Supramolecular photochemistry of 2-(3-benzoylphenyl)propionic acid (Ketoprofen). A study in the β-cyclodextrin cavity

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The photochemistry of 2-(3-benzoylphenyl)propionic acid (Ketoprofen) has been studied in the β-cyclodextrin cavity by stationary and time-resolved (picosecond and nanosecond) spectroscopic techniques. Conformational calculations of the inclusion complex were performed. Induced circular dichroism was measured and theoretically interpreted. Photodecarboxylation was found to occur with lower quantum yield than in aqueous medium. An additional photoreaction was evidenced. A mechanism in which the lowest triplet state of the Ketoprofen–β-cyclodextrin inclusion complex undergoes both intramolecular electron transfer and hydrogen abstraction is proposed. Adducts of reduced ketoprofen with β-cyclodextrin are the likely photoproducts of the additional reaction channel.

2-(3-Benzoylphenyl)propionic acid (Ketoprofen, KPF) is a non-steroidal anti-inflammatory drug frequently used for therapeutic topical applications. Despite its pharmaceutical use this compound is known to act as photosensitizer for biological substrates both *in vivo* and *in vitro*.[1,2] Lipid peroxidation, hemolysis of red blood cells and DNA cleavage were shown to be the most frequent dangerous effects. A complex mechanism involving free radicals, singlet oxygen and lytic photoproducts was invoked.[2] The toxic effects of KPF were drastically attenuated *in vitro* by β-cyclodextrin (β-CD) complexation.[3] This observation suggested the possible use of this host saccharide as a tool to minimize the photosensitizing action in therapeutic conditions.[3]

The process at the basis of the phototoxic reactions of KPF was recognized to be a very efficient photodecarboxylation (quantum yield: $\Phi_{dc} = 0.75$) occurring in aqueous solutions from the dissociated acid form and leading to (3-benzoylphenyl)ethane, (3-benzoylphenyl)ethyl hydroperoxide, (3-benzoylphenyl)ethanol and (3-benzoylphenyl)ethanone in the presence of oxygen and only (3-benzoylphenyl)ethane in the absence of oxygen.[1,4]

A recent review on photodecarboxylation of acids points out that in NSAID molecules several mechanisms may apply, depending on the structure of the arylacetic acid moiety.[5] A detailed study of the transients involved in the photochemistry of KPF in neutral aqueous solutions led us to propose an adiabatic mechanism as the main photodecarboxylation path.[6] The process is initiated in the triplet state of the dissociated acid form by a fast intramolecular electron transfer from the carboxyl to the carbonyl group (lifetime of the triplet: 250 ps). Release of carbon dioxide then proceeds in a triplet biradical with formation of a further triplet species, very reactive with oxygen, which converts to the final products.

To gain insight into the influence of the environment on the photodecarboxylation process and to contribute to the assessment of the molecular basis of the photoprotective action of β-CD, we carried out a study of the steady-state photodegradation of the KPF–β-CD inclusion complex and of the transients formed in the picosecond and nanosecond time domains. Induced circular dichroism (ICD) was measured and conformational calculations were performed. The production of singlet oxygen was investigated to shed light on the role of this species in the phototoxic effects.

As already reported for a large variety of systems,[7] β-CD was found to be able to modify the photochemistry of KPF. A rationale of the biological effects obtained by inclusion of the drug in the β-CD cavity is reached.

Experimental

Materials

Ketoprofen, as a racemic mixture of the enantiomers (Sigma), and β-cyclodextrin (Serva) were used as received. Benzophenone (Baker) was recrystallized three times from ethanol. D_2O was purchased from Carlo Erba. Water was purified by passage through a Millipore MilliQ system or purified by triple distillation. Phosphate buffer (10^{-2} mol l^{-1}, pH 7.4) was used.

Methods

Nuclear magnetic resonance (^{1}H NMR) spectroscopy was performed by means of a 200 MHz apparatus. Ultraviolet absorption spectra were measured on a Perkin–Elmer Lambda 9 spectrophotometer and circular dichroism spectra were obtained with a Jasco J-715 dicrograph. The experimental setup and the procedures used for the picosecond and nanosecond transient absorption experiments, as well as for the time-resolved IR emission, have been accurately described before.[5]

Steady-state photolysis. Steady-state irradiation was performed with light of 310–390 nm. The fluence at the sample position was 800 μW cm^{-2} and the photon flux incident on a 3 ml solution in the quartz cuvettes (1 cm optical path) was 3×10^{16} quanta s^{-1}. The light intensity was measured with a ferrioxalate actinometer[8] and a digital radiometer spectroline Model DRC-100X equipped with a sensor DIX-365, having a spectral range of 320–380 nm. All the experiments were carried out at 25 °C. The experimental procedures for irradiation and light intensity measurements have been described previously.[1] The quantum yields were measured in samples (3 ml) consisting of Ar saturated solutions of KPF (0.25×10^{-3}

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mol l^{-1}) alone or in the presence of increasing amounts of β-CD, in phosphate buffer, irradiated so as to achieve *ca.* 20% conversion of the starting material. After irradiation the samples were acidified with addition of 0.5 mol l^{-1} HCl up to pH 3.5 and extracted with chloroform (5 × 3 ml). With this procedure β-CD remained almost quantitatively in the aqueous phase. The extracts were evaporated at 40 °C and stored *in vacuo*. Quantitative analysis of KPF and its photoproducts was performed by high performance liquid chromatography (HPLC). The separation of the photoproducts were achieved on a Hypersil column (100 × 4.6 mm, 5 μm packing) using as the mobile phase 95 : 5 ethyl acetate–methanol for the starting compound and 20 : 80 ethyl acetate–cyclohexane for the photoproducts. For further details about the separation of KPF and its photoproducts see ref. 1. HPLC analysis was performed on a Water 600 E multisolvent delivery system fitted with a Jasco 820 FP intelligent spectrofluorimeter and a Water 490 E programmable multiwavelength UV detector. The uncertainty, related to the degree of reproducibility of the whole experiment, was within 20%.

Results

Absorption and induced circular dichroism spectra

The absorption spectrum of KPF in phosphate buffer is characterized by a band with λ_{max} at 260 nm ($\varepsilon = 15\,700$ l mol^{-1} cm^{-1}) attributed to the $S_0 \rightarrow S_2$ transition of π,π* nature, and by a lower energy shoulder in the 300–350 nm region corresponding to the $S_0 \rightarrow S_1$ forbidden transition of *n*,π* parentage. Addition of β-CD (10^{-2} mol l^{-1}) causes both a shift to the blue of the maximum by *ca.* 3 nm and a decrease of the molar absorption coefficients of the $S_0 \rightarrow S_2$ band and leaves the $S_0 \rightarrow S_1$ band almost unchanged. These spectral variations, very similar to those observed in the case of benzophenone (BP),[9] point to a less polar environment for the carbonyl group consistent with the formation of an inclusion complex.[3] Accordingly, in the presence of β-CD an ICD signal arises, both in the π,π* and *n*,π* absorption bands (Fig. 1), closely similar to that of the parent molecule.[9] The sign is in fact positive for both of the lowest energy transitions (intensity ratio *ca.* 4) and the signal depends on the β-CD concentration indicating the formation of a complex with 1 : 1 stoichiometry. Application of the Benesi–Hildebrand treatment[10] gave an association constant of 2700 l mol^{-1},[3] a value somewhat higher than those of 1500 l mol^{-1} or 800 l mol^{-1} reported for BP.[9,11]

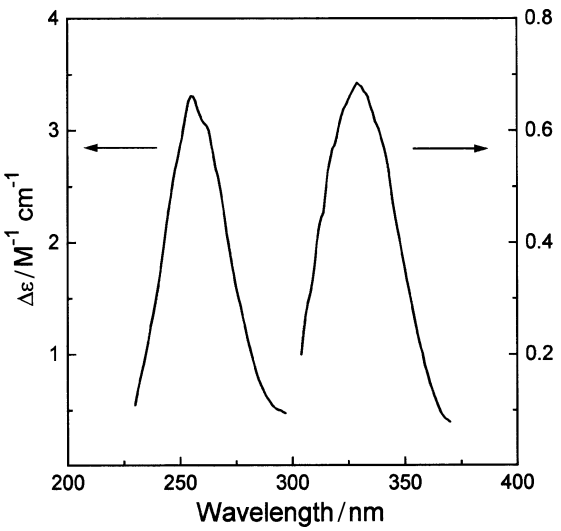


Fig. 1 Induced circular dichroism spectrum of the KPF–β-CD complex in phosphate buffer (10^{-2} mol l^{-1}) at pH 7.4

Conformation of the KPF–β-CD inclusion complex

In order better to relate the spectroscopic and photochemical properties of KPF and the parent molecule BP to the structure of the β-CD complexes, a series of conformational calculations were carried out. The geometries of the isolated molecules were first optimized using the MM3-92 molecular mechanics program of Allinger *et al.*[12] These geometries were then combined with those of β-CD as the starting point for the geometry optimization of the inclusion complexes. The latter was performed by using a Monte Carlo procedure to find low energy associates (potential energy and solvation energy); during this procedure the conformational space was restricted introducing the Metropolis criterion[13] in a simulated annealing algorithm.[14] The solvent accessible surface was calculated using the MSEED program along the procedure discussed in previous papers.[15] A display of two probable inclusion complexes is shown in Fig. 2: they refer to a minimum in the potential energy of 70.9 kcal mol^{-1} with an inclusion distance (defined as the mean distance between the glycosidic oxygens of β-CD and the center of the aromatic moiety of the guest) of 1.01 Å for KPF and an energy of 75.8 kcal mol^{-1} and a distance of 1.2 Å for BP. A further test of the calculated geometries was given by the calculation of the sign and relative intensity of the ICD of these complexes. The calculation of the ICD spectrum was carried out using the Tinoco–Kirkwood approach, a simplified version of the original dipole-dipole interaction term obtained introducing the polarizability of the macrocyclic bonds.[16] This method, combined with the topological information obtained from the procedure described above, has proven to be a sensitive one for reproducing the experimental ICD spectra of several chromophores included in cyclodextrins.[16] Such a calculation was performed on the 100 structures of lowest energy: the calculation is able to reproduce the right sign and intensity ratio for the two lowest energy bands in the ICD spectrum for 39 BP structures, but for only six in the case of KPF. This seems to be related to a larger steric hindrance in the case of KPF, which is deeply embedded in the host cavity with only the aromatic part of the molecule, while the propionic group tends to remain outside the macrocycle. This feature increases the degree of freedom of the whole molecule with a larger number of potential energy minima available, corresponding to different orientations of the propionic substituent. However, only a few structures give rise to the correct sign and intensity in the ICD spectrum.

Steady-state photolysis

In deaerated aqueous solutions (3-benzoylphenyl)ethane is the largely dominant photoproduct so that the photodecarboxylation and the photodegradation quantum yield practically coincide ($\Phi_{dc} = \Phi_{-KPF} = 0.75$[1]). In the inclusion complex the situation is different. The quantum efficiency of KPF photodecarboxylation was measured as a function of the β-CD concentration (Fig. 3). From these data a value of $\Phi_{dc}^{CD} = 0.42 \pm 0.08$ was derived for the inclusion complex on the basis of the association constant determined by ICD.[3] Correspondingly, the quantum yield of KPF disappearence is $\Phi_{-KPF}^{CD} = 0.85 \pm 0.15$.

The photoproducts extracted from the samples containing β-CD were (3-benzoylphenyl)ethane and unreacted KPF. In addition, there was a fraction of photoproducts, accounting for *ca.* 50% of the degradation of the starting compound, that could not be extracted from water. The absorption spectrum of the aqueous solution after the extraction procedure revealed a very intense band with a maximum at *ca.* 205 nm (Fig. 4, curve a). We checked both the UV absorption of the aqueous solution after the extraction of KPF from a non-irradiated KPF–β-CD solution (curve b) and after the irradiation of and extraction of the decarboxylated photoproduct

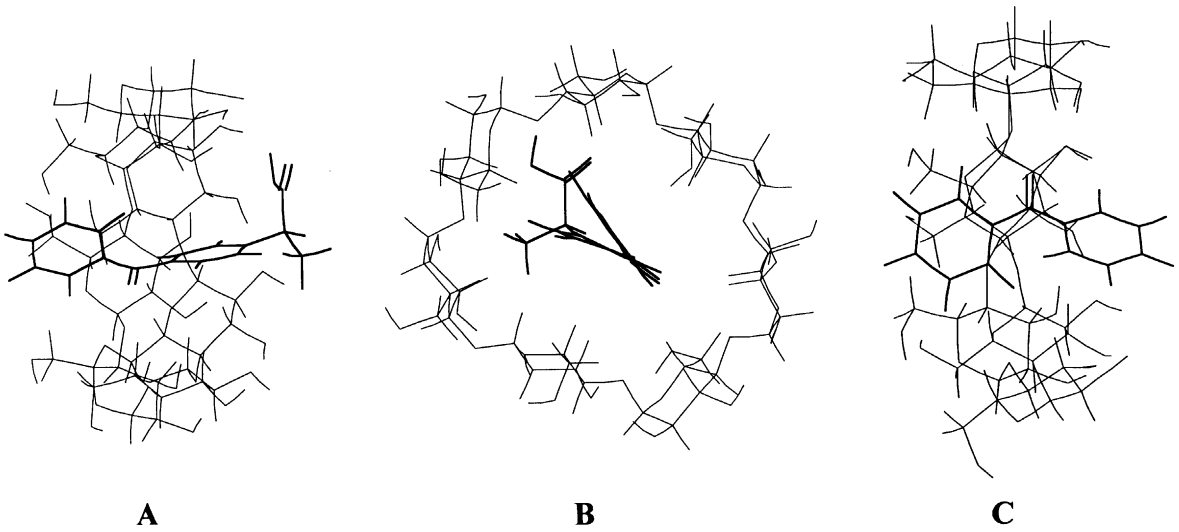


Fig. 2 (A, B) Structure of the KPF–β-CD complex corresponding to the energy minimum at 70.9 kcal mol^{-1}. (C) Structure of the BP–β-CD inclusion complex corresponding to the energy minimum at 75.8 kcal mol^{-1}

and unreacted KPF from a solution without β-CD (curve c). The absence of any significant absorption at 205 nm in the control experiments (b and c) indicates that spectrum a is due to photoproducts likely containing the CD macrocycle as a covalently linked moiety. The maximum at 205 nm is indicative of a structure in which the KPF carbonyl group has been reduced.[4] A better characterization of these photoproducts by NMR is complicated by the low solubility of the drug in aqueous solutions and the need to keep the conversion percentages in the irradiated samples low, in order to avoid significant light absorption by the decarboxylated photoproduct, which could undergo photoreduction in turn. By keeping the concentrations as high as possible [KPF (10^{-3} mol l^{-1}) in the presence of β-CD (10^{-2} mol l^{-1}) in deoxygenated phosphate buffer (10^{-1} mol l^{-1}), pH 7.4] and the conversion at 15%, the NMR spectrum of the irradiated sample, after the extraction of the decarboxylated derivative and the starting compound, was taken in D_2O. It showed *both* the signals of the cyclodextrin protons *and* weak signals of aromatic protons at 7.3 ppm, corresponding to the addition of an aromatic moiety to the sugar. The typical signals of the aromatic ethyl substituent (CH_3 triplet at 1 ppm and CH_2 quartet at 2.5 ppm) were not observed, thus excluding that the adduct results from the photoreaction of the decarboxylated derivative.

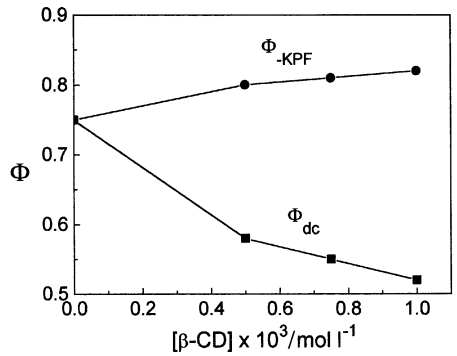


Fig. 3 Quantum yields of the disappearence and decarboxylation of KPF in phosphate buffer as a function of the β-CD concentration

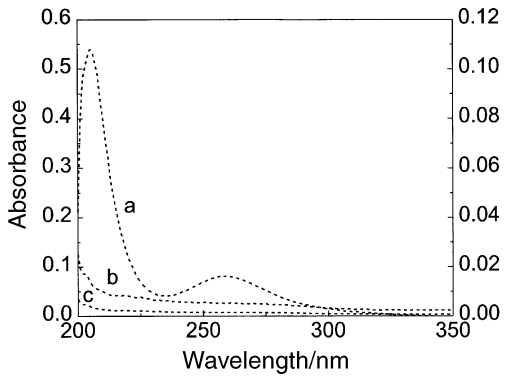


Fig. 4 Absorption spectrum of the aqueous phase remaining after product extraction from (a) a solution containing KPF (10^{-3} mol l^{-1}) and β-CD (10^{-3} mol dm^{-3}), irradiated up to 15% KPF transformation (left scale); (b) the same mixture non-irradiated (right scale); (c) a solution of KPF (10^{-3} mol^{-1}) without β-CD, irradiated up to 15% KPF transformation (right scale). All the samples were diluted three times and measured in a 1 cm cell

Laser flash photolysis

Excitation of a KPF solution (7.0×10^{-5} mol l^{-1}), containing β-CD (10^{-2} mol l^{-1}, *i.e.* under conditions of 96% complexation) with a laser pulse of 35 ps at 266 nm leads to the difference spectrum of Fig. 5. It exhibits a well-defined maximum at 526 nm with intensity similar to that in the absence of β-CD[6] and is attributed to the triplet state of the complex. The time evolution during the following 3 ns consists in a slight decrease of the absorbance at wavelengths shorter than 540 nm while a shoulder increases at *ca.* 600 nm. The

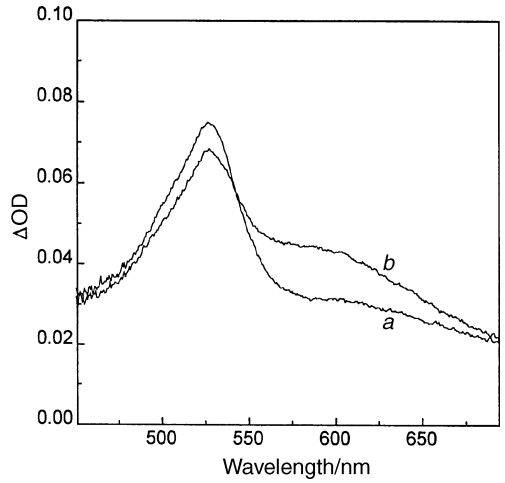


Fig. 5 Absorbance changes (1 cm cell path) detected in a KPF (7.0×10^{-5} mol l^{-1}) solution in aerated phosphate buffer containing β-CD (10^{-2} mol l^{-1}), upon excitation with a laser pulse of 35 ps at 266 nm. (*a*) 99 ps; (*b*) 3 ns after time ' zero'

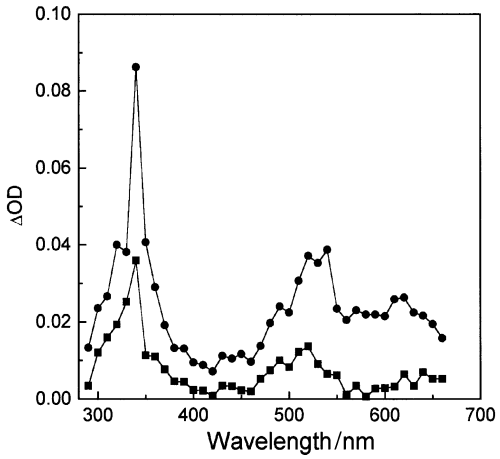


Fig. 6 Absorbance changes (1 cm cell path) observed in a KPF (3.5×10^{-5} mol l^{-1}) solution in argon saturated phosphate buffer containing β-CD (10^{-2} mol l^{-1}), upon excitation with a 35 ps, 4.5 mJ laser pulse at 266 nm. (●) 4 ns, (■) 800 ns after the pulse

maximum wavelength remains at 526 nm. No traces of the hydrated electron absorption are evidenced. Absorption changes in the nanosecond time domain are reported in Fig. 6. The difference spectrum taken 4 ns after the pulse is characterized by maxima at 340 and *ca.* 530 nm and by a shoulder at *ca.* 600 nm. The visible part is similar to that observed by means of the picosecond detection system. The time evolution over the whole wavelength range is well-described by a double exponential function: $\Delta A(t) = a_1\exp(-t/\tau_1) + a_2\exp(-t/\tau_2) + a_3$ with time constants of about 100 and 250 ns (Fig. 7A, B). The decay kinetics are the same in KPF (1.2×10^{-3} mol l^{-1}) solutions containing β-CD (10^{-2} mol l^{-1}, percentage of complexation *ca.* 95%) excited at 355 nm.

The difference spectrum taken 800 ns after the pulse is characterized by maxima at 340 and 520 nm. The further evolution, if analysed by a biexponential function, is characterized by time constants of *ca.* 15–25 and *ca.* 55–90 μs in both the UV and visible regions, somewhat varying by changing the analysis wavelength (Fig. 7C, D). Oxygen influences the faster rate according to the occurrence of a bimolecular quenching reaction characterized by $k_q \approx 2 \times 10^9$ l mol^{-1} s^{-1} and the slower one according to $k_q \approx 7 \times 10^8$ l mol^{-1} s^{-1}.

A solution of KPF (3.8×10^{-5} mol l^{-1}) in deuterated phosphate buffer does not give any detectable signal of $^1O_2$ emission.[6] The same behavior is observed in the presence of β-CD (10^{-2} mol l^{-1}).

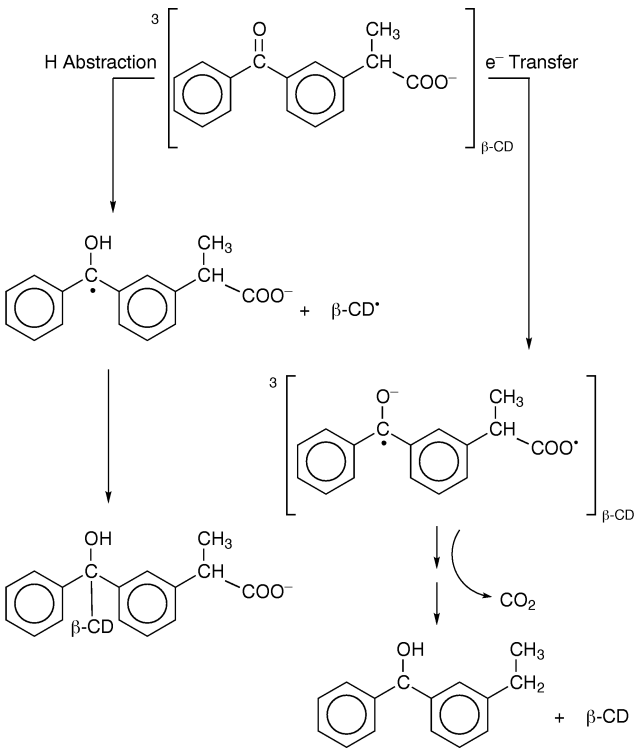
Discussion

The association of KPF with β-CD was established by both absorption and ICD spectroscopy.[3] The modification of the electronic spectrum and the shape of the ICD signal in both the n,π^* and the π,π^* bands point to an inclusion geometry similar to that of benzophenone, *i.e.* with the carbonyl group experiencing a 'non-aqueous' environment. The association constant is somewhat higher (2700 l mol^{-1} for KPF[3] *versus* 1500 l mol^{-1} for BP[15]), showing that the propionic acid group tends to stabilize the complex. Conformational calculations combined with the theoretical interpretation of the ICD sign and intensity confirm that the the carbonyl group is deeply inserted in the interior of the macrocycle, while the substituent remains outside the cavity (Fig. 2). Such calculations are reliable since they proved to be very useful in predicting the structure of CD complexes of several phenol and methoxybenzene derivatives and in interpreting their spectroscopic and photochemical properties.[17]

The predicted location of the propionic acid substituent in an 'aqueous' environment makes the assumption that the pK_a of the carboxyl group (4.7 in aqueous medium[4]) is not drastically affected by the CD reasonable, so that at neutral pH the inclusion complex is mainly in the dissociated form. Accordingly, efficient photodecarboxylation still takes place. However, the quantum yield of the process is much lower (0.42) than that measured in aqueous medium (0.75). A similar effect was observed in the photolysis of the drug bound to bovine serum albumine.[18]

An additional efficient photodegradation path is active in the complex, consistent with the reduction of the ketone (Fig. 3) and the formation of adducts with β-CD. Similar photochemistry was observed in the 3-benzoylpyridine-[19] and the benzophenone–β-CD[20] inclusion complexes and was attributed to the occurrence of cage recombination of radicals formed upon H abstraction from a glucose unit by the ketone triplet state.

The time-resolved experiments support the two reactivity paths (Scheme 1). The subnanosecond difference spectrum shows that the triplet state of the KPF inclusion complex is formed with a quantum efficiency close to unity. Accordingly, the triplet-triplet absorption has a maximum at 526 nm and its shape and intensity are quite similar to those found in aqueous medium under the same conditions.[6] However, contrary to what happens in water, where a fast electron transfer from the carboxylate to the carbonyl group makes the triplet lifetime quite short ($\tau = 250$ ps),[6] no subnanosecond decay is observed.[21] The triplet state lifetime becomes *ca.* 100 ns. It is concluded that the electron transfer process initiating the decarboxylation is thermodynamically disfavoured by the location of the C=O in the apolar CD interior and H abstraction from a CD glucose unit can compete. H abstraction by BP carboxylate derivatives in the triplet state is a known process in micellar systems[24] and was found to occur on the nanosecond time scale in the β-CD cavity.[9,19] The transient with 250 ns lifetime, not observed in the absence of CD, is reasonably attributed to the reduction path and assigned to the triplet radical pair ketyl–CD$^{\bullet}$, recombining (after ISC) to form the adduct. The first-order kinetics and the spectral properties (absorption maxima at 330 and 520–530 nm) are consistent with such an assignement[9,19] However, due to



Scheme 1

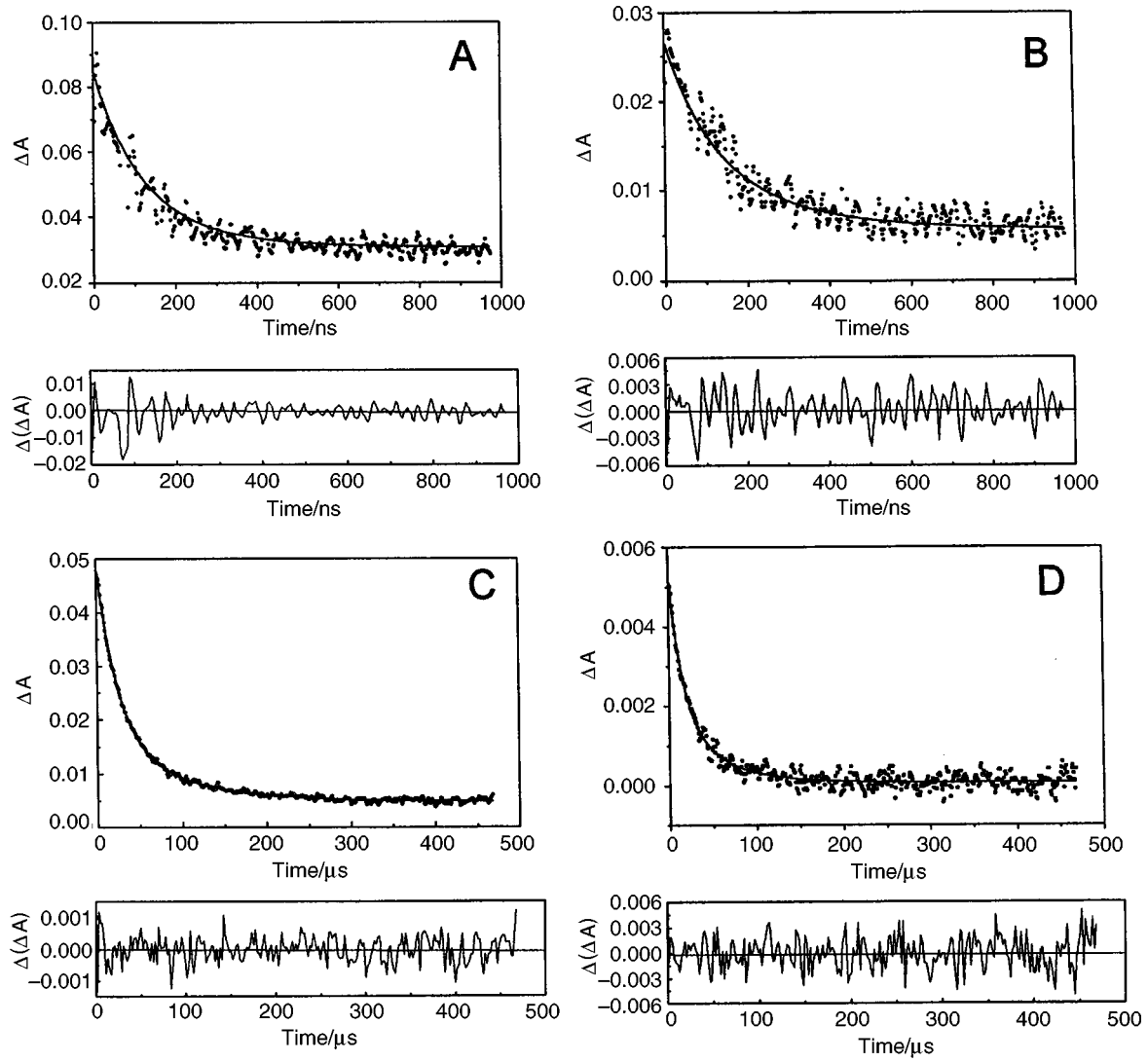


Fig. 7 Time profiles and best fitting parameters of the absorbance changes observed in KPF (3.5×10^{-5} mol l^{-1}) solutions in argon saturated phosphate buffer containing β-CD (10^{-2} mol l^{-1}), upon excitation with a 35 ps, 4.5 mJ laser pulse at 266 nm: (A) 340 nm ($a_1 = 4.57 \times 10^{-2}$, $\tau_1 = 100$ ns, $a_2 = 8.50 \times 10^{-3}$, $\tau_2 = 245$ ns, $a_3 = 3.06 \times 10^{-2}$). (B) 620 nm ($a_1 = 1.23 \times 10^{-2}$, $\tau_1 = 105$ ns, $a_2 = 8.76 \times 10^{-3}$, $\tau_2 = 242$ ns, $a_3 = 5.85 \times 10^{-3}$); (C) 330 nm ($a_1 = 3.20 \times 10^{-2}$, $\tau_1 = 23$ μs, $a_2 = 1.26 \times 10^{-2}$, $\tau_2 = 89$ μs, $a_3 = 4.76 \times 10^{-3}$); (D) 520 nm ($a_1 = 3.66 \times 10^{-3}$, $\tau_1 = 16$ μs, $a_2 = 1.61 \times 10^{-3}$, $\tau_2 = 56$ μs, $a_3 = 6.54 \times 10^{-5}$)

the scarce spectral characterization of the transients involved in the photochemistry of this system (the intermediates of the decarboxylation path all contain the ketyl radical center),[6] we cannot exclude that the dissociation of carbon dioxide, which in water was shown to occur with a time constant of 120 ns, may contribute to the observed time evolution.

The two microsecond time constants (15–25 and 55–90 μs) also pertain to transients characterized by close spectral similarities (maxima at *ca.* 330–340 and *ca.* 520 nm for both species). They have to be compared with the lifetimes of 4 and 12 μs, pertaining to the long-lived, oxygen-sensitive intermediates of the decarboxylation pathway in aqueous medium.[6] The spectral features and the sensitivity to oxygen are consistent with assignement to the same intermediates, complexed to the CD.

Finally, the absence of detectable solvated electron absorption upon excitation with the picosecond pulse does not exclude that a minor contribution to the decarboxylation process may come from photoionization of the inclusion complex. In the free molecule this process is observed only with nanosecond laser pulses, being mainly biphotonic and requiring extensive population of the triplet state as an intermediate step for the absorption of the second photon.[6]

Conclusions

This study shows that the photochemistry of Ketoprofen is extremely sensitive to the environment. While in aqueous environment the triplet decay is determined by a fast intramolecular electron transfer between the carboxylate and the carbonyl groups, ultimately leading to decarboxylation, in the β-CD inclusion complex the above process occurs with a reduced rate and an additional reductive channel is open. The overall photostability of the drug appears lower. The results are consistent with the formation of adducts of KPF with the β-CD macrocycle.

The modifications of the KPF photochemistry by inclusion in the β-CD cavity can provide a rationale for the decreased phototoxic activity of the drug *in vitro*. The damaging role of the decarboxyaled photoproducts is decreased because they are formed in lower yield. This effect was already noted in a previous work[3] where it was reported that pre-irradiated KPF–β-CD solutions are less lytic toward the membrane than solutions where β-CD is absent. The formation of ketyl radicals by reaction with β-CD is not likely to be responsible for the cell damage because of the competition of β-CD itself in a fast addition processes. Moreover, the production of singlet

oxygen by the decarboxylated photoproducts is reduced in the system, so that photosensitization *via* the type II mechanism, even if of secondary importance,[2] is depressed. However, the novel KPF photoreactivity evidenced in a hydrophobic, constrained environment in the presence of H-donating groups could be of toxicological relevance, leading to irreversible binding of the drug to cell components.

Acknowledgements

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21 The slight evolution of the spectral features during the first 3 ns are tentatively attributed to relocation of the excited state within the cavity.[22] On the other hand, exit of the excited molecule from the cavity is unlikely, being that the dipole moment of the excited state is closely similar to that of the ground state, so that no significant decomplexation should occur.[6] Moreover, the time scale involved seems to be too fast for complex dissociation.[23]
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