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Enhanced Photosafety of Cinacalcet upon Complexation with Serum Albumin

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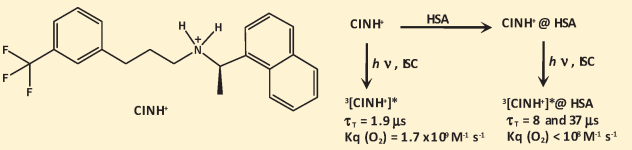
ABSTRACT: Cinacalcet (CIN) is a calcimimetic drug, which contains a naphthalene chromophore and binds almost quantitatively to human serum albumin (HSA). In the present work, the excited states of CIN have been characterized in order to obtain relevant information about complexation of CIN with HSA. The fluorescence spectrum in acetonitrile, at λ_{exc} = 290 nm, displayed two bands with maxima at 332 and 439 nm, assigned to the monomer and exciplex emission. Upon protonation of the amino group, the exciplex band disappeared, with a concomitant increase of the monomer emission intensity. Time-resolved fluorescence evidenced an intramolecular dynamic quenching, attributed to exciplex formation and/or photoinduced electron transfer, in agreement with the favorable thermodynamics predicted by the Rehm−Weller equations. Diffusion controlled dynamic quenching of $CINH^+$ fluorescence by oxygen was observed. The emission properties in PBS were similar to those obtained for $CINH^+$ in acetonitrile. Laser flash photolysis (LFP) of CIN and $CINH^+$ in acetonitrile/N_2, at λ_{exc} = 308 nm, gave rise to the naphthalene-like triplet excited states, with maxima at 420 nm and lifetimes of 4 and 7 μs; they were efficiently quenched by oxygen. No significant singlet excited state interaction was observed in $CINH^+$/HSA complexes, as revealed by the emission spectra, which were roughly explained taking into account the relative contributions of drug and protein in the absorption spectra. Upon LFP of the complexes, triplet excited states were generated; the decays monitored at 420 nm were satisfactorily fitted using a function containing two monoexponential terms, corresponding to a short-lived (τ_1 = 8 μs) and a long-lived (τ_2 = 37 μs) component. This indicates that the drug is incorporated into two different binding sites of HSA. Despite the long triplet lifetimes of the $CINH^+$/HSA complexes, the rate constant of quenching by oxygen was found to be 2 orders of magnitude lower than that determined in acetonitrile, which can be attributed to the relative slower diffusion rates in this microheterogeneous system. Therefore, the protein microenvironment protects cinacalcet from attack by oxygen; this prevents the phototoxic effects caused by formation of singlet oxygen and results in an enhanced photosafety of the drug.

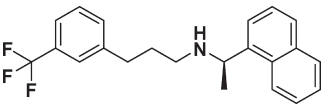


INTRODUCTION

Cinacalcet (CIN, Chart 1) is a recently marketed calcimimetic drug,[1,2] which activates the calcium receptor (CaR) and inhibits parathyroid hormone (PTH) secretion, acting as a positive allosteric modulator of the CaR located on the surface of parathyroid cells.[3] This calcimimetic agent is used for treatment of persistent hyperparathyroidism in renal transplanted patients, with chronic kidney disease and renal failure in dialysis.[4−6] In addition to lowering serum PTH and calcium levels, clinical trials in patients with uncontrolled secondary hyperparathyroidism of uremia have demonstrated that CIN decreases serum phosphorus and combined calcium/phosphorus levels.[7] It is also used for the treatment of hypercalcemia in patients with parathyroid carcinoma, by signaling the need to produce lower amounts of PTH in order to decrease the calcium level in blood.[8−10] The pharmacological activity of CIN is mainly attributed to the (R) isomer.

Photophysical and photochemical studies on drugs containing a naphthalene (NP) chromophore, such as naproxen, propanolol, or nabumetone, have previously been reported.[11−16] Singlet oxygen generation has been considered to be responsible for the phototoxic reactions associated with NP-derived drugs, as this reactive species is able to produce damage to key biomolecules, such as proteins, lipids, or nucleic acids.[17,18] Cinacalcet also contains a NP unit; therefore, it could in principle produce photobiological damage. However, no literature report on the possible phototoxicity of CIN has appeared as yet.

Chart 1. Chemical Structure of (*R*)-Cinacalcet



Cinacalcet binds almost quantitatively to human serum albumin (HSA), the major transport protein in humans.[3] Since binding of a drug to serum proteins plays a significant role in pharmacokinetics, pharmacodynamics, toxicity, and clinical therapy,[19−27] a detailed

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understanding of CIN-HSA interactions becomes an important issue.

With this background, the goal of the present work is to characterize the excited states of CIN and to obtain relevant information about the complexation of CIN with HSA, using photophysical techniques such as fluorescence and laser flash photolysis. Specifically, it will be shown that the protein microenvironment protects the CIN triplet excited state from attack by oxygen, which implies a less efficient formation of singlet oxygen, thus reducing the photobiological risk.

EXPERIMENTAL METHODS

Materials and Solvents. Cinacalcet hydrochloride was extracted from commercial Mimpara 60 mg (Amgen, Spain). The content of six pills was powdered in a mortar, suspended in NH_4OH 1 M (50 mL) and extracted with CH_2Cl_2 (4×50 mL). The combined organic layers were washed with brine (3×150 mL) and water (3 × 150 mL), dried over $MgSO_4$, and evaporated under reduced pressure. The residue was purified by column chromatography through silica gel 60 (dichloromethane:methanol:ammonium hydroxide 98:2:0.1 *v*/*v*/*v*) to give the corresponding free base of CIN as a colorless oil.

Human serum albumin (HSA, min. 99% fatty acid free) was purchased from Sigma−Aldrich (Steinheim, Germany). Phosphate buffered saline solution (PBS, pH 7.4, 0.01 M) was prepared by dissolving Sigma tablets in the appropriate amount of deionized water. All other solvents used were of HPLC grade.

Absorption and Fluorescence Measurements. UV spectra were recorded on a Cary 300 (Varian) spectrophotometer. Steady-state fluorescence experiments were carried out using a Photon Technology International (PTI, Germany) LPS-220B spectrofluorometer, equipped with a monochromator in the wavelength range of 200−700 nm. The time-resolved fluorescence determinations were performed with a Time Master fluorescence lifetime spectrometer TM 2/2003 from PTI, using a hydrogen/nitrogen flash lamp as the excitation source. The kinetic traces were fitted by one or two monoexponential terms decay functions, with using a reconvolution procedure to separate from lamp pulse profile. For fluorescence measurements, concentrations were fixed by adjusting the absorbance of the solutions at the arbitrary value of 0.1 at the excitation wavelength of 290 nm. Fluorescence quantum yield (ϕ_F) of CIN in the presence of acetic acid was determined in acetonitrile, by comparison with 1-methylnaphthalene as standard (ϕ_s=0.19 in acetonitrile)[28] under anaerobic conditions, using eq 1

$$\phi_F = \phi_s \frac{n^2}{{n_s}^2} \frac{I_i}{I_s} \frac{(1 - 10^{-A_s(\lambda_{exc})})}{(1 - 10^{-A_i(\lambda_{exc})})} \quad (1)$$

where A is the absorbance at the excitation wavelength, I is the area under the corrected fluorescence spectrum, and n is the refractive index of the solvent. The subscripts "i" and "s" refer to CIN and the standard respectively. Emission measurements were performed in the region 300−560 nm, and the excitation in the range 200−310 nm. All measurements were performed at room temperature using 10 × 10 mm^2 quartz cells with 4 mL capacity under anaerobic conditions.

Laser Flash Photolysis. Laser flash photolysis (LFP) experiments were carried out with a pulsed XeCl excimer laser (λ_{exc}= 308 nm, ca. 17 ns pulse width, < 100 mJ per pulse) or a Nd:YAG SL404G-10 Spectron Laser Systems (λ_{exc} = 266 nm, ca. 10 ns pulse width, < 10 mJ per pulse). In general, samples received between 1 and 3 pulses for all the kinetic experiments. A pulsed Lo255 Oriel Xenon lamp was used as detecting light source. The observation wavelength was selected with a 77200 Oriel monochromator, and the signal amplified by an Oriel photomultiplier tube (PMT) system made up of a 77348 side-on tube, 70680 housing, and a 70705 power supply. The signal was registered with a TDS-640A Tektronix oscilloscope and subsequently transferred to a personal computer. Concentrations of the samples were fixed by adjusting the absorbance of the solutions at the arbitrary value of 0.3 at the excitation wavelength. All transient spectra were recorded using 10 × 10 mm^2 quartz cells with 4 mL capacity and were bubbled for 10 min with N_2, O_2, or air before acquisition. Decay traces were registered at 420 nm. Experiments conducted in the presence of different protein concentrations were carried out on air-saturated PBS. Solutions were incubated 1 h in the dark before acquisition. In order to obtain deaerated solutions of CIN/HSA, experiments were performed using sodium sulfite (ca. 2×10^{-3} M) for chemical deoxygenation.[29] Triplet lifetimes of CIN in acetonitrile in the presence or absence of acid were obtained from the monoexponential fitting, whereas for CIN/HSA, a function with two monoexponential terms was needed. Triplet quantum yields (ϕ_T) of CIN and $CINH^+$ were determined in acetonitrile by comparison with 1-methylnaphthalene as standard (ϕ_T = 0.58 in deaerated cyclohexane).[28] The $T-T$ molar absorption coefficients were estimated by the energy transfer method, using benzophenone as donor. They were found to be very similar to that reported for 1-methylnaphthalene (11 200).[30] It was assumed that this parameter does not change significantly in aqueous media. All measurements were performed at room temperature.

Singlet Oxygen Measurements. The singlet oxygen phosphorescence decay traces after the laser pulse were registered at 1270 nm employing a Peltier-cooled (−62.8 °C) Hamamatsu NIR detector operating at 588 V, coupled to a computer-controlled grating monochromator. A pulsed Nd: YAG L52137 V LOTIS TII was used at the excitation wavelength of 266 nm. The single pulses were ca. 10 ns duration, and the energy was lower than 10 mJ/pulse. The laser flash photolysis system consisted of the pulsed laser, a 77250 Oriel monochromator and an oscilloscope DP04054 Tektronix. The output signal from the oscilloscope was transferred to a personal computer. All measurements were made at room temperature, air atmosphere, using acetonitrile or D_2O (pD 7.4) as solvents in 10 × 10 mm^2 quartz cells with 4 mL capacity. The absorbance of the samples was 0.30 at the laser wavelength. The singlet oxygen quantum yield (ϕ_Δ) was determined for each compound using 1-methylnaphthalene in acetonitrile (ϕ_Δ = 0.33)[31] or naproxen in D_2O at pD 7.4 (ϕ_Δ = 0.25)[17] as standards. Singlet oxygen formations was calculated from the slope of the plots of signal intensity at zero time versus laser light intensity according to eq 2 using a set of neutral density filters to obtain different laser intensities.

$$\phi_{\Delta(sample)} = \frac{I_{sample}}{I_{standard}} \phi_{\Delta(standard)} \quad (2)$$

where I_{sample} is the emission intensity for the sample, $I_{standard}$ is the emission intensity for the standard (1-methylnaphthalene

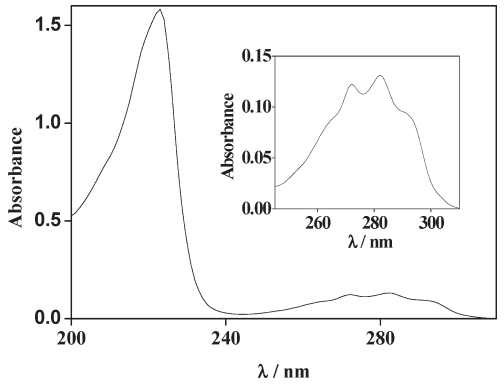

Figure 1. UV−vis absorption spectrum of CIN in acetonitrile 2×10^{-5} M. Insert: Magnification of the long-wavelength absorption region.

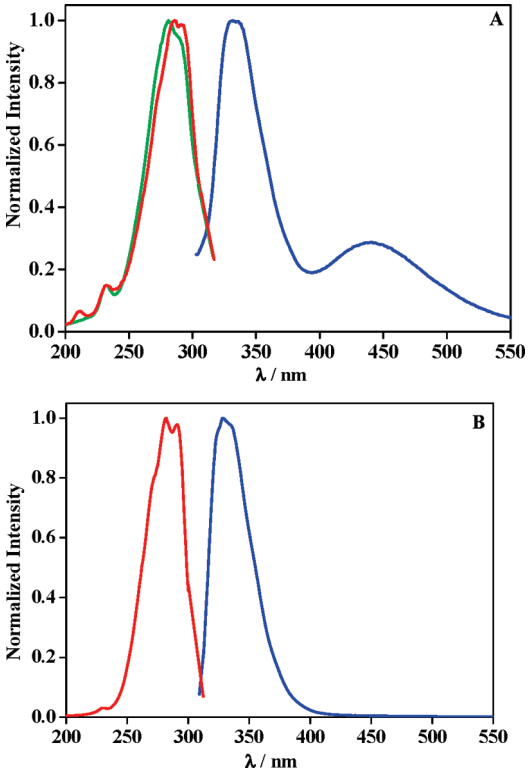

Figure 2. (A) Normalized emission and excitation spectra of CIN (2×10^{-5} M) in acetonitrile/N_2. Blue: emission, λ_{exc} = 290 nm, A_{290} = 0.1. Green: excitation, λ_{em} = 320 nm. Red: excitation, λ_{em} = 420 nm. (B) Normalized emission and excitation spectra of CIN in acetonitrile (2×10^{-5} M) /acetic acid (2×10^{-2} M)/N_2. Blue: emission, λ_{exc} =290 nm. Red: excitation, λ_{em} = 320 nm.

or naproxen), and $\phi_{\Delta(standard)}$ is the quantum yield of singlet oxygen formation for the standard.

In general, the experiments were repeated at least three times with fresh samples. For each set of experiments, the obtained values were coincident within ±5% error margins.

RESULTS AND DISCUSSION

Photophysics of Cinacalcet in Acetonitrile. The absorption spectrum of CIN in acetonitrile is shown in Figure 1. It exhibits a fine-structured band reaching beyond 300 nm, typical of naphthalene derivatives.[13,14]

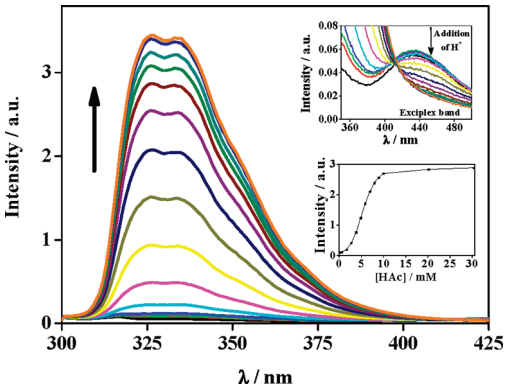

Figure 3. Fluorescence spectra of CIN in acetonitrile/N_2 (λ_{exc} = 290 nm, A_{290} = 0.02) in the presence of increasing amounts of acetic acid (0−3 × 10^{-2} M). Upper insert: magnified traces between 350 and 500 nm. Lower insert: emission intensity at 332 nm vs acetic acid concentration.

Emission studies were performed by excitation at 290 nm in deaerated acetonitrile. The spectrum displayed two bands with maxima at 332 and 439 nm (Figure 2A); their intensity was very low compared with emission of 1-methylnaphthalene as standard, suggesting efficient intramolecular quenching by the amino group. The long wavelength band was in principle attributed to an intramolecular exciplex, based on the fact that the excitation spectra for the two bands were nearly identical.

Interestingly, in the presence of acetic acid (2×10^{-2} M), where the amino group should be completely protonated, the longer wavelength band disappeared (Figure 2B), and the intensity of the monomer band was much higher. Actually, addition of increasing amounts of acetic acid (0−30 mM) to an acetonitrile solution of CIN resulted in a dramatic increase of the 332 nm band, accompanied by the progressive disappearance of the exciplex emission at 439 nm (Figure 3). The absorption spectrum did not show any significant difference upon addition of acetic acid. This in good agreement with an intramolecular singlet excited state interaction between the naphthalene moiety and the amino group, which is disrupted in acidic medium.

From the intersection between normalized excitation and emission bands, a singlet energy value of 91 kcal/mol was obtained, both for the protonated ($CINH^+$) and the neutral forms (Table 1). Fluorescence quantum yield (ϕ_F) of CIN under different conditions was determined using 1-methylnaphthalene as standard (ϕ_F = 0.19); the values are also shown in Table 1. In the case of $CINH^+$, ϕ_F was comparable to that of the standard; however, in the absence of acid the value was 2 orders of magnitude lower. Time-resolved fluorescence experiments evidenced an intramolecular dynamic quenching, as expected for exciplex formation. Thus, decay traces at λ_{em} = 320 nm in acetonitrile under N_2, fitted to a monoexponential function with a lifetime (τ_F) of 40 ns in acidic medium; by contrast, τ_F was remarkably shorter in the absence of acetic acid (Table 1).

The nature of monomer fluorescence quenching by the amine moiety could be attributed either to photoinduced electron transfer (which is a typical process in naphthalene derivatives)[14,32] or to exciplex formation.[33] Using the Rehm−Weller equations (eqs 3 and 4), where E_{ox} is the oxidation potential of the donor (diethylamine, 1.01 V),[34] E_{red} is the reduction potential of acceptor (1-methylnaphthalene, −2.58 V), E_{0-0} is the singlet energy of CIN (91 kcal/mol), ε is the acetonitrile dielectric constant (37.5 F/m), μ^2/ρ^3 is taken as 0.75,[13] the

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Table 1. Photophysical Parameters Obtained for CIN in Acetonitrile, under Different Conditions[a]

parameters	CIN	$CINH^+$
E_S (kcal/mol)	91	91
ϕ_F (N_2)	<0.01	0.14
ϕ_F (air)	<0.01	0.07
ϕ_F (O_2)	<0.01	0.02
τ_F (ns)	<1	40
k_{Fq} (M^{-1} s^{-1})[b]		1.7×10^{10}
ϕ_T (N_2)	0.60	0.53
ϕ_T (air)	0.53	0.49
ϕ_T (O_2)	0.34	0.43
τ_T (μs)	4.0	7.0
k_{Tq} (M^{-1} s^{-1})[b]	1.6×10^9	9.8×10^8
ϕ_Δ (air)	0.35	0.35

[a] Relative errors lower than 5% of the stated values. [b] Rate constant for fluorescence or triplet quenching by oxygen.

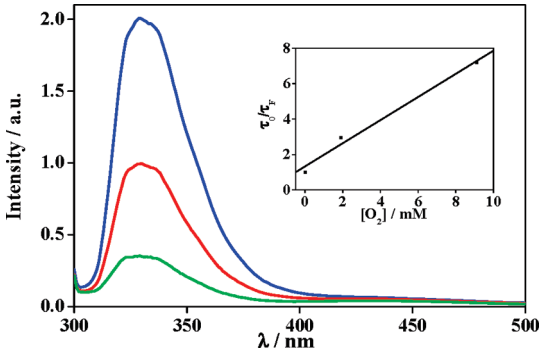

Figure 4. Quenching of $CINH^+$ fluorescence by oxygen. Emission spectra recorded in acetonitrile (2×10^{-5} M) /acetic acid (2×10^{-2} M) (λ_{exc} =290 nm) under nitrogen (blue), air (red), and oxygen (green). Insert: Stern−Volmer plots obtained from lifetime measurements.

estimated values of ΔG_{ex} and ΔG_{et} were found to be −4.6 and −9.9 kcal mol^{-1}, respectively.

$$\Delta G_{ex} = E_{ox} - E_{red} - \frac{E_{0-0}}{23} - \frac{\mu^2}{\rho^3}\left(\frac{\varepsilon - 1}{2\varepsilon + 1} - 0.19\right) + 0.38 \quad (3)$$

$$\Delta G_{et} = E_{ox} - E_{red} - \frac{E_{0-0}}{23} + \frac{2.6}{\varepsilon} - 0.13 \quad (4)$$

The favorable ΔG_{ex} estimation is in agreement with experimental observation of the 439 nm exciplex band; however, the low intensity of this long wavelength emission does not account for the almost complete disappearance of the monomer band. This suggests that the contribution of exergonic intramolecular electron transfer to the observed quenching must be significant.

Furthermore, quenching of $CINH^+$ fluorescence by oxygen was observed to take place (Figure 4). Basically, this was found to be dynamic in nature, as indicated by the shortening of fluorescence lifetimes in the presence of increasing amounts of oxygen. The rate constant for this process was obtained from the Stern−Volmer plots (see Insert in Figure 4) and found to be nearly diffusion controlled (value in Table 1).

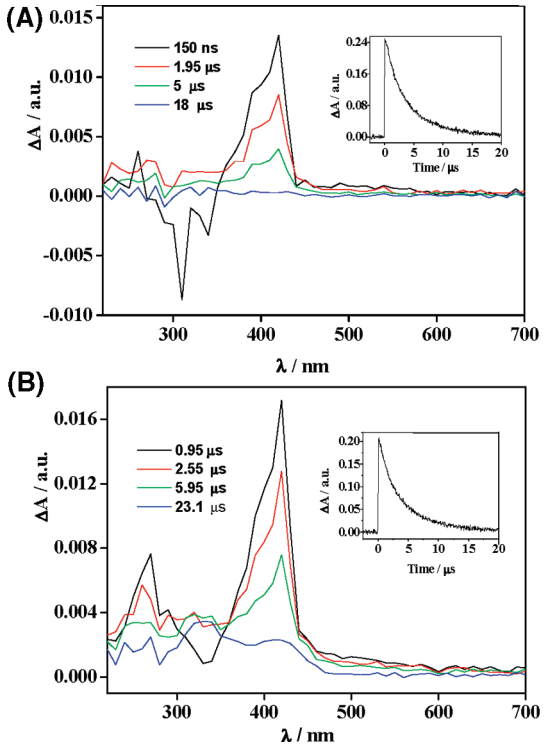

Figure 5. Laser flash photolysis of CIN (A) and $CINH^+$ (B) in acetonitrile/N_2. Absorbance of the samples was 0.3 at the laser excitation wavelength (308 nm). Spectra obtained at different delay times after the laser pulse. Inserts: decay traces monitored at λ_{max} = 420 nm.

Laser flash photolysis studies were performed on CIN and $CINH^+$ in acetonitrile/N_2, at λ_{exc} = 308 nm. In both cases, the naphthalene-like triplet excited states were detected as transient absorptions with maxima at 420 nm (Figure 5, panels A and B), which were efficiently quenched by oxygen. The decay kinetics of these transient species (^{3}CIN* and $^3CINH^{+}$*) are shown in the corresponding inserts. Their fitting led to triplet lifetime values of 4 and 7 μs for CIN and $CINH^+$, respectively.

The intersystem crossing quantum yields of CIN and $CINH^+$ were determined using 1-methylnaphthalene in cyclohexane as standard (ϕ_T = 0.58).[28] The obtained values are shown in Table 1. It is remarkable that triplet formation for the neutral form of CIN was unusually efficient, considering the extensive intramolecular quenching of its singlet excited state precursor. This suggests that alternative pathways are available to populate ^{3}CIN*, namely back electron transfer at the radical ion pair or intersystem crossing at the exciplex. On the other hand, the lack of correspondence between the ϕ_F and ϕ_T values of $CINH^+$ under N_2, air and O_2 (Table 1) indicated that fluorescence quenching by oxygen contributes to formation of $^3CINH^{+}$*, via a charge-transfer complex.[31,35] Scheme 1 summarizes the different mechanistic pathways interconnecting the excited states of CIN and $CINH^+$.

In this context, it is known that oxygen quenching of singlet excited states may yield triplet states by enhanced intersystem crossing.[31,36] As a result, $^1O_2$ may be produced, provided that the singlet−triplet energy gap is higher than 23 kcal/mol. In naphthalene derivatives, although this requirement is fulfilled (31 kcal/mol in the case of $CINH^+$) triplet quenching has been proven to be the almost exclusive source of $^1O_2$. As a matter

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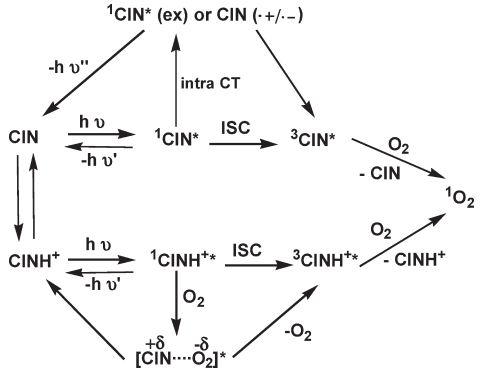
Scheme 1. Mechanistic Pathways of the CIN and $CINH^+$ Excited States



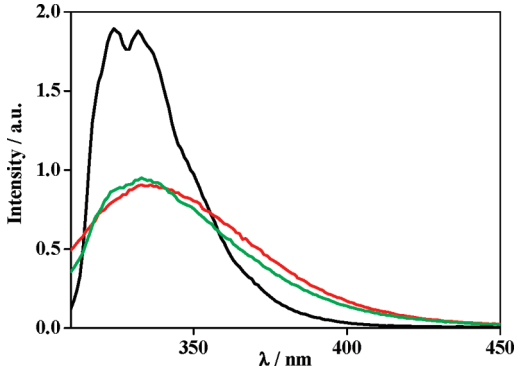


Figure 6. Fluorescence spectra of $CINH^+$ in PBS (black) and in PBS/HSA at 1:1 drug/protein molar ratio (green). Protein emission in the absence of $CINH^+$ is also shown (red trace). In all cases, the absorbance of the sample at 290 nm was fixed at 0.1.

of fact, the excited triplet states of CIN and $CINH^+$ were quenched by oxygen; the corresponding rate constants were determined using the Stern−Volmer relationships and are given in Table 1. The singlet oxygen quantum yields were determined for the NIR phosphorescence emission at 1270 nm and found to be 0.35 (see Table 1).

Photophysics of Cinacalcet/Protein Complexes in Neutral Aqueous Medium. The emission spectrum of $CINH^+$ in PBS was measured at 290 nm (A_{290} = 0.1), under air. As the reported pK_a value of CIN is 8.72,[37] basically all the compound is protonated under the employed experimental conditions (pH 7.4). Parallel measurements were made in the presence of equimolar amounts of HSA, and a control experiment was performed with the protein alone (Figure 6 and Table 2).

As expected, the protein displayed the typical emission maximum at 340 nm, corresponding to its only tryptophan residue;[38] hence, both HSA and $CINH^+$ absorb and emit in the same wavelength range, making it difficult to determine ϕ_F and τ_F in an accurate way. The ϕ_F = 0.06 given in Table 2 corresponds to the combined emission of the drug and the protein; however the intrinsic ϕ_F of the $CINH^+$@HSA complex must be very similar to the value found in the absence of protein (0.08). Actually, the emission of a 1:1 $CINH^+$/HSA mixture (Figure 6) roughly matched the simulated trace, taking into account the relative contributions of free protein and drug in the absorption spectrum. Thus, no protein-to-drug energy transfer was observed in

Table 2. Photophysical Parameters Obtained for $CINH^+$ in Aerated PBS[a]

parameters	$CINH^+$	$CINH^+$/HSA
ϕ_F	0.08	0.06
τ_F (ns)	30	ND[b]
ϕ_T	0.49[c]	0.56
τ_T (μs)	1.9[c]	8 and 37
k_{Tq} (M^{-1} s^{-1})[d]	1.7×10^9	5.4×10^7
ϕ_Δ (air)	0.35	*ca.* 0

[a] Relative errors lower than 5% of the stated values. [b] ND: not determined, as emission contained both tryptophan and $CINH^+$ contributions. [c] Excitation wavelength: 266 nm. [d] Rate constant for triplet quenching by oxygen.

the complex. Similar behavior has been observed for related naphthalene derivatives.[22]

Transient absorption spectroscopy was carried out in air-saturated PBS solutions. Unfortunately, the solubility of $CINH^+$ in this medium was found to be very limited, so it was not possible to prepare clear solutions of the appropriate absorbance (0.3) at 308 nm. Therefore, measurements were also performed using 266 nm as excitation wavelength. Interestingly, this problem was circumvented in the presence of equimolar amounts of HSA, where the drug was completely soluble, indicating efficient formation of the expected complexes.

After incubation for 30 min in the dark, transient absorption spectra were obtained (Figure 7A); they were assigned to the triplet−triplet transition, by comparison with the spectra of CIN in acetonitrile (see above). The decays monitored at 420 nm (Figure 7B) were satisfactorily fitted using a function containing two monoexponential terms, corresponding to a short-lived (τ_1 = 8 μs) and a long-lived (τ_2 = 37 μs) component. This indicates that the drug is incorporated into two different binding sites of HSA, with relative contributions (estimated from the preexponential factors) of 45% and 55%, respectively. To rule out that the short-lived component is due to free $CINH^+$ in the bulk solution, increasing amounts of HSA were added; as a matter of fact, the 8 μs contribution remained unaltered, confirming the initial assignment.

Determination of binding constants was achieved from laser flash photolysis experiments at λ_{exc} = 266 nm on solutions of CIN in PBS (5×10^{-5} M), varying HSA concentrations in the range $(4-6) \times 10^{-5}$ M . For a reversible binding to HSA leading to a 1:1 complex ($CINH^+$@HSA), the equilibrium is represented by

$$CINH^+ + HSA \overset{K_B}{\rightleftarrows} CINH^+@HSA$$

$$K_B = \frac{[CINH^+@HSA]}{[CINH^+][HSA]}$$

where K_B is the binding constant, [$CINH^+$@HSA] is the concentration of the complex, and [$CINH^+$] and [HSA] are concentrations of the noncomplexed species in solution. The required concentrations were obtained by fitting the triplet decay traces at 420 nm, as the contribution of three terms with different lifetimes (free, site-I bound, and site-II bound $CINH^+$). Since the relative population of the two protein sites was comparable (see above), the affinities were assumed to be similar; hence, the percentages of the two bound components were combined to obtain an average K_B value that was found to be 6.1×10^5 M^{-1}.

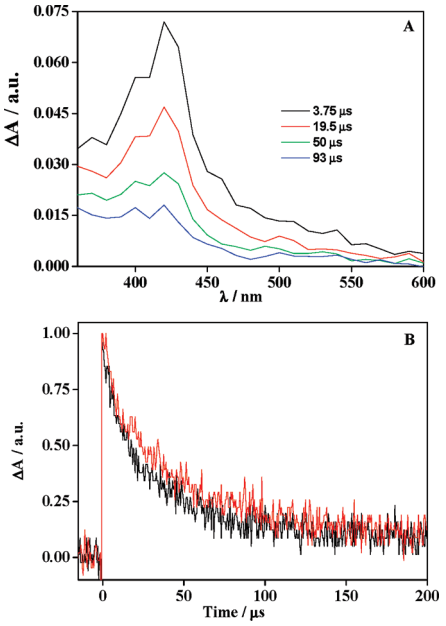


Figure 7. Laser flash photolysis of $CINH^+$ in the presence of HSA, at 1:1 molar ratio, under aerobic conditions. Absorbance of the samples was 0.3 at the laser excitation wavelength (308 nm). (A) Spectra obtained at different delay times after the laser pulse. (B) Normalized decay traces monitored at λ_{max} = 420 nm for $CINH^+$ in the absence (black) and in the presence of sulfite (red).

From the intensity of the triplet signal immediately after the laser pulse, an intersystem crossing quantum yield of 0.56 was determined for $CINH^+$ within the protein binding sites, under aerobic conditions, which is similar to that found in acetonitrile. To investigate triplet quenching by oxygen in the protein microenvironment, $CINH^+$/HSA solutions were submitted to chemical deoxygenation by addition of sodium sulfite (ca. 2×10^{-3} M). Despite the long triplet lifetimes in this medium, the rate constant was found to be 2 orders of magnitude lower than that determined in the bulk solution (5.4×10^7 $M^{-1}s^{-1}$). This can be attributed to the relative diffusion rates, which tend to be slower in microheterogeneous systems. As a consequence, the singlet oxygen quantum yield inside the protein was found to be nearly negligible, while its value was ϕ_Δ = 0.35 in the bulk aqueous solution.

CONCLUSIONS

Cinacalcet is a naphthalene-containing photoactive drug. Both in organic and aqueous media, its most remarkable photophysical property from the biological point of view is formation of the triplet excited state, with a relatively high quantum yield. Upon administration to patients, this drug is known to bind almost completely to human serum albumin; in the resulting complexes, the triplet lifetime is remarkably longer than in the bulk solution. More importantly, the rate constant of triplet quenching by oxygen is 2 orders of magnitude lower than in solution, which can be attributed to the relative slower diffusion rates in this microheterogeneous system. Therefore, the protein microenvironment protects cinacalcet from attack by oxygen; this prevents the phototoxic effects caused by formation of singlet oxygen and results in an enhanced photosafety of this drug.

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