enantiomeric excess (*ee*) for the former but the (*M*)-enantiomer in 77% yield and 97% *ee* for the latter.**

Supramolecular photochirogenesis mediated by chiral biomolecular host is one of the most intriguing, but less investigated, areas in current photochemistry.[1] Combining photochemistry with biomolecules is attractive as an environmentally benign yet potentially highly efficient access to optically active molecules, enabling us to utilize their inherently chiral binding sites without using metal catalyst or high temperature that could denature or damage the original structures.[1b,f,g] Indeed, recent exploratory studies have revealed that such biomolecules that possess inherently chiral binding site(s), such as DNA,[2] cyclic oligosaccharides[3-5] and proteins,[6-11] are highly promising supramolecular mediators for uni- and bimolecular photochirogenic reactions. Using biomolecular hosts in supramolecular photochirogenesis however accompanies some inherent complicatedness in mechanism elucidation to better understand the origin of enantioselectivity and also in host design and modification to improve the photochirogenic performance, which is in contrast to the supramolecular photochirogenesis mediated by simple chiral hosts such as hydrogen-bonding template.[12-15]

Serum albumin, the most abundant plasma protein that possesses well organized, inherently chiral hydrophobic binding site(s) for organic molecules,[16] has been used as a mediator for photochirogenic transformations. Zandomeneghi *et al*. employed bovine serum albumin (BSA) as a mediator for photochemical kinetic resolution of *rac*-1,1'-binaphthol[6] in (unbuffered) water to retrieve the (*R*)-enantiomer in 99.5% enantiomeric excess (ee) at 77% conversion, which turned out to be less efficient in a phosphate buffer at pH 7, affording 98% *ee* only upon 99% conversion.[7] Recently, Yokoyama *et al.* examined the enantiodifferentiating photocyclization of photochromic diarylethenes mediated by human serum albumin (HSA) to obtain the cyclization products in up to 71% *ee*.[9]

We also investigated the photocyclodimerization of 2-anthracenecarboxylate (AC) (Scheme 1) mediated by BSA to obtain chiral *syn-head-to-tail* (*syn*-HT) dimer (**2**) and *anti-head-to-head* (*anti*-HH) dimer (**3**) in 29% and 41% *ee*, respectively, along with achiral *anti*-HT dimer (**1**) and *syn*-HH dimer (**4**) (Scheme 1).[8a] Interestingly, the use of HSA as a mediator greatly improved the enantioselectivities for both **2** and **3** to 82% and 90% *ee*, respectively.[8b] However, HSA differs significantly from BSA not only in photochirogenic performance but also in substrate binding behavior, despite the relatively high homology of the amino acid sequence (75.6%,[17] see Table S1 in ESI†). Thus, the high enantioselectivities reported are attractive and promising, but the lack of clear criteria or the difficulty to find the right albumin for mediating specific photochirogenic reaction deters photo-, bio-, organic and supramolecular chemists from exploiting albumins as bio-supramolecular mediators.

In this study, we wanted to establish the reliable criterion and practical protocol for choosing photochirogenically most effective one(s) from a pool of mammalian serum albumins and also to optimize both the chemical and optical yields, by employing the enantiodifferentiating photocyclodimerization of AC as a benchmark photochirogenic system and BSA, HSA, SSA (sheep), RSA (rabbit), PSA (porcine) and CSA (canine) as bio-supramolecular mediators.




**Scheme 1.** Enantiodifferentiating photocyclodimerization of 2-anthracenecarboxylate (AC) mediated by mammalian serum albumins.

The AC-binding behavior of BSA was examined by fluorescence and circular dichroism (CD) spectral titrations and Job analyses to show the existence of four independent AC-binding sites that accommodate 1, 3, 2 and 3 AC molecules in the order of decreasing affinity (Table 1). The

affinity of AC to the first (strongest) binding site was independently determined as $K_1$ = 5.3 x $10^7$ $M^{-1}$ by fluorometric titration at μM concentrations,[8a] where the binding of AC to the weaker (higher order) sites was negligible. Similar examinations with HSA revealed the existence of four binding sites, which accommodate 1, 1, 3 and 5 ACs (Fig. 1), and the $K_1$ value was determined as 3.0 x $10^8$ $M^{-1}$ by the fluorometric titration.[8b] Further binding study with mutant HSAs revealed the essential role of Arg410, which is preserved throughout the mammalian SAs,[8g,16g] upon complexation of AC in the first site.

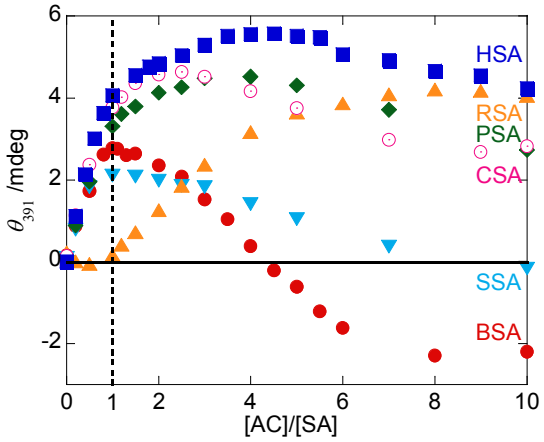



**Fig. 1** Ellipticity changes at 391 nm ($\theta_{391}$) induced upon addition of 1-10 equivalents of AC to a 60 μM solution of BSA (bovine), HSA (human), SSA (sheep), RSA (rabbit), PSA (porcine) and CSA (canine) in phosphate buffer (pH 7.0) at 25 °C; see Figs. S1-S7 in ESI† for the plots at higher AC/SA ratios.

In the present study, essentially the same techniques were used to determine the $K_1$ value and the number and stoichiometries of AC-binding sites for the newly employed SSA, RSA, PSA and CSA. Thus, the gradual addition of AC to an aqueous solution of serum albumin (SA) induced CD signals to the $^1L_a$ and $^1L_b$ bands of AC (*ca.* 320-400 nm), ellipticity ($\theta$) of which was plotted against the AC/SA ratio to give the titration profile of $\theta$ monitored at 391 nm (Fig. 1) (for those monitored at 330, 360 and 420 nm, see Figs. S1-S8 in ESI†). For all of the examined SAs (including BSA[8a] and HSA[8b]), a clear inflection point was observed at AC/SA = 1, indicating the 1:1 stoichiometry for the first binding site; note that the first binding site of RSA did not show any appreciable CD upon addition of AC, which however does not mean the lack of binding in this region since the AC fluorescence was efficiently quenched upon addition of RSA to give $K_1$ = 3.4 x $10^7$ $M^{-1}$ (for the fluorescence titration data and the binding constants, see Figs. S9-S12 and Table S2 in ESI†). The strongest AC-binding site of HSA, which was assigned to the Sudlow's drug site II by the inhibition experiment with warfarin,[8b] appears to be well preserved throughout the mammalian SAs. However, the subsequent AC-binding profiles significantly differed from SA to SA, exhibiting additional inflections at higher AC/SA ratios (see Figs. S1-S8, which more clearly reveal some of the inflection points). The binding stoichiometries of SAs thus obtained are summarized in Table 1 (left columns). In comparison to BSA and HSA, SSA, RSA, PSA and CSA possess smaller numbers of AC-binding sites.

From the photochemical point of view, the AC molecule in the first site, which is strongly bound and isolated by the hydrophobic walls of the binding pocket from the other ACs in higher binding sites or in the bulk solution, is difficult to encounter and react with one of them in its excited-state lifetime.[8] This means that the unimolecular binding site does not contribute to the photocyclodimerization (but may function as a reservoir), while the higher binding sites that accommodate multiple AC molecules are "productive," allowing intra-site attack of an excited AC to other AC.[8] Thus, the first productive site is the second strongest AC-binding site for most SAs but the third one for HSA.

Photoirradiation of AC (0.6 mM) was performed at >320 nm in a phosphate buffer at pH 7 in the presence and absence of SA. The initial AC/SA ratio was set low at 1.3 or 3.0 (for HSA and CSA) to keep AC being populated only to the first productive site[8a] (in addition to the non-productive

**Table 1.** Binding stoichiometry of 2-anthracenecarboxylate (AC) at each site of mammalian serum albumin (SA) and the results of enantiodifferentiating photocyclodimerization of AC mediated by SA[a]

| SA | complexation stoichiometry (AC/site) | | | | AC/SA | tempera-ture/°C | conver-sion[b]/% | photoreaction yield[c]/% (*ee*[d]/%) | | | | *syn/anti* | | HT/HH |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1st | 2nd | 3rd | 4th | | | | **1** | **2** | **3** | **4** | **2/1** | **4/3** | |
| none | | | | | | 25 | 80 | 43 | 36 (*0*) | 14 (*0*) | 7 | 0.8 | 0.5 | 3.8 |
| bovine | 1 | 3 | 2 | 3 | 1.3 | 25 | 5 | 14 | 21 (-25) | 32 (*44*) | 33 | 1.5 | 1.0 | 0.5 |
| | | | | | | 0 | 2 | 21 | 23 (*-10*) | 30 (*43*) | 26 | 1.1 | 0.9 | 0.8 |
| human[e] | 1 | 1 | 3 | 5 | 3.0 | 25 | 20 | 42 | 41 (*79*) | 11 (*88*) | 6 | 1.0 | 0.5 | 4.9 |
| | | | | | | 5 | 13 | 42 | 45 (*82*) | 8 (*90*) | 5 | 1.1 | 0.6 | 6.7 |
| sheep | 1 | 4 | | | 1.3. | 25 | 19 | 27 | 21 (*-18*) | 26 (*47*) | 26 | 0.8 | 1.0 | 0.9 |
| | | | | | | 0 | 14 | 32 | 19 (*-11*) | 29 (51) | 20 | 0.6 | 0.7 | 1.0 |
| rabbit | 1 | 3 | 5 | ~5 | 1.3 | 25 | 12 | 50 | 20 (*54*) | 17 (24) | 13 | 0.4 | 0.8 | 2.3 |
| | | | | | | 0 | 6 | 58 | 18 (*47*) | 13 (*28*) | 11 | 0.3 | 0.5 | 3.2 |
| porcine | 1 | 2 | ~4 | | 1.3 | 25 | 3 | 23 | 38 (*-53*) | 19 (*4*) | 20 | 1.7 | 1.1 | 1.6 |
| | | | | | | 0 | 4 | 16 | 69 (*-89*) | 10 (*25*) | 5 | 4.3 | 0.5 | 5.7 |
| canine | 1 | 2 | | | 3.0 | 25 | 31 | 23 | 71 (*94*) | 3 (*27*) | 3 | 3.1 | 1.0 | 16 |
| | | | | | | 0 | 42 | 19 | 77 (*97*) | 2 (*18*) | 2 | 4.1 | 1.0 | 24 |

[a] Irradiated at λ >320 nm for 1 h under Ar in aqueous phosphate buffer at pH 7.0; [AC] = 0.6 mM (fixed). [b] Consumed AC; error <±3%. [c] Relative yield; error <±1%. [d] Enantiomeric excess; error <±3%; the positive/negative *ee* indicates predominant formation of (*M*)/(*P*)-enantiomer, respectively (reference 20). [e] Data from reference 8b.

unimolecular site(s)), which allows a direct comparison of the photochirogenic performance of each SA. The conversion (consumption of AC) was determined by monitoring the absorbance change of AC and the relative yield and *ee* by chiral HPLC analysis[18] to reveal the exclusive formation of cyclodimers **1**-**4**, along with a trace amount (<1%) of 9,10-anthraquinone-2-carboxylate.[8]

As shown in Table 1 (right columns; see also Table S3 in ESI† for the results at higher AC/SA ratios), the addition of SA substantially decelerated the photocyclodimerization of AC. Thus, the conversion at 25 °C was 80% for free AC, but was reduced to 3-31% in the presence of SA. This is reasonable, as roughly 33% (CSA) to 67-77% (other SAs) of the added AC are trapped in the non-productive unimolecular site(s) and only the remainder is populated to the productive site, where the mobility of bound AC is inevitably reduced, when compared to free AC.

The product distribution was also altered substantially in the SA-mediated photocyclodimerization. As can be seen from Table 1, free AC photocyclodimerizes to HT and HH dimers in a 79:21 ratio, as a result of the electrostatic and steric repulsion between the carboxylate anions in reacting ACs.[8] Crucially, the HT:HH ratio was a critical function of the SA and temperature employed, varying from modest HH preference (65% HH) for BSA (at 25 °C) to extreme HT preference (96% HT) for CSA (at 0 °C), which undoubtedly reflects the binding geometry in the productive site of each SA. A closer examination of the product distribution reveals that one of the four cyclodimers is specifically favored upon photoirradiation with RSA and CSA to afford **1** in 58% yield and **2** in 77% yield (both at 0 °C), respectively. These results suggest that the relative geometry of AC molecules is stereochemically well controlled upon binding to the productive site and practically preserved in the subsequent "intra-site" photocyclodimerization.

The same is true for the enantioselectivities of the chiral cyclodimers obtained with various SAs. Thus, the *ee* values of both **2** and **3**, varying widely from –89% to +97% for the former (for the corresponding chiral HPLC traces, see Fig. S13 in ESI†) and from +4% to +90% for the latter, are highly specific to the SA employed (Table 1), which supports our claim that the stereochemical outcomes are governed by the relative geometry of multiple AC molecules accommodated in an identical site. This seems reasonable, since the photocyclodimerization is considered to proceed in the hydrophobic pocket of a productive site through the attack of an excited AC (AC*), which is released from the binding partner (lysine or arginine residue) upon excitation (leading to an increase of pKa by 2.4[19] to loosen the binding), to an ground-state AC which is immobilized in the same site.[8a-c,f] Hence, which enantiotopic face of immobilized AC is exposed absolutely crucial in determining the enantioselectivity of subsequent photocyclodimerization. The attack of AC* from its *re* face to the *re* face of immobilized AC leads to the (*P*)-enantiomer of **2** or **3** and the *si*-*si* attack affords the antipode, while the *re*-*si* and *si*-*re* attacks lead to achiral **1** or **4**.[8c,f,20] As the selective photoexcitation is not feasible for ACs in the same site, high enantioselectivity is achievable only when all the ACs accommodated in the same site expose the identical enantiotopic face.

Another important issue to be considered is the *anti/syn* and HT/HH selectivities, which also reflect the binding geometry but not straightforwardly, since AC* released upon photoexcitation may rotate before attacking other AC immobilized in the same site. In this regard, the HT/HH, rather than *syn/anti*, ratio appears to better retain the initial orientation, as the longitudinal rotation of AC requires a much larger space to occur than the lateral one. Indeed, the *syn* preference in the HT dimers is modest (**2**/**1** = 4.1) even when the HT preference is high (HT/HH = 24) upon photocyclodimerization with CSA (Table 1).

When three or more ACs are immobilized in a single site, their relative orientations are not necessarily coherent to each other and hence the *anti/syn* and HT/HH selectivities will not be very high intrinsically. However, if only two ACs are immobilized in the same site, a single relative orientation is automatically achieved between them and the only factor that would deteriorate the product selectivity is the rotation of AC* before attaching the remaining AC in the same site.

These considerations lead us to a convincing rationale for the chemical and optical yields optimized by using particularly PSA and CSA as mediators, both of which bind two ACs in the first productive site (Table 1). Thus, in the photocyclodimerization mediated by PSA, the second site is deduced to bind two ACs preferentially in *syn*-HH fashion with the *re*-face being exposed. Upon excitation, one of the ACs is released and attacks another AC in the same site with some longitudinal and/or lateral rotations to give (*P*)-**2** of 89% *ee* in 69% yield (at 0 °C). In the CSA case, the two ACs bound to the second site are also *syn*-HH oriented but expose the *si*-face to afford antipodal (*M*)-**2** of up to 97% *ee* in 77% yield.

In the present study, we have not only achieved the bio-supramolecular photochirogenesis in unprecedentedly high chemical and optical yields but also found a simple strategy for choosing a best suitable bio-supramolecular mediator from the mammalian serum albumin library, both of which will stimulate research on this less explored interdisciplinary area of photochemical, supramolecular and biological sciences.

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## Notes and references

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† Electronic Supplementary Information (ESI) available: experimental details, a list of the amino acid residues for SAs, CD and fluorescence spectral titration data, association constants for the first binding sites of SAs, chiral HPLC chromatograms and a full list of the photochemical results. See DOI: 10.1039/b000000x/

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## TOC Graphic