FLASH PHOTOLYSIS - ELECTRON SPIN RESONANCE STUDIES OF THE DYNAMICS OF PHOTOSYSTEM I: III TEMPERATURE DEPENDENCE OF THE DECAY OF SIGNAL I[1]

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Summary: Using the technique of flash photolysis - electron spin resonance (FPESR), we have confirmed that a portion of the ESR Signal I (P700) of Photosystem I in green-plant photosynthesis responds reversibly to light at low temperatures. In the temperature range 150K to 270K the decay of Signal I follows a normal first-order Arrhenius behavior with an activation energy of 5.5 $\pm$ 0.5 kcal mole^{-1}. Below ~150K most of the light-induced signal is "frozen in"; however, 10-30% of the Signal responds reversibly to light and exhibits a decay halflife of ~0.8s. This halflife is temperature independent from 5K to 150K. This phenomenon is interpreted in terms of a quantum-mechanical tunnelling model for the reverse electron transfer in the reaction center of Photosystem I.

It is well known that P700 can be photooxidized at low temperatures (~77K) with the associated formation of the electron spin resonance (ESR) Signal I***[4-9]. This is the principal evidence for identifying $P700^+$ as one of the primary photochemical products of Photosystem I (PSI). However, there is considerable disagreement in the literature concerning the dark decay characteristics of $P700^+$ (or Signal I) at low temperatures. Some authors[4-7] claim that at ~77K the decay of $P700^+$ is either very small and slow or that the P700 bleaching (or Signal I formation) is "frozen in" after illumination However, Mayne and Rubinstein[8] reported that the bleaching of P700 at ~77K recovers

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*** Hence forth we will consider $P700^+$ and Signal I as synonymous since the assignment of the ESR Signal I to the optical $P700^+$ moiety is now well established[2,3].

by ~50% in Anacystis nidulans and by ~10% in spinach chloroplasts. Recently Yang and Blumberg[9] showed that about 10-15% of Signal I decays in minutes at ~77K but that the decay can continue for days so that eventually ~50% of the signal decays.

We have examined the decay of Signal I following a pulse of red light from milliseconds to minutes and at temperatures from 5K to 300K in spinach chloroplasts and Photosystem I subchloroplast particles and in intact cells of the blue-green alga Cyanidium caldarium. We find that part of the Signal I decay is reversible at very low temperatures but the decay exhibits several components at different temperatures. We propose a quantum-mechanical tunnelling model for the temperature-independent decay components found at low temperatures.

MATERIALS AND METHODS

Spinach chloroplasts were prepared by the method of Sane et. al.[10] from market spinach. Three types of photosystem I subchloroplast preparations were used: RC160 particles prepared by the method of Sane et. al.[10], TSF1 particles by the method of Vernon and Shaw[11], and D144 particles by the method of Anderson and Boardman[12].

Cyanidium caldarium was grown on Allen's medium[13] at 37°C and harvested one week after innoculation from a slant.

Actinic illumination was provided either by a Synergetics Chromabeam 1070 dye laser using Rhodamine B (10^{-4}M in ethanol) at 620 nm (~3×10^{17} photons per flash), or by a Spectra-Physics Model 164-01 Krypton CW Laser operating at 647.1 nm (light output ~250 mW). The CW laser was shuttered with a Vincent Associates Model 300-B "Uniblitz" Electronic Programmable Shutter Drive and Control with a Model 26 6 mm shutter. Both the flash and CW lasers were sequenced by an electronic programming unit described elsewhere[14]. Kinetic data was accumulated and time averaged with a Fabritek Model 1072 Computer of Averaged Transients (CAT) manufactured by Nicolett Corp. The electron spin resonance spectrometer was a Varian Model E-12.

Low temperatures were achieved either by a Varian Model E257 Variable Temperature apparatus (100K - 300K) or an Air Products Model LTD-3-110 "Heli-tran" liquid transfer system (4K - 100K).

RESULTS

As described in Part II of this series[1] spinach chloroplasts blocked with DCMU exhibit two decay components of Signal I at room temperature: a fast component (halflife ~20ms) and a slow component (halflife ~0.2 - 1.0 s). Similar results are obtained with Photosystem I subchloroplast particles (see Part I of this series[1]). We have postulated that the fast component represents direct return of an electron from the primary acceptor X^- to $P700^+$. We have now studied the temperature dependence of the fast component in D144 particles. Below ~0°C the slow component is "frozen in" so that the only light reversible decay component was the fast component. Above 0°C the slow component was subtracted out before determining the first-order rate constant for the fast component. An Arrhenius plot for the fast component decay rate constant is given in Fig. 1 yielding an activation energy of ~5.5 $\pm$ 0.5 kcal. mole $^{-1}$.

Now if we extrapolate the Arrhenius plot to ~90K, even with an activation energy as low as 4 kcal. mole $^{-1}$, the halflife for Signal I decay would exceed 100 hours! But Fig. 2a shows that at 90K the decay is much faster with a halflife of ~0.8 s. All three types of Photosystem I subchloroplast particles, whole chloroplasts and intact cells of Cyanidium exhibit virtually identical Signal I decay at ~90K. Furthermore, studies at ~5K with TSF1 particles and RC160 particles show essentially the same decay of Signal I as seen in the case of D144 particles at 90K. We have examined this decay at several different temperatures and between 5K and ~150K, and in this temperature range the decay rate of Signal I is virtually temperature independent (Fig. 2a and 2b).

Not all of Signal I forms and decays reversibly at low temperatures. About (70-90%) of Signal I is "frozen in" while the remaining (10-30%) is

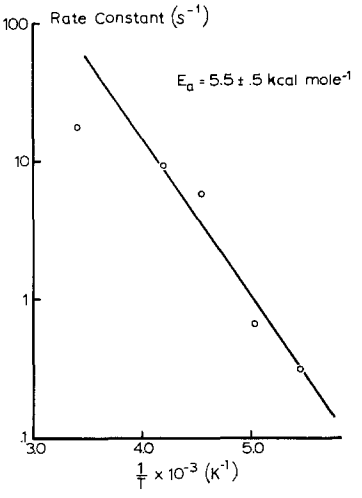

Figure 1 Arrhenius plot of the logarithm of the first-order decay rate constant of Signal I vs T^{-1} for D144 photosystem I subchloroplast particles. Each point is the average of 128 flashes (Xenon Corp. Suntron 6C with a Corning CS2-64 filter). Actinic intensity was difficult to determine; however, a single flash did not elicit a full response.

light reversible. At temperatures above 150K the "frozen in" portion begins to decay and complex decay patterns are obtained (Fig. 2c - 2f).

DISCUSSION

The observation of a temperature-independent decay component of Signal I can be accounted for reasonably within a simple quantum-mechanical tunnelling model. Similar behavior has been found for Signal B1 in photosynthetic bacteria[15,16]. This type of electron transfer in biological systems was first suggested by Chance et. al.[17,18].

In photosynthetic bacteria about 50% of Signal B1 is "frozen in"[7]; the reversible part has a halflife of ~20 ms.[15,16] between 1.7K and ~150K. In green plants and algae Signal I of photosystem I has a halflife of ~0.8s in the temperature range 5K to 150K. If we assume that this decay is due to

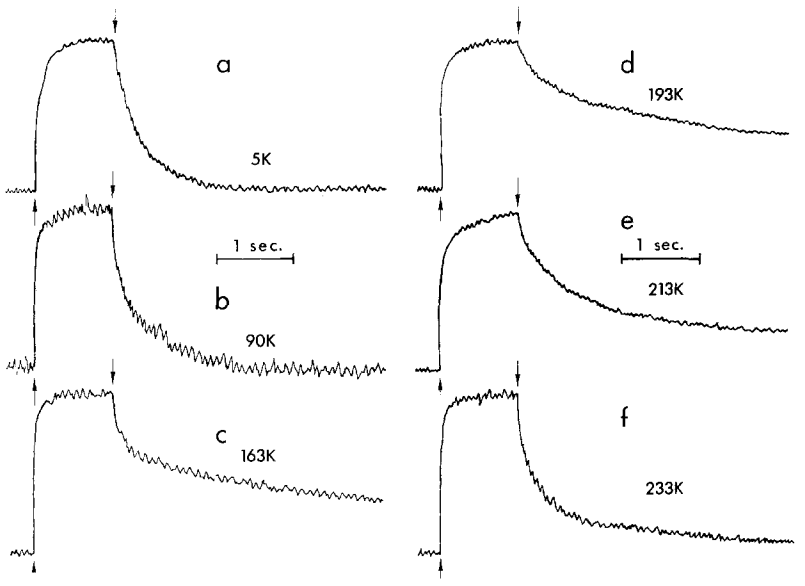

Figure 2 Time course of the formation and decay of Signal I in TSFI photosystem I subchloroplast particles at various temperatures. Actinic illumination was by a CW Spectra-Physics Krypton Laser at 647.1 nm at 50 mW power. The shutter was open for 1.0 s. Each trace is the average of 32 scans. Modulation amplitude 10G, time constant 4 ms. The magnetic field was set at the low-field extremum of Signal I. At temperatures below ~150K only about 20% of Signal I decays in the dark. The amplitude of the light-induced signal has been normalized for each trace. Note that for traces a and b the kinetic curves represent the change from dark-after-light state to the light state and back again. At these temperatures 70 to 90% of Signal I is "frozen in."

direct electron return from X^- to $P700^+$ then a simple square-well calculation indicates that the barrier width (i.e. separation of X and P700) is ~45 - 55 Å. This distance is well within the estimated dimensions of the

photosynthetic unit[19] and may well represent a dimension within a Photosystem I reaction-center protein similar to that found in photosynthetic bacteria[15,16].

The question of why part of Signal I is "frozen in" presents somewhat of a puzzle. It may indicate two types of P700 entities, one type in which a secondary acceptor can be reached at low temperatures but no return is possible and another type in which the electron can go no further than the primary acceptor X and then returns via the tunnelling process. Or it may be that the freezing process separates X from P700 for most of the centers so that the electron may reach X but cannot return because the barrier width is too great.

Our findings raise the question as to what is the primary acceptor for PSI? We believe that we have detected an ESR signal which is the kinetic counterpart to the reversible part of Signal I. This work will be reported later[20]. Another question raised concerns the optical component P430 which has been postulated as arising from the primary acceptor by Ke[21]. Does this component show a reversible decay behavior at low temperatures?

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