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 20.5 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 OCH₂NCH₂C₆H₅, 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-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. Similar elimination by cycloreversion was observed for related chlorins, under liquid secondary ion mass spectrometry (LSIMS) and ESI-MS conditions.

The MALDI-MS spectrum of the tetrasubstituted 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. Overall, the molecular envelope corresponds to the overlap of both isotopic patterns of the [M+H]⁺ and [M+2H]⁺ ions.

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 OCH₂NCH₂C₆H₅ (-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-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.

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 transient difference absorbance 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 \(\text{CH}_2\text{NCH}_2\text{C}_6\text{H}_5 (-119 \text{ Da})\).

2 - Determination of triplet molar extinction coefficients

**Method A**

The sequence of reactions involved in this process may be summarized as:

\[ \beta\text{-car} \rightarrow \text{FC} \text{ (decay to ground state, rate } k_1) \]
\[ \beta\text{-car} + \text{FC} \rightarrow \beta\text{-car + FC} \text{ (energy transfer, rate } k_t) \]
\[ \beta\text{-car} \rightarrow \text{FC} \text{ (decay to ground state, rate } k_3) \]

The energy transfer from \(\beta\text{-car}\) to ground state \(\text{FC}\) is shown on Fig. 3A where the characteristic absorbance of \(\beta\text{-car}\) at about 500 nm in toluene is clearly observed 2 and 10 \(\mu\text{s}\) after the pulse, this time corresponding to maximal \(\beta\text{-car}\) transient absorbance \(\text{Abs}_{\text{max}}\) (Fig. 3B). Stern-Volmer kinetics applied to the \(\beta\text{-car}\) decay in presence of 1.6 and 6.6 \(\mu\text{M}\) \(\beta\text{-car}\) yield a \(k_t\) value of \(5.0 \times 10^5 \text{ M}^{-1} \text{s}^{-1}\) 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 \(\mu\text{M}\) \(\beta\text{-car}\) is due to the marked inner filter effect of \(\beta\text{-car}\) corresponding to maximal ground state absorbance of \(\beta\text{-car}\) in this spectral region (\(\varepsilon \approx 130,000 \text{ M}^{-1} \text{cm}^{-1}\) at 450 nm) while \(\varepsilon\) is only 16,500 \(\text{M}^{-1} \text{cm}^{-1}\) 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, \(\text{Abs}_{\text{max}}\) is \(\approx 0.07\) at 520 nm. However due to its short lifetime \(\tau = 6-7 \mu\text{s}\), substantial decay of \(\beta\text{-car}\) during energy transfer process. As a result \(\text{Abs}_{\text{max}}\) must be corrected to account for the true \([\beta\text{-car}]\) produced by the transfer, the \(\text{Abs}_{\text{max}}\) value being given by the relationship: \(\text{Abs}_{\text{max}} = \text{Abs}_T \times \exp[\ln (k_2/k_3) \times (1-k_2/k_3)]\) (equation 1) where \(\text{Abs}_T\) is the \(\beta\text{-car}\) transient absorbance that would be observed in the absence of decay and \(k_2 = k_1 + k_t [\beta\text{-car}]\). Applying equation 1 yields \([\beta\text{-car}]\approx 3.1 \mu\text{M}\) at plateau.

The transient difference absorbance \(\Delta\text{Abs} = 0.09\) at 450 nm) observed on Fig. 5B of the manuscript one \(\mu\text{s}\) after the laser pulse is due to both the long-lived \(\beta\text{-car}\) absorbance rise and ground state FC absorbance depletion. Since the molar extinction coefficient of ground state FC in toluene is 3,300 \(\text{M}^{-1} \text{cm}^{-1}\) at 450 nm, the molar extinction coefficient of triplet FC at its wavelength of maximum absorbance can be estimated from \(\Delta\text{Abs}\) to be \(\approx 32,000 \text{ M}^{-1} \text{cm}^{-1}\).

**Method B**

At any time \((t)\) after the laser pulse and at any wavelength \((\lambda)\), the transient difference absorbance \(\Delta\text{Abs}\) must be for simple first order processes: \(\Delta\text{Abs}_t = \text{Abs}(3\text{FC})_0 - \alpha \times \text{Abs}^{(\text{FC})}_0\) (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 \(\text{Abs}(3\text{FC})_{t=0} = 0.007\) at 675 nm where FC does not absorb and \(\text{Abs}(3\text{FC})_{t=0} = 0.0055\) at 625 nm where the ground state FC molar extinction coefficient is very low (i.e. \(= 660 \text{ M}^{-1} \text{cm}^{-1}\)).

Taking advantage of these spectral properties and assuming that there is no sharp transient absorbance attributable to \(3\text{FC}\) in the 625-675 nm absorbance region of the first Q band, it can be tentatively estimated that the upper limit attributable to the \(\text{Abs}(3\text{FC})_{t=0}\) at 650 nm is \(\approx 0.007\). As a result, applying equation 2, 12 \(\mu\text{M}\) FC produce an \(\text{Abs}(\text{FC})_{t=0} = 0.52\) in toluene whereas \(\Delta\text{Abs}_{\text{max}} = -0.0948\) (Fig. 5B of the manuscript) leads to \(\alpha = 0.19\) corresponding to an initial \([3\text{FC}]\) of 2.3 \(\mu\text{M}\). Using this value, \(\varepsilon\) of \(3\text{FC}\) at 450 nm is found to be 42,000 \(\text{M}^{-1} \text{cm}^{-1}\).

References

1. D. O. Konn, J. Murrell, D. Despeyroux and S. J. Gaskell, Comparison of the effects of ionization mechanism, analyte concentration, and ion "cool-times" on the internal energies of...


