Photochemistry in Biological Matrices: Activation of Racemic Mixtures and Interconversion of Enantiomers

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Abstract: The antipodes of 1,1'-bi-2-naphthol (**1**) interact quite differently with bovine serum albumin (BSA), a transport protein to which they bind very strongly. This difference is reflected in their absorption and circular dichroism spectra. In particular, the (−)-*S*-isomer, bound to its higher affinity site in BSA, presents an absorption/CD band in the 350–400-nm spectral region which is 5-fold more intense than the (+)-*R*-isomer band. This results in a particularly efficient process of photoenrichment in the *R*-antipode when racemic **1**, complexed to BSA, is irradiated with near-UV light. Moreover, irradiation of the (−)-**1**/BSA complex at 365 nm also results in photoinversion to (+)-**1**. Photoinversion in the opposite direction, i.e. (+)-**1** to (−)-**1**, has not been observed, however. Thus, the photointerconversion of (−)-**1** in BSA contributes, with preferential photodestruction of this antipode, to (+)-**1** enrichment during the photolysis of racemic **1**/BSA. The law of kinetics presented is followed, at least up to 77% photodestruction of **1**, with 99% enantiomeric excess obtained, showing that chiral discrimination properties of BSA remain unaltered during prolonged photolyses. The irradiation of (+)-**1** in basic CH_3CN also leads to photoinversion. The reactivity and spectroscopic data presented here show that (−)-**1** in BSA is located in a basic binding site where its reactivity resembles that of the naphtholate anion.

Introduction

The serum albumins have been recognized as the principal transport proteins in plasma. Their structure is adapted to the binding of a wide variety of substrates, such as fatty acids, amino acids, hormones, bilirubins, etc., and also "foreign" molecules such as aspirin, heroin, and drugs in general.[1] Bovine serum albumin (BSA) and human serum albumin (HSA) also possess the ability to recognize chirality in their interaction with racemic, also nonphysiological, substrates;[2] this encouraged the application of BSA and HSA to resolve racemates using both heterophasic systems, such as chromatographic systems with the protein bonded to the stationary phase,[3] and "homophasic" procedures.[4] In the latter, the enantiomers of a racemic mixture in solution bind to BSA to different degrees. Hence, separation of the free substrate from the BSA/ligand complex by means of ultrafiltration results in partial resolution.[4] Most probably, weak interactions are involved in chiral chromatography, while stronger binding is required in the above complexation–separation process.

Strong interactions with enantiomeric substrates may also be important in chemical reactions involving the bound antipodes which, because of the influence of the hosting asymmetric biological matrix, can evolve in different ways. Recently[5] it has been reported that the near-UV irradiation of (±)-1,1'-bi-2-naphthol ((±)-**1**) complexed to BSA leads to the strongly preferential phototransformation of the (−)-(*S*)-**1** antipode, thus kinetically enriching in (+)-(*R*)-**1** the residual **1** with only moderate destruction of the substrate.

In this paper, we report on (−)-**1** and (+)-**1**, which, when complexed to BSA, phototransform differently upon near-UV irradiation. In fact, (−)-**1** significantly photoinverts to (+)-**1**, while the phototransformation of (+)-**1** in the opposite direction was not observed. In addition, (−)-**1** transforms into photoproducts much more rapidly than does (+)-**1**. It is worth noting that photoinversion is not detectable in common solvents. The above phenomena quantitatively explain the strong optical photoactivation of racemic **1** when complexed to BSA, at the acceptable level of destruction observed. In this study, spectroscopic measurements together with photochemical experiments were undertaken in order to define the physicochemical conditions present in the binding sites of the protein which might explain the above-mentioned extraordinary differences in photoreactivity between the two complexes.

Experimental Section

Materials. Essentially fatty acid free BSA (A6003) was purchased from Sigma; the solid sample was conserved at 0 °C under N_2. (+)-(*R*)-1,1'-Bi-2-naphthol, (+)-**1**, (−)-(*S*)-**1**, and (±)-**1**, from Aldrich, were 99% chemically pure: the active samples proved to have an equal enantiomeric excess of ~99.5%. The solvents (from C. Erba) were of HPLC quality.

Instrumentation. A Cary 219 spectrophotometer and a Jasco J40AS spectropolarimeter were used for the UV and CD absorption measurements, respectively. The Jasco instrument was modified in order to measure with a precision greater than 10% the difference in ellipticity between the **1**/BSA complexes and BSA with an identical concentration of the protein, in the 250–215-nm spectral region, where the strong protein signal largely dominates. In this region, the difference, which is attributable to complexed **1**, was well above the instrumental error (0.8 mdeg at 230 nm), while, in the original J40AS machine, the measurements were severely affected by registration noise. In essence, after the instrument modifications in the baseline offset section,[6] elongations up to ±200 cm, i.e. 8 times the original range, became measurable with a precision which was sufficient to control the registration noise.[6]

Cylindrical quartz cells with an optical path length of 5–0.01 cm were used; thus the necessity of diluting the solutions was avoided. The spectral bandwidths used were 1 nm for UV and 2 nm for CD. A Jasco 880-Pu HPLC pump was utilized equipped with a Jasco 875-UV spectrophotometric detector connected to an RP C18, 25 cm × 4.6 mm i.d. column or, in the case of the ee determinations, to an analytical chiral TC-DNBPG column (25 cm × 4.6 mm i.d.). Ultrafiltrations were carried out with a Spectra/por stirred cell (S25-10), pressurized under nitrogen at about 3 bar and equipped with a Spectrum C20K (20000-Da cutoff) membrane.

Preparation of the Proteic Complexes. **1** is highly soluble in CH_3OH, CH_3CN, or diethyl ether and almost insoluble in water. Crystalline (−)-**1**, (+)-**1**, or (±)-**1** was slowly dissolved at room temperature in aqueous BSA solutions under gentle agitation. The solutions were filtered

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(0.2 μm) to separate excess solid **1**. Under comparable conditions, the speed of dissolution was found to be (−)-**1** > (+)-**1** > (±)-**1**; a few hours were required to reach the molar ratio r = [**1**]/[BSA] ≈ 1, with [BSA] ca. 0.000 15 M (about 10 mg/mL). We were unable to load the protein with more than 2 molecules of **1**. In irradiated samples, the initial r ranged between 0.4 and 0.8 and [BSA] ≈ 0.000 15 M. The above complexes could be stored at ca. 0 °C under N_2 almost indefinitely.

When the solutions of **1**/BSA were ultrafiltered, no free **1** was detected in the permeate by UV or HPLC analysis: indeed, in practice the concentration of free **1** in **1**/BSA solutions should be much lower than its saturation concentration in water. Racemic **1** dissolves in BSA as a racemate, since diethyl ether extraction from the filtered complex led to the recovery of authentic racemate. Notice that the above described method of preparation guarantees an identical [BSA] in the **1**/BSA complex solution and in the protein mother solution which was used as the reference solution for the UV and CD measurements. The pH of the aqueous **1**/BSA solutions was neutral (6.95–7.05), as was that of the original BSA mother solution.

Recovery of 1 from 1/BSA Complexes. **1** was quantitatively recovered from the **1**/BSA complexes by 3-fold extractions with twice the volume of diethyl ether at room temperature under gentle agitation, each extraction lasting 2 h. No preferential extraction of antipodes from the racemic **1** complexes was observed.

Irradiations. Irradiations were carried out at room temperature on 2–5-mL samples in 12 mm i.d. cylindrical, sealed, pyrex tubes submerged in distilled water and externally irradiated by a medium-pressure Hg lamp (100 watts), placed 3 ± 1 cm from the plasma of the light source. Under these conditions, the fluence of light, at 365 nm, falling on the sample was relatively high, thus reducing the irradiation time. On the other hand, unavoidable small geometry variations and the instability of light emission at 365 nm (linked to the cooling of the lamp by the forced ventilation of the light filter) made the absolute quantum yield measurements irreproducible. Therefore, only the ratio of the quantum yields obtained in the same experiment are reported here. The 365-nm emission lines of Hg were isolated with a Schott-type filter (Melles-Griot 03 FCG003, 78% maximum transmission at 385 nm, 70-nm transmittance spectral bandwidth), cooled with forced ventilation. The fluence of light falling on a 3-mL sample was generally ca. 3×10^{-7} einstein/min. Magnetic stirring was used, and the samples were kept under N_2.

Photoconversion Determination. HPLC injection of the above **1**/BSA aqueous solutions, RP18 column and mobile phase CH_3CN or aqueous CH_3CN (generally H_2O/CH_3CN, 20/100 v/v ratio), led to the quantitative determination of **1**. The latter complex had a retention time and peak shape identical to that obtained when **1**, dissolved into CH_3CN, was injected. Good reproducibility is guaranteed if the injected sample is allowed to remain for 3 s in the injection loop, where **1** is completely extracted from the protein. It remains entrapped in the guard column in a denatured state; consequently the pressure could increase, a few bars/injection, if a constant flow of the mobile phase is maintained. With 100/20 CH_3CN/H_2O and a flow of 0.5 mL/min, the retention time of **1** is ca. 4.8 min. The photoconversion of **1** was determined by injecting, in sequence, the irradiated and nonirradiated samples. The peak area proved to be constant in successive injections, varying by no more than 5%; therefore, at least three pairs of injections were performed to determine the extent of conversion.

Enantiomeric Excess Determination. HPLC measurement of the ee was carried out on **1** which had been purified by semipreparative HPLC and then dissolved in hexane or hexane/2-propanol (IPA) mixtures. The working conditions were typically the following: rt., mobile phase *n*-hexane/IPA, 100/20 (v/v), and flow 1 mL/min. Under these conditions, the chromatographic parameters were found to be Tr(−) = 13 min 15 s, Tr(+) = 16 min 20 s (solvent T = 2 min 30 s), selectivity factor α = 0.79, and resolution R_s = 1.28. Measurement of the peak area by graphical integration required evaluation of the tail of (−)-**1** in the (+)-**1** peak zone because of residual overlap. This was necessary when the concentration of (+)-**1** was low with respect to that of (−)-**1**, as was the case in the irradiation of (−)-**1**/BSA. The evaluation was carried out using the peak shape of pure (−)-**1**. This procedure proved to be reliable: the measured ee of the standards, obtained by mixing solutions of pure enantiomers, fitted their actual values (0%, −75%, and −90%).

Optical Purity (OP) Determinations. Where possible, i.e. in the presence of sufficient **1** in solution, the CD value for the couplet amplitude, at ca. 230 nm, was measured and correlated with that of the pure antipode at equal concentration, obtaining a good estimate of the OP. In such cases, the difference between ee (from chiral chromatography) and OP (from CD) was marginal (±2%).

Results

Spectroscopy of 1/BSA Complexes. As mentioned in the above section, the concentration ratio r = [**1**]/[BSA] of our complexes never exceeded 2. From these solutions with $r \approx 2$, solutions with values of r as small as 0.3 could be obtained by diluting the original samples with the BSA "mother" solution. In this 0.3–2 range, the absorption and CD spectra of the complexes turned out to be significantly different from the analogous spectra of the BSA "mother" solution in the 420–215-nm spectral region. This difference is attributable to the absorption and circular dichroism of the **1** bound to the protein.[6] Thus, the molar CD ($\Delta\epsilon$) of bound **1** was calculated by taking the difference of the CD elongations of the complex and of the protein and the HPLC concentration of **1** (it must be remembered that the free **1** in the **1**/BSA solutions was under the detectability limit, i.e. some 10^{-8} M).

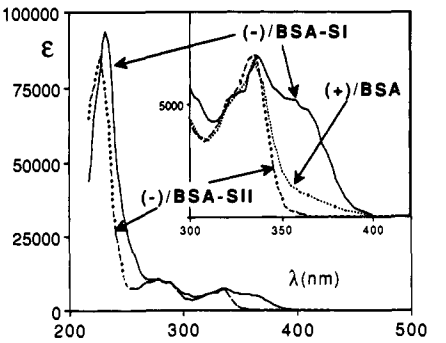

Figure 1. Molar UV of (−)-(*S*)-**1** in the BSA SI binding site (solid line) and in the SII site (dashed line). The spectrum of (+)-(*R*)-**1** complexed to BSA is also shown for λ > 300 nm.

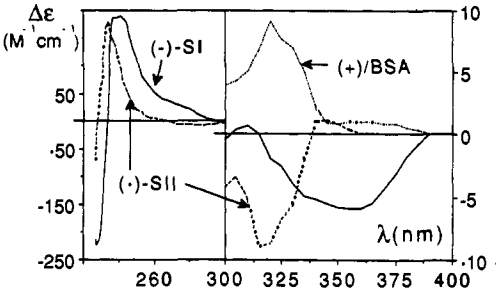

Figure 2. CD spectra of (−)-(*S*)-**1** in the BSA SI binding site (solid line) and in the SII site (dashed line). The spectrum of (+)-(*R*)-**1** complexed to BSA is also shown for λ > 300 nm.

The spectra were measured by varying both r and the concentration of BSA in the 3–12-mg/mL range. These spectra revealed interesting features. In fact, while the ϵ and $\Delta\epsilon$ of (+)-**1** in BSA were almost independent of the ratio r, in the case of (−)-**1**, a definite trend was observed, which could be represented by the equations:[6]

$$\epsilon(\lambda) = p\epsilon^{I}(\lambda) + (1 - p)\epsilon^{II}(\lambda) \quad (1)$$

$$\Delta\epsilon(\lambda) = p\Delta\epsilon^{I}(\lambda) + (1 - p)\Delta\epsilon^{II}(\lambda) \quad (2)$$

where $\epsilon(\lambda)$ and $\Delta\epsilon(\lambda)$ are the spectra of (−)-**1** in BSA and p is an r-dependent parameter which tends to 1 as r approaches 0. When r = 0.5, $p \approx 0.8$. Finally, we attributed $\epsilon^{I}(\lambda)$ and $\Delta\epsilon^{I}(\lambda)$ to the absorption and CD of (−)-**1** in a higher affinity site, SI, of BSA. Consequently, $\epsilon^{II}(\lambda)$ and $\Delta\epsilon^{II}(\lambda)$ were the values of (−)-**1** spectra in a second binding site, SII. Figures 1 and 2 report ϵ^{I}, ϵ^{II}, and ϵ((+)-**1**/BSA) and $\Delta\epsilon^{I}$, $\Delta\epsilon^{II}$, and $\Delta\epsilon$((+)-**1**/BSA), respectively. Clearly, p represents the fraction of bound (−)-**1** in the SI site. In our opinion, the validity of the interpretation of the experimental spectra presented here depends on the constancy of above site spectra with varying r and on consistency with the photochemical results.

The case of the racemate bound to BSA appears to be more complex; in fact, both the UV and CD spectra were found to be the sum of (+)-**1** and (−)-**1** contributions, the latter being r-dependent as previously explained.[6] In Figure 3 the experimental absorption and CD spectra of (±)-**1** in BSA (r = 0.58) are reported. Notice that the contribution of (−)-**1**, virtually all located in SI, dominates the CD and UV spectra in the region λ > 350

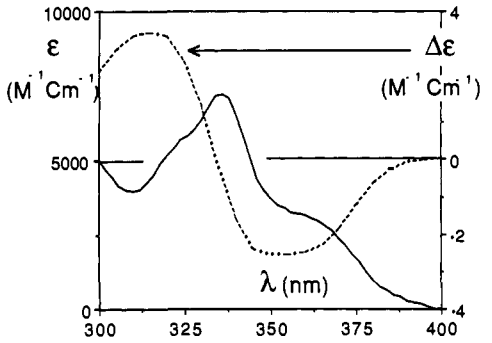

Figure 3. UV and CD spectra of the (±)-1/BSA complex, [1]/[BSA] ≈ 0.58.

nm. Hence, light of λ > 350 nm effectively illuminates the (−)-1 antipode. This is the basic observation which led to the preferential photodestruction experiments performed on (±)-1/BSA (vide infra).[5]

The interactions of BSA with (−)-1 in the two binding sites SI and SII are very different; this is also evident when the high-energy spectral region is considered (Figure 2). Here the CD couplet centered at 234 nm in SI is 4–5-nm red shifted and the exciton splitting almost doubled with respect to the SII one. It is worth noting that the UV and CD spectra of (−)-1 in the second binding site appear very much like the spectra of (−)-1 in CH_3CN, or in CH_3OH. Some differences can be noted when the (+)-1 spectra in BSA are compared with those in CH_3CN.[6]

Photoinversion to (+)-1 of (−)-1 in BSA. When (−)-1/BSA solutions are photolyzed with 365-nm light, the genuine formation of (+)-1 is observed, it being found in amounts well in excess of the small quantity initially present as an optical impurity in commercial batches of (−)-1. In a typical experiment, after irradiation of a 3-mL solution of (−)-1/BSA for 240 min, the ee passed from an initial ee value of ≈−99.5% to −32%, with accompanying destruction of ca. 85% of the substrate. Samples of (−)-1/BSA subjected to the same experimental operations, excluding the irradiation step, i.e. aging at room temperature, exhaustive extraction with diethyl ether, HPLC purification, vacuum evaporation of the solvent, and chiral chromatography, revealed no variation in ee. The ratio of the quantum yield of photoinversion to photodestruction was found to be ca. 0.06. As will be shown below, the ee after irradiation, calculated on the basis of a simple kinetic model, vide infra, is −28%.

Similar photolysis experiments were performed on (+)-1/BSA, with ee = 99.5% and $r \sim 1$. No photoinversion was detected. A precise determination of the (−)-1 content in the mixture obtained after an irradiation which produced 25% photodestruction of 1 was not possible because of the insignificance of the (−)-1 peak, as revealed by chiral HPLC. In other words, the irradiation of (+)-1/BSA resulted in an enhanced ee, a result consistent with the kinetic equations (vide infra). The calculated ee after irradiation is 0.9994, although we could not experimentally verify this value.

In order to measure sizeable ee variations, a sample of (+)-1/BSA solution with ee 96.9% was obtained by mixing 10 mL of the original (+)-1/BSA (ee 99.5%) with an appropriate quantity of (−)-1/BSA (identical [BSA]). Two 3-mL samples were photolyzed for 80 and 280 min obtaining 5% and 26% photodestruction, respectively. The ee of 1 in these samples was found to be 98.4% and 99.5%, respectively (error ±0.3%), which is in satisfactory agreement with the calculated values (97.9% and 99.7%, respectively) (vide infra). In Figure 4 the chiral HPLC traces of the 1 which was recovered and chemically purified, before and after the 280-min photolysis, are reported for comparison.

As in the case of (−)-1/BSA, in (+)-1/BSA, no thermal variation in ee at room temperature was detected. Photolysis of (+)-1, 0.000 45 M in CH_3OH, with an unfiltered quartz Hg lamp, (keeping in mind that 1 dissolved in the usual solvents does not absorb light with λ > 350 nm) until 22% photodecomposition, revealed no photoinversion. Thus, (−)-1 photoinversion is a

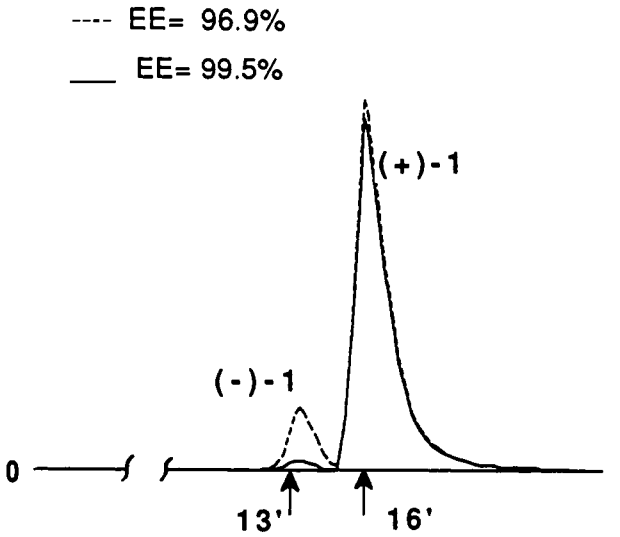

Figure 4. Chiral HPLC of (+)-1 (ee = 96.9%) and of (+)-1 (ee = 99.5%), the latter obtained after 280-min irradiation of the (+)-1/BSA complex (ee = 96.9%) and workup of the mixture. The peaks at 13 min were amplified 8-fold with respect to the (+)-1 peaks at 16 min.

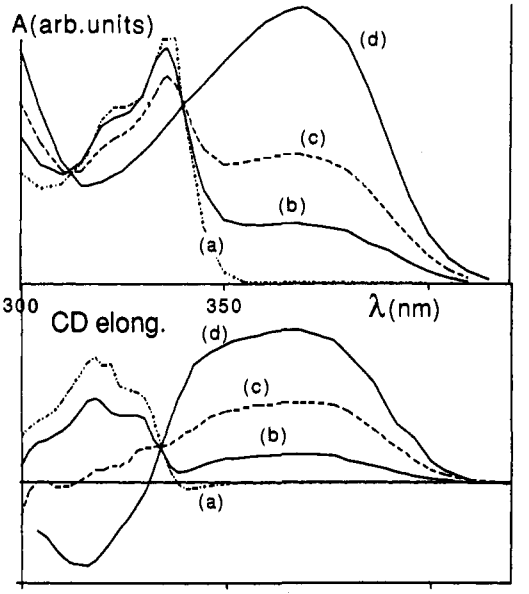

Figure 5. CD and UV spectra of 0.0038 M (+)-1 in CH_3CN: (a) neutral; (b) with 0.5 equiv of 1 N aqueous NaOH added; (c) with 1.5 equiv of NaOH added; (d) with 3.5 equiv of NaOH added. (1 mol of 1 is considered 1 equiv.)

BSA-induced reaction which demonstrates the protein's ability to discriminate enantiomers also in their excited-state reactivity.

Spectroscopy and Photochemistry of (+)-1 in Basic CH_3CN. In a previous work,[5] the hypothesis was formulated that when (−)-1 is bound to the BSA SI site, it is present, at least partially, in an ionized form as a naphtholate ion. This hypothesis was based on the similarity between the UV/CD spectra in SI and in basic CH_3CN. The spectroscopic differences between 2-naphthol, $pK_a \sim 9.5$, in basic and acid solutions are well known,[7] and a similar base-dependence is to be expected for 1,1'-bi-2-naphthol. In Figure 5 the CD and UV spectra in the 300–400-nm spectral region for (+)-1, in anhydrous CH_3CN at different base concentrations, are reported and can be compared with the SI and SII spectra (Figures 1 and 2).

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(8) For racemization of 1 in the dark and under much more drastic conditions: Kyba, E. P.; Gokel, G. W.; de Jong, F.; Koga, K.; Sousa, L. R.; Siegel, M. G.; Kaplan, L.; Sagah, G. D. Y.; Cram, D. J. *J. Org. Chem.* **1977**, *42*, 4173–4179.

A new absorption/CD band, at $\lambda > 350$ nm, emerged on the addition of 1 N NaOH, and in the meantime, the bands on the left-hand side of the isosbestic point (342 nm for absorption and 333 nm for CD) were progressively reduced. The same trend in the absorption spectra was observed during the neutralization of 2-naphthol with base.[7] In practice, the addition of ca. 1.5 equiv of 1 N NaOH to the 0.0038 M solution of (+)-**1** in anhydrous CH_3CN led to CD and UV spectra which almost overlapped with the SI spectra (Figure 5 curves c and Figures 1 and 2). In the above situation, mono-deprotonation of (+)-**1** was incomplete, since at least 3 equiv of NaOH are required to transform **1** into its mononaphtholato ion (spectra d, Figure 5). Comparing the value of ϵ_{max} for the naphtholate at 368 nm (Figure 5, spectra d) with that of (−)-**1** in SI at the same wavelength, we estimated the degree of ionization of (−)-**1** in SI to be roughly 4900/8300, i.e. 59%. Moreover, 365-nm photolysis (47 min long) of a 5-mL sample of (+)-**1**, 0.000 25 M in CH_3CN, to which NaOH(aq), 0.2 N, had been added in 4-fold excess with respect to the concentration of **1** led to 39% disappearance, while the ee changed from 99.5% to 90.3%. On the contrary, racemization of (+)-**1** in nonirradiated basic samples, under N_2 as above, proved to be undetectable, at least over a several day period. The value of the inversion-to-destruction ratio of the quantum yields was evaluated to be ca. 0.1. One should recall that here both antipodes photoinvert and phototransform in conformity with the same kinetic law, i.e. differently than in BSA.

Enantiomeric Photoenrichment on Irradiation of (±)-1/BSA. As previously reported,[5] the photolysis of (±)-**1**/BSA complexes is a very efficient procedure for photolytic optical enrichment. In that study, a quantitative treatment of the kinetic process was presented, taking into account solely the preferential photodestruction of (−)-**1** in BSA. Actually, however, one must also take into account the photoinversion phenomenon. On the basis of our present findings, using the simplest kinetic model for the photolysis of a mixture of (+)-**1** and (−)-**1** with initial ee_0, complexed to BSA and irradiated at a specific wavelength, the following equations hold:

$$\frac{dc^+}{dt} = -KI\frac{\epsilon^+c^+\phi^+}{\epsilon^+c^+ + \epsilon^-c^- + R} + KI\frac{\epsilon^-c^-\chi}{\epsilon^+c^+ + \epsilon^-c^- + R} \quad (3)$$

$$\frac{dc^-}{dt} = -KI\frac{\epsilon^-c^-\phi^-}{\epsilon^+c^+ + \epsilon^-c^- + R} - KI\frac{\epsilon^-c^-\chi}{\epsilon^+c^+ + \epsilon^-c^- + R} \quad (4)$$

where ϵ^-, c^-, ϕ^-, χ are the absorption at λ_{irr}, the concentration, and the quantum yield of chemical phototransformation and of photointerconversion, respectively, of (−)-**1** in BSA. K and R account for the volume of irradiated solution and for the light absorption by other molecules, and, finally, I is the intensity of light absorbed. Division of eq 3 by eq 4 leads to

$$\frac{dc^+}{dc^-} = \frac{\epsilon^+c^+\phi^+ - \epsilon^-c^-\chi}{\epsilon^-c^-(\phi^- + \chi)} \quad (5)$$

This equation, with the introduction of the variable $V = c^+/c^-$, reduces to

$$\frac{\epsilon^-(\phi^- + \chi)}{[\epsilon^+\phi^+ - \epsilon^-(\phi^- + \chi)]V - \epsilon^-\chi}\,dV = \frac{dc^-}{c^-} \quad (6)$$

in which variables V and c^- are mathematically "separated". Equation 6 can then be integrated, leading to a variety of equivalent solutions. In the following form

$$\ln\frac{1 - ee}{1 - ee_0} + \ln\frac{c}{c_0} - \frac{2 - G}{2G}\ln\frac{F\left(\frac{1 - ee}{1 + ee}\right) - 1}{F\left(\frac{1 + ee_0}{1 - ee_0}\right) - 1} = 0 \quad (7)$$

a relationship is expressed among the experimental quantities (ee_0, c_0, ee, and c, initial and actual ee and **1**-concentration, respectively) and the two parameters G and F to be determined. G and F are defined in terms of ϵ^+ and ϵ^-, the absorption of (−)-**1** and of (+)-**1** in BSA at the irradiation wavelength, and the quantum yields

$$G = \frac{\epsilon^+\phi^+ - \epsilon^-(\phi^- + \chi)}{0.5[\epsilon^+\phi^+ + \epsilon^-(\phi^- + \chi)]} \qquad F = \frac{\epsilon^+\phi^+ - \epsilon^-(\phi^- + \chi)}{\epsilon^-\chi}$$

When $\epsilon^+\phi^+ = 0$, we have $G = -2$, the case of perfect chiral discrimination, and eq 7 considerably simplifies (curve b in Figure 6). To test the validity of eq 7 and to extract the parameters G and F from experimental data, a set of 12 photolysis experiments on (±)-**1**/BSA was performed; r was 0.53, and (−)-**1** was assumed to be entirely located in SI (therefore $\epsilon^- = \epsilon^l$). The irradiation time in the experiments ranged from 0 min to more than 5 h, with decomposition from 0% to ≈76%. Curve a in Figure 6 reports the results obtained. The mean G and F were thus obtained (−1.6 and −10, respectively), and from these and the measured ϵ^+ and ϵ^-, the ratios $\chi/\phi^- = 0.08$ and $\phi^-/\phi^+ = 1.7$ were calculated. We consider this χ/ϕ^- ratio to be close to the value of 0.1 obtained in the photodestruction/photoinversion of basic (+)-**1**.

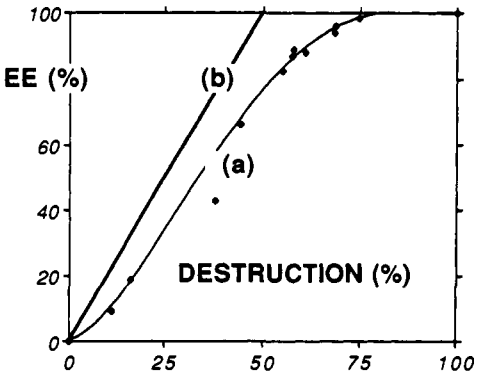

Figure 6. Plot of % ee vs % destruction: (a) experimental; (b) calculated, with $\epsilon^+\phi^+$ set to 0.

From the χ/ϕ^- value, we deduce that photoinversion is a *minor* source of enantiomeric enrichment during the photolyses of (±)-**1**/BSA; on the other hand, it is *the* source of (+)-**1** in the photolyses of (−)-**1**/BSA. Finally, the application of eq 7, and the above values for F and G, to photolysis experiments on commercially pure enantiomers complexed to BSA essentially gives the experimentally obtained ee's, as mentioned above.

Conclusions

A satisfactory fit of the experimental ee versus substrate decomposition was obtained for irradiations of both racemic and quasi-enantiomerically pure **1** complexed to BSA. Equation 7 is an accurate representation of the kinetics of photoenrichment. The preferential photodestruction of the (−)-**1** antipode is due to both the higher (4900/950) light absorption of (−)-**1** in SI and the higher quantum yield of photodestruction ($\phi^-/\phi^+ \sim 1.7$). In addition, a mechanism of photointerconversion of (−)-**1** into (+)-**1** is at work, producing yet further enrichment in (+)-**1**. Thus, the curve describing the optical enrichment of the initial racemic **1** complexes (curve a in Figure 6) is quite close to curve b, which represents what can be defined as the "perfect photodiscrimination" case.

In essence, starting with a racemate, one can obtain almost any ee by means of a predetermined, acceptable also from a preparative point of view, extent of substrate photoconversion.[9] In this regard, the photolyses of proteic complexes described above surpass the ordinary asymmetric reactions, where the ee obtained generally depends on variables which are either hidden or difficult to control.

We observe that the validity of eq 7 at each conversion degree indicates an unaltered chiral discrimination capability of BSA

(9) Photolysis of (±)-**1** with circularly-polarized light would, in theory, lead to any ee, but the photodecomposition required would be certainly "intolerable"; for example, to reach ee = 1%, 97% photodestruction is required, using an optimal wavelength of irradiation light. For the optical activation of racemates with circularly-polarized light see: Cavazza, M.; Festa, C.; Veracini, C. A.; Zandomeneghi, M. *Chirality* **1991**, *3*, 257–262 and references therein.

even at the prolonged irradiation times required to obtain high ee. That is, apparently neither noxious energy transfers to BSA chromophores nor inhibition from photoproducts develop. This agrees well with the previous observation[5] that the protein recovered after irradiation is equivalent to the native BSA. Thus, each protein site represents a kind of microlaboratory which remains essentially undamaged by the chemical and photochemical operations performed on the substrates selected and worked-on by it. While it is difficult, on the basis of the present experimental evidence, to define with any certainty the position of these sites in the macromolecular sequence, there is no doubt that the SI site offers an environment for (-)-1 comparable to basic CH_3CN, with a similar evolution of the lowest lying electronically-excited (-)-1 state. It is worth noting that the inversion of excited (+)-1 in its (basic?) site is not allowed by the protein.

It would be premature to extrapolate from the present case any general conclusion regarding other substrates. Most probably, photointerconversion remains a rare event in organic molecules.[10] Not so rare is the acid-base interaction between substrates and binding sites of biological macromolecules, or other forms of strong interaction: this, coupled to chiral discrimination, offers much latitude for explorations using the present technique.

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Photoinduced Electron Transfer Reactions: Nitrogen-Oxygen Bond Cleavage in Reduced *N*-(Aryloxy)pyridinium and *N,N'*-Dialkoxy-4,4'-bipyridinium Salts

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Abstract: *N*-(Aryloxy)pyridinium cations and *N,N'*-dialkoxy-4,4'-bipyridinium dications form charge-transfer complexes with neutral hydrocarbons. Irradiation of these charge-transfer complexes leads to the cleavage of the nitrogen-oxygen bond and the formation of an aryloxy radical in the first case, but no reaction is observed for the second. In contrast, electron transfer to the dialkoxybipyridinium cation from the triplet state of 9-acetylanthracene leads to nitrogen-oxygen bond cleavage and the formation of an alkoxy radical. The rate constants for nitrogen-oxygen bond cleavage (k_{BC}) in the reduced pyridinium salts were estimated by time-resolved laser spectroscopy. For *N*-(4-cyanophenoxy)pyridinium tetrafluoroborate, $k_{BC} > 10^{11}$ s^{-1}, and for *N,N'*-diethoxy-4,4'-bipyridinium (bis)hexafluorophosphate), $k_{BC} = 1.4 \times 10^4$ s^{-1}. The effects of structure on the dynamics of the excited charge-transfer complexes and on k_{BC} are discussed.

Introduction

The study of single electron transfer reactions that result in bond cleavage is presently a field of active investigation.[1-8] The electron transfer may be thermally activated[9] or, more commonly, may be stimulated by light.[10,11] In the latter instance, irreversible chemical reactions must compete with the very exothermic back electron transfer which regenerates the ground-state reagents. The rates of back electron transfer in excited charge-transfer complexes and in solvent-separated ion pairs have recently been examined for several reaction classes.[12] It is clear from this work that the electronic coupling matrix element, which, in part, governs the rate of back electron transfer, is considerably larger in excited charge-transfer complexes (also known as contact ion pairs) than it is when the donor and the acceptor exist as a solvent-separated ion pair. Consequently, the rate of back electron transfer in contact ion pairs can be extraordinarily large. This postulate has been confirmed by direct measurement of electron transfer rates by spectroscopic means. Nevertheless, Kochi and co-workers discovered that some bond cleavage reactions initiated by irradiation of charge-transfer complexes can compete succesfully with energy-wasting back electron transfer.[10] Also, several recent reports describe bond cleavage reactions that compete with the back electron transfer in photogenerated solvent-separated ion pairs.[11]

The thermal chemistry of *N*-(aryloxy)pyridinium cations has been studied extensively,[13] but the photochemistry of these cations has not been reported. The absorption spectra of charge-transfer complexes of substituted *N*-alkylpyridinium salts are well known, since they form the basis of the widely used Kosower *Z*-value solvent microscopic polarity scale.[14] Similarly, *N,N'*-dimethyl-4,4'-bipyridinium dication (methyl viologen) has been extensively studied. Its reduction leads to a stable radical cation that does

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