LOCATION OF FIELD-SENSITIVE CAROTENOID MOLECULES IN THE CHLOROPLAST MEMBRANE. ARGUMENTS FROM LOW-TEMPERATURE STUDIES

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1. Introduction

Excitation by light of photosynthetic structures from green plants induces several absorption changes peaking around 515 nm. One of them, whose duration is in the millisecond time range, has been well characterized and attributed to a light-induced electric field acting on the photosynthetic pigments [1,2]. It has been proposed that the main part of the absorption change at 520 nm is due to an electrochromic effect on carotenoid molecules [3], an hypothesis which is well supported by theoretical evaluations [4]. Schiephake et al. showed that both System-1 and System-2 photoreactions contribute equally to the carotenoid shift, in chloroplasts at physiological temperatures [5]. Around −50°C also, both photosystems contribute nearly equally to the 520 nm absorption change [6–8]. At lower temperature the Photosystem-2 contribution keeps practically the same magnitude (at least down to liquid N_2 temperature) whereas the Photosystem-1 contribution changes dramatically, as it is nearly zero at −170°C [9]. In this letter we describe a more detailed study of the effect of low temperatures on the 520 nm absorption change linked to Photosystem-1 activity, in an effort to better account for the field-induced carotenoid shift.

2. Materials and methods

Spinach chloroplasts were prepared by a standard technique [9]. Photosystem-1 particles ('144 000 × *g*' fraction) were obtained as described by Boardman et al. [10]. Both preparations were resuspended in a mixture of 65% glycerol–35% extraction buffer and stored in liquid N_2. Chloroplast suspensions were supplemented with DCMU (20 μM) and hydroxylamine (100 μM) and particle suspensions with ascorbate (1 mM) and dichlorophenol–indophenol (20 μM).

Absorption changes were measured as previously described [11]. Before being cooled in the cuvette, chloroplast suspensions were dark-adapted for 2 min at 20°C and then received two short saturating flashes of white light [9] in order to block Photosystem-2 reaction centers in an inactive state [9,12]. Cuvettes containing Photosystem-1 particles were dark-adapted before cooling. A cuvette temperature between −30 and −145 ± 5°C was obtained by a flow of cold N_2 gas. A temperature of −170°C was obtained as previously [9,11]. Absorption changes were induced by a single saturating flash from a dye laser (duration, 1 μs; wavelength, about 600 nm). For each measurement we stored and averaged the effect of 5–10 experiments.

3. Results

3.1. *Absorption changes at −50°C*

With suspensions kept at −50°C, flash-induced absorption changes have been measured between 465 and 540 nm, as well as at 703 and 820 nm. In all cases we observed an immediate flash-induced change, followed by a recovery which has the same kinetics at all the wavelengths studied. A few examples are shown in fig.1 for chloroplasts and for particles. Absorption changes were fully reversible, although some variation was observed from batch to batch in the kinetics of decay. The absorption increase at 820 nm is attributed to P^+700 (see also refs. [13,14].

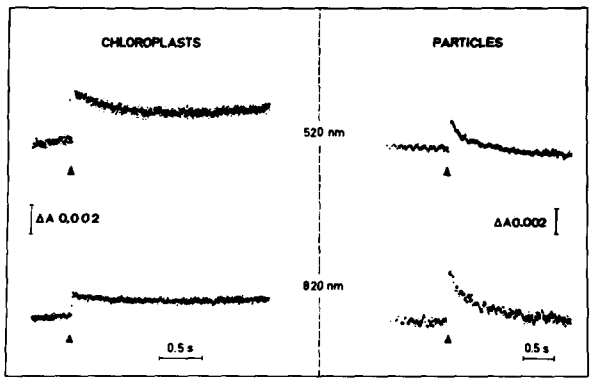

Fig.1. Time course of flash-induced absorption changes at −50°C, in suspensions of spinach chloroplasts (a) or Photosystem-1 particles (b), at 520 nm and 820 nm. Optical path in the cuvette: 1.4 mm. Chlorophyll concentration: (a) 500 $\mu g \cdot ml^{-1}$, (b) 400 $\mu g \cdot ml^{-1}$.

The difference spectra of the flash-induced absorption changes around 500 nm are reported in fig.2, for chloroplasts and for Photosystem-1 particles. With particles the spectrum is broad and of small amplitude. It can be attributed to P^+700 only (compare refs. [13 and 9]). With chloroplasts the spectrum presents the usual features of the 520 nm absorption change. The positive peak is relatively large compared to the negative one. This may be due to a contribution of P^+700 (as in particles) and also to some actinic effect

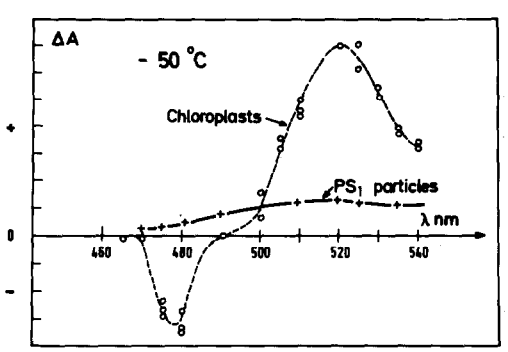

Fig.2. Difference spectra of the absorption changes induced by a saturating flash, at −50°C, in suspensions of chloroplasts or of Photosystem-1 particles. Relative values obtained as in fig.1, the concentrations in the two suspensions being adjusted so as to obtain an identical absorption change at 703 nm (same concentration of Photosystem-1). Monochromator bandwidth: 5 nm.

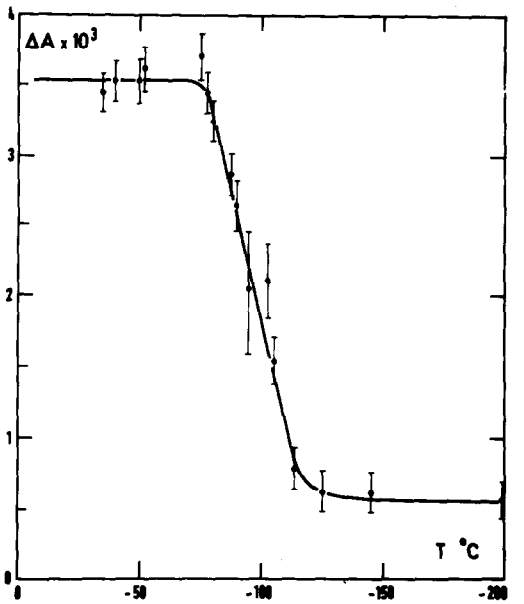

Fig.3. Amplitude of the absorption change induced by a saturating flash in suspensions of spinach chloroplasts, at various temperatures. Chlorophyll concentration: 500 $\mu g \cdot ml^{-1}$

of the measuring beam under 490 nm, although this effect has been largely minimized. The 520 nm change is absent in particles either because it is not created at all or because it decays in less than a millisecond.

3.2. *Absorption changes between −30°C and −170°C*

We have studied the evolution with temperature of the flash-induced absorption change at 520 nm, with chloroplasts whose Photosystem-2 activity was blocked. The result is shown in fig.3. At temperatures above −70°C the amplitude of the absorption change is nearly constant and not very different from its value at room temperature. It decreases abruptly between −75 and −110°C (half point at −95°C) and attains below −150°C a constant low value. The spectrum of this residual effect has been reported previously [9] and attributed to a small band of P^+700 [13]. The abrupt variation of the 520 nm change around −95°C is not correlated with an important change in the magnitude of flash-induced oxidation of P^+700 detected at 703 or 820 nm. A curve similar to that of fig.3 has also been obtained upon excitation of chloroplasts with saturating continuous light (result not shown).

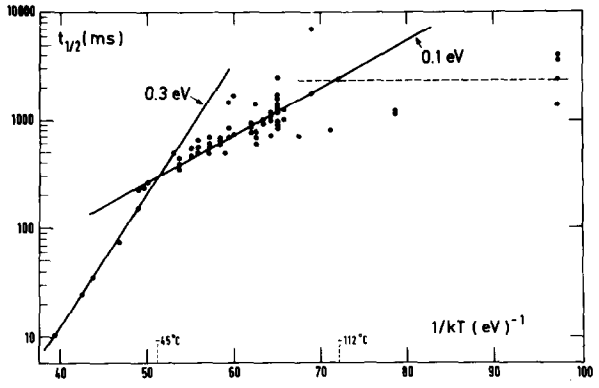

Fig.4. Arrhenius plot of the half-time of decay of the absorption change at 520 nm induced by a flash in chloroplast suspensions at various temperatures. The values in eV correspond to the activation energies which can be deduced from such a plot in two different temperature ranges, assuming an exponential decay.

Following flash excitation, the absorption change at 520 nm decays with complex kinetics. Our measurements were rather imprecise because of fluctuating base-line slopes, and for that reason we simply express our results in terms of only one decay half-time. Between −35 and −80°C the decay half-times were the same at 520 and at 703 nm, indicating that the carotenoid shift disappears as P^+700 is reduced. Half-times are plotted in fig.4, in an Arrhenius plot, which indicates three temperature ranges with different activation energies for the decay: 0.3 eV over −45°C, 0.1 eV between −50 and −110°C, and practically zero below −110°C. The kinetics of the back reaction of reduction of P^+700 have been studied at low temperature by several investigators [15–17]. Our results are in reasonable agreement with these previous studies and altogether do not indicate any special break around −95°C.

4. Discussion

At low temperatures the primary photoinduced electron transfer occurs in both photosystems of chloroplasts, but it is a striking difference that the 520 nm absorption change occurs with a nearly normal amplitude for Photosystem-2 at −170°C [4] whereas it is of negligible amplitude in the case of Photosystem-1. In the frame of the electrochromic hypothesis [1,4] we would like to discuss two hypotheses which account for that different behaviour. In a first one, we suppose that the field-sensitive molecules (probably carotenoids) are homogeneously located in the membrane with respect to both photosystems and that the abrupt decrease of the 520 nm absorption change linked to Photosystem-1 activity is due to an important change in the geometry of the Photosystem-1 reaction center and/or of nearby carotenoids. This geometrical change can be related to a change of the chemical nature of the electron acceptor, in a manner similar to that discussed by Mc Intosh et al. on the basis of EPR measurements in Photosystem-1 particles [18]. This model is a two-state model, which can be interpreted in terms of chemical equilibria; the results of fig.3 can be plotted in a Van't Hoff plot, which gives thermodynamic values for the inferred transition: ΔH° = 9 kcal.mol^{-1} and ΔS° = 20 cal·mol^{-1}. These values are compatible with a simple chemical reaction.

In a second hypothesis we suppose that field-sensitive carotenoid molecules are inhomogeneously located and are close to the Photosystem-2 reaction center. A carotenoid shift related to Photosystem-1 activity (as observed around −50°C) would require a charge delocalization, probably by ionic movements. Such a delocalization can be inferred from recent results by Amesz and de Grooth [19]. We suppose that lowering the temperature hinders the charge delocalization, so that the charges remain localized on rather primary electron donors or acceptors, and that the light-induced field is a rather localized dipole field. A carotenoid shift would thus be observed for Photosystem-2 (see also ref. [9]) but not for Photosystem-1. Our two hypotheses have direct implications on the arrangement of pigments and reaction centers in the chloroplast membrane.

A choice between them is presently difficult. Two of our results are in favor of the second one: (i) the absence of carotenoid shift in Photosystem-1 particles and (ii) the absence of any clear break in the kinetic behaviour (fig.4) in the region where an abrupt change is observed in the magnitude of the carotenoid shift. The occurrence of a membrane conformational change around −100°C might be a rather general property since a break similar to the one we observe has been reported by Chance et al. in the case of membrane

cytochrome oxidase [20] and by several authors in the case of Photosystem-2 reactions [21,22].

Acknowledgement

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