Chiroptical Switch Based on Photoisomerization of Bilirubin-IIIα Bound to Human Serum Albumin

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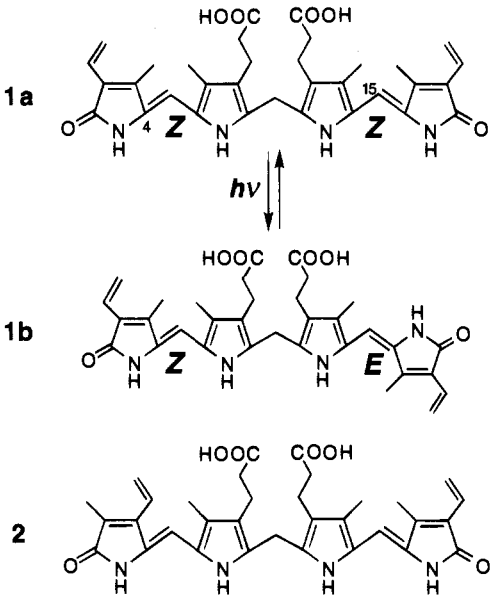
Feringa and co-workers recently described the first chiroptical molecular switch.[1] Their system was based on rapid reversible photochemical interconversion of two helical, intrinsically chiral, alkene diastereomers with opposite helicities. Different wavelengths of UV light were used to interconvert photostationary mixtures of the two chiral diastereomers, and the interconversions were detected by circular dichroism (CD) spectroscopy. We describe here another chiroptical switch, based on an analogous yet different principle, which is sensitive to visible light. In this system, light is used to interconvert two diastereomers of a bichromophoric complex in which the relative orientation and proximity of the two conjoined chromophores results in strong exciton coupling[2] and large chiroptical effects. Interconversion between the two diastereomers is manifested by substantial changes in CD absorption.

The bichromophore in the complex is bilirubin-IIIα (**1**), a symmetrically-substituted isomer of the naturally-occurring yellow pigment bilirubin-IXα (**2**). In solution, **1** and **2** show strong broad absorption bands in the visible centered near 450 nm. These bands correspond to overlapping exciton components from electric transition dipole–dipole interaction of the two methylene-linked dipyrrinone units present in each molecule.[3] When irradiated within these absorption bands, both pigments undergo rapid configurational $Z \rightleftarrows E$ isomerization (e.g., **1a** $\rightleftarrows$ **1b**, Scheme 1).[4] The *Z,Z* and *Z,E* isomers have extensively overlapping UV–vis absorption spectra, and a photostationary mixture of both isomers, along with a relatively small amount of the *E,E* isomer, is formed on photoexcitation of either isomer. The composition of the *Z,Z* $\rightleftarrows$ *Z,E* mixture at the photostationary state is strongly dependent on the wavelength of irradiation, particularly for wavelengths in the long-wavelength edge of the absorption band.[5]

Bilirubins **1** and **2** form water-soluble stoichiometric 1:1 complexes with human serum albumin (HSA). Surprisingly, binding to HSA does not hinder $Z \rightleftarrows E$ isomerization, and the *Z,E* isomers, which revert rapidly to the parent *Z,Z* isomers in water, are stable in the dark when complexed with HSA. Although *(Z,Z)*-**2** is not optically active in solution, its complexes with HSA exhibit characteristic intense bisignate CD spectra.[3] It has been reported that the CD spectrum of *(Z,Z)*-bilirubin-IXα/HSA changes markedly on photoisomerization, because of

Scheme 1. Structures of Bilirubins IIIα (**1**) and IXα (**2**) and $Z \rightleftarrows E$ Photoisomerization of **1**



a CD sign inversion associated with the $Z \rightarrow E$ change in configuration.[6] This being so, bilirubin-IXα/HSA could, in principle, act as a chiroptical switch controllable by visible light. Bilirubin IXα, however, is unstable to light because it undergoes, in addition to $Z \rightleftarrows E$ isomeriation, competing photocyclization involving the *endo* exocyclic vinyl group.[7] Therefore, we studied instead the symmetrical isomer bilirubin IIIα (**1**), which lacks an *endo* exocyclic vinyl group and does not undergo competing photocyclization.

The CD spectrum of *(Z,Z)*-bilirubin-IIIα/HSA at pH 7.4 shows negative and positive maxima at 406 and 458 nm, respectively, with an amplitude difference between the two maxima of 138 M^{-1} cm^{-1} ($\Delta\epsilon_{406} = -58$ M^{-1} cm^{-1}, $\Delta\epsilon_{458} = +80$ M^{-1} cm^{-1}) (Figure 1). The CD spectrum of the corresponding *Z,E* isomer in HSA solution is also bisignate, but the signed order of the two maxima is reversed and the spectrum is weaker in intensity with an amplitude difference between the two maxima of 42 M^{-1} cm^{-1} ($\Delta\epsilon_{421} = +21$ M^{-1} cm^{-1}, $\Delta\epsilon_{477} = -21$ M^{-1} cm^{-1}).[8,9] The large difference between the two CD spectra and the sign inversion presumably reflect a change in the relative helical orientation (***P*** $\rightarrow$ ***M***) of the dipole transition moments that are associated with the dipyrrinone units of each bound diastereomer.

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(2) Harada, N.; Nakanishi, K. *Circular Dichroic Spectroscopy—Exciton Coupling in Organic Stereochemistry*; University Science Books: Mill Valley, CA, 1983.

(3) Person, R. V.; Peterson, B. R.; Lightner, D. A. *J. Am. Chem. Soc.* **1994**, *116*, 42. Lightner, D. A.; Wijekoon, W. M. D.; Zhang, M. *J. Biol. Chem.* **1988**, *263*, 16669. Lightner, D. A.; Gawroński, J. K.; Wijekoon, W. M. D. *J. Am. Chem. Soc.* **1987**, *109*, 6354.

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(8) CD spectra for *(Z,E)*-bilirubin-IIIα/HSA were determined both by difference spectroscopy and by direct measurement. Similar, though not totally identical,[9] curves were obtained by both methods. For the difference measurements, solutions of the *Z,Z/HSA* isomer were irradiated at λ_{max} to a photostationary state and the *Z,Z/Z,E/HSA* composition at the photostationary state was measured by HPLC[5] with the HPLC detector set at an isosbestic point for the $Z,Z \rightleftarrows Z,E$ reaction in the HPLC solvent. For direct measurements, **1** was irradiated anerobically in $CHCl_3/Et_3N$ (1:1)[4] to a photostationary state and the solvent was rapidly removed under reduced pressure on a rotary evaporator at room temperature. The residue was extracted rapidly with ice-cold methanol, in which the *Z,E* isomer is soluble but the *Z,Z* isomer is not, and the residue remaining after flash evaporation of the methanol extract was dissolved in HSA solution to give a pigment: protein mole ratio of about 1:2. The curve depicted in Figure 1a is a mean of three determinations by the difference method.

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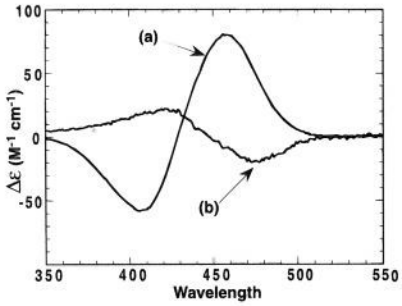


Figure 1. Circular dichroism spectra of (a) (4*Z*,15*Z*)-bilirubin-IIIα/HSA and (b) *(Z,E)*-bilirubin-IIIα/HSA in 0.1 M phosphate buffer at pH 7.4. The HSA blank (which showed $\Delta\epsilon_{350-550} \approx 0$) has been omitted for clarity.

Irradiation of *(Z,Z)*-bilirubin-IIIα/HSA with broad-band blue light (λ_{max} 430 nm) produced a photostationary mixture containing 32% *(Z,E)*-bilirubin-IIIα/HSA, estimated by HPLC with detection at a $Z \rightleftarrows E$ isosbestic point; irradiation with green light (λ_{max} 544 nm) yielded a mixture containing 20% *(Z,E)*-bilirubin-IIIα/HSA.[10] Through switching between the two states was hard to detect by absorbance measurements, it was readily demonstrable by CD monitoring. Figure 2 shows cycling between the two states using alternating 1-min irradiations with blue and green light. The change in $\Delta\epsilon_{458}$ with each cycle was about 15%. Even in non-degassed solutions the blue−green cycle could be repeated at least 10 times with only a slight (~1.5%) photooxidative loss of pigment. Since the amplitude and rate of the CD changes are dependent on irradiation wavelength, light intensity, and irradiation time, the response of the switch can be varied by adjusting these parameters. The switch is, therefore, a chiroptical dimmer switch.

While this switch has no conceivable practical value, because of the requirement for a thermally unstable protein to selectively bind a chiral conformer, protein-free chiroptical molecular switches based on the same exciton-coupling principle can be envisaged. These would consist of chiral molecules containing pairs of photoisomerizable chromophores held, by steric constraints, at a suitable distance and relative orientation for exciton coupling. Potential examples from the tetrapyrrole literature would be optically active compounds such as **3** and **4** (Scheme 2) which show strong dual Cotton-effect CD spectra in organic solvents because of exciton coupling.[11,12]

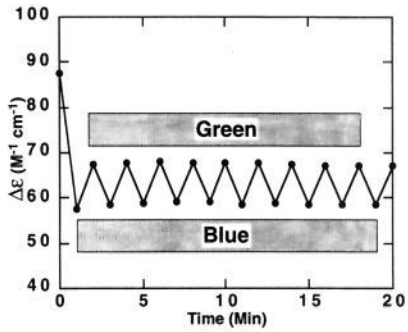
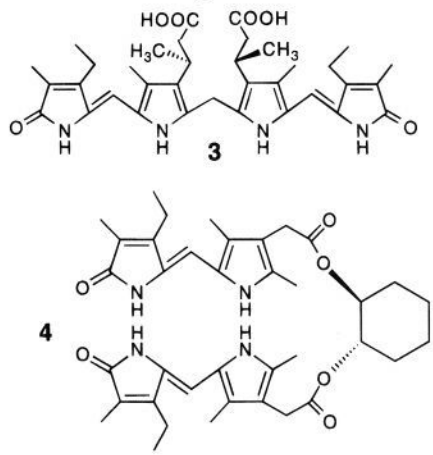


Figure 2. Changes in molar circular dichroism, $\Delta\epsilon(M^{-1}\ cm^{-1})$, at 458 nm of a (**1a** + **1b**)/HSA mixture during alternating irradiations with blue and green light. Irradiation times were 1 min.

Scheme 2. Examples of Inherently Dissymmetric Photoisomerizable Bichromophoric Molecules



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(9) Although the two dipyrrinone chromophores of **1** are chemically identical, their local environments will not be identical in the **1**/HSA complex. Consequently, $Z \rightarrow E$ isomerization of **1** bound to HSA could in principle yield two isomers, depending on which particular dipyrrinone undergoes isomerization. These isomers would differ only in the orientation of the *(Z,E)*-**1** within the binding pocket on the protein. Similar orientational disorder could lead to formation of two diastereomeric complexes when synthetic *(Z,E)*-**1** is added to HSA solutions. Presently, it is not known whether photoirradiation of **1**/HSA yields a single orientational diastereomer or a mixture.

(10) Solutions containing 25.1 μM **1** and 48.8 μM HSA in phosphate buffer (pH 7.4) were irradiated alternately with blue and green light in 1-cm path length cuvettes with magnetic stirring in the cell compartment of a spectropolarimeter, and the CD signal at the 458-nm maximum was measured. Light was delivered to the cuvette via a 5-mm optical fiber connected to a 150-W tungsten lamp illuminator, filtered by broad-band glass filters. The spectral shape and intensity of the light impinging on the cuvette were measured by a Li-Cor spectroradiometer (LI-1800) using a fiber optic probe. The blue light peaked at 430 nm, with a half-height bandwidth of 70 nm, and the irradiance in the 350−550-nm range was 2.77 mW/cm^2. The green light peaked at 544 nm, with a half-height bandwidth of 90 nm and irradiance of 6.58 mW/cm^2 in the 350−550-nm range. CD values were recorded as the mean of five data acquisition periods each lasting 10 s (10 points/s), during which time the irradiation beam was blocked.

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