Spectroscopic characterization and photochemical behavior of host–guest complexes between β-cyclodextrin and drugs containing a biphenyl-like chromophore

Salvatore Sortino,†*[ab] Salvatore Giuffrida,[a] Sandro Fazio[ab] and Sandra Monti*[b]

[a] *Dipartimento di Scienze Chimiche, Università di Catania, Viale Andrea Doria 8, 95125 Catania, Italy. E-mail: ssortino@mbox.unict.it*

[b] *Istituto di Fotochimica e Radiazioni d'Alta Energia, Area della Ricerca, Via Pietro Gobetti 101, 40129 Bologna, Italy. E-mail: monti@frae.bo.cnr.it*

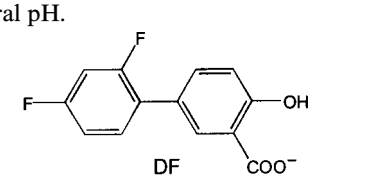
Received (in Montpellier, France) 15th January 2001, Accepted 14th March 2001
First published as an Advance Article on the web 18th April 2001

The effects of the addition of β-cyclodextrin (β-CD) on the light absorption and emission properties and on the photoreactivity of two non-steroidal anti-inflammatory drugs, diflunisal (DF) and fenbufen (FB), containing a biphenyl skeleton, have been investigated in aqueous media. The formation of host–guest inclusion complexes with 1 : 1 stoichiometry was indicated by steady state absorption, induced circular dichroism, NMR and fluorescence spectroscopy. The emitting properties of DF and FB were changed by β-CD complexation in a well-differentiated manner, in agreement with a particular sensitivity of the respective fluorescent states to the microenvironment. The interaction with the host cage is also responsible for remarkable changes in the photoreactivity of both drugs. The encapsulated molecules exhibit a significantly higher photochemical stability if compared to the free drugs. Moreover, in the case of DF, the β-CD microenvironment influences the distribution of the stable photoproducts by controlling the fate of the photogenerated radical intermediates. A rationale for the β-CD induced photochemical changes is proposed.

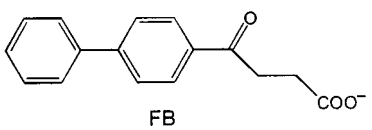
Cyclodextrins (CDs) are cyclic oligosaccharides consisting of six (α-CD), seven (β-CD) or eight (γ-CD) units of α-D-glucose linked together by α-(1,4) bonds. They are shaped like truncated cones, with a smaller and larger rim opening at the primary hydroxyl and the secondary hydroxyl faces of the cyclic sugar network, respectively.[1,2] These molecules are characterized by a hydrophobic cavity and a relatively hydrophilic periphery due to the hydroxyl groups of the receptor edges. Such features confer on CDs the ability to form water soluble inclusion complexes with several organic and inorganic substrates.[1–5] A number of factors influence complexation. The "goodness of fit" between host and guest and the hydrophobic effect are probably the most important.[6] Encapsulation and photochemical investigations in such organized assemblies is an extremely active area of research in the wide arena of supramolecular chemistry for both fundamental studies and practical purposes.[7–12] As already reported for a large variety of systems, CDs have extensively proven their potential as media for controlling photochemical reactions.[8–12] Polarity, steric constraints, specific interactions, mode and stoichiometry of complexation are the main factors that are responsible for changes in the photoreactivity of the included molecules. All of them can in fact modify profoundly the properties of the lowest excited state of the guest molecule, the efficiency of its deactivation pathways and the evolution of the reaction intermediates.

Due to their molecular structure, many non-steroidal anti-inflammatory drugs (NSAIDs) are suitable guests for the CD macrocycle. With respect to this, photophysics and photochemistry of inclusion complexes of NSAIDs with CDs have received growing attention, especially during the last few years.[13–17] The use of CDs as complexing agents represents a useful strategy to minimize the biological damage photoinduced by NSAIDs as well as a tool to increase drug photostability.[17–20] Moreover, the weak binding forces responsible for association to the CD cavity provide a useful model to mimic the interactions of drugs with hydrophobic pockets of biological substrates. Finally, beyond the concerns of both drug photostability and phototoxicity, the present investigation offers the opportunity to gain more insight into the fundamental aspects related to the binding of simple aromatic molecules to CDs, as well as into the mechanisms of photoinitiated reactions in organized and constrained media. Indeed, many NSAIDs contain chromophores (*e.g.*, benzophenone-, naphthalene- and biphenyl-like moieties) that have been favorites of photochemists over the years.

With this in mind we have investigated the spectroscopic and photochemical behavior of the inclusion complexes of β-CD with two NSAIDs containing the biphenyl-like skeleton, diflunisal (DF), 2′,4′-difluoro-4-hydroxy-(1,1′-biphenyl)-3-carboxylic acid and fenbufen (FB), γ-oxo-(1-1′-biphenyl)-4-butanoic acid. Both compounds are well known to act as efficient photosensitizers, inducing a large variety of photoinduced damage on several biological substrates.[21–23] Due to the values of their pK_a, DF and FB exist in the carboxylate form at neutral pH.



DF



FB

† Correspondence should be addressed to Dr Sortino at the Università di Catania address.

Experimental

DF and FB, from Sigma Chemical Company (St. Louis MO, USA), and β-CD from Serva (Heidelberg, Germany) were used as received. Water was purified through a Millipore Milli-Q system. All the experiments were performed in 10^{-2} M phosphate buffer at pH 7.4. The pH of the solutions was measured with a glass electrode.

Absorption and induced circular dichroism spectra (ICD) were recorded with a Beckman 650 DU spectrophotometer and a Jasco J-715 dichrograph, respectively. Fluorescence emission spectra and fluorescence polarization measurements were recorded with a Spex Fluorolog-2 (model F-111) spectrofluorimeter.

^{1}H NMR spectra of DF and FB, both in the absence and in the presence of β-CD, were recorded in D_2O using the residual HOD signal (δ 4.82) as internal reference.

High performance liquid chromatography (HPLC) was performed on a Hewlett Packard 1100 chromatograph equipped with an on-line photodiode array detector (DAD). The analysis of the irradiated mixtures was achieved on a LiChroCart RP-18 column (5 μm packing, 4 × 250 mm, Hewlett Packard) by eluting with a linear gradient of CH_3CN in 0.01 M phosphate buffer (pH 7) from 0 to 75% over 25 min, at a flow rate of 1 mL min^{-1}.

Irradiations were performed using monochromatic light obtained from a Series 200 He–Cd 325 nm laser (Liconix, St. Clara, CA, USA). The incident photon flux was *ca.* 5×10^{15} quanta s^{-1}. The experimental procedures of irradiation and the light intensity measurements have been described previously.[24,25]

The photodegradation quantum yields were determined by HPLC analysis, from the disappearance of the starting compound up to 12% conversion. The UV traces were monitored at 250 and 310 nm. The UV spectra (DAD) were recorded between 200 and 400 nm. A quantitative evaluation of injected material in the presence of 10^{-2} M β-CD assured that the column retained no inclusion products.

The setup for the nanosecond absorption measurements has been described previously.[26] The laser beam (Nd-YAG, JK Lasers, pulse 20 ns FWHM, 266 nm pulse) was focused on a 3 mm high and 10 mm wide rectangular area of the cell and the first 2 mm were analyzed at a right angle geometry. Spectral resolution was 2 nm. Oxygen was removed by vigorously bubbling the solutions with a constant flux of argon, previously passed through a water trap to prevent evaporation of the sample. Care was taken to renew the solution at each laser shot. The temperature was maintained at 295 ± 2 K.

Results and discussion

Absorption properties

Fig. 1 shows the absorption spectra of DF and FB recorded in the presence of increasing amounts of β-CD. In both cases a decrease in the molar absorption coefficients upon addition of β-CD was observed. In the case of FB, a slight blue shift in the maximum was also noticed, consistent with a "polarity" effect by the cavity on the main absorption band of π,π* nature (*vide infra*). These changes provide a first indication of the incorporation of the drugs in the hydrophobic compartment of the macrocycle. A more direct proof of the host–guest association is provided by induced circular dichroism (ICD, Fig. 2). Indeed, due to the absence of chiral centers the compounds are not optically active by themselves in aqueous solutions. Addition of β-CD induces optical activity as a consequence of the inclusion complexation within the chiral macrocycle. It is noteworthy that the ICD bands are characterized by maxima

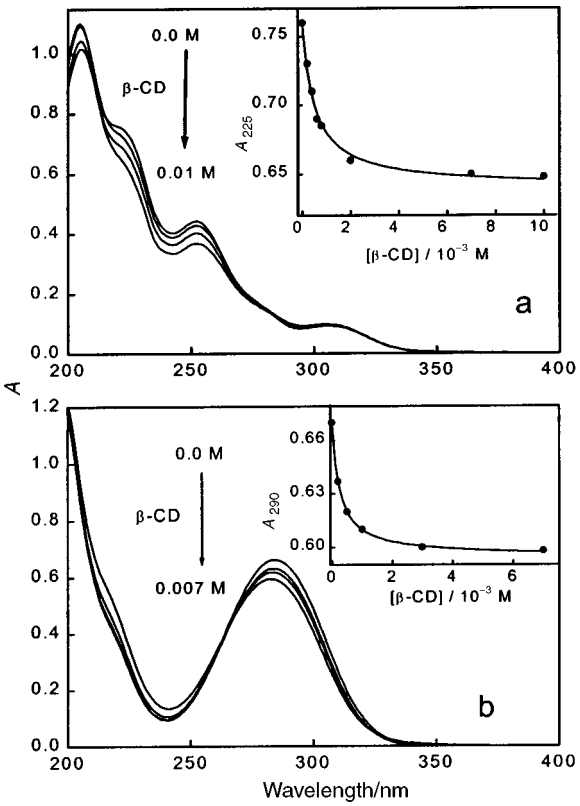


Fig. 1 Absorption spectra of 3.3×10^{-5} M (a) DF and (b) FB in 10^{-2} M phosphate buffer solution, pH 7.4, in the presence of increasing amounts of β-CD in the range 2×10^{-4}–10^{-2} M (some spectra are omitted for the sake of clarity). Cell path 1 cm. The insets show the dependence of the absorbance on [β-CD] and the non-linear fits according to eqn. (1): DF at 225 nm, $K_{ass} = 1920$ M^{-1}, $\varepsilon_c/\varepsilon_0 = 0.82$; FB at 290 nm, $K_{ass} = 3200$ M^{-1}, $\varepsilon_c/\varepsilon_0 = 0.88$.

corresponding well to those of the absorption bands, thus ruling out the occurrence of exciton splitting due to the formation of complexes with 2 : 2 stoichiometry, observed in the case of other biphenyl derivatives.[27] In order to obtain the association constants related to the host–guest systems, we preferred to apply a non-linear fitting procedure because in this way all the data are equally weighted. The analysis of the dependence of the absorption (A) and ICD signals on the β-CD concentration (Insets in Fig. 1 and Fig. 2) was performed according to eqn. (1) and (2), respectively (corresponding to conditions where the β-CD concentrations are in large excess with respect to the guest concentration):

$$A = \varepsilon_0 c_0 \frac{1 + \frac{\varepsilon_c}{\varepsilon_0} K_{ass}[\beta\text{-CD}]}{1 + K_{ass}[\beta\text{-CD}]} \quad (1)$$

$$\text{ICD} = \frac{10\ \theta c_0 K_{ass}[\beta\text{-CD}]}{1 + K_{ass}[\beta\text{-CD}]} \quad (2)$$

here c_0 is the initial concentration of the drug, ε_0 the molar absorption coefficient of the free molecule, ε_c that of the complex, θ is the molar ellipticity of the complex and K_{ass} is the association constant. Both absorption and ICD are consistent with the formation of complexes with 1 : 1 stoichiometry according to the following equilibrium:

$$\text{DF(FB)} + \beta\text{-CD} \rightleftarrows \text{DF(FB)–}\beta\text{-CD} \quad (3)$$

Association constants of 1700 ± 300 M^{-1} and 2700 ± 500 M^{-1} were found for DF and FB, respectively, with both methods. Such binding constants are in good agreement with the values reported for biphenyl derivatives.[27] Despite the fact

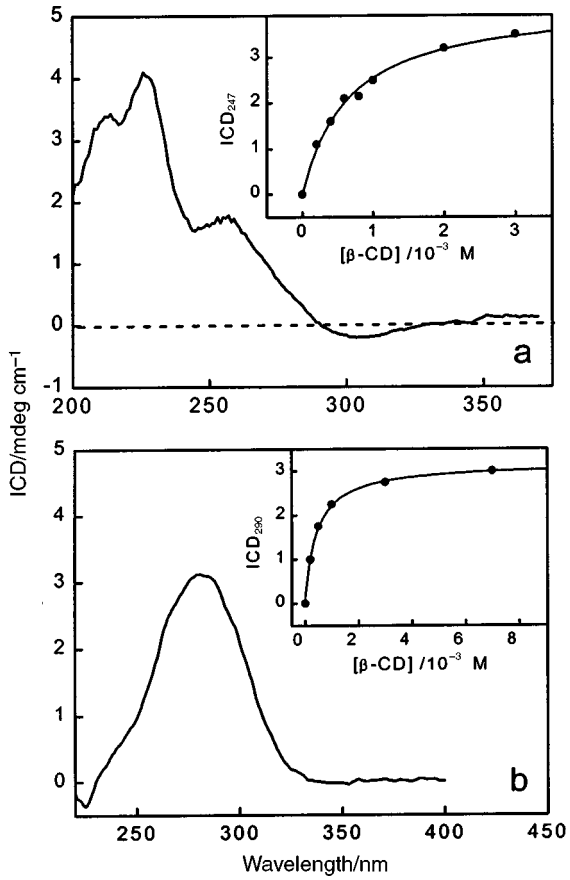


Fig. 2 Induced circular dichroism of the (a) DF–β-CD and (b) FB–β-CD inclusion complexes in 10^{-2} M phosphate buffer solution, pH 7.4: [β-CD] = 10^{-2} M; [drug] = 3.3×10^{-5} M; cell path 1 cm. The insets show the dependence of the ICD signals on [β-CD] and the non-linear fits according to eqn. (2): DF at 247 nm, $K_{ass} = 1690$ M^{-1}, $\theta = 5.4 \times 10^3$ deg decimol^{-1} cm^2; FB at 280 nm, $K_{ass} = 2200$ M^{-1}, $\theta = 1.0 \times 10^4$ deg decimol^{-1} cm^2.

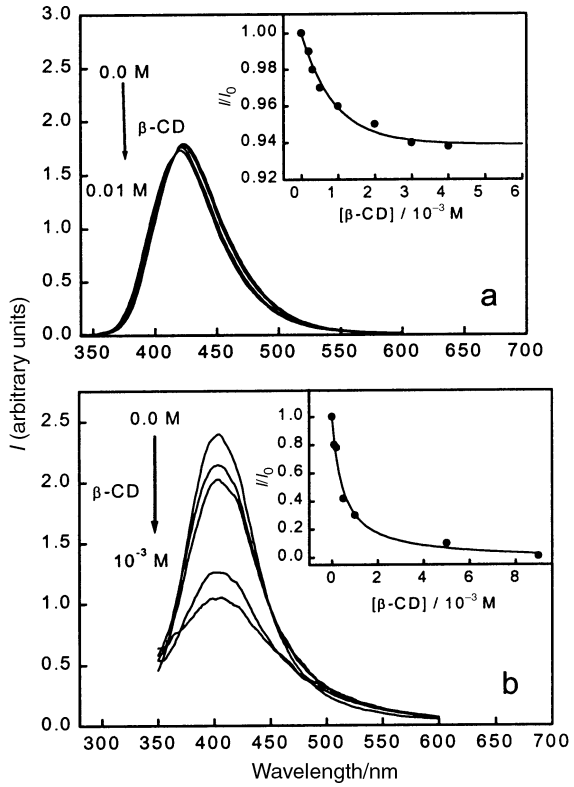


Fig. 3 Fluorescence spectra of (a) DF ($\lambda_{exc} = 318$ nm) and (b) FB ($\lambda_{exc} = 290$ nm) in 10^{-2} M phosphate buffer solution, pH 7.4, in the presence of increasing amounts of β-CD (some spectra are omitted for the sake of clarity). The insets show the dependence of the I/I_0 ratio on [β-CD] and the non-linear fits according to eqn. 4: DF, $K_{ass} = 1400$ M^{-1}, $\Phi_c/\Phi_0 = 0.9$; FB, $K_{ass} = 2690$ M^{-1}, $\Phi_c/\Phi_0 = 0.01$.

that the two molecules are characterized by the presence of the same aromatic unit, the somewhat higher association constant obtained for FB indicates a better affinity of this drug for the β-CD cavity, not surprising in the light of the more hydrophobic nature of FB if compared with DF.

Fluorescence emission studies

The addition of β-CD affects the fluorescence emission of the two compounds in a well-differentiated manner. As can be seen from Fig. 3, addition of β-CD provokes only a slight decrease of the fluorescence intensity of DF, accompanied by a small blue shift in the emission maximum. In contrast, in the case of FB a remarkable decrease in the emission intensity was noticed upon complexation but no shift in the spectra was observed. These results are consistent with the formation of a non-emissive inclusion complex. The fluorescence changes observed were analyzed by non-linear fitting using eqn. (4):

$$\frac{I}{I_0} = \frac{1 + \frac{\Phi_c}{\Phi_0} K_{ass}[\beta\text{-CD}]}{1 + K_{ass}[\beta\text{-CD}]} \quad (4)$$

where I and I_0 are the integrated fluorescence intensities in the presence and in the absence of β-CD, and Φ_c and Φ_0 are the quantum yields of the bound and free molecule, respectively. The results of the fitting procedure, shown in the insets of Fig. 3, gave association constants of *ca.* 1400 and 2700 M^{-1} for DF and FB, respectively, in very good agreement with the values found by absorption and ICD measurements.

A recent study on the excited state properties of DF in aqueous medium[28] evidenced a large Stokes shift between the absorption and the emission maxima, pointing to a large geometry difference between the ground and the relaxed excited state. Biphenyl derivatives are known to undergo a change in the angle between the planes of the phenyl rings (twisted in the ground state and coplanar in the excited state) upon light excitation.[29] However, the Stokes shift in DF is *ca.* 20 nm bigger than that observed for 4-biphenylcarboxylic acid, considered as a suitable model compound. This suggests that a change in the degree of molecular planarity cannot be the only structural consequence of the excitation. An additional contribution could originate from an intramolecular proton transfer. Indeed, the proton of the hydroxyl group involved in the hydrogen bond to the carboxyl moiety in the ground state might shift toward the carboxylic oxygen in the excited state, according to the behavior of other salicylic acid derivatives.[30,31] The inclusion of DF in the cavity could in principle influence both the twisting motion around the central single bond and the excited state prototropic shift. However, as observed in other hydroxybiphenyl and phenol derivatives,[27,32] the hydrophilic moiety containing the hydroxyl and carboxylate groups is not expected to be deeply included. Thus, significant perturbation of the intramolecular proton transfer by the macrocycle is not likely. This is also consistent with the changes in the absorption spectra [Fig. 1(a)], which are significant in the 260 nm band, corresponding to transitions localized on the phenyl rings.[33] On the basis of these considerations, the blue shift observed in the fluorescence spectra in the DF–β-CD inclusion complex is attributed mainly to steric hindrance, *i.e.* reaching a planar conformation once the two phenyl rings are constrained by the cavity, similarly to what was proposed for the 4-biphenylcarboxylic acid–β-CD inclusion complex.[34]

This proposal was corroborated by fluorescence polarization measurements, a direct means to assess the hindrance to rotational motions in the excited state. The degree of polarization is expressed by eqn. (5):

$$p = \frac{I_{VV} - GI_{VH}}{I_{VV} + GI_{VH}} \quad (5)$$

where the first and the second indexes refer to the vertical or horizontal orientations of the excitation and emission polarizers. The factor G is a correction for both partial light polarization and unequal transmission of excitation through the sample and is calculated from the ratio I_{HV}/I_{HH} with horizontal orientation of the excitation polarizer.[35] As shown in Fig. 4, the degree of polarization of the DF emission was *ca.* 1.5-fold higher in the presence of 10^{-2} M β-CD than in the absence of it, reaching a constant value of *ca.* 0.06 over most of the excitation range. This finding indicates that the tumbling motion of the excited chromophore finds a partial obstacle during its lifetime due to the constraints exerted by the host. It is thus reasonable to attribute some hindrance to the twisting motion about the central single bond.

In order to rationalize the emitting behavior of the FB–β-CD inclusion complex we have to take into account the value of the fluorescence quantum yield Φ_f in aqueous medium in the absence of the host receptor (*ca.* 0.001),[36] a very low value if compared to that of biphenyl in polar solvents (*ca.* 0.2). This fact accounts for a low-lying and poorly emitting singlet state and is not surprising given the molecular structure of FB. Recent works on the photophysics and photochemistry of this molecule have indeed shown that FB behaves as a biphenyl perturbed by a carbonyl substituent.[36,37] The latter moiety plays a substantial role by providing low-lying n,π* excited states.[36,37] On the basis of the ZINDO/S method a n,π* nature was assigned to the lowest excited singlet state, calculated to lie at 288.3 kJ mol^{-1}.[36] Such a state, less polar than the ground state (2.6 *vs.* 6.93 D, respectively), is expected to be stabilized by the low polarity of the β-CD cavity. This fact can lead to a strong spin–orbit coupling with the close-lying T3(π,π*) state, calculated to lie at 280.3 kJ mol^{-1} and probably somewhat destabilized by the inclusion, thus promoting an increase in the ISC efficiency, similar to what is observed in solvents less polar than water or when the drug is incorporated in micellar media.[36] This mechanism, requiring that the carbonyl group (which the n,π* state is mainly localized on) resides in a hydrophobic microenvironment, is actually supported by NMR results and by the photochemical behavior of the FB–β-CD complex, described in the following sections.

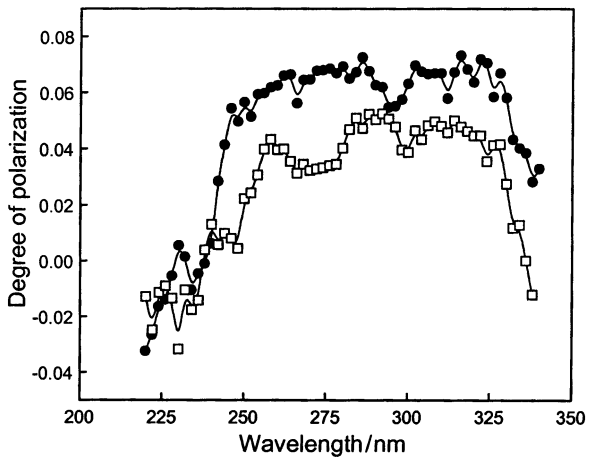


Fig. 4 Excitation polarization spectra of 3×10^{-5} M DF in 10^{-2} M phosphate buffer solution, pH 7.4, (□) in the absence and (●) in the presence of 10^{-2} M β-CD. Cell path 1 cm; $\lambda_{em} = 420$ nm.

NMR experiments

In order to gain more insight into the geometry of the inclusion complexes, we performed ^1H experiments in 2×10^{-4} M DF or FB solutions in D_2O in the absence and in the presence of 10^{-2} M β-CD. Under these conditions, *ca.* 90% of the drug molecules are complexed with the macrocycle.

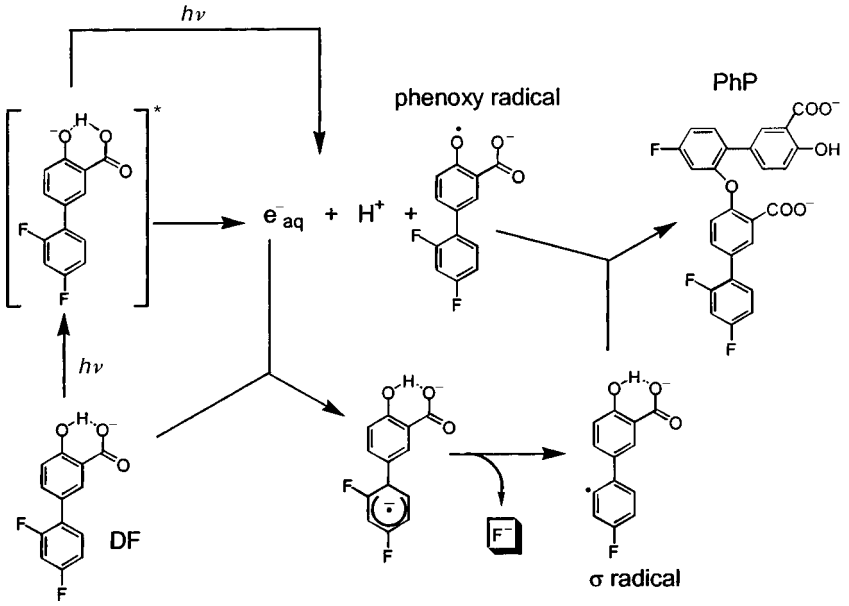
In the case of the DF–β-CD system, the resonances of H-2, H-5 and H-6 of the unfluorinated ring were markedly shifted upfield by 0.12, 0.53 and 0.59 ppm, respectively. By constrast, the multiplet related to H-3, H-5 and H-6 of the fluorinated ring was shifted downfield by *ca.* 0.50 ppm. Such effects, commonly observed for aromatic guests incorporated in CD cavities, are generally caused by the van der Waals interactions with the cavity walls,[38] which are extremely short-range, being proportional to $1/r^6$, where r is the distance between the interacting groups. In our case hydrogen bonds involving the fluorine substituents with the hydrophobic cavity of the macrocycle could significantly contribute to the shifts observed. These findings suggest that both phenyl rings are deeply incorporated in the host cavity.

The resonances of the FB protons were also significantly shifted in the presence of β-CD. In particular, the multiplet related to the unsubstituted aromatic ring was shifted upfield by *ca.* 0.26 ppm; H-2 and H-6 of the substituted phenyl moved downfield by 0.12 ppm whereas H-3 and H-5 of the same ring were only slightly affected. Furthermore, whereas the two aliphatic H close to the carboxyl group were basically unperturbed in the presence of β-CD, the two H adjacent to the carbonyl group moved downfield by 0.03 ppm. On the basis of these results it is reasonable to propose that the FB molecule is deeply included into the cavity and that H-2 and H-6 are closer to the β-CD wall than H-3 and H-5. The carbonyl group has to be located in a hydrophobic microenvironment. Such an inclusion geometry helps to rationalize the photophysical behavior of the FB–β-CD complex, discussed in the previous section, as well as its photochemical behavior reported below.

Photodegradation of the host–guest complexes

DF–β-CD complex. DF was irradiated in the presence of 10^{-2} M β-CD (DF incorporation >90%) in a nitrogen-saturated solution. The chromatographic analysis of the irradiated mixture, performed to 12% conversion of the starting compound, revealed a *ca.* 4-fold reduction of the photodegradation quantum yield, if compared to that observed in the absence of β-CD.[21] Moreover, it is interesting to note that the formation of the product 2′-[2‴,4‴-difluoro-3″-carboxy-(1″,1‴-biphenyl)-4″-oxy]-4′-fluoro-4-hydroxy-(1,1′-biphenyl)-3-carboxylic acid (PhP), the main photoproduct in the absence of oxygen, was suppressed. This result, beyond its photochemical interest, is remarkable in the light of the highly toxic activity displayed by PhP towards cell membranes, leading to this compound being cited as the main species responsible for *in vitro* photoinduced damage.[21]

For the understanding of the photochemical behavior of the inclusion complex, we recall in Scheme 1 the results of our previous studies concerning the photoreactivity of DF in aqueous solution.[21,28] Under UVA excitation DF undergoes photodefluorination, an uncommon reaction in fluoroaromatics due to the strength of the C–F bond (dissociation energy *ca.* 523 kJ mol^{-1}). It was shown that the primary photoprocess is photoionization occurring from the excited singlet state *via* mixed mono- and bi-photonic pathways and leading to the formation of a phenoxy radical and hydrated electrons.[28] Defluorination takes place from a radical anion intermediate, formed by efficient trapping of the photoejected electrons by the ground state DF, and a σ-aryl radical is formed. A cross-combination reaction involving the phenoxyl



Scheme 1

and the σ-aryl radical centers was proposed to be responsible for the formation of PhP. From a strictly mechanistic point of view, the almost exclusive formation of the cross-combination product provides an example of a reaction controlled by the so-called "Fisher–Ingold persistent radical effect".[39,40] Indeed, according to this theory, when one radical (*i.e.* the σ-aryl radical) is less persistent than another (*i.e.* the phenoxyl radical) and its self-termination reaction takes place, the concentration of the more persistent species will increase in time to high levels and steer the system towards cross-termination. Basically, the self-termination of the less persistent radical (fast process) will be suppressed by the slow self-termination of the more persistent radical.

The potential reasons for the enhanced photostability of the inclusion complex, as well as for the suppression of the persistent radical effect, might lie in the peculiar role played by the β-CD in (i) modulating the photoionization pathways, (ii) controlling the efficiency of the electron scavenging process by the ground state DF and (iii) providing a suitable microenvironment to trap the two radical intermediates involved in the cross-combination reaction.

It is known that the efficiency of the one-photon photoionization involving phenol derivatives is particularly sensitive to the environment and that the structure of the solvent around the OH group plays a dominant role. In particular, the formation of H-bonded supramolecular structures in which the OH binds two water molecules by acting as both H-donor and H-acceptor is believed to be a key factor.[41–44] In our case, although the photoionization of DF occurs by mixed biphotonic and monophotonic mechanisms,[28] only the latter dominates under steady state irradiation by low intensity light sources. Therefore, compartmentalization of the phenolic moiety of DF in regions characterized by lower water content than the bulk solution could lead to a decrease in the photoionization efficiency, similarly to what was observed for the phenol derivatives.[32] Nevertheless, a plot of the absorbance changes at 720 nm (absorption maximum of the hydrated electron) as a function of the laser pulse energy, performed both in the presence and in the absence of 10^{-2} M β-CD in argon-saturated solution, did not show any significant difference in the efficiency of the overall photoionization process [Fig. 5(a)]. Moreover, the lifetime of the hydrated electrons was not affected by the presence of β-CD. These results provide direct evidence that the enhanced photostability of DF when encapsulated in β-CD is not related to either hypotheses (i) or (ii) proposed above. Thus, possibility (iii) is considered to be most likely. Indeed, in the light of the more hydrophobic character of the phenoxyl radical compared to DF, it is reasonable to assume that a fraction of these radicals, produced by electron photoejection, remain trapped in the

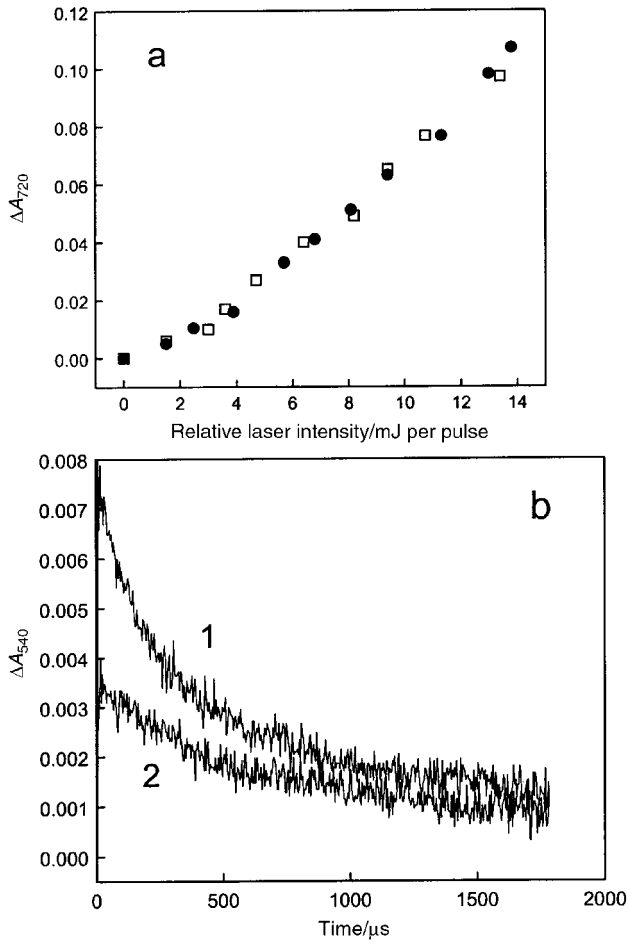
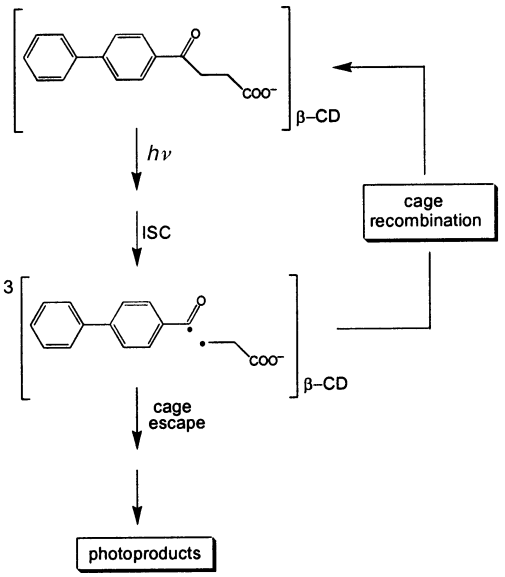


Fig. 5 (a) Laser intensity dependence for the formation of solvated electrons observed in an argon-saturated 3×10^{-5} M DF solution in 10^{-2} M phosphate buffer, pH 7.4 (□) in the absence and (●) in the presence of 10^{-2} M β-CD (ΔA taken 50 ns after the pulse). (b) Kinetic traces for the decay of the phenoxyl radical at 540 nm recorded in the same solution (1) in the absence and (2) in the presence of 10^{-2} M β-CD. Laser excitation at 266 nm, *ca.* 2.5 mJ per pulse.



Scheme 2

cavity. It is well-documented in the literature[45–47] that such species, generally characterized by low reactivity for H-abstraction,[48] can become much more reactive in β-CD, given the presence of 14 available hydrogen atoms bonded to secondary carbons and in close proximity to the radical center. Such a pathway would lead back to the starting compound, thus accounting for the higher photostability of the inclusion complex. The sensibly lower yield of the phenoxyl radical, decaying over a long time scale, observed in the presence of 10^{-2} M β-CD [Fig. 5(b)], is fully consistent with the fast disappearance of a significant fraction of this intermediate within the CD cavity. Moreover, the disappearance of the phenoxy radical in the presence of β-CD is described by second-order kinetics with $k/\varepsilon \approx 2.5 \times 10^5$ cm s^{-1}, a value *ca.* two times smaller than that observed in homogeneous medium. This behavior presents close analogies with that observed in non-ionic micelles.[49]

The total inhibition of the formation of the cross-combination product PhP can also be related to an intra-cavity reaction involving the σ-aryl radical. Given the high H-abstraction ability of this species[50,51] it is reasonable to assume that it reacts promptly before exiting. Unfortunately, due to the optical transparency of the σ-aryl radical in the monitored wavelength range[50,51] it was not possible to gain direct evidence for this transient.

FB–β-CD complex. Irradiation of FB in the presence of 10^{-2} M β-CD (FB incorporation >90%) in nitrogen-saturated solution provoked a significant decrease in the photodegradation quantum yield, which dropped *ca.* 3-fold with respect to that observed in aqueous solution.[22,35] However, the final photoproducts did not change. In a recent study we have shown that FB photodegradation is consistent with an α-cleavage process (Norrish Type I fragmentation) occurring in an efficiently produced, long-lived triplet state and leading to 4-biphenylcarboxyaldehyde as the main stable product in the absence of oxygen.[22,36] It is well known that a confined environment favors radical pair recombination (upon ISC) with respect to diffusion of the radical moieties into the bulk solvent.[11,52–54] Thus, a rationale for the lower photoreactivity of FB in β-CD could be a "cage effect" by the cavity walls on the decay of the radical pair produced in the photocleavage process. Pictorially represented in Scheme 2, this mechanism requires deep inclusion of the carbonyl moiety. That the CO of FB is included deep within the β-CD cavity accords well with both the fluorescence and NMR experiments (*vide supra*).

Conclusions

We studied the encapsulation of DF and FB in β-CD cavities by different spectroscopic techniques. Both compounds are efficiently included in the macrocycle although the more hydrophobic character of FB makes its binding more favorable than that of DF. Accommodation of the guest molecules in the optically active, hydrophobic β-CD cage leads to new properties, that is optical activity and modified fluorescence and photochemistry. The two drugs display a well differentiated sensitivity to the host microenvironment upon light excitation. A non-emissive complex is formed in the case of FB, whereas only a slight decrease of the fluorescence quantum yield, accompanied by an increase in the energy of the emitting state, is noticed for the DF–β-CD complex. The associated guests are characterized by a higher photostability than the free ones. In the case of DF, a drastic change in the nature of the photoproducts is observed. The multifaceted role of β-CD in influencing the efficiency of the primary photochemical act and in interfering with secondary radical reactions was discussed.

The results confirm that CD complexation represents a valid tool for controlling excited state properties of the guest molecules. As already shown in previous work,[17,18,20] the use of CDs as drug-carriers may sometimes represent a simple, cheap and effective strategy to increase drug photostability as well as to minimize drug photoinduced toxic effects on biosubstrates. However, no generalizations are possible in this respect and it is pertinent to stress that a specific investigation of the photobehavior of each particular drug–CD system has to be performed in order to exclude enhanced photolability of the inclusion complex (see the example of the ketoprofen–β-CD system in ref. 14).

Acknowledgements

Financial support from MURST "cofinanziamento di programmi di ricerca di rilevante interesse nazionale" (Progetto: Meccanismi di Processi Fotoindotti in Sistemi Organizzati) and from Istituto Superiore di Sanità (Progetto: Proprietà Chimico-Fisiche dei Medicamenti e loro Sicurezza d'Uso) is gratefully acknowledged. The authors thank Professor G. Condorelli for a critical reading of the manuscript.

References

1 M. L. Bender and M. Komiyana, *Cyclodextrin Chemistry*, Springer–Verlag, New York, 1977.
2 W. Saenger, *Angew. Chem., Int. Ed. Engl.*, 1980, **19**, 344.
3 I. Tabushi, *Acc. Chem. Res.*, 1982, **15**, 66.
4 I. Tabushi and Y. Kuroda, *Adv. Catal.*, 1983, **32**, 417.
5 C. Sirlin, *Bull. Soc. Chim. Fr., Ser. II*, 1984, **1**, 5.
6 J. Sztejtli, *Cyclodextrins and Their Inclusion Complexes*, Akademiai Kiado, Budapest, 1982.
7 G. Wenz, *Angew. Chem., Int. Ed. Engl.*, 1994, **33**, 803.
8 K. Kalyanasundaram, *Photochemistry in Microheterogeneous Systems*, Academic Press, New York, 1987.
9 V. Ramamurthy and D. F. Eaton, *Acc. Chem. Res.*, 1988, **21**, 300.
10 V. Ramamurthy, R. G. Weiss and G. S. Hammond, *Adv. Photochem.*, 1993, **18**, 67.
11 P. Bortolus and S. Monti, *Adv. Photochem.*, 1996, **21**, 1.
12 M. H. Kleinman and C. Bohne, *Organic Photochemistry: Molecular and Supramolecular Photochemistry*, Marcel Dekker Inc., New York, 1997.
13 Y. H. Kim, D. W. Cho, S. G. Kang, Y. Minjoong and D. Kim, *J. Lumin.*, 1994, **59**, 209.
14 S. Monti, S. Sortino, G. De Guidi and G. Marconi, *New J. Chem.*, 1998, **22**, 599.
15 S. Sortino, G. De Guidi, G. Marconi and S. Monti, *Photochem. Photobiol.*, 1998, **67**, 603.
16 S. Monti, S. Sortino, S. Encinas, G. Marconi, G. De Guidi and M. A. Miranda, in *Drugs Photochemistry and Photostability*, ed. A. Albini and E. Fasani, The Royal Society of Chemistry, Cambridge, UK, 1998, pp. 150–161.

17 S. Sortino, J. C. Scaiano, G. De Guidi and S. Monti, *Photochem. Photobiol.*, 1999, **70**, 549.
18 G. De Guidi, G. Condorelli, L. L. Costanzo, S. Giuffrida, S. Monti and S. Sortino, in *Drugs Photochemistry and Photostability*, ed. A. Albini and E. Fasani, The Royal Society of Chemistry, Cambridge, UK, 1998, pp. 194–210.
19 T. H. Hoshino, K. Ishida, T. Irie, F. Hirayama and M. Yamasaki, *J. Inclusion Phenom.*, 1988, **6**, 415.
20 G. De Guidi, G. Condorelli, S. Giuffrida, G. Puglisi and G. Giammona, *J. Inclusion Phenom. Mol. Recognit. Chem.*, 1993, **15**, 43.
21 G. De Guidi, R. Chillemi, S. Giuffrida, G. Condorelli and M. Cambria Famà, *J. Photochem. Photobiol., B: Biol.*, 1991, **10**, 221.
22 G. De Guidi, L. L. Costanzo, G. Condorelli, S. Giuffrida, P. Miano, S. Sortino and A. Velardita, *Gazz. Chim. Ital.*, 1996, **126**, 719.
23 F. Castelli, G. De Guidi, S. Giuffrida, P. Miano and S. Sortino, *Int. J. Pharm.*, 1999, **184**, 21.
24 G. De Guidi, R. Chillemi, L. L. Costanzo, S. Giuffrida, S. Sortino and G. Condorelli, *J. Photochem. Photobiol., B: Biol.*, 1994, **23**, 125.
25 G. Condorelli, L. L. Costanzo, G. De Guidi, S. Giuffrida and S. Sortino, *Photochem. Photobiol.*, 1995, **62**, 155.
26 S. Monti, N. Camaioni and P. Bortolus, *Photochem. Photobiol.*, 1991, **54**, 577.
27 (*a*) P. Bortolus, G. Marconi, S. Monti, B. Mayer, G. Kohler and G. Grabner, *Chem. Eur. J.*, 2000, **6**, 1578; (*b*) P. Bortolus, G. Marconi, S. Monti, G. Grabner and B. Mayer, *Phys. Chem. Chem. Phys.*, 2000, **2**, 2943.
28 S. Sortino, J. C. Scaiano and G. Condorelli, *J. Phys. Chem. B*, 1999, **103**, 9279.
29 E. C. Lim and Y. H. Li, *J. Chem. Phys.*, 1970, **52**, 6416.
30 A. Weller, *Elektrokhimiya*, 1956, **60**, 1144.
31 P. J. Kovi, C. L. Miller and S. G. Schulman, *Anal. Chim. Acta*, 1972, **61**, 7.
32 S. Monti, G. Kohler and G. Grabner, *J. Phys. Chem.*, 1993, **97**, 13011.
33 I. Berlman, *J. Chem. Phys.*, 1970, **52**, 5616.
34 D. W. Cho, Y. H. Kim, S. G. Kang, M. Yoon and D. Kim, *J. Phys. Chem.*, 1994, **98**, 558.
35 J. R. Lacowicz, *Principles of Fluorescence Spectroscopy*, Plenum Press, New York, 1983.
36 S. Sortino, L. J. Martinez and G. Marconi, *New J. Chem.*, submitted.
37 S. Navaratman and S. A. Jones, *J. Photochem. Photobiol, A: Chem.*, 2000, **132**, 175.
38 P. V. Demarco and A. L. Thakkar, *Chem. Commun.*, 1970, 2 and references therein.
39 H. Fisher, *J. Am. Chem. Soc.*, 1986, **108**, 3925.
40 D. Griller and K. U. Ingold, *Acc. Chem. Res.*, 1976, **9**, 13.
41 G. Kohler and N. Getoff, *Chem. Phys. Lett.*, 1974, **26**, 525.
42 J. Zechner, G. Kohler, G. Grabner and N. Getoff, *Can. J. Chem.*, 1980, **58**, 2006.
43 J. Zechner, G. Kohler, G. Grabner and N. Getoff, *Chem. Phys. Lett.*, 1976, **37**, 297.
44 G. Kohler and N. Getoff, *J. Chem. Soc., Faraday Trans. 1*, 1976, **72**, 2101.
45 M. S. Symala, B. Nageswer Rao and V. Ramamurthy, *Tetrahedron*, 1988, **44**, 7234.
46 A. V. Veglia, A. M. Sanchez and R. H. de Rossi, *J. Org. Chem.*, 1990, **55**, 4083.
47 A. V. Veglia and R. H. de Rossi, *J. Org. Chem.*, 1993, **58**, 4941.
48 V. W. Bowry and K. U. Ingold, *Acc. Chem. Res.*, 1999, **32**, 27 and references therein.
49 S. Sortino, G. De Guidi, S. Giuffrida, A. Belvedere and G. Condorelli, *J. Phys. Chem. A*, 2000, **104**, 11918.
50 J. C. Scaiano and L. C. Stewart, *J. Am. Chem. Soc.*, 1983, **105**, 3609.
51 V. Madhavan, R. H. Schuler and R. W. Fessenden, *J. Am. Chem. Soc.*, 1978, **100**, 888.
52 N. J. Turro, M. Graetzel and A. M. Braun, *Angew. Chem., Int. Ed. Engl.*, 1980, **19**, 675.
53 N. J. Turro and W. R. Cherry, *J. Am. Chem. Soc.*, 1978, **100**, 7431.
54 N. J. Turro and B. Kraeutler, *J. Am. Chem. Soc.*, 1978, **100**, 7432.