Excited state absorption properties in Cytochrome C at pico- and femtosecond regime.

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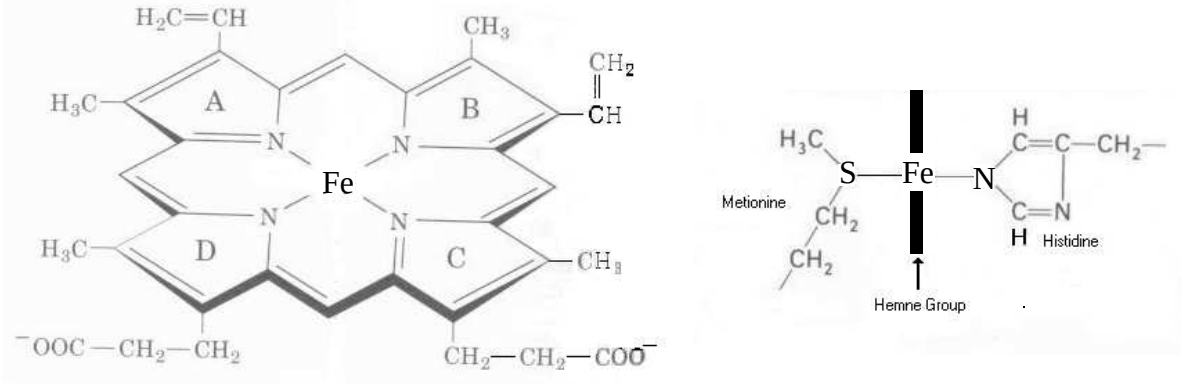
Abstract

Here we present a nonlinear absorption study on oxidized state of Cytochrome C molecule water solution, using the Z-scan technique. The measurements were carried out with picosecond pulses at 532 nm, and femtosecond pulses ranging from 460 nm to 640 nm. Using picosecond pulses we have observed a dynamic excited state saturable absorption (SA) process occurring during the pulse interaction. In addition, using femtosecond pulses, we have observed two-photon absorption (2PA) bands below 520 nm and above 565 nm, and a ultrafast saturable absorption (SA) band between then.

Introduction

The Cytochrome C is a protein used for transporting electron in the cellular respiratory process that contain a covalent heme group linked to polypeptide chains. The heme group is an iron porphyrin molecule with some peripherals groups bonded to pyrrole rings (Fig. 1a) and the polypeptide chains are polymers made by the amino acid residues linked by peptide bonds. In the Cytochrome C the iron atom of heme group is axially bonded to sulfur atom of a methionine and the nitrogen atom of a histidine (Fig. 1b). To work like electron carrier during cellular respiration, the iron ion, presents in the heme group, can change its oxidation state from reduced (Fe^{2+}) to oxidized (Fe^{3+}) [1], depending the cellular respiration step. As a consequence, the comprehension of physical chemical properties of this molecule is very important to provide understanding of these complex mechanisms.



(a) (b)

Figure 1: a) the Heme group and 1b) the side view of Cytochrome C molecule showing the axial boding of iron ion

In this work we report measurements of nonlinear absorption in Cytochrome C molecule solutions in their oxidized form (Fe^{+3}) using open aperture Z-scan technique [2] with pico and femtosecond laser pulses. Using a picosecond laser, a saturable absorption (SA) process in the resonant region (532 nm) was observed. We have also used the Z-scan pulse trains technique (VZTP) [3] with a Q-switched and mode-locked laser to study some cumulative triplet absorption process [4], however, no signal have been observed. Using a femtosecond laser, saturable absorption (SA) processes were observed for the resonant Q-band region

(between 520 nm and 565 nm) while a two-photon absorption (2PA) have been observed above 565 nm and below 520 nm.

Experimental Setup

The Cytochrome C was diluted in water at different concentrations. Their linear absorption spectra were obtained with a Carry–17 spectrophotometer.

We have used two commercial lasers, a frequency doubled Q-Switched and modelocked Antares laser from Coherent that delivery a train of pulses with 70 picosecond at 532 nm and a Ti:Sapphire CPA 2001 from Clark MRX, delivering pulses with 150 femtosecond at 775 nm operating at a 1 KHz repetition rate. The CPA 2001 is used to pump a commercial OPA TOPAS from Quantronix that convert the 775 nm pump pulse into tunable femtosecond pulses. The tuning range of TOPAS is from 460 nm up to 2600 nm.

The Z-scan measurements using a picosecond pulses have been performed with open aperture at 10 Hz repetition rate to avoid thermal effects. The beam was focused with a 12 cm focal length lens onto the sample placed in a quartz cuvette with 0.2 cm path length. The spot size at the focal plane was about 50 μm. Although this laser delivers a train of modelocked pulses, a single modelocked pulse can be extracted with a Pockell's cell, from the Q-Switched envelope. In the femtosecond experiments, the tunable pulse was focused by a 12 cm focal length lens into the same quartz cuvette. In this experiment we have tuned the wavelength from 460 nm to 640 nm. The spot size at the focal plane for 640 nm was about 14 μm. For both, SA or 2PA measurements we have also performed open aperture Z-scan.

Results and Discussions

The absorption spectrum showed in the Fig. 2 depicts the bands in the visible region for the Cytochrome C molecules. These bands are due to the iron porphyrin complexes (heme group). The oxidized state could be determined by its characteristic absorption spectrum. The characteristic B (Soret) and Q band of metalloporphyrin complex can be seen around 400 nm and 550 nm, respectively. The Fig. 2 also shows the five-level energy diagram for porphyrins.

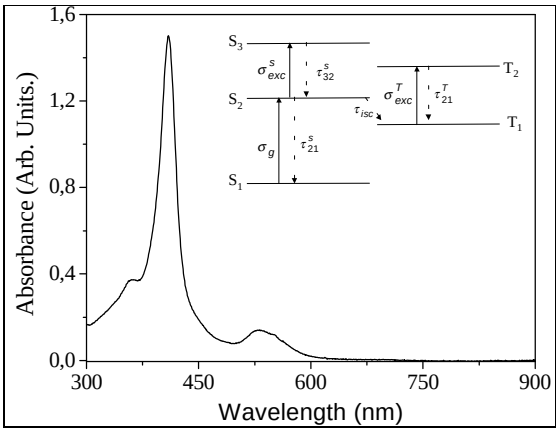


Figure 2: Absorbance spectrum of Cytochrome C/Water solution. The figure inserted in is the five-level diagram for porphyrin complex

Using the Beer's Law for a molecular concentration of 4 x 10^{16} molecules/cm^3 we are able to obtain the fundamental absorption cross-section. For 532 nm, the value obtained was σ_g = 4.1 x 10^{-17} cm^2. No fluorescence emission was observed for this excitation wavelength.

In order to investigate the effect of the excited singlet state population on the optical nonlinearity, we performed the picosencond pulse Z-scan as function of pulse intensity. The results are showed in Fig. 3, which contains a typical saturable absorption Z-scan curve inserted.

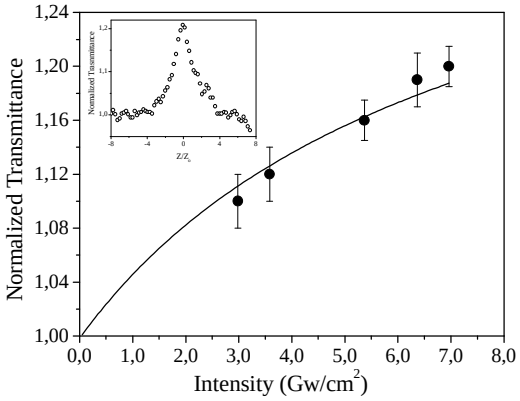


Figure 3: Normalized transmittance versus pulse intensity for picosecond Z-scan experiments at 532 nm. The solid line is the best fitting obtained from the three-level model system.

The solid black line in the Fig. 3 represents the fitting obtained using just the singlet states of the diagram inserted in Fig. 2, which corresponds to a three-level energy system. This assumption is correct once we have assumed that the pulse duration is faster than intersystem crossing time or, in other words, the triplet states are not populated during the pulse duration. The fitting gave us the values of excited singlet absorption cross-section σ_{exc}^{s} = 3.7 x 10^{-17} cm^2 and the singlet decay time τ_{21}^{s} = 2 ps, approximately. Since the pulse duration is relatively long compare to the lifetime (≈ 2 ps), this SA process is dynamic and occurs during the 70 ps pulse duration. In addition, the excited singlet state S_2 lifetime is assumed to be very short to present any appreciable population buildup. Also, the 2PA process is neglected in our model, once 532 nm is in the resonant region. To obtain information about triplet systems we performed the pulse train z-scan measurements, however no accumulative process was observed.

To observe nonlinear absorption effects in Cytochromo C using ultra fast pulses we performed the open aperture Z-scan measurements using 150 fs pulses, ranging from 460 nm (resonant Q-band) to 640 nm (non resonant region). For femtosecond regime, at constant pulse energy (0.1μJ), we have observed a 2PA process at non-resonant wavelength from 460 nm to 520 nm and 565 nm to 640 nm, with a maximum signal around 490 nm and 590 nm, respectively. While a SA effect for resonant wavelengths from 520 nm to 565 nm, was observed. In the wavelengths around 520 nm and 565 nm, the signals obtained were almost zero. Probably the 2PA and SA contributions have similar magnitudes and opposite signs so they nearly cancel. In Fig. 4 we show plot of Z-scan curves versus the wavelength, where the two types of nonlinear process can be observed.

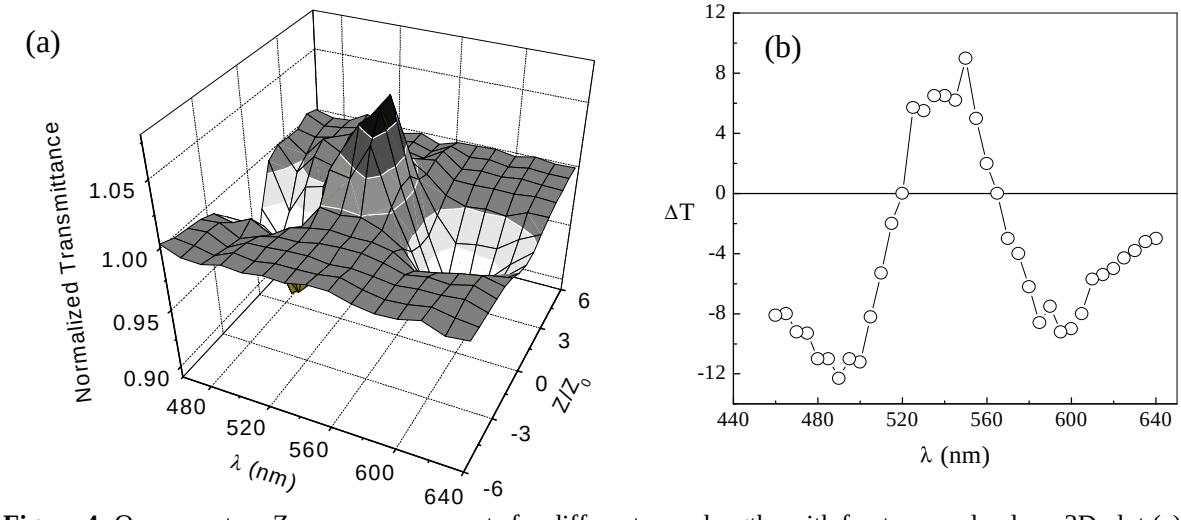


Figure 4: Open aperture Z-scan measurements for different wavelengths with femtosecond pulses, 3D plot (a) and the maximal ΔT (b) for same laser intensity.

Conclusions

In summary, we have performed open aperture Z-scan measurements with pico- and femtosecond pulses in oxidized state of Cytochrome C solutions. We have measured excited state saturable absorption (SA) spectrum and two-photo absorption (2PA) spectraum in resonant and non-resonant conditions, respectively. By fitting the data obtained with picosecond pulse we were able to determine the first singlet excited state absorption cross-section and its decay time. Using femtosecond pulses we have observed 2PA from 460 nm to 520 nm and from 565 nm to 640 nm, with a maximum signal around of 490 nm and 590 nm, respectively, and a SA processes from 540 nm up to 565 nm. At 520 nm and 565 nm the signal obtained were zero, probably due to the fact that 2PA and SA cancel each other.

References

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Acknowledgements

The authors thank FAPESP and CNPq for the financial support of this work.