Photophysical properties of a photocytotoxic fluorinated chlorin conjugated to four β-cyclodextrins

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Electronic Supplementary Information

1 - Characterization of the chlorins by mass spectrometry

Mass spectra were recorded using electrospray ionization, ESI-MS and Matrix Assisted Laser Desorption Ionization, MALDI-MS techniques, in positive mode. MALDI-MS spectra were acquired with a MALDI-TOF/TOF Applied Biosystems 4800 Proteomics Analyser (Applied Biosystems, Framingham, MA, USA). This instrument is equipped with a nitrogen laser emitting at 337 nm and operating in reflectron mode. Spectra were acquired using as a matrix a solution of α-cyano-4-hydroxycinnamic acid (Aldrich), prepared in a 1:1 mixture of acetonitrile with water containing 0.1% trifluoroacetic acid. The ESI-MS spectra were acquired with a Linear Ion trap LXQ (ThermoFinnigan, San Jose, CA, USA) after averaging 1500 shots. Samples were introduced into the mass spectrometer at a flow rate of 10 μL min^{-1}. Typical ESI conditions were: nitrogen sheath gas 20.7 kPa, spray voltage 5.5 kV, heated capillary temperature 350 °C, capillary voltage 1 V and tube lens voltage 40 V. Data acquisition was carried out with Xcalibur data system.

The ESI-MS of the chlorin FC (Fig. 1) shows the $[M+H]^+$ at *m/z* 1110, and a low abundance ion at *m/z* 975, formed by loss of a neutral species of 135 Da and molecular formula $OCH_2NCH_2C_6H_5$, due to cycloreversion by retro-1,3-dipolar cycloaddition. The same ions are observed in the MALDI-MS spectrum of FC (Fig. 1), but in this case the ion $[M+H\text{-}135Da]^+$ shows higher relative abundance than the $[M+H]^+$ ion at *m/z* 1110, indicating that the cycloreversion process occurred more extensively under MALDI ionization conditions. Although MALDI is a mild ionization method, the energy transfer to the protonated molecules during the ionization process is higher than in ESI process.[1] Similar elimination by cycloreversion was observed for related chlorins, under liquid secondary ion mass spectrometry (LSIMS) and ESI-MS conditions.[2,3]

The MALDI-MS spectrum of the tetrasubsituted chlorin CDFC showed a group of ions composed by several molecular envelopes (Fig. 2). The one with the highest *m/z* value (*m/z* 5571.4), although in low abundance, corresponds to the tetrasubstituted chlorin CDFC. Since the calculated isotopic mass for the $[M+H]^+$ ion is 5570.5 the observation of the ion at *m/z* 5571.4 indicates that an ion $[M+2H]^+$ could be formed under MALDI conditions. Similar behaviour was observed for fluorinated porphyrins and porphyrin dimers under LSIMS ionization conditions.[4-7] Overall, the molecular envelope corresponds to the overlap of both isotopic patterns of the $[M+H]^+$ and $[M+2H]^+$ ions.

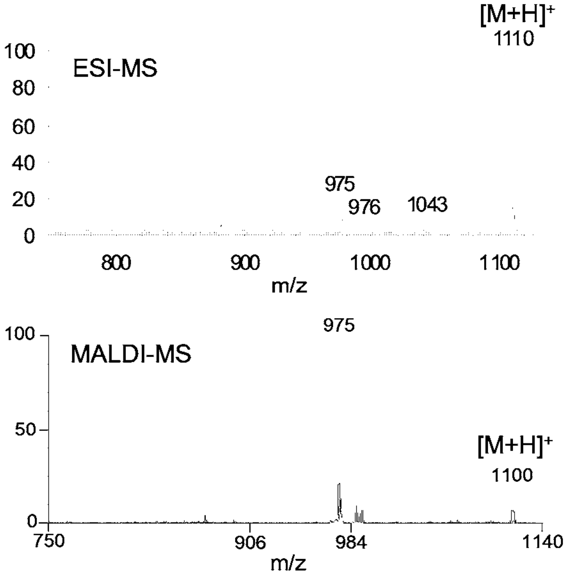

Fig. 1 ESI-MS and MALDI-MS spectra of the non-substituted chlorin FC

In the MALDI-MS spectrum of CDFC (Fig 2), the most abundant ion in the cluster of ions, is the one at *m/z* 5436. As observed with FC, this ion presumably results from the loss of a neutral species with molecular formula $OCH_2NCH_2C_6H_5$ (-135 Da) by retro-1,3-dipolar cycloaddition. In the spectrum of CDFC (Fig. 2), the ratio of the ions $[M+H]^+$ and $[M+H\text{-}135Da]^+$ is similar to the one observed in the MALDI-MS from chlorin FC (Fig. 1). This indicates that the cycloreversion process takes place under the ionization conditions.

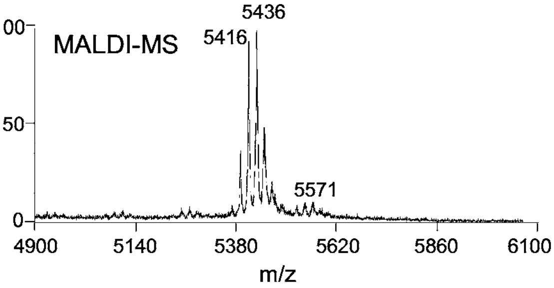

Fig. 2 MALDI-MS spectrum of CDFC

The second most abundant ion of the MALDI-MS spectrum appearing at *m/z* 5416, is due to the loss of HF from the ion at *m/z* 5436. The loss of HF fragment was previously observed with other porphyrins bearing fluorine atoms in the phenyl rings.[5,7,8] Another abundant ion was observed at *m/z* 5452; its formation corresponds to the partial loss of the isoxazolidine ring and it can be justified by loss of a neutral fragment $CH_2NCH_2C_6H_5$ (-119 Da).

2 - Determination of triplet molar extinction coefficients

Method A

The sequence of reactions involved in this process may be summarized as:
^{3}FC → FC (decay to ground state, rate k_1)
^{3}FC + β-car → ^{3}β-car + FC (energy transfer, rate k_t)
^{3}β-car → β-car (decay to ground state, rate k_3)
The energy transfer from ^{3}FC to ground state β-car is shown on Fig. 3A where the characteristic absorbance of ^{3}β-car at about 500 nm in toluene is clearly observed 2 and 10 µs after the pulse, this time corresponding to maximal ^{3}β-car transient absorbance (Abs$_{max}$) (Fig. 3B). Stern-Volmer kinetics applied to the ^{3}FC decay in presence of 1.6 and 6.6 µM β-car yield a k_t value of 5.0×10^9 M^{-1} s^{-1} indicating that this transfer is nearly a diffusion controlled process. The apparently new structure appearing on Fig. 3A in the 460-480 nm region in the presence of 10 µM β-car is due to the marked inner filter effect of β-car corresponding to maximal ground state absorbance of β-car in this spectral region (ε ~ 130,000 M^{-1} cm^{-1} at 450 nm) while ε is only 16,500 M^{-1} cm^{-1} at 355 nm, the excitation wavelength. It may be noted that saturation effects were negligible considering that this inner filter effect obeyed the Beer-Lambert law. Data in Fig. 3B allow an estimate of the molar extinction coefficient of the FC first triplet state at 450 nm. At plateau, Abs$_{max}$ is ~ 0.07 at 520 nm. However due to its short lifetime ($k_3 = 1.4 \times 10^5$ s^{-1}; τ = 6-7 µs), substantial decay of ^{3}β-car occurs during the energy transfer process. As a result Abs$_{max}$ must be corrected to account for the true [^{3}β-car] produced by the transfer, the Abs$_{max}$ value being given by the relationship: $\mathrm{Abs_{max}} = \mathrm{Abs_T} \times \exp[(\ln k_2/k_3)/(1-k_2/k_3)]$ (equation 1) where Abs$_T$ is the ^{3}β-car transient absorbance that would be observed in the absence of decay and $k_2 = k_1 + k_t$ [β-car].[9] Applying equation 1 yields [^{3}β-car] ~ 3.1 µM at plateau. The transient difference absorbance (ΔAbs = 0.09 at 450 nm) observed on Fig. 5B of the manuscript one µs after the laser pulse is due to both the long-lived ^{3}FC absorbance rise and ground state FC absorbance depletion. Since the molar extinction coefficient of ground state FC in toluene is 3,300 M^{-1} cm^{-1} at 450 nm, the molar extinction coefficient of triplet FC at its wavelength of maximum absorbance can be estimated from ΔAbs to be ~ 32,000 M^{-1} cm^{-1}.

Method B

At any time (t) after the laser pulse and at any wavelength (λ), the transient difference absorbance ΔAbs$_t$ must be for simple first order processes: ΔAbs$_t$ = Abs(^{3}FC)$_t$ − α × Abs(FC$_0$) (equation 2).[10,11] An interesting feature of chlorins such as FC or CDFC is the presence of the intense first Q band at the edge of the ground state absorbance spectrum (see Table 1 of the manuscript) and of weak transient triplet absorption bands beyond this ground state absorption. Figure 3A shows that Abs(^{3}FC)$_{t=0}$ = 0.007 at 675 nm where FC does not absorb and Abs(^{3}FC)$_{t=0}$ = 0.0055 at 625 nm where the ground state FC molar extinction coefficient is very low (*i.e.* = 660 M^{-1} cm^{-1}). Taking advantage of these spectral properties and assuming that there is no sharp transient absorbance attributable to ^{3}FC in the 625-675 nm absorbance region of the first Q band, it can be tentatively estimated that the upper limit attributable to the Abs(^{3}FC)$_{t=0}$ at 650 nm is ~ 0.007. As a result, applying equation 2, 12 µM FC produce an Abs(FC$_0$) = 0.52 in toluene whereas ΔAbs$_{t=0}$ = –0.0948 (Fig. 5B of the manuscript) leads to α = 0.19 corresponding to an initial [^{3}FC] of 2.3 µM. Using this value, ε of ^{3}FC at 450 nm is found to be 42,000 M^{-1} cm^{-1}.

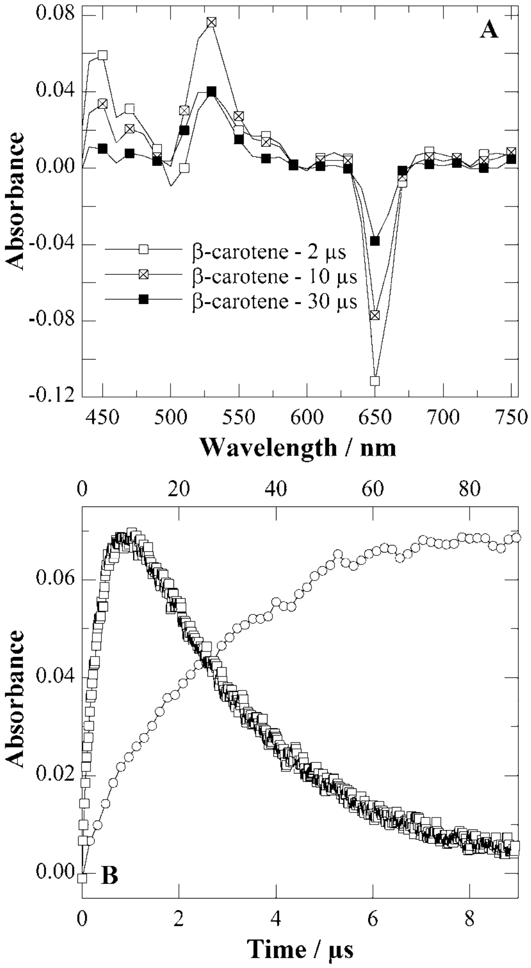

Fig. 3 **A** Transient difference absorbance spectra obtained by 355 nm-laser flash photolysis of a mixture of 12 µM FC plus 10 µM β-carotene; 2 µs (□) 10 µs (☒) and 30 µs (■) after the laser pulse. All the spectra were obtained with N_2-saturated solutions in toluene. **B** Growth and decay of the β-carotene transient triplet state absorption (□, upper time scale) which superimposes to the decay of the transient absorbance of FC at 520 nm after 355 nm laser flash photolysis of a mixture of 12 µM FC plus 10 µM β-carotene in N_2-saturated toluene solutions. (○, lower time scale) Extended time scale to show the growth of the β-carotene triplet state.

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