THE TEMPERATURE DEPENDENCE OF INITIAL EVENTS IN PHOTOPHOSPHORYLATION OF SPINACH CHLOROPLASTS

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1. Introduction

In the photosynthetic membrane a variety of reactions are dependent on temperature, e.g., electron transport [1–6], proton movement [7,8], phosphorylation [1,13], decay of the membrane potential [9,10] and delayed light emmission [11,12]. When data of these and other membrane processes are drawn as Arrhenius plots, a break in the slope of straight lines is seen. This change in activation energy is often discussed as an effect of the phase transition of the membrane lipids which is mainly dependent on the lipid composition of the membrane. Activities mentioned above measured with chloroplasts of spinach or other higher plants display this break point between 15–20°C.

This inflection point varies with growth temperature, a fact well documented with algae grown at different temperature. The dependence of photophosphorylation on temperature has been investigated with membranes from the cyanobacteria *Anacystis nidulans* [1] and *Mastigocladus laminosus* [13]. Here, the influence of temperature on photophosphorylation of spinach chloroplast particles measured under steady state illumination (30 s) and during the initial first 50 ms is described.

2. Materials and methods

Chloroplasts were prepared as in [14]. Phosphorylation in the long range (usually 30 s) and in the ms range were done with the equipment described in [14]. The incubation medium contained: Tricine–NaOH buffer (pH 8, 50 mM), $MgCl_2$ (5 mM), NaCl (10 mM), PMS (50 μM), Na-ascorbate (1 mM), BSA (1 mg/ml), ADP (2 mM), P_i (^{32}P, 1 mM), chloroplasts equal to 0.05 mg chl/ml. The light intensity was 500 mW/cm^2 for the ms experiments, 50 mW/cm^2 for the 30 s experiments. The reaction vessel was kept at constant temperature by a thermostatic water jacket.

The reaction was stopped with perchloric acid and the reaction products analysed according to [15,16]: filtration through active charcoal retains the adenine nucleotides, these are eluted by ethanol–NH_3. The eluate containing the labelled ATP is then separated on a Dowex 1-X4 column and the labelling in the ATP measured in a scintillation counter.

3. Results and discussion

The initial kinetic of phosphorylation with 2 preparations of chloroplasts are given for several temperatures in fig.1. After a lag of a few ms from the onset

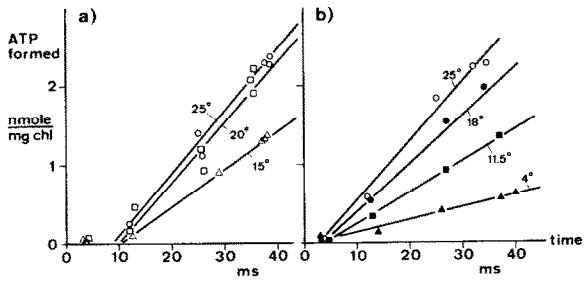

Fig.1. Temperature dependence of the initial ATP synthesis by spinach chloroplast fragments in the light. Reaction conditions see section 2. (a) and (b) are two different chloroplast preparations. The steady state phosphorylation rate (30 s, 25°C) was 290 μmol/h . mg chl for (a) and 280 μmol/h . mg chl for (b).

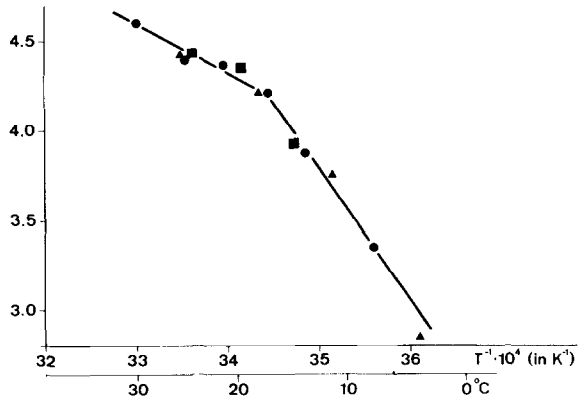

Fig.2. Arrhenius plot of the phosphorylation rate of spinach chloroplast fragments: abscissa, $1/T$; ordinate, ln of phosphorylation rate; (○,●) values from steady state phosphorylation (30 s); (□,■) values from fig.1(a); (△,▲) values from fig.1(b).

of light the rate of phosphorylation reaches a constant value and shows the usual dependence on temperature. Depicted as Arrhenius plot (fig.2) a break at ~18°C is observed, indicating a change in activation energy probably due to a transition in the membrane fluidity controlling one or more steps in photophosphorylation or a conformational change of a protein active in this process.

However, the duration of the lag phase seems independent within the experimental error on the temperature of the experiment, but varies largely from preparation to preparation of chloroplasts. In the first experiment shown, the calculated lag time is 9.57 ± 0.52 ms [extreme values: 9.1 ms for 25°C, 10.3 ms for 15°C], in the second 4.33 ± 0.35 ms [4.0 ms for 25°C, 4.1 ms for 4°C]; in other preparations the lag varied from 2.8–11.3 ms under otherwise identical experimental conditions. Similar lag times have also been found by other groups [18,19,23].

The temperature dependence of the steady state phosphorylation correlates with the behaviour of other membrane coupled photosynthetic processes measured with chloroplasts or algae such as electron transport, proton translocation, decay of membrane potential and others. The break found at ~18°C for our material after growth under unknown and undefined conditions fits well with published data ranging from 15–20°C [1–12]. Furthermore, the calculated activation energy of 59.2 kJ/mol below and 22.3 kJ/mol above the transition point for the short time as well as for the 30 s phosphorylation agree well with [5,8].

The temperature independence of the short lag is rather unexpected. So far with similar techniques or with flash accumulation the lag was found dependent on light intensity, coupling state of the phosphorylation process or presence of permeable ions known to abolish the membrane potential [17,18]. This suggests a dependence of the initial lag on the building up of a minimal value of the protonmotive force. These experiments further demonstrate that in chloroplasts photophosphorylation is driven initially more by the $\Delta\psi$, while under steady state conditions the ΔpH is the main driving force [19–21]. The decay of the membrane potential is clearly temperature dependent [9,10], however, its formation is probably rather independent of temperature in the physiological range. It seems unlikely that an activation process of the ATP-synthetase such as conformational changes of the protein itself [22–25], removal of an inhibitor protein [26], binding of nucleotides [27] and others would be independent of temperature and of the state of the lipids. However, the activation may be induced by the formation of the membrane potential directly or by some interaction with the primary electron transport [23,24].

Thus it can be summarized that the lag time consists of the sum of:

(a) The time for minimal energisation and build up of the protonmotive force;
(b) The time for activation of the ATP-synthetase; and
(c) The time for the initial part of a single turnover of the phosphorylation process to produce 1 ATP in the bound state.

It seems unlikely that the processes (b) and especially (c) are independent on the temperature. An overall independence on temperature would be observed if only (a) is temperature independent and its time is long compared to (b) and (c).

Assuming a coupling factor concentration of 1.3 nmol/mg chl [28] a turnover time for the enzyme can be calculated from the different rates of phosphorylation. Such a calculated turnover time ranges from 9–87 ms which is by far longer than the observed time lag. In addition there is no correlation between time lag, phosphorylation rate and calculated turnover time. This discrepancy may be explained by the hypothesis [23] that only a fraction of the ATP synthetases is active under certain conditions. By such a mechanism, the real turnover time of the enzyme would be much shorter than the apparent one and could reach

a value below the observed lag time. However, if, as suggested in [29], the release of the bound ATP is the energy-dependent step and if this step is rate limiting, the reaction leading to the bound ATP [our experimental analysis does not differentiate between bound and free ATP] may be by far more rapid than the calculated time of formation of free ATP.

These considerations support the above suggestion that the physiological processes (b) and (c) may need less time than (a), the build up to the protonmotive force. As a consequence the latter would be determining the lag and simulate the temperature independence of the initial process leading to bound ATP.

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