A TEMPERATURE-DEPENDENT CONFORMATIONAL CHANGE IN PHOTOSYSTEM-II THYLAKOID MEMBRANE

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1. Introduction

A photosystem-II subchloroplast fragment (TSF-2a) fractionated from spinach chloroplasts by Triton X-100 treatment is highly enriched in cytochrome b_{559} (cyt b_{559}) ($\sim$1/40 total chlorophyll (chl) molecules) [1]. The cytochrome in this subchloroplast fragment is present in the low-potential form, with $E_{7,m}$ ~60 mV [2]. The oxidized form of cyt b_{559} in the isolated subchloroplast fragments readily undergoes photoreduction, which is followed by a slower reversal to the oxidized form in the dark [2].

Cyt b_{559} in isolated chloroplasts has rather anomalous properties and its physiological function is yet unclear ([3–6] also cf. [7]). However, it is generally considered that cyt b_{559} is closely associated with photosystem II. Detergent-fractionated subchloroplast fragments, in which the state of cyt b_{559} has probably been modified as a result of exposure to the detergent, do provide useful vehicles for investigating its physico-chemical and biochemical properties, particularly the relationship between them, which may be relevant to the functional role of the cytochrome in photosynthesis.

This note reports the finding of a temperature-dependent change in the oxidation kinetics of cyt b_{559} which indicates the effect of a possible conformational change in the microenvironment in the thylakoid membrane.

2. Experimental

TSF-2a fragments, prepared as in [2], has 1 cyt b_{559}/34 chl molecules, no detectable *P*-700, and a DCIP-reduction activity of 1920 μmol/mg chl . h. The reaction mixture contained TSF-2a fragments at 15 μg chl/ml in 0.02 M Na-phosphate buffer (pH 7.0) in 2 ml final vol. $MgCl_2$, when present, was at 0.5 mM.

Light-induced ΔA due to cyt b_{559} reactions were measured in a dual-wavelength spectrophotometer [8], either at 560 or 430 nm, using 570 nm as the reference wavelength. This wavelength is devoid of changes due to C550, pheophytin, or other photosynthetic pigments. To avoid the appearance of and interference by the irreversible bleaching of the bulk chlorophyll at 430 nm, the illumination period was limited to 3 s by an electronic shutter.

Saturating red actinic light with an intensity of 5×10^5 ergs/cm^2 . s was isolated by Corning filters (2-58) and 2 in. $CuSO_4$ solution. An EMI 9558 photomultiplier tube shielded by two Corning 4-96 filters was used for signal detection.

Temperature of the reaction mixture was regulated to 0.1°C by flowing thermostated water through an insulated cuvette jacket (Cary model 1444100). Temperature was monitored directly with a diode temperature probe (Kettering model KDT-200).

3. Results

Light-induced rapid reduction of cyt b_{599} followed by a slower reversal in the dark, monitored at either 560 or 430 nm using 570 nm as the reference wavelength, have identical first-order kinetics. Figure 1 plots the normalized changes at the two wavelengths for cyt b_{599} oxidation. The ratio of the ΔA amplitudes at these two wavelengths and the difference spectrum

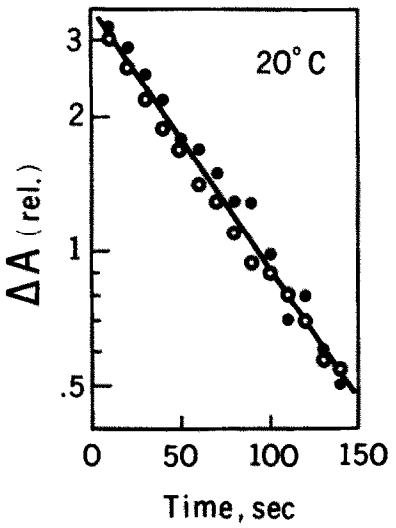

Fig. 1. Kinetics of cyt b_{559} oxidation after illumination of TSF-2a particles at 20°C. (-○-) ΔA_{430}; (-●-) ΔA_{560}.

plotted for these changes are consistent with the reduction of cyt b_{559} (cf. [2]). Because of the better signal-to-noise ratio, only the 430 nm data are presented below.

The dark oxidation of cyt b_{559} is highly dependent on temperature. The Arrhenius plot for the 6–28°C range in fig. 2 shows a discontinuity near 16–18°C.

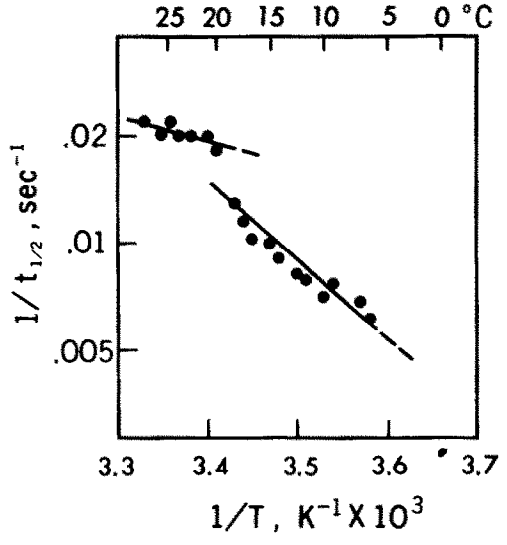

Fig. 2. The Arrhenius plot for cyt b_{559} oxidation.

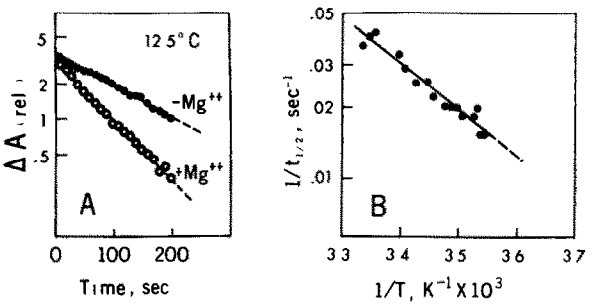

Fig. 3. Kinetics of cyt b_{559} oxidation in the presence and absence of Mg^{2+} (A), and the effect of Mg^{2+} on the Arrhenius plot for cyt b_{559} oxidation (B). Mg^{2+} was 0.5 mM.

Although the results shown in fig. 2 represent a series of experiments conducted by lowering the temperature from 28–6°C, spot check on reversing the temperature showed the reaction to be completely reversible. The activation energy calculated from the Arrhenius equation are 4.2 and 10 kcal/mol, respectively, for the upper and lower temperature segments.

Cations such as Mg^{2+} affect the oxidation rate of cyt b_{559} and the discontinuity in the Arrhenius plot. When 0.5 mM $MgCl_2$ was added to the TSF-2a reaction mixture, cyt b_{559} reoxidation becomes accelerated (fig. 3A), and the discontinuity in the Arrhenius plot is almost abolished (fig. 3B).

4. Discussion

A large number of examples exist in the literature for enzyme reactions of both plant and animal origin that show a discontinuity in the plot of the logarithm of the reaction-rate constant versus the reciprocal of the absolute temperature (Arrhenius plot), within the range of physiological temperatures. Such a discontinuity, representing a change in the activation energy, is often attributed to a change in the physical properties of the membrane.

Among the more prominent examples related to photosynthesis are:

(a) Contrasting behavior in the temperature dependence of the rate of NADP-reduction by water in chloroplasts from chill-sensitive and chill-resistant plants [9];

(b) A light-induced electrochromic shift in the absorption of membrane pigments in spinach chloroplasts attributed to a light-generated transmembrane electric field [10];
(c) The electron-transport reaction measured by $P\text{-}700^+$ re-reduction or the rate of photosynthesis measured by O_2-evolution in *Anacystis* [11];
(d) Light-induced quenching of fluorescence by the probe molecule atebrin [12];
(e) Delayed light emission from *Chlorella* cells [13] and bush-bean chloroplasts [14];
(f) Light-induced H^+ transport in spinach chloroplasts [15].

The presence of a discontinuity in the Arrhenius plots in all above-mentioned cases has been correlated with the mobility of the chloroplast-membrane lipids as measured by EPR spin-label spectroscopy [14,16,17] and by differential scanning calorimetry [14].

Thus, our observation on the oxidation kinetics of cyt b_{559} in the TSF-2a particles, with the point of discontinuity in the Arrhenius plot at 15–18°C (fig. 2), may also be attributed to some temperature-dependent conformational change in the thylakoid membrane. The lipids present in the TSF-2a retain a similar overall composition as in unfractionated chloroplasts [1]. The nature of the lipids is such that, although no true phase transition is expected to occur near ambient temperature, they could provide substantial fluidity in the thylakoid membrane. Furthermore, the thylakoid membrane is probably not homogeneous in lipid distribution, thus some local environments may have a temperature-dependent mobility different from that of the bulk lipids. Such a heterogeneous distribution has been shown by fractionation experiments [18,19], and also inferred from kinetic experiments suggesting that certain phenomena depending on the microenvironments may not be readily detected in whole thylakoid membrane [20].

The other possibility is a temperature-dependent conformational change in cyt b_{559} itself. This suggestion has been made plausible in view of the finding that cyt b_{559} is a complex lipoprotein with mol. wt 117 000 and composed of 56% of non-covalently bound lipids, including two unknown polar lipids [21]. The lipoprotein molecule could conceivably alter its accessibility relative to the electron-acceptor molecule as a result of a temperature-dependent conformational change.

The acceleration of the rate of oxidation of cyt b_{559} and the elimination of the discontinuity in the Arrhenius plot by Mg^{2+} are apparently related to some modification of control of energy transfer by the cation. Metal cations are known to regulate the distribution of excitation energy between the two photosystems [22]. More recently, it has been found that mono- or divalent cations can also regulate energy transfer within photosystem II [23]: either acting on the light-harvesting chl-*a*/*b* protein, or on the 'core' complex itself. As both TSF-2a and cyt b_{559} itself contain substantial amount of polar lipids, cations may enhance the fluidity of the membrane in addition to its other site-specific effects.

Acknowledgements

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References

[1] Vernon, L. P., Shaw, E. R., Ogawa, T. and Raveed, D. (1971) Photochem. Photobiol. 14, 343–357.
[2] Ke, B., Vernon, L. P. and Chaney, T. H. (1972) Biochim. Biophys. Acta 256, 345–357.
[3] Cramer, W. A. and Whitmarsh, J. (1977) Annu. Rev. Plant Physiol. 28, 133–172.
[4] Levine, R. P. (1969) Annu. Rev. Plant Physiol. 20, 523–540.
[5] Radmer, R. and Kok, B. (1975) Annu. Rev. Biochem. 44, 409–433.
[6] Bendall, D. S. and Sofrova, D. (1971) Biochim. Biophys. Acta 234, 371–380.
[7] Butler, W. L. (1978) FEBS Lett. 95, 19–25.
[8] Ke, B., Sahu, S., Shaw, E. R. and Beinert, H. (1974) Biochim. Biophys. Acta 347, 36–48.
[9] Shneyour, A., Raison, J. K. and Smillie, R. M. (1973) Biochim. Biophys. Acta 292, 152–161.
[10] Gräber, P. and Witt, H. T. (1974) Proc. 3rd Int. Congr. Photosynthesis, (Avron, M. ed.) pp. 951–956, Elsevier, Amsterdam, New York.
[11] Murata, N., Troughton, J. H. and Fork, D. C. (1975) Plant Physiol. 56, 791–796.
[12] Kraayenhof, R. and Katan, M. B. (1971) Proc. 2nd Int. Congr. Photosynthesis, pp. 937–949, Junk, The Hague.
[13] Jursinic, P. and Govindjee (1972) Photochem. Photobiol. 15, 331–348.

[14] Jursinic, P. and Govindjee (1977) Photochem. Photobiol. 26, 617–628.
[15] Yamamoto, Y. and Nishimura, M. (1976) Plant Cell Physiol. 17, 11–16.
[16] Raison, J. K. (1973) J. Bioenerget. 4, 285–309.
[17] Torres-Pereira, J., Mehlhorn, R., Keith, A. D. and Packer, L. (1974) Arch. Biochem. Biophys. 160, 90–99.
[18] Vernon, L. P. and Shaw, E. R. (1971) Methods Enzymol. 23, 277–289.
[19] Anderson, J. M. and Boardman, N. K. (1978) Biochim. Biophys. Acta 112, 403–421.
[20] Conjeaud, H., Michel-Villaz, M., Vermeglio, A. and Mathis, P. (1976) FEBS Lett. 71, 138–141.
[21] Garewal, H. S. and Wasserman, A. R. (1974) Biochemistry 13, 4072–4079.
[22] Murata, N. (1969) Biochim. Biophys. Acta 172, 242–250.
[23] Davis, D. J., Janovitz, E. B. and Gross, E. L. (1977) Arch. Biochem. Biophys. 184, 197–203.