Characterisation of the lowest singlet and triplet excited states of S-flurbiprofen

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The photophysical properties of S-flurbiprofen [S-2-fluoro-α-methyl-4-biphenylacetic acid], a nonsteroidal anti-inflammatory drug, have been examined using steady-state and time-resolved spectroscopic techniques. The energy of its first singlet excited state is 99 kcal mol^{-1}. The fluorescence quantum yields and lifetimes (at 300 nm) have been determined in acetonitrile, methanol, hexane and PBS; they are in the range $0.15<\phi_F<0.33$ and $0.7<\tau_F<2.0$ ns. The intersystem crossing quantum yields are between 0.45 and 0.71; the λ_{max} of the T-T absorption is 360 nm, and the triplets live from 15 to 106 μs. Steady state photolysis in aqueous medium leads to S-2-hydroxy-α-methyl-4-biphenylacetic acid *via* photonucleophilic aromatic substitution, in addition to the photodecarboxylation products observed in organic solvents.

Introduction

S-Flurbiprofen [S-2-fluoro-α-methyl-4-biphenylacetic acid] (Chart 1) is a nonsteroidal anti-inflammatory drug (NSAID). It has been widely used for several medical purposes, such as treatment of inflammation and pain caused by rheumatoid arthritis,[1] osteoarthritis[2] and soft tissue injuries (tendinitis and bursitis),[3] prevention of migraine headache,[4] treatment of sunburn[5] and acute gout[6] or pain control after excimer laser photorefractive keratectomy.[7]
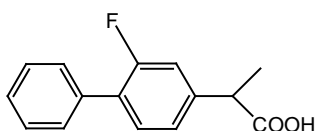

Chart 1

The pharmacological effects of S-flurbiprofen (FBP) are associated with inhibition of prostaglandin H$_2$ synthase (also named cyclooxygenase or COX), which exists in two isoforms (COX-1 and COX-2) and is the enzyme responsible for the catalytic conversion of arachidonic acid into prostaglandin H2, a key intermediate in the biosynthetic pahtway of prostaglandins, prostacyclins and thromboxanes.[8]

S-Flurbiprofen has been used as a probe to displace fluorescent cyclooxygenase inhibitors from the active sites; emission variations provide valuable information on the interactions of such inhibitors with COX-1 and COX-2.[9] Moreover, the intrinsic fluorescence of FBP has been shown to be a useful tool for the study of the interaction between the drug and human serum albumin, as well as for the characterisation of the binding sites.[10] This is an important issue, as formation of non-covalent complexes with proteins is a key process in the transport and biodistribution of nonsteroidal antiinflammatory drugs in treated patients.

In this context, although the photophysical properties of a number of antiinflammatory 2-arylpropionic acids (*i. e.* naproxen, benoxaprofen, carprofen, ketoprofen, tiaprofenic acid and suprofen) have been previously described,[11] the first singlet excited state of FBP has not yet been fully characterised. Besides, there is no report on the lowest lying FBP triplet, in spite of its potential interest as a reporter of the physicochemical properties of its environment when complexed with biomolecules. A detailed investigation on the photophysical properties of FBP would also be helpful to understand its photoreactivity, which involves initial cleavage of the C-C bond α to the carbonyl group, ultimately leading to decarboxylation products.[12]

In the present paper, the lowest singlet and triplet excited states of S-flurbiprofen have been examined, using both steady-state and time resolved spectroscopic techniques.

Experimental

Materials and solvents

S-Flurbiprofen, benzophenone, acetophenone, chloranil, biacetyl and 1,3-cyclohexadiene were purchased from Aldrich. Their purity was checked by ^{1}H NMR and HPLC analysis.

The reagent grade solvents (methanol, hexane or acetonitrile) were obtained from Scharlau and used without further purification. Solutions of PBS 0.01 M (pH = 7.4) were prepared by dissolving Sigma phosphate buffered saline tablets in deionized water.

Absorption spectra

Optical spectra in different solvents were measured on a Perkin Elmer Lambda 35 UV/Vis Spectrometer.

Fluorescence measurements

Emission spectra were recorded on an Edinburgh FS900 spectrofluorimeter system, provided with a M300 monochromator in the wavelength range 200-900 nm. The samples were placed into 10 x 10 mm^2 Suprasil quartz cells with a septum cap. The solutions were purged with nitrogen or oxygen for at least 15 min before the measurements. Fluorescence quantum yields were determined using β-methylstyrene as reference ($\phi_F = 0.35$

Table 1 Photophysical properties of S-flurbiprofen in deaerated media

Solvent	$\phi_F{}^a$	τ_F (ns)	k_F (s^{-1})	ϕ_{ISC}	τ_T (μs)	k_{ISC} (s^{-1})	k_{NR} (s^{-1})
MeCN	0.20 (0.22)	1.7	1.2×10^8	0.71	31	4.2×10^8	4.1×10^7
MeOH	0.27 (0.27)	1.5	1.8×10^8	0.70	33	4.7×10^8	2.0×10^7
C$_6$H$_{14}$	0.32 (0.33)	2.0	1.6×10^8	0.65	15	3.3×10^8	1.0×10^7
PBS	0.15 (0.15)	0.7	2.1×10^8	0.45	106	6.4×10^8	5.0×10^8

^{a} The data have been obtained upon excitation at 254 nm or 281 nm (in brackets)

at 281 nm and 0.30 at 254 nm in hexane).[13] The absorbance of the samples at the excitation wavelength was kept below 0.1. Excitations and emission slits were maintained unchanged during the emission experiments. For time resolved fluorescence decay measurements, the conventional single photon counting technique was used.

Laser flash photolysis experiments

Laser flash photolysis experiments were performed by using a Q-switched Nd:YAG laser (Quantel Brilliant, 266 nm or 355 nm for photosensitized experiments, 4 mJ per pulse, 5 ns fwhm) coupled to a mLFP-111 Luzchem miniaturized equipment. All transient spectra were recorded employing 10 x 10 mm^2 quartz cells with 4 mL capacity and were bubbled during 30 min with N$_2$, air or O$_2$ before acquisition. The absorbance of the samples was kept between 0.20 and 0.25 at the laser wavelength.

All the experiments were carried out at room temperature (22 °C).

General irradiation procedure

Solutions of FBP (5 mM) in hexane, acetonitrile, methanol or PBS were irradiated for 16 h through quartz, inside a Luzchem multilamp photoreactor, with the light from 4 8W-lamps emitting mainly at 254 nm. The course of the reaction was followed by GC, GC-MS and ^{1}H-NMR; the degrees of conversion, the product distributions and the mass balances were determined using adequate standards. Photoreaction quantum yields were determined using phenylglyoxylic acid as actinometer.[14]

Results and discussion

Fluorescence characteristics of S-flurbiprofen

Figure 1 shows the fluorescence spectrum of S-flurbiprofen in PBS, when excited at 254 nm. The excitation spectrum for the emission at 300 nm is also shown in the figure. From the intersection between the two bands, after normalisation, a singlet energy of 99 kcal mol^{-1} was obtained.
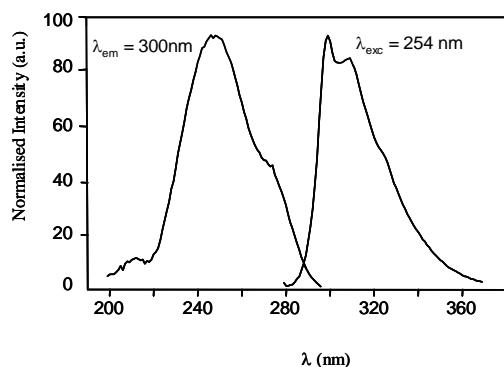

Fig. 1 Normalised excitation and emission spectra of S-flurbiprofen in PBS.

No major changes in the shape of the peaks or in the position of the absorption maxima were observed when methanol, hexane or acetonitrile were employed as solvents. Likewise, changing the excitation wavelength from 254 to 281 nm did not result in significant variations. Fluorescence quantum yields and lifetimes under different conditions are reported in Table 1.

The fluorescence of FBP was sensitive to the presence of oxygen. Thus, when the samples were purged with O$_2$ a significant decrease of the quantum yields and the lifetimes was observed; for instance, in acetonitrile solution (excitation at 281 nm) these values were found to be $\phi_F = 0.15$ and $\tau_F = 1$ns.

Laser flash photolysis measurements

Figure 2 shows the transient absorption spectra of S-flurbiprofen (2.5×10^{-5} M) in PBS after 266 nm laser excitation. The band with maximum at 360 nm was assigned to the T-T absorption of FBP and, as expected for a triplet, it was quenched by oxygen.
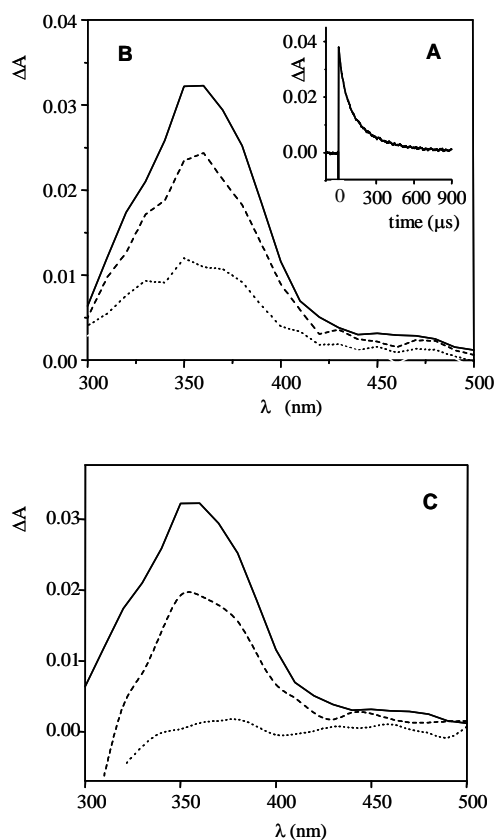
Fig. 2 Laser flash photolysis (266 nm excitation) of S-flurbiprofen 2.5×10^{-5} M in PBS. **A**. Transient decay monitored at 360 nm, under N$_2$. **B** . Spectra recorded under the same conditions, 14.4 μs (——), 100 μs (– –) and 310 μs (······) after the laser pulse. **C**. Comparison between the spectra generated under N$_2$, air and O$_2$.

The triplet lifetimes in deaerated media were found to be between 15 and 106 μs, depending on the solvent (see Table 1). The highest value was found in neutral aqueous buffer. The table also shows the intersystem crossing quantum yields that ranged between 0.45 and 0.71; they were calculated by the comparative method, based on energy transfer from known donors. The triplet lifetimes did not depend on the laser intensity (between 4 and 20 mJ/pulse), ruling out a possible annihilation process. However, they decreased with increasing FBP concentration; the self-quenching constant in MeCN is given in Table 2.

The T-T transient absorption of S-flurbiprofen was also generated by photosensitisation with benzophenone (BZP) and acetophenone. Thus, laser flash photolysis of BZP at 355 nm produced the typical BZP triplet with maximum at ca. 525 nm. In the presence of FBP, a fast disappearance of this band was observed, concomitantly with the growth of the 360 nm band, characteristic of FBP triplet (see Fig. 3). The rate constant of this energy transfer process was found to be 6.3×10^8 M^{-1} s^{-1} in MeCN at room temperature. The molar absorption coefficient of flurbiprofen triplet was determined using benzophenone as standard and assuming a value of 7800 mol L^{-1} cm^{-1} at 525 nm for this compound.[15] In MeCN, it was found to be 32000 mol L^{-1} cm^{-1} at 360 nm.
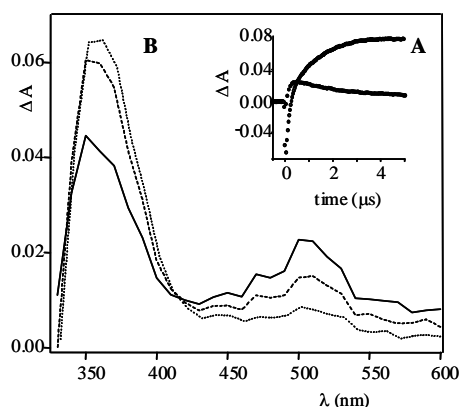
Fig. 3 A. Growth and decay profiles monitored at 360 nm and 520 nm upon 355 nm laser flash photolysis of benzophenone (2×10^{-3} M) in the presence of S-flurbiprofen (5×10^{-4} M) under N_2-saturated conditions. **B.** Spectra of the transient intermediates generated 4 μs (——), 5.3 μs (– –) and 8.5 μs (·····) after the laser pulse.

The ISC quantum yields of S-flurbiprofen in different solvents were calculated from the ΔA of the T-T bands of isoabsorptive solutions of BZP and FBP, the molar absorption coefficients of their triplets in acetonitrile (see above) and the known ISC quantum yield of BZP (ca. 1); they are shown in Table 1. From the values of ϕ_F, τ_F and ϕ_{ISC}, it was possible to determine the rate constant of the different photophysical process (k_F, k_{ISC} and k_{NR}); they are also included in Table 1.

Thus, the energy of the lowest triplet excited state of FBP must be lower than 69 kcal mol^{-1} (the triplet energy of BZP).[16] In order to obtain a narrower range for this parameter, different triplet quenchers (i. e. 1,3-cyclohexadiene, chloranil, and biacetyl) were used, and their interaction with the T-T absorption band was studied; the rate constants for these processes are given in Table 2.

The above data indicate that, as expected for a biphenyl derivative, the triplet energy of FBP must be

close to 65 kcal mol^{-1}. Actually, triplet-triplet energy transfer to all the acceptors in Table 2 (E_T between 50 and 57 kcal mol^{-1}) would be thermodynamically favoured.

Table 2 Reactivity of S-flurbiprofen triplet in MeCN

Substrate	k_q (M^{-1} s^{-1})
Self-quenching	5.7×10^8
O_2	8.2×10^8
1,3-Cyclohexadiene	1.0×10^{10}
Chloranil[a]	9.0×10^9
Biacetyl	5.0×10^9

[a] Absorption from both the chloranil triplet and its radical cation were observed

Product studies

When FBP was irradiated in different solvents, in the presence of oxygen, four products were detected in the photoreaction mixtures (see Chart 2 and Table 3). In general, the photoreaction quantum yields were low (0.03 in PBS and < 0.005 in the organic solvents).
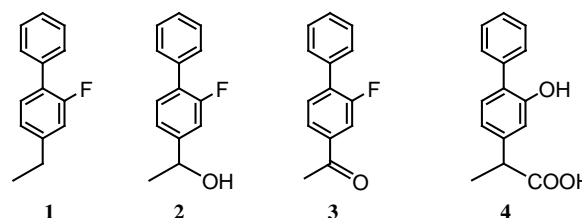

Chart 2

Table 3 Photochemistry of FBP at 254 nm

Solvent	Conversion	1	2	3	4
MeCN	71	-	3	97	-
MeOH	33	-	13	87	-
C_6H_{14}	65	-	25	75	-
PBS	100	50	-	23	27

The results obtained in the three organic solvents were in good agreement with the previously reported photochemical behaviour of FBP in methanol.[12] However, formation of phenol **4**[17] in aqueous solution was unexpected. Clearly, this photoproduct must arise *via* photonucleophilic aromatic substitution. A simplified reaction scheme is shown in Fig. 4. Similar processes have been described for other fluoroaromatics, including fluoroquinolone antibiotics.[18] In general, deaeration of the irradiated solutions did not result in an enhanced photodegradation efficiency; this points to the first excited singlet as the reactive state. Accordingly, triplet sensitisation with benzophenone derivatives did not lead to significant amounts of products. Further support in favour of photoreaction from the excited singlet was provided by comparing the photophysical and the photochemical data. It is specially noteworthy that the fluorescence quantum yields and lifetimes, as well as the efficiency of intersystem crossing, were lower in aqueous media, where the photoreaction was faster and gave rise to photosubstitution as well as photodecarboxylation products.
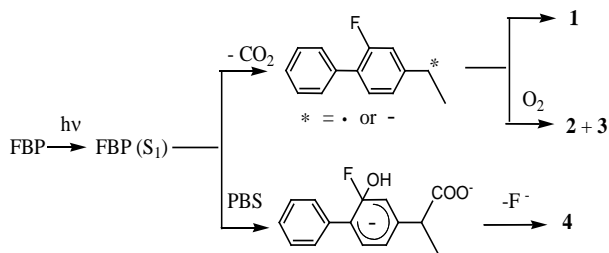
Fig. 4. Mechanistic rationalisation for the formation of the FBP photoproducts.

Conclusions

In summary, the first excited singlet and triplet states of S-flurbiprofen have been fully characterised, and the main processes leading to their deactivation have been identified. The excited state lifetimes and the quantum yields of the involved photophysical processes have been determined. Finally, steady state photolysis in aqueous medium has provided evidence for photonucleophilic aromatic substitution as an important photochemical pathway, in addition to the photodecarboxylation previously observed in organic solvents.

When the photophysical behaviour of FBP is compared with that of biphenyl (BP, the parent chromphore), a number of properties are closely simirlar. They include the singlet and triplet energies (95-100 and 65-70 kcal/mol, respectively), the position of the fluorescence band (λ_{max} *ca* 300 nm) and the T-T absorption maxima ($\lambda_{max} = 360$ nm). The quantum yields of fluorescence and intersystem crossing of BP are known to be 0.15 and 0.84 respectively.[19] This is in reasonable agreement with the values obtained for FBP (Table 1). However, the main differences between FBP and BP were the singlet and triplet lifetimes, that were found to be $\tau_F = 15$ ns and $\tau_T = 130$ μs for the latter.[19] The values determinded for FBP (Table 1) were clearly shorter, probably due to excited state deactivation *via* hydrogen bonding interactions involving the carboxylic group. As regards the comparison with related NSAIDs, FBP shares with naproxen (NPX) and carprofen (CP) the ability to generate detectable singlet and triplet excited species. By contrast, ketoprofen (KP), tiaprofenic acid (TPA) and suprofen (SP) do not exhibit reasonable flourescence, owing to a very efficient intersystem crossing. On the other hand, FBP (as NPX and CP) decarboxylates for its singlet excited state with low quantum yield (< 0.01), while in the case of KP, TPA and SUP decarboxylation occurs from the triplet excited state much more efficiently ($\phi_{DEC} > 0.4$). Finally, FBP and CP undergo dehalogenation as an additional photoreaction pahtway.

As a general conclusion, the results obtained for FBP in the present work suggest that the singlet and triplet excited states of FBP can be used as reporters to gain insigth into the nature of the binding sites of NSAIDs to receptors and transport proteins. The careful characterisation of both species and their medium/dependent properties provide the basis for future investigations in this field.

Acknowledgements

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