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Enantioselective Photochromism of Diarylethenes in Human Serum Albumin

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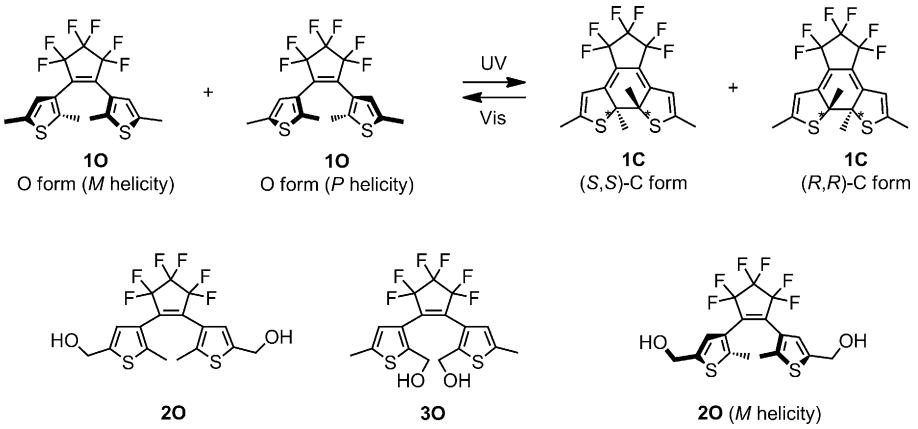
Enantioselective photochromic ring-closing reactions of three bisthienylethenes, possessing either no or two hydroxy groups, were carried out in the hydrophobic pockets of human serum albumin (HSA) in aqueous media. When 10 equivalents of HSA were used, the reaction of 1,2-bis(5-hydroxymethyl-2-methyl-3-thienyl)hexafluorocyclopentene predominantly produced the *S*,*S* closed form in 63% *ee* at RT and 71% *ee* at −4°C upon irradiation with 313 nm light.

Diarylethenes (DAEs) are photochromic compounds with a high potential for applications in switches with functions based on 6π-electrocyclization–cycloreversion reactions between a 1,3,5-hexatriene and a 1,3-cyclohexadiene.[1] We have focused our efforts on achieving high diastereoselectivity in the photochromism of diarylethenes with the aid of chiral factors such as asymmetric stereogenic carbon atoms or an axially or facially chiral unit, introduced within the molecules themselves.[2] So far, we have achieved completely diastereoselective ring-closing reactions with several compounds.[2a,b,d] However, although the highly enantioselective photochromic reactions of diarylethenes in a chiral crystalline state have been reported,[3] those in solution have yet to be achieved.[4]

The application of photochromic compounds to biological systems has recently attracted considerable attention.[5] To apply photochromic compounds based on 6π-electrocyclization in a biological environment, the solubility of the compounds in water is essential. Therefore, since water-insoluble materials can be delivered to cells of living creatures with the aid of serum albumin peptides, we can convey less-water-soluble photochromic compounds to even the farthest periphery of biological systems by using serum albumin as the carrier in aqueous media.

We report herein on the enantioselective photochromism of diarylethenes incorporated in human serum albumin (HSA) in buffered aqueous media to elucidate the effect, on the photochromism of diarylethenes, of the chiral peptides in HSA,[6] an abundant simple protein in human beings that can incorporate a remarkably wide range of chemical species within its hydrophobic pockets. Since HSA consists of optically active amino acids, compounds introduced into the HSA pockets are surrounded by a fully chiral environment. However, although research into the induction of chirality in photochemical dimerization products has been reported,[7] photochromic reactions have yet to be carried out in HSA.

As a basic diarylethene, we employed 1,2-bis(2,5-dimethyl-3-thienyl)hexafluorocyclopentene (**1O**), and prepared its derivatives **2O** and **3O** (Scheme 1). These compounds



Scheme 1. Photochromism of bisthienylethene **1O** and its derivatives **2O** and **3O** employed in this study.

were designed to be small enough for secure incorporation into HSA. In addition, they gained the ability to form hydrogen bonds with the chiral peptide walls of the HSA pockets in two different ways by the introduction of the hydroxy groups at different positions in **1O**.

Photochromic reactions of **1O**–**3O** in the absence of HSA were first carried out in acetonitrile.[8] The conversion ratio (c.r.) values to the closed form (C-form) of **1O**–**3O** at their photostationary states (pss) in acetonitrile were 68, 58, and 59%, respectively.

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We then carried out photochromic reactions of **1O**–**3O** in the presence of HSA in buffered aqueous solution. They exhibited photocyclization in the presence of HSA with 313 nm light. For all of the diarylethenes employed, we detected two enantiomers of the C-form by use of an HPLC machine equipped with a chiral column. Fortunately, we had previously determined the absolute stereochemistry of optically resolved enantiomers of **2C** by X-ray crystallographic analysis[9] so that the slower moving major enantiomer of **2C** was identified as the *S*,*S* configuration. Absorption spectral changes starting from **2O** in the presence of an equimolar amount of HSA are shown in Figure 1.

Table 1 shows the enantiomeric excess (*ee*) and c.r. values of diarylethenes in HSA. These enantioselectivities were apparently induced through the following processes: 1) the open-form molecules of diarylethene entered the HSA pockets to avoid exposure to the aqueous medium; 2) due to the shape and chirality of the HSA inner walls, a biased distribution of the enantiomeric helical conformations of the open form was induced; and 3) the UV irradiation "locked" the enantiomeric ratio of the open form by generating its enantiomeric C-form molecules.

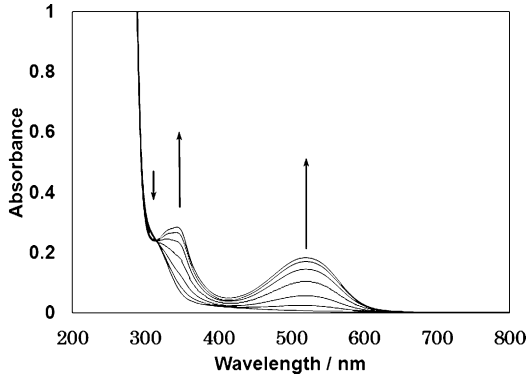

Figure 1. Absorption spectral changes of **2O** in the presence of HSA upon UV irradiation. HSA/**2O**: 1.0 (mol/mol) in phosphate buffer/acetonitrile (99:1 v/v); concentration of **2O**: 4.23×10^{-5} mol dm^{-3}; temperature: RT; cell length: 1 cm; irradiation wavelength: 313 nm; light intensity: 0.322 mW cm^{-2}; irradiation time: 0, 0.5, 1.5, 3.5, 6.5, 10.5, 16 min.

Table 1. Enantiomeric excess and conversion ratio of **1O**–**3O** at the pss with irradiation by 313 nm light in the presence of HSA in phosphate buffer/acetonitrile (99:1 v/v).

HSA/DAE	**1O** to **1C**		**2O** to **2C**		**3O** to **3C**	
	ee [%]	c.r. [%]	*ee* [%]	c.r. [%]	*ee* [%]	c.r. [%]
10	42	67	63	57	27	12
5	40	46	61	57	21	14
1	33	22	45	53	7	25
0.2	8	17	18	27	3	31

As can be seen in Table 1, when the concentration of HSA is increased, the *ee* and c.r. values increase for diarylethenes **1** and **2**. A value as high as 63% *ee* was observed for **2C** when the HSA/**2O** ratio was 10. This indicates that the more HSA there is in the reaction medium, the more **2O** binds to the pocket in which **2O** can take the most stable enantiomeric helical conformation. On the other hand, for **3**, the c.r. value decreases although the *ee* value increases as the concentration of HSA increases.

Since the order of magnitude of the *ee* values was found to be **3C** (27%) < **1C** (42%) < **2C** (63%) when the HSA/DAE ratio was 10, we have tentatively concluded that the shape of the molecule is an important factor in determining its conformation within the HSA pockets. Moreover, the hydrogen bonds formed between the hydroxy groups on the diarylethenes and the inner walls of HSA can either increase or decrease the stereoselectivity depending on the position of the hydroxy groups.

To determine how **2O**, which showed the best *ee* and c.r. values, and HSA interact with each other in the aqueous solution, we used ^{19}F NMR spectroscopy, which has previously been used to investigate the interaction of macromolecules with a ^{19}F-labelled ligand due to its high sensitivity. Since there are almost no ^{19}F atoms in biological materials, the background signals can be eliminated.[10]

Sharp ^{19}F NMR signals due to the perfluorocyclopentene moiety in **2O** were observed at $\delta=-109.0$ (side 4F) and -130.5 ppm (center 2F) in the phosphate buffer solution. When five molar equivalents of HSA were added, the signals appeared at $\delta=-107.8$ and -129.8 ppm, respectively, as shown in Figure 2. The signals broadened and shifted to lower magnetic fields, which indicates that **2O** is bound to the HSA pockets so that its movement is strongly dominated by the large HSA molecule.

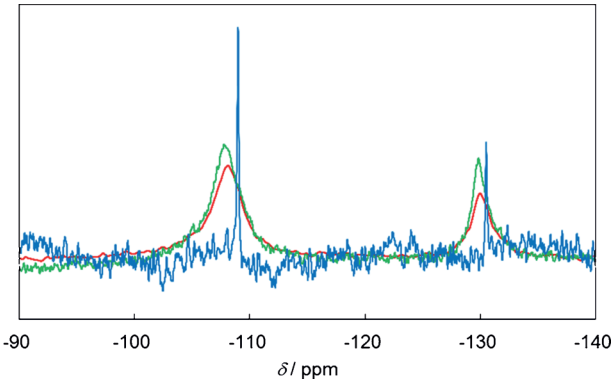

Figure 2. ^{19}F NMR spectra of **2O** in phosphate buffer/acetonitrile (99:1 v/v for HSA/**2O**, 98:2 v/v for **2O** only). Blue: **2O** only; red: HSA/**2O** = 1; green: HSA/**2O** = 5.

The use of ^{19}F NMR spectroscopy enabled us to obtain the binding constants of **2O** to HSA by titrating solutions of 4×10^{-4} mol dm^{-3} **2O** with HSA. Since the change in chemical shifts and changes in *ee* and c.r. values are monotonic, we carried out our analysis on the premise that there is one major pocket that accepts **2O** preferentially. The spectroscopic shifts of the ^{19}F signals of **2O** on addition of HSA were plotted against the HSA concentration. A nonlinear

least-squares method by using KaleidaGraph software gave a binding constant of $8.2\times10^3\ mol^{-1}\,dm^3$ with HSA.[8]

To obtain information on the binding sites of **2O** in HSA, inhibition experiments were carried out by using warfarin (War) and 4-iodobenzoic acid (4IB), both of which have large binding constants to specific binding sites in HSA. Warfarin binds to site I[6a,11] with a binding constant of $3.3\times10^5\ mol^{-1}\,dm^3$, whereas 4-iodobenzoic acid binds to site II[6a,11] with a binding constant of $1.8\times10^5\ mol^{-1}\,dm^3$.[5a] If the binding site of **2O** is either site I or II, **2O** will be squeezed out of the binding site by the addition of an inhibitor due to its comparatively small binding constant ($8.2\times10^3\ mol^{-1}\,dm^3$) so that the *ee* and/or c.r. values may change dramatically after UV irradiation. The results of these experiments are shown in Table 2. Before the addition of an inhibitor, the *ee* was 45% and the c.r. was 53%. When one equivalent of either of these inhibitors was added, both the *ee* and c.r. values decreased slightly. However, although the addition of more 4IB did not affect either the *ee* or the c.r. values, the addition of more warfarin caused a continuous and steady decrease of both the *ee* and the c.r. values. These data indicate that **2O** binds to neither site I nor II, but binds to the site with which warfarin binds secondarily. The initial decrease in *ee* and c.r. values on addition of the inhibitors may be due to an allosteric effect.

Table 2. Enantiomeric excess and conversion ratios of diarylethene **2O** in the presence of HSA and its inhibitors in phosphate buffer/acetonitrile (99:1 v/v).

HSA/inhibitor[a]/**2O**	*ee* of **2C** [%]	c.r. of **2C** [%]
1:0:1	45	53
inhibitor = 4IB		
1:1:1	39	48
1:5:1	39	49
inhibitor = War		
1:1:1	39	48
1:2:1	29	39
1:3:1	21	35
1:4:1	20	29
1:5:1	18	26

[a] 4IB: 4-iodobenzoic acid; War: warfarin.

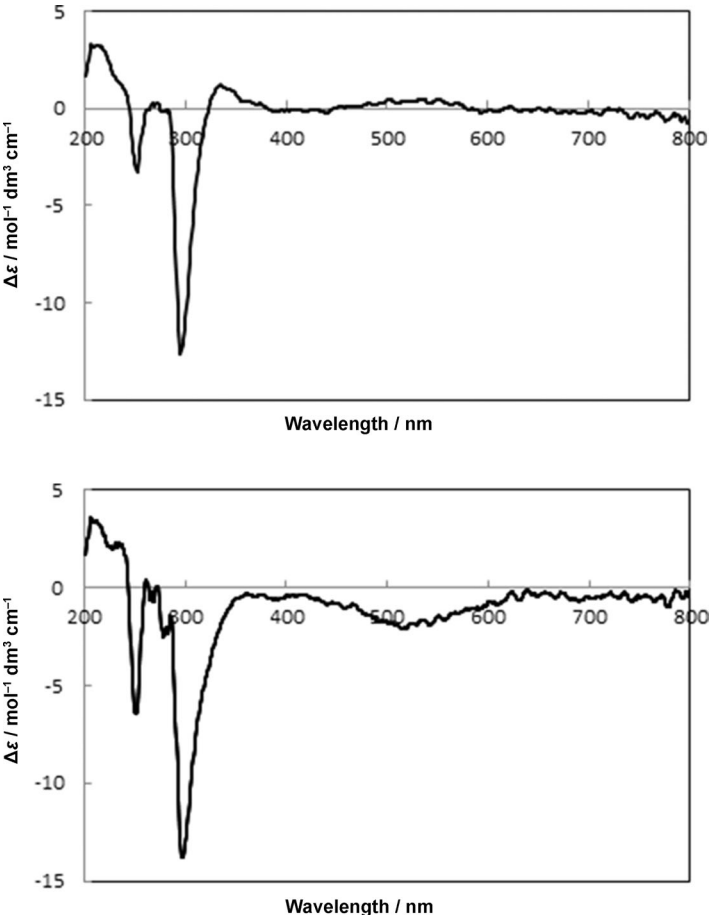

Figure 3. CD spectra of diarylethenes **1O** and **2O** at pss. Top: HSA/**1O** = 5 (*ee*: 40%: c.r.: 46%); bottom: HSA/**2O** = 5 (*ee*: 61%, c.r.: 57%).

We measured the CD spectra after UV irradiation to determine the stereochemistry of the major C-forms of diarylethenes that were formed in the HSA pockets (Figure 3). For **2O**, the spectrum showed a negative Cotton effect in the visible region, indicating that the major enantiomer of **2C** has an *S*,*S* configuration, as already mentioned.[9] On the other hand, **1O** showed a slight positive Cotton effect in the visible region. Matsuda et al. have reported that the sign of rotatory strength for an *R*,*R* enantiomer of the C-form available by the photoirradiation of 1,2-bis(5-substituted-2-methylthienyl)hexafluorocyclopentenes is positive when the π-conjugating substituent is no larger than a vinyl group, whereas it is negative when the conjugating group is larger than a vinyl group, such as in the case of phenyl or formyl groups.[12] The substituents on both thiophene rings are methyl groups in **1** and hydroxymethyl groups in **2**, neither of which are π-conjugated systems. Thus, the major enantiomer of **1C** has an *R*,*R* configuration,[13] unlike that of the major enantiomer of **2C**.

This brings into question whether **1O** and **2O** are accommodated in the same pocket in HSA. Thus a phosphate buffer solution containing equimolar amounts of **1O**, **2O**, and HSA was irradiated with 313 nm light and the *ee* values were determined. Only a drastic decrease in the *ee* value of **1C** was observed (Table 3). We, therefore, concluded that **1O** and **2O** competed to occupy the same pocket in HSA and that the binding constant of **2O** must be larger than that of **1O**.

Although **1O** and **2O** are incorporated in the same HSA pocket, their major C-forms, formed by photochemical ring closure, have opposite absolute stereochemistry. This means

Table 3. Enantiomeric excess values of **1C** and **2C** obtained as a result of competition between **1O** and **2O** to occupy the pocket in HSA.

DAE	*ee* [%]	
	HSA/DAE = 1:1	HSA/**1O**/**2O** = 1:1:1
1O to **1C**	33	13
2O to **2C**	45	39

that the hydrogen bonds between the hydroxy groups of **2O** and the inner walls of the HSA pocket have a strong directing power for the helicity of the hexatriene moiety of the bisthienylethene core, which can even reverse the helicity of a diarylethene molecule with no hydroxy groups (**1O**).

Finally, we examined the effect of temperature on the *ee* and c.r. values. Namely, **2O** and 10 equivalents of HSA were mixed in a buffer solution that was then kept at −4 °C for one day and then irradiated with 313 nm light. The temperature effect was remarkable; a 71 % *ee* and 52 % c.r. were achieved.

In conclusion, we have succeeded in transferring the chirality of human serum albumin effectively to diarylethene **2O** in aqueous media. Diarylethene **2O** showed an excellent enantiomeric excess of 63 % to form (*S*,*S*)-**2C** predominantly at RT with a HSA/**2O** ratio of 10. When the operation was carried out at a temperature as low as −4 °C, the enantiomeric excess rose dramatically to 71 %. Various facets of the underlying mechanisms behind the incorporation phenomena and enantioselective photocyclization reaction were investigated.

Experimental Section

Photochromic reactions: The photochromic reactions of diarylethenes in the presence of HSA were carried out in phosphate buffer solution (pH 6.8) with different HSA/diarylethene ratios (0.2:1 to 10:1) and constant concentrations of diarylethene (4.23×10^{-5} mol dm^{-3}) by irradiation with 313 nm light for a constant period (16 min, 0.3 mW cm^{-2}). Conversion ratios to the closed form were determined by UV/Vis absorption spectra and the enantiomeric excesses were determined by use of an HPLC machine equipped with a chiral column.

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Keywords: chirality transfer · diarylethenes · enantioselectivity · human serum albumin · photochromism

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[13] The CD spectrum of the **1C** isolated from HSA showed a slightly positive Cotton effect, whereas that of the **2C** isolated showed a negative Cotton effect.

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