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Monitoring electron transfer by photoacoustic spectroscopy

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Abstract. The electron transfer process between octaethylporphin and quinone molecules dispersed in a polymeric matrix was studied by the photoacoustic technique. It was observed that there was an enhancement of the octaethylporphin photoacoustic signal with an increase of the quinone concentration in the films. This increase appeared to be complementary to octaethylporphin fluorescence quenching and was associated with the electron transfer process. The data were analyzed according to the theory developed by Kaneko for fluorescence data (Kaneko 1992).

Key words: Electron transfer – Photoacoustic spectroscopy

Introduction

A simple artificial system was used to study intermolecular electron transfer in a polymeric medium. These model systems are very useful in the study of complicated biological processes, such as photosynthesis. Several theoretical aspects were developed (Marcus 1965; Marcus and Suttin 1985; Onuchic and Beratan 1987) and a series of experimental techniques (Miller et al. 1982; Fox and Chanon 1988) were successfully employed to investigate the electron transfer phenomenom. However, whereas in the natural reaction centers the various chromophores are held in place by non-covalent interactions (Breton and Vermeglio 1988), most of the studies were in model systems where the electron donor was linked to the acceptor (Gust et al. 1990; Wasielewski 1992). Therefore, studies on non-covalently linked photosynthetic model systems could be interesting. Recently, time-resolved photoacoustics were used to study electron transfer reactions (Feitelson and Mauzerall 1993). In this paper this process was examined using conventional photoacoustic spectroscopy.

Electron donor and acceptor molecules were dispersed in a polymeric film and photoexcited to induce the electron transfer process. Since this mechanism competes with the fluorescence and the non-radiative decay, it can be monitored by emission quenching or by photoacoustic spectroscopy. Photoacoustic spectroscopy is a way of detecting the non-radiative decay (Rosencwaig 1980) and it is used in the determination of optical and thermal parameters of materials (Tam 1986).

An enhancement of the octaethylporphin (the electron donor) photoacoustic signal was observed with an increase of the quinone (the acceptor) concentration in the films. This behavior appeared to be complemetary to the known fluorescence quenching due to electron transfer in rigid media (Guarr et al. 1985; Fischer and Bronstein-Bonte 1985; Gasyna et al. 1985). The data were, therefore, analyzed on the basis of the model developed by Kaneko for fluorescence quenching (Kaneko 1992).

Experimental

Octaethylporphin from Sigma was chosen as the donor molecule. It was dissolved in chloroform and its optical absorption spectrum, shown in Fig. 1, was recorded on a Varian-Cary 2315 spectrophotometer. Its concentration was obtained using the extinction coefficient $1.38 \times 10^4\ M^{-1}cm^{-1}$ at 498 nm (Gong and Dolphin 1985). The electron acceptor molecules, duroquinone (DQ) or 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) from Sigma were dissolved in the same solvent and their concentrations were determined by weight. A fixed amount of the donor solution and varying amounts of one of the quinone solutions were mixed with poly(methyl-methacrylate) and then poured onto a glass plate and extended, forming a film about 100 μm thick. After drying for about 12 h in a N_2 environment, 3 pieces were cut and the photoacoustic spectra obtained. The averaged spectrum was considered in the analysis. The acceptor concentration in the films was calculated using the molecular weight of the quinone and the polymer density (1.19 g/cm^3) (Weast and Astle 1978). The donor concentration in the films was kept fixed at 1.4×10^{-4} M.

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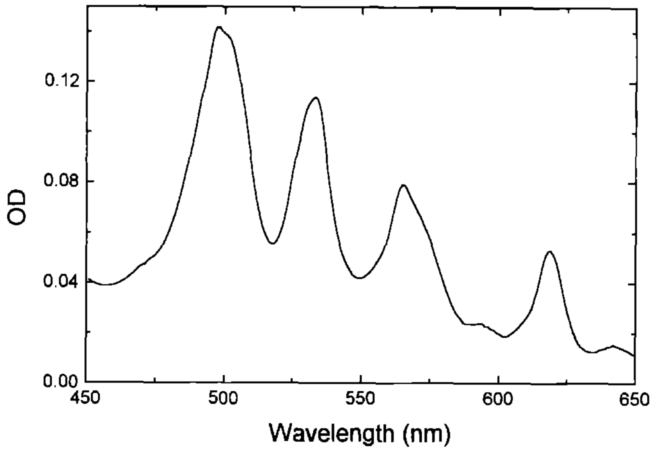

Fig. 1. Optical absorption spectrum of octaethylporphin in chloroform

The photoacoustic spectra were obtained at 5 Hz in a spectrometer assembled in the laboratory. A description of the equipment was given previously (Melo et al. 1993). The spectra were normalized using a piece of carbon paper as reference.

Theory

The analysis of electron transfer based on emission quenching proposed by Kaneko considers that the photoexcited state of the donor can decay by one of the three processes: Fluorescence, non-radiative decays or electron transfer (Energy transfer is ignored because there is no overlap of the octaethylporphyn fluorescence and the quinone's absorption spectra). When the donor and acceptor molecules are immobilized in a matrix the quenching is called static, and it occurs when the acceptor molecule is present in a quenching sphere around the donor molecule. Considering that during the film formation there is a stepwise incorporation of acceptor molecules into the quenching sphere, and that the backward rate constant at each incorporation step is proportional to the number of acceptor molecules present in the sphere, the following relation can be obtained for the relative emission intensity as a function of the acceptor concentration (Kaneko 1992):

$$\ln(F/F_0) = \frac{-K_1}{1+K_1[D]}[A]$$

where F_0 and F are the emission intensities of the donor in the absence and in the presence of acceptors, respectively, [A] and [D] are the concentrations of acceptor and donor in the films, respectively, and K_1 is the equilibrium constant for the first incorporation step.

Results and discussion

To verify how the acceptor influences photoacoustic spectrum of the donor, the band around 620 nm was chosen, since in this region the acceptors don't absorb. For each film, three different pieces were measured and averaged. This procedure was used to confirm the random distribution of donor and acceptor molecules in the film and also to improve the signal to noise ratio. It was observed that the band position was unaffected by the presence of the quinones; however, the band intensity increased with the acceptor concentration in the films, as shown in Fig. 2. This behavior can be explained by considering that when the donor alone is present in the film, once it is excited, there are two competing processes that bring it back to the ground state: luminescence and non-radiative decay. When acceptor molecules are also present in the films, the electron can be transferred from the donor to the acceptor, and this process is a pathway for non-radiative decay of the excited state. On increasing the acceptor concentration, electron transfer is favored and there is an increase in non-radiative decays and, as a result, there is a larger photoacoustic signal. If this is true, the fluorescence quenching and the photoacoustic signal increase are expected to be complementary processes. Considering the low concentration of acceptors used in the films, the photoacoustic signal should follow the relation:

$$\frac{I}{I_0} = 1 + \frac{F_0}{I_0}\frac{K_1}{1+K_1[D]}[A]$$

where I and I_0 are the intensity of the photoacoustic band around 620 nm obtained for films with and without acceptors, respectively. However, the fluorescence quantum yield in the octaethylporphin film is close to 0.5 (Albuquerque 1992), therefore, $F_0 \approx I_0$.

The intensity of the band around 620 nm was obtained from the fitting of 3 Gaussians to the spectra in the range 550–650 nm, as shown in Fig. 3 for the spectrum of a sample with a concentration of DDQ 20 times larger than the concentration of octaethylporphin. The positions of the three bands were 566, 599 and 619 nm and these remained constant (within 1 nm) for all spectra. For the widths a 3 nm variation was observed. The plot of the ratio I/I_0 as a function of the acceptor concentration in the films is shown in Fig. 4. The linear fittings to the data are also shown in Fig. 4 and they provide K_1. For DDQ, the value obtained for K_1 was 106 M^{-1} and for DQ, 51 M^{-1}. The value of K_1 obtained by Kaneko, from fluorescence quenching measurements for a (Zn)porphyrin-benzoquinone pair in liposomes, was 52 M^{-1} (Kaneko 1992).

From these K_1 values, the distribution of the acceptor molecules in the quenching sphere around D can be calculated, assuming it obeys Poissonian statistics. The average number of acceptor molecules incorporated in a quenching sphere is given by:

$$m = \frac{K_1[A]}{1+K_1[D]}$$

and the proportion of spheres that incorporate one A molecule is given by:

$$P_1 = me^{-m}$$

At an acceptor concentration of 4 mM, the average number of DDQ molecules incorporated in a quenching sphere is m=0.42, and 28% of the spheres have one DDQ molecule incorporated. When DQ is used as acceptor, m=0.20

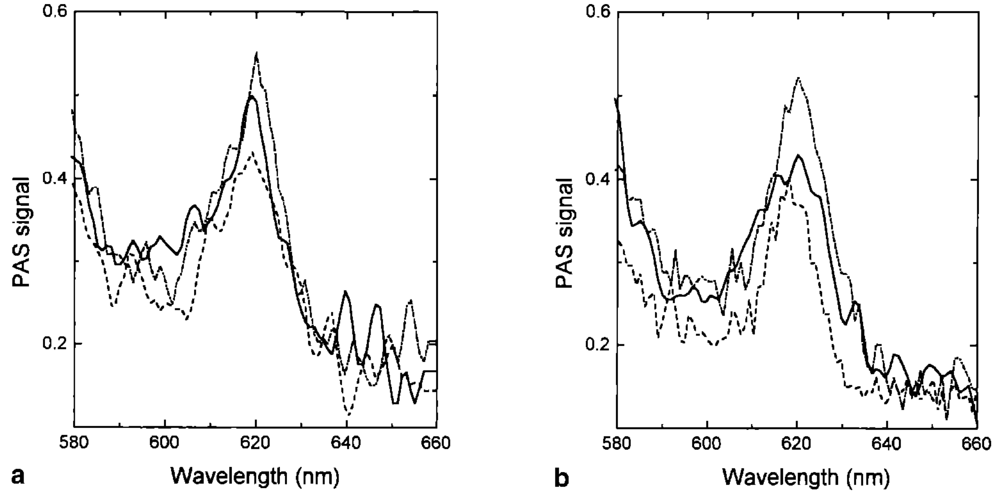

Fig. 2a, b. Photoacoustic signal (PAS) from films with the octaethylporphin concentration equal to 1.4×10^{-4} M and different concentrations of acceptor. **a** The DQ concentration was 10 (– – –), 20 (——) and 40 (– . . – . .) times larger than the donor concentration **b** The DDQ concentration was 0 (– – –), 20 (——) and 30 (– . . – . .) times larger than the donor concentration

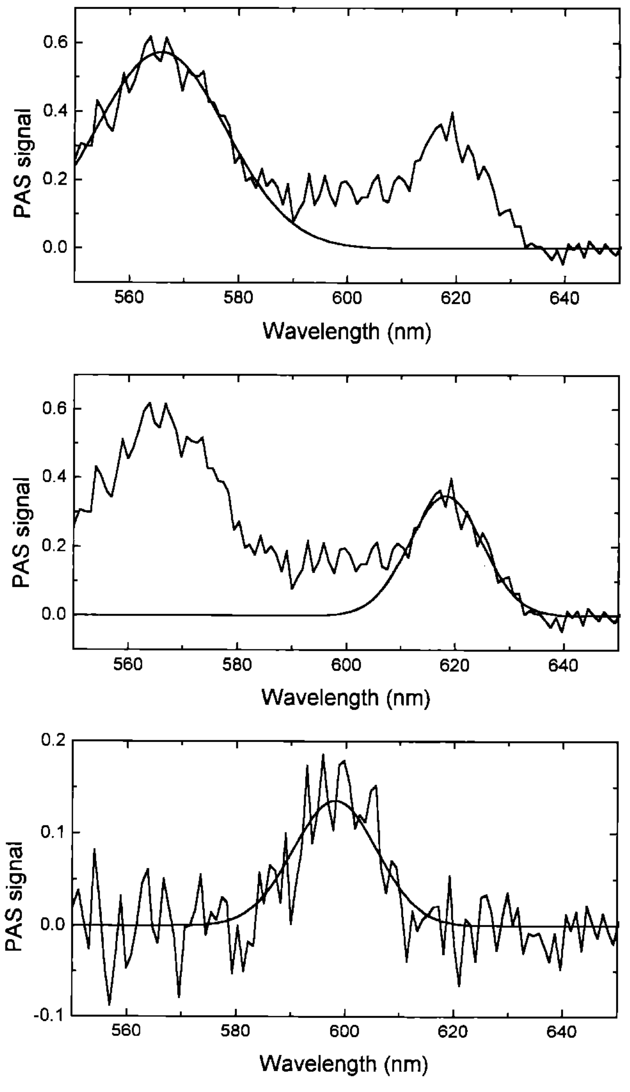

Fig. 3. 3 Gaussians fitted to one of the photoacoustic spectra. The concentration of DDQ in the film was 20 times larger than the concentration of octaethylporphin. The spectrum in the last part is the subtraction from the experimental data of the two previously fitted Gaussians

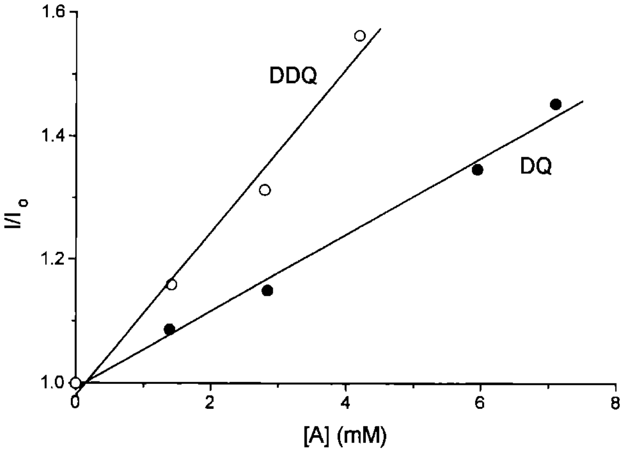

Fig. 4. The relative intensities of the photoacoustic signal at 619 nm as a function of the acceptor concentration in the films. I and I_0 are the intensities with and without acceptors, respectively

and $P_1 = 17\%$. Since the same donor was used in the experiments, and the same donor and acceptor concentrations were used in the calculations, the difference in m and P_1 must be due to an intrinsic property of the acceptors. DDQ is known to have a larger electron affinity than DQ (Beitz and Miller 1979) and therefore its radius of action must be longer. In other words, the volume of the quenching sphere for DDQ must be larger than that for DQ. In a larger sphere one expects a larger average number of acceptor molecules and a higher probability of having spheres with one acceptor molecule.

In conclusion, this new way of monitoring the electron transfer process is shown to be easy and reliable. The validity of the procedure used was confirmed for a porphyn-quinone system from fluorescence and photoacoustic measurements (Cornelio and Sanches 1994). Comparing the two methods, photoacoustics has a great advantage for systems with low fluorescence yield or systems with highly quenched fluorescence.

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