SOME PHOTOREACTIONS OF SPINACH CHLOROPLASTS IN SUCROSE SYRUP AT HIGH AND LOW TEMPERATURE

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Received 20 November 1972†

1. Introduction

Primary photoreactions are thought to be occurring even at low temperatures (e.g. 110°K) while dark reactions should be blocked if they are diffusion limited. In green plant photosynthesis reactions of the following pigments have been described as occurring at low temperature: Chl-a_{I} (*P*-700) [1,2], Cyt *b*-559 [3-7], C-550 [5-8] and X-320 [9]. Cyt *f* was believed to be photooxidized at low temperature [1,2] but new results show that this might not be the case [4-6]. Furthermore a pigment with a band at 680 nm reacting at low temperature [4] has been assumed to be identical with the photoreactive chlorophyll-a_{II} (*P*-680) [10,11].

Most experiments have been carried out with normal suspensions of chloroplasts which increase straylight very much when frozen. Therefore the optical path through the cuvette is not known at low temperature. Thus a quantitative comparison of high and low temperature spectra is impossible. If, however, highly viscous liquids are used as 'solvents', which freeze glass-like, these troubles are overcome. In this work low temperature photoreactions of chloroplasts suspended in sucrose syrup have been studied in the chlorophyll band region. This syrup freezes glass-like.

2. Experimental

Chloroplasts were prepared from market spinach by the method of Winget et al. [12]. The sample contained chloroplasts, chlorophyll content 110 μg/ml, ferricyanide 2 × 10^{-4} M, sucrose approx 2.3 M in tricine buffer pH 7.2, 0.05 M. The rate of the Hill reaction with ferricyanide in the presence of NH_4Cl is reduced in sucrose syrup by a factor of 2–3 which is certainly due to kinetic effects. The viscosity of the sucrose solution at 295°K was 250 cp which reduced to 150 cp in the sample by the additions (estimated by the measured dependance of the viscosity on dilution with water).

Reversible and irreversible absorption changes excited by short flashes have been measured as described elsewhere [13]. The intensity of the measuring beam at the cuvette was 2.6 $\mu W/cm^2$ at 705 nm and reduced to smaller than 1 $\mu W/cm^2$ at 430 nm. Excitation of the light reactions by the measuring beam is certainly negligible as can be seen by the nearly horizontal zero line in fig. 3, the measuring beam being on for about 0.2 sec before the oscilloscope was triggered. The intensity of the exciting flash was just saturating photosynthesis.

For low temperature measurements a plexiglass cuvette was fastened on a copper holder and inserted into a quarz-Dewar: This Dewar is silvered up to half height and contains flat windows for the measuring beam. The sample is frozen by dipping it with the holder into liquid nitrogen at the bottom of the Dewar. When the desired temperature is reached (measured by a thermocouple of cromel–alumel inside the cuvette), the holder is brought into measuring position The holder then has no contact with the liquid nitrogen in order to avoid perturbations by the gently boiling liquid. A temperature of 110°K was well reproduced and seems to be low enough for trapping experiments.

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† Publication delayed for technical reasons.

North-Holland Publishing Company – Amsterdam

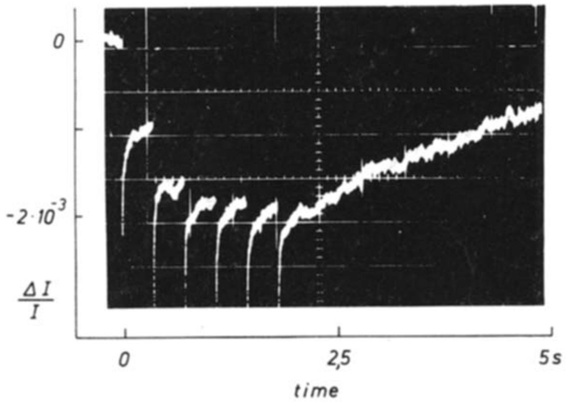

Fig. 1. Absorption changes of Chl-a_I at λ= 705 nm and T = 295° K during a flash group in spinach chloroplasts suspended in sucrose syrup. First flash fired 400 msec after switching on the measuring beam. Spikes on top of absorption changes are artifacts by flash-excited fluorescence. Chlorophyll content: 100 μg/ml, activity of O_2 production without sucrose 0.013 $A(O_2)/M(Chl) \times$ sec, electron acceptor ferricyanide 2×10^{-4} M, sucrose 2.3 M, optical path length 0.12 cm, excitation: duration 2×10^{-5} sec, measuring light: grating monochromator, half-width 2 nm, intensity 2.6 $\mu W/cm^2$, electrical band width 0–16 Hz.

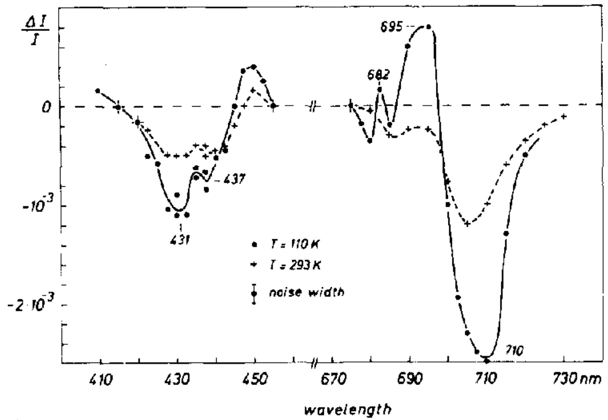

Fig. 2. Spectrum of absorption changes at high (T= 293°K) and low (T= 110°K) temperature. Depicted is the amplitude after the first flash. Further conditions see fig. 1.

3. Results and discussion

3.1. *Photochemical reactions in sucrose syrup at room temperature*

The use of sucrose syrup results in some differences of the absorption changes as compared with those in solutions without high concentrated sucrose. For discussion of absorption changes under normal conditions see a paper by H.T, Witt [14].

3.1.1. Chl-a_I

The amplitude of the Chl-a-reaction caused by the first flash is nearly half of the value under normal conditions, but appears in full after a group of short flashes (fig. 1). It is possible that the true amplitude after the first flash is as large as that after a flash group. Some fast decay kinetics might not have been resolved in these measurements. Haehnel et al. [15] show that under normal conditions the decay kinetics of Chl-a_I have two fast phases (10 μsec and 300 μsec) and a slow one (20 msec). The slow phase of Chl-a_I-reduction which indicates the rate limiting reaction of the electron transport chain of photoynthesis is slowed down by a factor of nearly 150 from about 20 msec to 1–3 sec (see fig. 1).

3.1.2. X-320

The amplitude is not altered, the decay kinetics are only slightly changed from 600 to about 900 μsec.

3.1.3. Plastoquinone

The amplitude of the plastoquinone-reaction increases 8–10 times in a group of short flashes as compared with that of the first flash. This indicates the existence of a pool of plastoquinone [16]. The decay kinetics are prolonged 150-fold (20 msec to 1–3 sec) as is the case with Chl-a_I.

3.1.4. Field

The amplitude of the absorption changes attributed to an electric field is not changed. The decay kinetics are accelerated from about 100 msec to 10 msec which is interpreted by the damages of the membrane due to the osmotic action of the high sucrose concentration [17].

These results indicated that the electron transport chain of photosynthesis is still working, however, the rate limiting reaction is slowed down by a factor similar to that of the increase of the viscosity (about 150). This is a hint that at a site between plastoquinone and Chl-a_I a collision-limited reaction is affected by viscosity. Probably one reactant is plastoquinone itself.

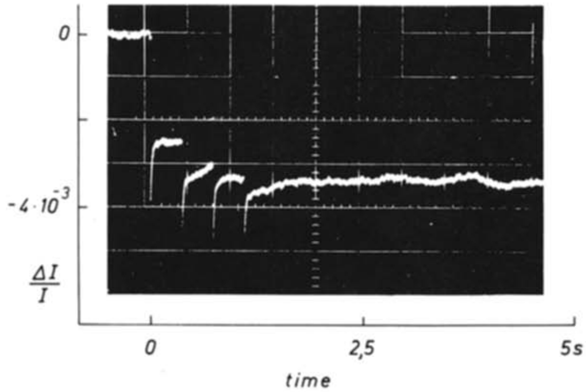

Fig. 3. Absorption changes at λ= 710 nm and T= 110°K during a flash group. Further conditions see fig. 1.

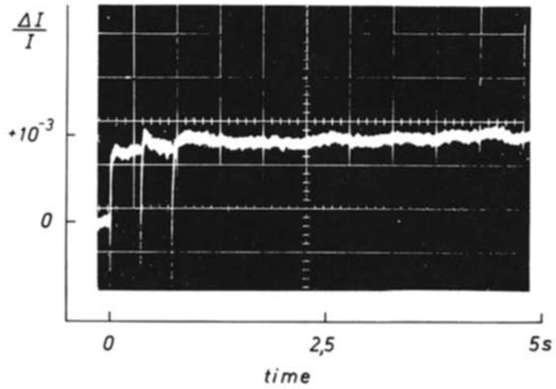

Fig. 4. Absorption changes at λ= 695 nm and T= 110°K during a flash group. Further conditions see fig. 1.

3.2. *Photochemical reactions in sucrose syrup at low temperature*

In fig. 2 flash-excited difference spectra are shown in the chlorophyll band region, the spinach chloroplasts being suspended in sucrose syrup. The full line indicates the amplitude of the first flash at T= 110°K, the dotted line at T= 295°K. The prominent feature of the low temperature as compared to the room temperature spectrum is the doubling of the amplitudes at 431 nm and 710 nm and a new positive band at 695 nm. Qualitatively similar negative bands have been found by different authors [1, 2, 4] and the positive band by Floyd et al. [4].

Flash group experiments at low temperature are shown in fig. 3 at 710 nm and fig. 4 at 695 nm. A comparison of figs. 1 and 3 shows that a flash group at room temperature increases the total amplitude compared to that after the first flash by 100% while at low temperature the value is only 50%, but the total amplitude at low temperature is by a factor of 1.7 larger than the high temperature amplitude. These comparisons have been made at the band maxima, which have been shifted from 705 (295°K) to 710 nm (110°K). This shift is not observed by Floyd et al. [4] who find the low temperature maximun at 705 nm. Furthermore they find an increase of the absorption change within ten flashes while here the total amplitude is obtained by three flashes. Other minor differences could be due to a different preparation technique (here clear sucrose sample, there scattering sample because of ice crystals).

The extinction coefficients of Chl-a_I at low temperature are not known, especially there is no information about possible band narrowing, concomitant increase of peak absorption or band shift at low temperature. Therefore a more qualitative comparison of high and low temperature spectra is in order. The apparent half-widths of the 431 and 710 nm bands at low temperature are nearly the same as at high temperature as can be seen in fig. 2. The appearance of a new positive band at 695 nm at low temperature is indicative for a new low temperature photoreaction of a special chlorophyll *a*. The most probable explanation of the spectrum is, that at low temperature a chlorophyll-a_O, is photoreactive with a positive band at 695 nm and negative bands near to 431 and 710 nm which are superimposed on the spectrum of Chl-a_I (negative bands near to 435, 685, 705 nm).

The nature of this Chl-a_O-reaction is not clear and only some speculative comments are possible. At low temperature only oxidoreductive reactions have been found is spinach chloroplasts which are mainly irreversible and thus indicating a trapping of electrons. Figs. 3 and 4 show the irreversibilty of the absorption changes with little superposition of a reversible phase after a flash group. As the spectrum of Chl-a_I indicating an oxidation is different from that of Chl-a_O, this could indicate a reduction of Chl-a_O. As a donator for this reduction Chl-a_I itself is possible. According to this assumption Chl-a_O operates at low temperature as an additional electron acceptor besides the natural one, which has been shown to operate at low temperature [18]. The flash-group experiments show nearly no increase of the amplitude at 695 nm (fig. 4) but a distinct increase at 710 nm (fig. 3). This increase is therefore attributable to Chl-a_I. The cause for this increase may be the same as discussed at room temperature (see above). The second photoreactive chlorophyll in photosynthesis Chl-a_{II} is characterised at room temperature by an absorption decrease at 685 nm [10,11]. Floyd et al. [4] observed negative changes at 680 nm which they attributed to Chl-a_{II}. But it was not shown why these changes should not belong to the minor peak of Chl-a_I at 682 nm. According to the above spectrum the possible low temperature reaction of Chl-a_{II} might be masked by the positive absorption change at 695 nm.

Acknowledgements

The stimulation of this work by Professor H.T. Witt is gratefully acknowledged. I thank Dr. A. Muller for some technical advice, Dr. H.-E. Buchwald for technical assistance of the electronics, Dipl.-Chem. Haehnel for discussions and Mrs. Jurgens for measurements of the Hill reaction in sucrose syrup.

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