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Stereodifferentiation in the Compartmentalized Photooxidation of a Protein-Bound Anthracene

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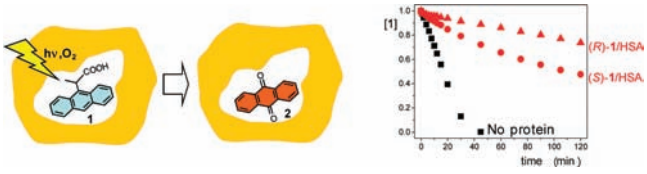
ABSTRACT



Encapsulation within transport proteins strongly reduces the photooxidation rate of (*S*)- and (*R*)-2-(9-anthracenyl)propanoic acid (1) and results in a significant stereodifferentiation. The most remarkable effects are observed within human serum albumin (HSA).

Asymmetric organic photochemistry has emerged as an important field in the past two decades.[1] Chiral host systems and synthetic assemblies such as cyclodextrins,[2] modified zeolites,[3] hydrogen-bonding templates,[4] nanoporous materials,[5] or crystal lattices,[6] as well as biomacromolecules, including DNA[7] and proteins,[8−10] have shown potential in inducing stereoselective photoreactions. Stereodifferentiating photoprocesses in the presence of carrier proteins, such as serum albumins (SAs) and α-1-acid glycoproteins (AAGs), are of special interest for the

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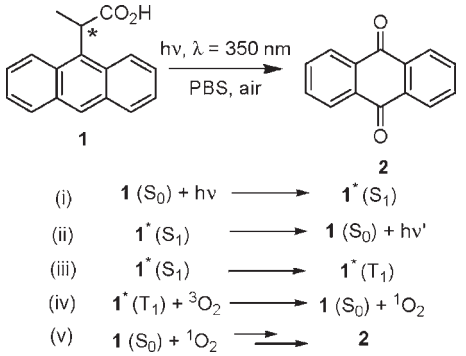
understanding of drug–biomolecule interactions. The photobehavior of included guests can be modified as a result of conformational restrictions imposed by the protein binding pockets. A number of examples of modified photoreactivity within protein cavities have been reported, including dehalogenation,[9] dimerization, cyclization, or isomerization.[10]

Scheme 1. Photooxidation of **1** and Key Mechanistic Steps



The main photochemical pathways for anthracene derivatives are dimerization[11] and oxidation,[12] depending on the reaction conditions. In the case of 9-substituted anthracenes, regioselective control of photocycloaddition in organic solvents and within confined media has been reported.[13] Moreover, stereoselective photodimerization of 2-anthracenecarboxylate within serum albumins at low protein/substrate ratios has also been investigated.[8a–c] We have previously described the photobehavior of a water-compatible anthracene derivative in solution and upon protein encapsulation.[14] Now, we wish to report on the sterodifferentiation in the compartmentalized photooxidation of (*S*)- and (*R*)-2-(9-anthracenyl)propanoic acid ((*S*)- and (*R*)-**1**) encapsulated within human and bovine serum albumins (HSA, BSA) and α-1-acid glycoproteins (HAAG, BAAG).

Long wavelength irradiation (λ_{max} = 350 nm, Gaussian distribution) of **1** in aerated phosphate buffered solution (PBS) afforded anthraquinone (**2**) as the only product (Scheme 1).

The mechanism for this type of process in anthracenes[15] involves triplet–triplet energy transfer to molecular oxygen, generating singlet oxygen ($^1O_2$); subsequent [4 + 2] cycloaddition of this reactive species to the central anthracene ring, followed by rearrangement of the resulting endoperoxide and cleavage of the 9-substituent, would lead to **2**. The photoreaction of **1** was monitored by UV absorption spectroscopy, following the decrease of the characteristic long-wavelength bands of the anthracene chromophore. In the absence of proteins, **1** was almost completely consumed after 20 min of irradiation. The reaction was also performed in the presence of equimolar amounts of SAs and AAGs. Upon protein encapsulation, photooxidation of **1** was much slower than in PBS. Moreover, when **1** was incorporated into HSA, a dramatic stereodifferentiation in the reaction rate was observed, with the photoreactivity being much higher for (*S*)-**1** than for (*R*)-**1**. This led to a kinetic resolution (Figure S1 in the Supporting Information (SI)), with a clear enhancement of the less reactive (*R*)-enantiomer (ee 34% after 120 min of irradiation). The reverse was true for **1** encapsulated within BSA, where a slower photooxidation of (*S*)-**1** was observed (Figure 1). Concerning the reactivity inside α-1-acid glycoproteins, no stereodifferentiation was detected in the presence of HAAG; by contrast (*S*)-**1** reacted faster in the presence of BAAG (Figure S2 in SI).

To assess the involvement of $^1O_2$ in the process, the irradiations were performed in D_2O, where this reactive oxygen species is much longer-lived than in H_2O.[16] As a matter of fact, a remarkable acceleration of the photooxidation rate was observed in all media (Figure 1, as well as Figure S2 in SI). As expected for a photooxidation process, a significant enhancement was noticed under oxygen. In addition, the photoreaction was found to be compartmentalized, i.e., $^1O_2$ reacts with **1** in the same microenvironment where it is generated. To investigate this issue, photosensitization with (*R*)-propranolol ((*R*)-PPN) and (*R*)-naproxen ((*R*)-NPX) was attempted, as these drugs do not bind to HSA and HAAG, respectively.[17] Furthermore, they can be selectively excited at 318 nm in the presence of **1** (see Figure S3 in SI) and are appropriate sources of $^1O_2$ (ϕ_Δ = 0.28 for NPX and 0.24 for PPN).[18] Actually, the photosensitized oxidation of **1** was efficiently achieved in the bulk solution, with both (*R*)-PPN and (*R*)-NPX. However, protein encapsulation provided an exceptional microenvironment protecting **1** from the attack by externally generated $^1O_2$, using the unbound photosensitizers (Figure S4 in SI).

After the photobehavior of **1** in the presence of proteins was established, the formation of **1**@SA and **1**@AAG complexes was investigated. Their stoichiometry was established by means of Job plot analysis.[19] Thus, the absorbance of **1**/protein mixtures was plotted versus the

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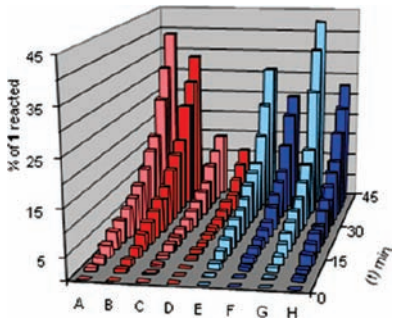

Figure 1. Kinetics for direct photooxidation of **1** (λ_{exc} = 350 nm) in protein media. (*S*)-**1**: (A) HSA/D_2O, (B) HSA/PBS, (E) BSA/D_2O, (F) BSA/PBS. (*R*)-**1**: (C) HSA/D_2O, (D) HSA/PBS, (G) BSA/D_2O, (H) BSA/PBS.

protein molar fraction. In all cases, a maximum at $\chi_{protein}$ = 0.5 was reached, revealing the formation of 1:1 complexes (see Figure S5 in SI).

The fluorescence behavior of **1**@SAs was markedly different from that of free **1**. For HSA, addition of increasing amounts of protein to a PBS solution of **1** resulted in a significant decrease of the fluorescence intensity. This process was clearly configuration dependent, with a more pronounced effect for (*R*)-**1** (Figure 2). Similar results were found for **1**@BSA complexes; by contrast, in the case of AAGs changes were less important (see Figure S6 in SI).

Laser flash photolysis (LFP) experiments were performed to explore the possibility of using the triplet excited states of **1** (**1*** (T_1)) as reporters for its binding to proteins. As in the case of 9-anthraceneacetic acid,[14] **1** showed a very complex transient absorption spectrum in aqueous solution (Figure 3, inset); however, for equimolar **1**/protein mixtures, only the band attributed to the first triplet excited state was observed (see Figure 3a for the case of (*S*)-**1** in HSA). Under aerobic conditions, the triplet lifetimes (τ_T) of both enantiomers, monitored at 420 nm, were found to be markedly longer in the protein media (see Figure 3b and Table 1, as well as Figure S7 in SI). The observed τ_T lengthening can be attributed to a slower deactivation of the species inside the protein pockets, where the microenvironment provides protection from attack by oxygen.[20]

As expected, triplet lifetimes were markedly longer under an inert atmosphere (see Table 1). Interestingly, in the case of SAs, two intraprotein microenvironments were revealed by two different triplet lifetimes; this was explained taking into account that SAs are reported to contain two main binding sites for small organic molecules, namely sites I and II.[21] Assignment was achieved based on the presence of a Trp residue at site I of the albumins. In

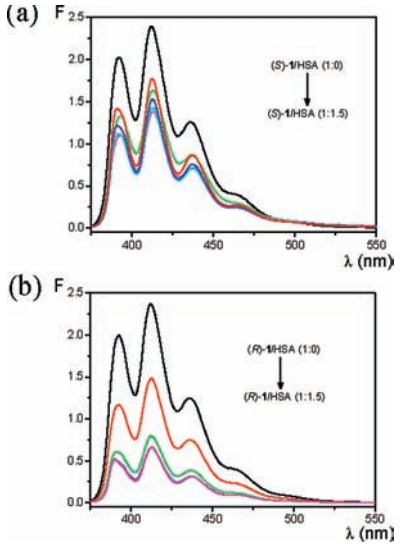

Figure 2. Fluorescence spectra of **1** in the presence of increasing amounts of HSA, up to a 1:1.5 molar ratio for (a) (*S*)-**1** and (b) (*R*)-**1**.

PBS solution this amino acid was found to quench **1*** (T_1) (Figure S8 in SI), with a rate constant of 1.6×10^8 M^{-1} s^{-1}. Therefore, the longer lifetime in the presence of SAs was ascribed to **1** located in site II, while the shorter one was safely assigned to **1** within the Trp containing site I. By contrast, only one lifetime was needed to fit the decays in the presence of AAGs, indicating only one binding site, in agreement with the expectations from the literature.[22]

According to the photooxidation mechanism (Scheme 1) a number of processes (i–v) should in principle be taken into account to anticipate the reactivity of **1**. The intersystem crossing quantum yields (Φ_T) and rate constants of quenching by oxygen (k_q O_2) would provide quantitative data related to steps iii and iv, respectively. The values obtained for these parameters are also given in Table 1. It is interesting to note that the highest Φ_T was found for PBS. In general, the presence of proteins resulted in a reduced Φ_T, which correlates well with the lower photoreactivity. As regards the accessibility of oxygen to **1** in protein media, quenching of **1*** (T_1) by oxygen was also investigated. Thus, the decay traces obtained at 420 nm under a nitrogen, air, and oxygen atmosphere were fitted to obtain the corresponding triplet lifetimes. The oxygen quenching constants (k_q O_2), obtained by Stern–Volmer analysis,[23]

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Table 1. Relevant Photophysical Parameters Obtained for the Two Enantiomers of **1** under Different Conditions

substrate	protein	Φ_F[a]	τ_F (ns)[a]	Φ_T[a]	τ_T (μs)[b]	τ_T (μs)[a]	k_q O_2 $\times$ 10^6 (M^{-1} s^{-1})
(*S*)-**1**	None	0.40	10.5	0.51	115	2.8	1100
(*S*)-**1**	HSA	0.25	9.8	0.35	50, 379	18, 107	110, 19
(*S*)-**1**	BSA	0.31	11.6	0.39	28, 237	20, 126	110, 16
(*S*)-**1**	HAAG	0.39	11.8	0.44	98	27	120
(*S*)-**1**	BAAG	0.39	11.2	0.33	124	28	120
(*R*)-**1**	None	0.40	10.5	0.51	115	2.8	1100
(*R*)-**1**	HSA	0.10	8.1	0.25	44, 311	20, 109	140, 23
(*R*)-**1**	BSA	0.34	12.1	0.39	32, 312	24, 139	55, 10
(*R*)-**1**	HAAG	0.37	12.2	0.45	99	29	120
(*R*)-**1**	BAAG	0.39	13.6	0.36	121	33	130

[a] Measurements performed under air. [b] Measurements performed under nitrogen.

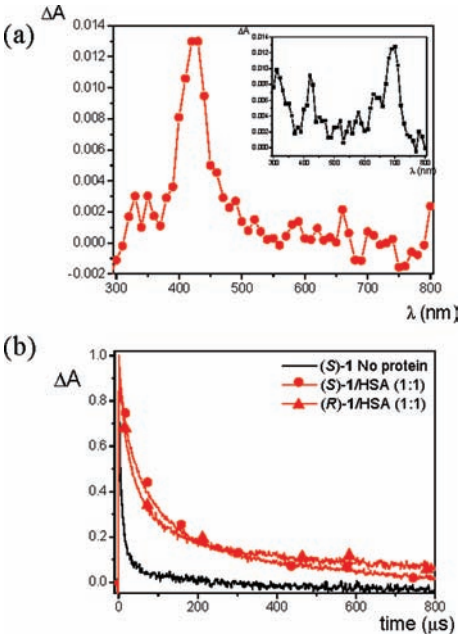

Figure 3. Laser flash photolysis in PBS, under air. (a) Transient absorption spectra of (*S*)-**1** in the presence of HSA, at 1:1 molar ratio, recorded 2.9 μs after laser pulse. Inset: Spectrum obtained in the absence of protein, 1 μs after the laser pulse. (b) Normalized decays monitored at 420 nm for (*S*)-**1** and (*R*)-**1**, in the absence and presence of HSA at a 1:1 molar ratio.

were in the range 1.0×10^7–1.4×10^8 M^{-1} s^{-1}, markedly lower than in PBS solution (Table 1). This is also consistent with the lower photooxidation rate inside the proteins. Furthermore, different quenching constant values were obtained within sites I and II for SAs; the photoprocess was in general much slower in the latter (see Figure S9 in SI).

In summary, a remarkable stereodifferentiation is observed for the photooxidation of **1** encapsulated within serum albumins and α-1-acid glycoproteins. The observed photoreactivity is markedly compartmentalized, with higher reaction rates in the bulk solution than within the protein microenvironments. The reaction is mediated by $^1O_2$ and only occurs in the compartment where this reactive oxygen species is generated. Finally, quenching of the triplet excited state of **1** by molecular oxygen, required to produce $^1O_2$, is much faster in site I than in site II of serum albumins.

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Supporting Information Available. Characterization data and experimental procedures for the synthesis of **1**, irradiation procedures, Job plot diagrams, fluorescence measurements, laser flash photolysis experiments, Stern–Volmer plots for oxygen quenching, and X-ray crystallographic data for the 2-bromophenyl ester of (*S*)-**1**. This material is available free of charge via the Internet at http://pubs.acs.org.