

Photosensitization by chiral drugs: Looking for stereodifferentiating photoprocesses in the presence of biomolecules*

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Abstract: Photoreactivity of chiral carprofen (CP) and ofloxacin in the presence of two biomolecules, namely, DNA and human serum albumin (HSA), has been reported. Analysis of the photosensitization of 2'-deoxyguanosine and thymidine (Thd) by high-performance liquid chromatography has shown that racemic ofloxacin and levofloxacin [its (*S*)-stereoisomer] acts by a mixed type I/type II mechanism, while CP does not lead to significant degradation of the nucleosides. Studies on DNA relaxation have revealed formation of single-strand breaks and specific alterations of DNA bases. Ofloxacin and levofloxacin photoinduce direct single-strand breaks and formation of purine and pyrimidine oxidative photoproducts; no Thd dimer has been detected. CP produces only photosensitized single-strand breaks. Moreover, DNA photosensitization has shown a weak enantiodifferentiation in favor of levofloxacin and (*S*)-CP.

In the case of HSA, a remarkable stereodifferentiation has been found in the interaction between the excited triplet state of CP and protein. Time-resolved laser flash photolysis measurements revealed the presence of two components with different lifetimes that have been assigned to complexation of CP to the two binding sites of albumin. Moreover, photobinding of the drug to protein and formation of the dehalogenated photoproduct of CP proceed via stereodifferentiating photoprocesses.

Keywords: stereodifferentiation; stereoselectivity; DNA photosensitization; human serum albumin; photobinding; carprofen; ofloxacin; levofloxacin.

INTRODUCTION/BACKGROUND

Asymmetric photochemistry is becoming an important field of organic chemistry as it can be considered an attractive alternative to basic asymmetric synthesis. The first enantiodifferentiating photolysis was reported at the end of the 18th century when production of an excess of a particular enantiomer was observed after irradiation of the racemic mixture with circularly polarized light. Since then, several strategies (use of chiral chemical sources, supramolecular systems, etc.) have been employed to induce chirality [1]. For the photobiologist, asymmetric chemistry is of special interest when chirality is provided by biomolecules.

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Chirality provided by biomolecules

Asymmetric photoreactions induced by nucleosides and DNA have been reported for the photoisomerization of *Z*-cyclooctene [2]. It was proven that the supramolecular complex formed in the ground state between DNA and *Z*-cyclooctene is an important factor to achieve high enantiomeric excess.

Albumins are also biomolecules of interest because of their ability to bind substrates in their hydrophobic pockets. The differences between the binding affinities of (*R*)- and (*S*)- ketoprofen (KP) and binaphthols to bovine serum albumin (BSA) have been exploited to achieve the photoresolution of racemic mixtures [3]. Very low photoenrichment has been observed with KP, while efficient photoresolution of binaphthols has been performed. More recently, supramolecular photochirogenesis using BSA and the prochiral 2-anthracenecarboxylate as substrate has been reported [4]. It has been shown that irradiation in the presence of the protein inverts the regioselectivity of the photocyclodimers formation and gives good enantiomeric excess.

Asymmetric photosensitization of biomolecules

Asymmetric photosensitization of biomolecules can help to improve the benefit-to-risk ratio of drugs. Nowadays, an increasing trend consists in introducing the enantiomerically pure form of drugs to minimize the toxic effect mediated by the less active enantiomer. Among the adverse effects, drug photosensitivity is described with increasing frequency. So, a possible stereoselective photoprocess during biomolecule sensitization would inform about the most photoactive stereoisomer, providing additional data to consider in the switching toward pure enantiomeric drugs.

In this aim, models mimicking interactions between photoactive drugs, namely, nonsteroidal anti-inflammatory drugs (NSAIDs), and biomolecules have been synthesized. The ability of KP to realize lipid peroxidation has been modeled by a bichromophoric compound, including a KP unit and a derivative of cyclohexadiene in the role of the fatty acid [5]. Stereoselective intramolecular hydrogen abstraction by the benzophenone part of KP has been observed, the (*S,R*)-enantiomer being more efficient than the (*S,S*). Another interesting example consists in the reproduction of the drug-protein preassociation by the bichromophores tiaprofenic acid-tryptophan and suprofen-tryptophan [6]. These two anti-inflammatory agents are regioisomers as they contain the same benzoylthiophene moiety substituted either at the thiophene part or at the benzoyl part. The intramolecular electron transfer was shown to occur regioselectively as well as stereoselectively. Similar results have been obtained with tiaprofenic acid-tyrosine and suprofen-tyrosine bichromophores [7].

OBJECTIVES

The objectives of this work were to look for a possible differentiation in the photosensitization of biomolecules by chiral drugs. Until now, investigation of stereoselective photoprocess has never been carried out in the case of whole biomolecules.

The fluoroquinolones, such as ofloxacin (the racemic mixture) and its commercially available (*S*)-stereoisomer (known as levofloxacin), are antibiotic drugs effective against a broad spectrum of bacteria. They have been reported for their ability to induce phototoxicity, photoallergy, and photocarcinogenicity [8]. Mechanistic DNA photosensitization by racemic ofloxacin has been described to proceed mainly via a type II (singlet oxygen) mechanism. Studies of isolated and cellular DNA showed a mixed type I/type II mechanism as alkali-labile sites, 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodGuo), and oxidized pyrimidines have been revealed [9].

The second drug is the NSAID carprofen (CP). NSAIDs are largely used in the treatment of pain. One of their adverse effects is their potential to induce gastrointestinal disorders. Nevertheless, their ability to produce skin photosensitivity should not be neglected, as severe cases of phototoxicity and

photoallergy have frequently been reported [10]. Although CP is one of the most photoactive NSAIDs [10], its DNA-photosensitizing properties have not been reported until recently [11].

In the present work, we will first present photosensitization of DNA and isolated nucleosides by chiral ofloxacin and CP. Then, the attention will be directed toward interaction between CP enantiomers and human serum albumin (HSA) with a study including photophysics, photochemistry, and drug (photo)binding to HSA.

DNA PHOTSENSITIZATION BY CHIRAL DRUGS [11]

Photosensitized DNA damage can be associated with mutagenic or carcinogenic effects. So, study of DNA photosensitization by sunlight-absorbing drugs is necessary to assess the photobiological risk of such compounds.

Ofloxacin and levofloxacin

Photoreactivity of ofloxacin and levofloxacin toward 2'-deoxyguanosine (dGuo) and thymidine (Thd) has been studied.

dGuo is an excellent model to assess the mechanisms involved in the photoreaction, as it contains the only nucleobase that can suffer both type I and type II mechanisms. The former mechanism, involving radical formation, gives mainly rise to the formation of an oxazolone, while singlet oxygen addition (type II mechanism) to the nucleobase leads to the formation of the diastereoisomers of spiroiminodihydantoin (dSp). Irradiation with UVA of dGuo in the presence of ofloxacin or levofloxacin led to consumption of the nucleoside (almost complete in 5 h) and to the formation of the oxazolone and dSp. So, a mixed type I/type II mechanism was occurring, but no significant difference has been found between the racemic mixture and the (*S*)-stereoisomer. Consumption of Thd (30 % after 5 h) was slower than that of dGuo and gave rise to the typical pattern of oxidation photoproducts of Thd [11]. Moreover, formation of thymine, resulting from the cleavage of the glycosidic bond of the nucleoside, has also been observed. In this case, a stereoselective photoprocess was occurring, levofloxacin being a slightly more efficient photosensitizer than ofloxacin.

Supercoiled circular DNA (pBR322) is a sensitive tool to detect structural alterations as single-strand breaks or specific DNA damage formation. DNA single-strand breaks can be observed directly, while specific DNA base damage is revealed by means of DNA repair enzymes. Endonuclease III, Formamidopyrimidine DNA-glycosylase (Fpg), and T4 endonuclease V are the commonly used enzymes to recognize oxidized pyrimidine, 8-oxodGuo (the major oxidative damage occurring by type I or type II photosensitization of DNA) and cyclobutane thymine dimers (derived from a triplet-triplet energy transfer mechanism), respectively.

UVA irradiation of pBR322 in the presence of racemic ofloxacin or levofloxacin induced single-strand break formation in a concentration-dependent way. Experiments with DNA repair enzymes showed that 8-oxodGuo was by far the most important photodamage followed by oxidized pyrimidines. By contrast, no thymine dimer formation has been observed. The distribution of the DNA damages is in agreement with the mixed type I/type II mechanism found for the photosensitization of nucleosides. The lack of thymine dimer is relevant of the fact that the ofloxacin triplet state might lie below that of thymine.

(*R*)- and (*S*)-CP

No degradation of Thd or dGuo was detected after UVA irradiation in the presence of CP stereoisomers, a plausible explanation being that polymerization of the drug [12] is a more efficient process than its photoreaction with the nucleosides.

A time-dependent, single-strand break formation has been observed, (*R*)-CP being somewhat more active than its (*S*)-stereoisomer. The use of repair enzymes did not clearly show formation of specific base damages.

PHOTOREACTIVITY OF CP IN THE PRESENCE OF HSA [13]

Interactions between chiral CP and HSA have been investigated by nanosecond laser flash photolysis. The same transient spectra ($\lambda_{\text{max}} = 450 \text{ nm}$) was present for both stereoisomers/HSA solutions. This transient was assigned, by comparison with the spectrum of CP in phosphate-buffered saline (PBS), to the triplet–triplet transition of the drug. Absorption corresponding to formation of the carbazolyl radical, generally detected upon photolysis of CP alone in PBS, has not been observed. The biphasic triplet decays exhibit a remarkable stereodifferentiation, which is more pronounced for the short-lived component than for the long-lived one. Moreover, a dramatic lengthening of the lifetime was observed when compared with CP alone in PBS. This can be explained by the more rigid surrounding of the drug when complexed to albumin, but also by the suppression of the typical self-quenching of carbazole derivatives [14]. Treatment of the biphasic decay clearly evidenced that a correlation can be done between the presence of two lifetime components and that of two binding sites in HSA. Dark binding of CP stereoisomers to HSA has previously been reported [15]. The high affinity site, namely, site II, is primarily populated, and a slightly higher constant affinity has been described for the (*S*)-CP. The excellent agreement between distribution of (*S*)- and (*R*)-CP in each binding site (II/I in table of Fig. 1) and the ratios of the two component lifetimes (A_2/A_1) suggested that the two components of the biphasic decay correspond to the CP triplet state in the two binding sites of the protein (scheme of Fig. 1).

It is noteworthy that tryptophan is the most efficient amino acid in the quenching of CP triplet state. In addition, it plays a particular role in HSA, which contains only one tryptophan situated in the low affinity site, namely, site I. So, the shortening of τ_1 if compared with τ_2 could be explained by the neighborhood of tryptophan. This data, together with the A_2/A_1 ratio, strongly supports the previous hypothesis of the distribution of CP triplet in each binding site of HSA (scheme of Figure 1).

	τ_1 (μs)	τ_2 (μs)	A_2/A_1	II/I
(<i>R</i>)-CP	8.9	40	4.8	5
(<i>S</i>)-CP	2.3	24	10.8	10

Site I

 $\left\{ \begin{array}{l} \tau_{1,R} = 8.9 \mu\text{s} \\ \tau_{1,S} = 2.3 \mu\text{s} \end{array} \right.$

HSA

Site II

 $\left\{ \begin{array}{l} \tau_{2,R} = 40 \mu\text{s} \\ \tau_{2,S} = 24 \mu\text{s} \end{array} \right.$

Fig. 1 The table reports photophysical and photochemical properties of (*R*)- and (*S*)-CP in the presence of albumin. τ_1 and τ_2 are the lifetimes measured under argon; A_1 and A_2 are the components with lifetimes τ_1 and τ_2 , respectively; II/I are the ratios of CP distribution in the binding sites of albumin (obtained from ref. [15]). The scheme represents the proposed distribution of CP triplet states in HSA.

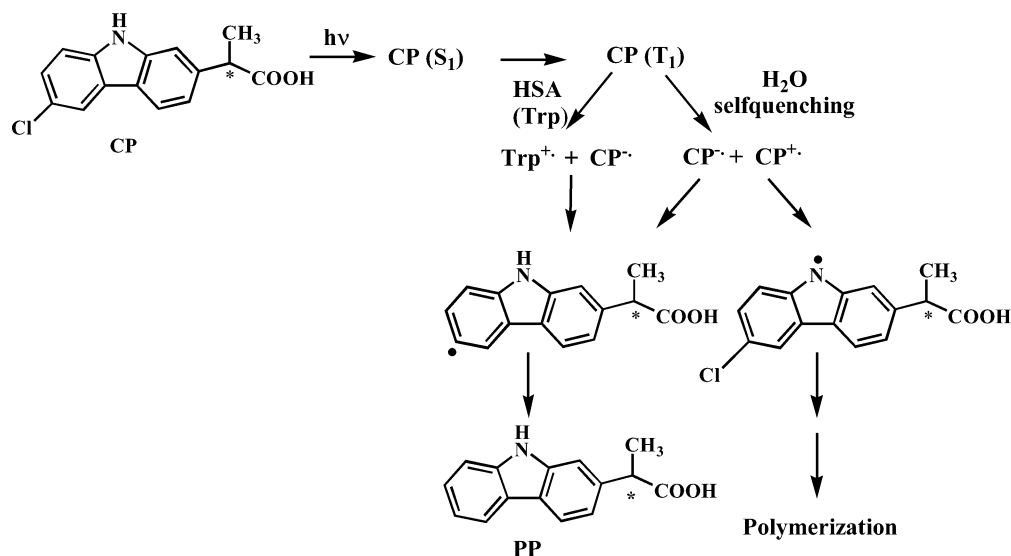
The possibility of a stereoselective process during protein photosensitization by chiral CP is strongly supported by the observed stereodifferentiating interaction between the CP triplet state and

albumin. Previous studies have demonstrated that fluorescence coupled with sephadex filtration was a method of choice to study the photobinding of CP to HSA [16]. Emission spectra of both stereoisomers in the presence of HSA are similar ($\lambda_{\max} = 360$ nm). Nevertheless, after UVA irradiation, an increase of the emission intensity has been observed, and (*S*)-CP showed a more intense and better-defined spectrum than the (*R*)-stereoisomer. After sephadex filtration, the fluorescence present in the protein fraction can be attributed to that of the covalent photoadducts CP-HSA. In this case, the fine structure was lost and the emission intensity of (*R*)-CP appeared to be somewhat higher than that of its (*S*)-enantiomer.

Before sephadex filtration, the increase of the emission intensity pointed to a particular role of the protein in the photolysis of the drug, as CP irradiated alone in PBS exhibits a very low emission due to the polymerization of the drug. Moreover, a special interest should be given to the difference of shape and intensity of the emission spectra of both stereoisomers. The well-defined structure of the spectrum can be correlated with that of the dechlorinated photoproduct of carprofen (PP). This hypothesis was checked by high-performance liquid chromatography (HPLC) and the only photoproduct detected has been identified as the dehalogenated PP. In accordance with the difference in the fluorescence intensity between (*R*)-CP/HSA and (*S*)-CP/HSA, photoproduct formation has shown a clear stereodifferentiation as PP is formed ca. 1.5 times more efficiently from (*S*)-CP/HSA mixtures.

Mechanism proposed

A plausible mechanism for CP photolysis includes the involvement of tryptophan (Scheme 1).



Scheme 1 Mechanism of PP formation.

In aqueous solution, CP undergoes an efficient self-quenching leading to the formation of the radical ion pair that will suffer deprotonation (cation) and dehalogenation (anion), respectively. Deprotonation of the radical cation will give rise to the carbazoyl radical observed during laser flash photolysis of CP in PBS. Further radical recombination will lead to the polymerization of the drug.

In the presence of HSA, CP photolysis follows a different pathway. CP triplet state will react with HSA, and particularly with tryptophan, avoiding CP self-quenching and leading to the formation of the radical anion of the drug. This hypothesis is supported by the lack of the carbazoyl radical formation

in the presence of albumin or of an excess of tryptophan. Dehalogenation process will finally give rise to the photoproduct PP.

CONCLUSIONS

Stereoselective photoprocesses during nucleosides and DNA photosensitization have appeared to be low. A slightly higher efficiency has been found for (*R*)-CP and levofloxacin.

The most salient feature in this work is the remarkable stereodifferentiation in the photophysical, photochemical, and photobiological studies of CP associated with HSA.

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