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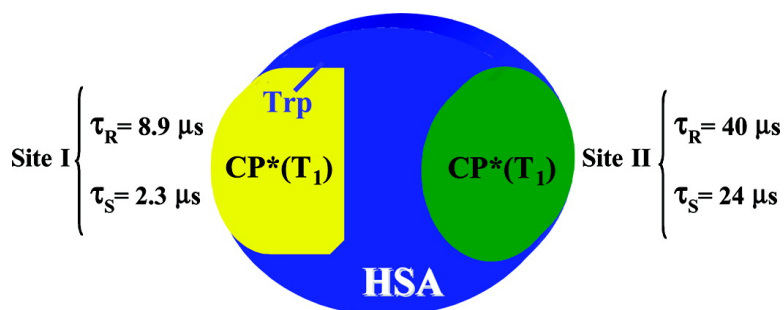
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Human Serum Albumin-Mediated Stereodifferentiation in the Triplet State Behavior of (*S*)- and (*R*)-Carprofen

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In the past decade, an increasing amount of research effort has been devoted to asymmetric photochemistry.[1,2] Photoresolution of racemic mixtures[3] and photochirogenesis[2] has attracted considerable attention; however, direct photophysical evidence for chiral discrimination in the triplet excited states has only been found in a few cases.[4–6]

Chiral environments provided by synthetic[7] or natural[8] host molecules have been shown to induce asymmetric photoreactions. Studies in the presence of biomolecules are of special interest, as a detailed knowledge of stereodifferentiating photoprocesses is essential for the design of new chiral drugs and therapeutic agents. On the basis of this knowledge, it should be possible to improve the efficiency of photodynamic therapy and to decrease the photosensitivity side effects induced by photolabile drugs such as the widely used nonsteroidal anti-inflammatory drugs (NSAIDs).[9] Despite the significance of such a study, the photobiological properties of chiral drugs remain practically unexplored.[10] Recently, bichromophoric compounds, designed to mimic interactions between chiral drugs and lipids or proteins, have exhibited a high diastereodifferentiation in the intramolecular quenching of the drug triplet state.[5] An analogous chiral recognition at the intermolecular level, i.e., the possibility of stereodifferentiating interactions between the drug triplet excited states and biomolecules, has not yet been reported.

In this context, we have now investigated the photoreactivity of the NSAID carprofen (Chart 1, CP) in the presence of human serum albumin (HSA). Unlike the other 2-arylpropionic acids, the major CP photodegradation pathway is not decarboxylation, but dehalogenation to PP; this allows preservation of the chiral center. Moreover, CP singlet and triplet states are efficiently formed and well-characterized.[11] Finally, the dark binding sites and affinity constants of CP stereoisomers to HSA have been previously reported.[12]

Dynamic studies on the interaction between HSA (25 μM) and (*R*)- or (*S*)-carprofen (25 μM) in phosphate buffered solution (0.137 M NaCl, pH = 7.4, PBS) were performed by nanosecond laser flash photolysis (LFP) using a 308-nm Xe/HCl/Ne excimer laser. The obtained transient absorption spectra were similar for both stereoisomers (λ_{max} = 450 nm, Figure 1 inset) and were assigned to the triplet–triplet transition of CP by comparison with data previously reported for the racemic drug in solution.[11] This assignment was further supported by oxygen quenching. It is noteworthy that the N-centered carbazolyl radical (λ_{max} = 640 nm), formed upon LFP of CP alone in PBS, was not observed under these conditions.

As shown in Figure 1, the presence of HSA induced a significant stereodifferentiation of CP triplet state lifetimes. The decays monitored at 450 nm appeared to be biphasic in both cases with a long-lived major component (τ_2, A_2) and a shorter-lived minor

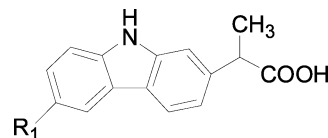**Chart 1.** Structures of Carprofen (CP, R_1 = Cl) and of Its Photoproduct (PP, R_1 = H)

Table 1. Photophysical and Photochemical Properties of (*R*)- and (*S*)-Carprofen in the Presence of Human Serum Albumin

	τ_1 (μs)[a]	τ_2 (μs)[a]	A_2/A_1[b]	II/I[c]	% PP[d]
(*R*)-CP	8.9	40	4.8	5	30
(*S*)-CP	2.3	24	10.8	10	43

[a] Lifetimes were measured under argon. [b] A_1 and A_2 were the components with lifetimes τ_1 and τ_2. They did not change significantly when [HSA] was increased up to 40 μM, keeping [CP] at 25 μM. [c] Distribution of CP in HSA-binding sites (site II /site I) calculated from the nK values given in ref 12, estimated by equilibrium dialysis. [d] Formation of the photoproduct PP determined by reverse-phase HPLC.
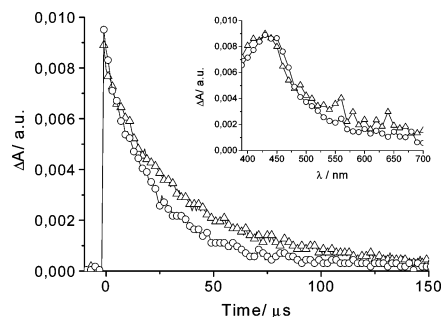

Figure 1. Decays monitored at 450 nm after LFP of anaerobic buffered solutions of (*R*)- or (*S*)-CP (25 μM) in the presence of HSA (25 μM). Inset: Transient spectra obtained 5 μs after laser excitation (308 nm). ($\triangle$) (*R*)-CP/HSA mixture. (○) (*S*)-CP/HSA mixture.

component (τ_1, A_1) (Table 1). The stereodifferentiation factor was more remarkable for the transients with shorter lifetimes ($\tau_{1,R}/\tau_{1,S}$ ca. 4).

Whatever the component considered, a dramatic lengthening of the lifetime was observed in comparison with CP alone in PBS (τ = 0.18 μs). This could be partially due to the more rigid surrounding and to the suppression of CP self-quenching, typical of chlorocarbazole derivatives.[13] The above findings, together with the relative contribution of the two components (A_1 and A_2, Table 1), can be clearly correlated with the presence of two binding sites in HSA.[12] Indeed, noncovalent binding (i.e., complex formation) of CP stereoisomers to HSA, in the absence of light, has been reported. The high affinity site, namely site II, is primarily populated with a slight preference for (*S*)-CP. Furthermore, the distribution of (*S*)- and (*R*)-CP in each site (see column II/I in Table 1) is in excellent agreement with the A_2/A_1 ratios under the employed nonsaturating
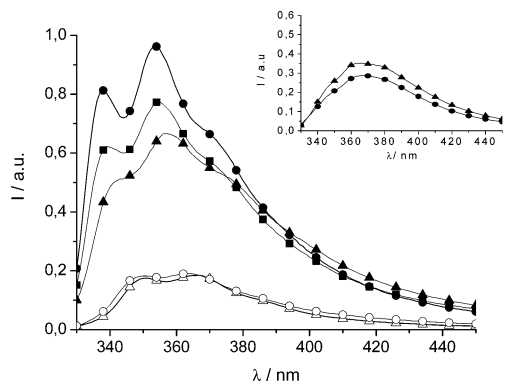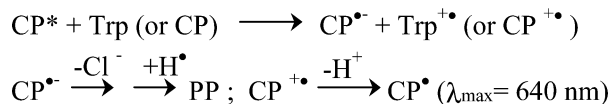
Figure 2. Emission spectra (λ_{exc}= 320 nm) of aerated (R)-CP/HSA (△), (S)-CP/HSA (○), or racemic CP/HSA (□) mixtures before (open symbols) and after UVA irradiation (closed symbols). Inset: emission spectra of (R)- and (S)-CP/HSA photomixtures after sephadex filtration.

Scheme 1. Mechanism of CP Quenching and PP Formation

$$\text{CP*} + \text{Trp (or CP)} \longrightarrow \text{CP}^{\bullet-} + \text{Trp}^{+\bullet} \text{ (or CP}^{+\bullet})$$

$$\text{CP}^{\bullet-} \xrightarrow{\text{-Cl}^-} \xrightarrow{\text{+H}^\bullet} \text{PP} \; ; \; \text{CP}^{+\bullet} \xrightarrow{\text{-H}^+} \text{CP}^\bullet \; (\lambda_{max}= 640 \text{ nm})$$

conditions, strongly suggesting that the two components of the biphasic decays correspond to the CP triplet state in the two binding sites.

In this context, a special role must be played by tryptophan (Trp), not only because it is the most efficient amino acid able to quench CP triplet (k_q ca. 6×10^8 M^{-1} s^{-1}) but also because HSA contains only one Trp unit located in site I. So, the observed shortening of τ_1 if compared to τ_2 could be explained by the neighborhood of Trp that would act as electron donor generating the radical anion of CP. Subsequent loss of chloride would lead to an aryl radical, the immediate precursor of the photoproduct PP (Scheme 1). In the absence of Trp, ground-state CP would be the electron-donating species (self-quenching), thereby giving rise to the radical ion pair CP$^{\bullet+}$/CP$^{\bullet-}$. This explains why the carbazolyl radical, generated upon LFP of CP alone (via deprotonation of the radical cation), is absent in the case of the CP/HSA complexes.

The observed stereodifferentiating interaction between CP triplet state and HSA pointed to the possibility of a stereoselective process during protein photosensitization by this chiral drug. It has previously been shown that fluorescence coupled with sephadex filtration is the method of choice to study photobinding of racemic CP to protein.[14] In the present work, no difference was found between the emission spectra of (R)- and (S)-CP in the presence of HSA. The lifetimes (1.2 ns for both isomers) were the same as in the absence of HSA. However, after UVA irradiation, (S)-CP gave rise to a more intense and better-structured spectrum than its (R)-enantiomer (Figure 2). After sephadex filtration, only the fluorescence attributable to covalent photobinding remained in the protein fraction. Besides, the fine structure was lost, and the emission intensity was somewhat higher in the case of (R)-CP (Figure 2, inset).

Irradiation of carprofen alone in PBS led to a decrease in the fluorescence intensity because of polymerization of the drug. In the presence of HSA, the observed increased emission spectra point to a major role of the protein in the photolysis of CP. The most salient features of Figure 2 are the different shapes and intensities of the emission spectra of (R)- and (S)-CP/HSA photomixtures prior

to sephadex filtration. The fine structure of the spectra can be attributed to the presence of dechlorinated carprofen (PP) whose emission is more intense and blue-shifted than that of CP.

To check this hypothesis and to investigate a possible stereodifferentiation in the formation of the dehalogenated photoproduct PP, aerated solutions of the CP-stereoisomers were UVA-irradiated in the presence of HSA and analyzed by chiral HPLC. The only photoproduct detected, concomitantly with consumption of CP, was actually identified as PP. A clear stereodifferentiation was observed in photoproduct formation that occurred ca. 1.5 times more efficiently from (S)-CP/HSA than from its (R)-counterpart (Table 1). The experiment was also performed with racemic CP. As expected, formation of (S)-PP was faster than that of (R)-PP (ratio ca. 1.4). This is consistent with the relative intensities of the spectra shown in Figure 2.

In summary, the most significant result of this work is the remarkable enantiodifferentiation in the interaction between CP triplet state and HSA. The appearance of two components with different lifetimes is explained by complexation to the two binding sites.

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