The effect of chloroplast pH on the distribution of excitation energy between photosystems I and II suggests the involvement of two kinases in state 1–state 2 transitions

Ora Canaani

Department of Biochemistry, The Weizmann Institute of Science, Rehovot, 76100 Israel

Received 7 June 1985

The effect of medium pH on state 1–state 2 transitions was investigated in an intact leaf using the photoacoustic technique. Oxygen evolution yield in a leaf adapted to state 2 was independent of external pH. Emerson enhancement of modulated oxygen evolution during adaptation to state 2 was found to be pH dependent, with minima at pH 6.0–6.5 and 8.0–8.5. These minima correspond to two maxima for the balance of excitation energy distribution between photosystems I and II. A similar pH dependence was found for the initial rate of ATP-induced fluorescence quenching in isolated thylakoids. These results suggest that protein phosphorylation is maximal at pH 6.0–6.5 and 8.0–8.5. It is proposed that these pH maxima in protein phosphorylation reflect the participation of two kinases in the transition to state 2.

State 1–state 2 transition *pH effect* *Protein phosphorylation* *Photoacoustics* *Chlorophyll fluorescence* *Photosynthesis*

1. INTRODUCTION

The distribution of absorbed excitation energy between the two photosystems under limiting light conditions is regulated [1–3]. The need for this regulation arises because PS II absorbs more excitation energy in the red spectral region ($\lambda < 680$ nm) than PS I and the opposite situation occurs in the far-red region ($\lambda > 680$ nm). The physiological changes reflecting this mechanism are known as state 1–state 2 transitions. These changes were demonstrated in algae [4,5] and intact leaves [6–8]. State 1 results from over-excitation of PS I by far-red light. During adaptation, excess energy of monitoring red light is redistributed to PS II resulting initially in a large energy imbalance between the two photosystems. State 2 results from over-excitation of PS II in which excess energy is redistributed to PS I until a balanced distribution is approached. Experiments in isolated thylakoids [9,10] and in intact leaves [11] have shown that phosphorylation by an activated kinase and dephosphorylation by a phosphatase of chloroplast membrane proteins regulates state 1–state 2 transitions. This regulation was postulated to occur by a reversible diffusion of phosphorylated LHC which changes the absorption cross-sections and photochemical activities of the two photosystems, respectively [9–16]. In addition to LHC, phosphorylation of several PS II-associated proteins, including 9-, 32- and 45-kDa polypeptides, has also been shown [2,3,12]. Finally it has been reported that protein phosphorylation also leads to modification of PS II activity [17,18]. To try to understand better the role of phosphorylation of thylakoid proteins in vivo, I investigated the pH dependence of both the transition to state 2 in an intact leaf and the ATP-induced energy redistribution toward PS I in isolated thylakoids. I found that each of these activities have a similar pH dependence characterized by two maxima occurring at pH 6.0–6.5 and 8.0–8.5. These results suggest a more complex mechanism for the re-

Abbreviations: PS, photosystem; LHC, light-harvesting chl *a*/*b*-protein complex; a.u., arbitrary units

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/85/$3.30

distribution of excitation energy during transition to state 2 than previously anticipated [9] reflecting the participation of 2 kinases each having a different pH optimum.

2. MATERIALS AND METHODS

Intact tobacco leaves (*Nicotiana tabacum* L. var. *xanthi*) were used for the photoacoustic experiments. Tobacco chloroplasts were used for ATP-induced fluorescence quenching measurements [10]. Chloroplasts were prepared according to a standard method [19].

Photoacoustic measurements were conducted with the apparatus described in [20]. The photoacoustic signal of an intact leaf arises from the release of modulated heat and modulated photosynthetic oxygen evolution in response to the absorption of modulated light. The two contributions can be separated [20]. The photothermal signal generated by the thermal conversion of modulated light is directly proportional to the absorbed light. The photoacoustic oxygen signal divided by the photothermal signal is proportional to the quantum yield of oxygen evolution [8]. For monitoring the effect of pH on the leaf photoacoustic signal, a leaf disc (1 cm diameter) was soaked for 10 min in 10 ml of 100 mM potassium phosphate buffer or Mes-Tricine buffer at different pH values and inserted into the photoacoustic cavity.

Room-temperature chlorophyll fluorescence was measured as described [21]. Fluorescence yield changes caused by addition of 1 mM ATP were measured in 1 cm^2 cuvette using chloroplasts at a concentration of 20 μg of chlorophyll/ml in 1.4 ml reaction medium, containing 0.5 μM nigericin, 0.5 μM valinomycin and 10 mM KCl to suppress fast ATP-induced changes in fluorescence yield [10] and 5 mM NaF to inhibit chloroplast phosphatase activity [10]. The reaction medium also contained 50 mM Mes-Tricine or phosphate buffer and 5 mM $MgCl_2$. Phosphorylation of thylakoids resulted in 20–30% attenuation of the PS II fluorescence yield [14].

3. RESULTS

A leaf incubated in a particular pH in phosphate buffer was adapted to state 2 by illumination with modulated 650 nm light for 10 min as reported [8].

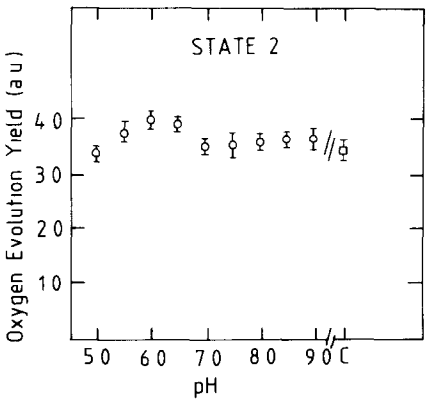

Fig.1. Relative quantum yield of oxygen evolution from an intact tobacco leaf adapted to state 2 as function of medium pH. Leaf discs were incubated for 10 min in potassium phosphate buffer (100 mM) and adapted to state 2 by 10 min illumination with 650 nm modulated light (5 W/m^2). Modulation frequency, 22 Hz.C, control, untreated leaf.

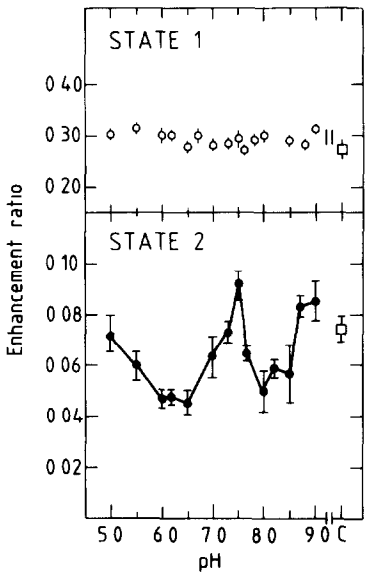

Fig.2. The effect of pH on Emerson enhancement of modulated oxygen evolution measured in state 1 and state 2. Enhancement was caused by addition for 30 s of far-red (710 nm, 18 W/m^2) constant background light to modulated 650 nm light. State 1 was obtained by illuminating the leaf disc for 10 min with excess far-red light on top of modulated light. Other conditions as in fig.1. Each value is the average of 4 measurements.

The state 2 relative oxygen evolution yield as a function of pH is shown in fig.1. It did not change significantly except for a small peak at about pH 6.0 and a slight rise at about pH 8.5. These small increases reflect the adaptation to maximum balance at these pH values resulting in higher quantum yields of oxygen evolution in state 2 (see below). At each specific pH, the distribution of excitation energy between the 2 photosystems was probed by measuring the enhancement of modulated oxygen evolution yield following the addition of nonmodulated background far-red light to the exciting 650 nm modulated light. This is the Emerson enhancement which was previously demonstrated in an intact leaf by the photoacoustic technique [7,8,11]. The extent of the immediate far-red light effect indicates the degree of over-excitation in PS II relative to PS I. The Emerson enhancement δE is equal to the ratio of $(\phi 2-\phi 1)\phi 1$ where $\phi 2$ and $\phi 1$ are the oxygen evolution quantum yields in the presence and absence of 710 nm non-modulated background light. The enhancement ratio is minimal at state 2 ($\delta E \rightarrow 0$) and maximal at state 1 [9]. In fig.2, δE was measured as a function of the pH in state 1 (top) and state 2 (bottom). It is observed that δE is in the range 0.04–0.09 in state 2 and 0.30 in state 1. In state 2, the Emerson enhancement ratio exhibits two troughs, one in the pH range 6.0–6.5 and another in the range pH 8.0–8.5 (fig.2, bottom). Similar results were also observed with other buffers (e.g. Tricine, Mes) but the largest differences in enhancement ratios were obtained with potassium phosphate buffer. At the lower pH trough, δE = 0.045 was smaller than at the higher pH trough (δE = 0.052). Outside the range of any trough (e.g. pH 7.5 or 9.0), δE increased to about 0.087. At pH 6.5 and 8.0, δE decreased by a factor of 1.9 and 1.7, respectively. An untreated leaf disc (fig.2C) exhibited a similar δE to a leaf disc incubated in a buffer of pH 7.3. A minimum in enhancement reflects a situation in which the balance of distribution of excitation energy between the 2 photosystems is maximum. It is therefore concluded that the 2 enhancement ratio minima at pH 6.5 and 8.0 correspond to 2 maxima of the activities leading to a greater balance of energy distribution during adaptation to state 2. When the enhancement ratio was analysed in state 1, it did not change markedly as function of pH (fig.2, top). This result indicates that molecular events responsible for the imbalance in energy distribution in state 1 are not significantly dependent on the surrounding pH.

Next I measured the ATP-induced change in excitation energy distribution in isolated thylakoids. This change is analogous to a transition to state 2 in intact leaves. The distribution was measured by ATP-induced fluorescence quenching [10]. Fluorescence induction of isolated tobacco thylakoids incubated at pH 6.5, had an F_m/F_0 ratio of 4.3 where F_m and F_0 indicate maximal and minimal fluorescence yields, respectively. The F_m/F_0 ratio is a parameter for the electron-transfer reactions [21]. It was found that this ratio did not change appreciably in the pH range 5.0–8.0 but decreased at higher pH (not shown). Addition of ATP, resulted in an immediate fluorescence quenching, as reported [10]. The initial rate of ATP-induced fluorescence quenching as a function of pH of the buffer and in the presence of uncouplers and the phosphatase inhibitor NaF is shown in fig.3. ATP-induced fluorescence quenching was maximal at pH 6.3 and 8.5, respectively. When NaF was excluded from the reaction it was very difficult to measure ATP-induced fluorescence quenching because of its extremely small initial rate, due to strong phosphatase activity in thylakoids [10].

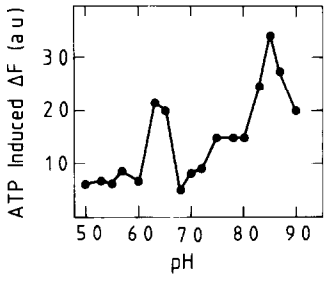

Fig.3. The effect of pH on the initial rate of ATP-induced fluorescence quenching due to protein phosphorylation in isolated tobacco chloroplasts. Chloroplasts were resuspended in a medium comprised of Tricine-Mes buffer of different pH values, NaF and uncouplers. Extent of fluorescence decrease during 4 min incubation with 1 mM ATP was recorded at each pH. Complete fluorescence quenching occurred in 8 min.

4. DISCUSSION

Here, I analyzed the effect of pH on the Emerson enhancement ratio during adaptation to state 2 in intact leaves and on ATP-induced fluorescence quenching in isolated thylakoids. The balance in energy distribution of both activities showed very similar pattern of 2 maxima around pH 6.0–6.5 and 8.0–8.5 in leaves and in thylakoids. ATP-induced fluorescence quenching in thylakoids and state 1–state 2 transitions in intact leaves were previously shown [10,11] to be directly correlated with changes in energy distribution due to the reversible phosphorylation of membrane proteins. Therefore, I propose that the balance in energy distribution shown here to be maximal in 2 pH ranges reflect the corresponding maximal phosphorylation of membrane proteins. I further suggest that the activity of 2 phosphokinases with maximal initial rates at pH 6.0–6.5 and 8.0–8.5, respectively, results in the pattern of energy distribution observed here.

In intact leaves, to obtain the particular external pH, a high concentration (100 mM) of potassium phosphate buffer was used in the incubation medium. The exact internal pH at the stroma side of leaf cells is unknown and is assumed to be close to external buffer pH due to equilibration by the two following mechanisms. First, diffusion of H^+ into stroma from the medium. Although the proton permeability of the chloroplast envelope is slow [22], changes in the pH of the external medium of intact chloroplast resulted in pH alterations within the stroma [23]. Second, transfer of H^+ indirectly through the protonated dihydrophosphate ion. In illuminated leaf cells, there is a rapid exchange of anionic metabolites between chloroplasts and cytosol, due to a specific translocator within the chloroplast envelope for the transport of phosphate, 3-phosphoglycerate and triose phosphate [22]. Therefore, the inclusion of a phosphate buffer in the external medium could have been a significant factor in promoting pH equilibration across the leaf's membranes.

It was found that the quantum yield of oxygen evolution in an intact leaf incubated in phosphate (P_i) buffer was refractory to pH changes (fig.1). This is probably due to the partial inhibition of CO_2 fixation in intact chloroplasts exposed to external P_i [24] resulting from substrate depletion of the carbon cycle by a P_i counter exchange. In this case and probably also in an intact leaf exposed to high P_i concentration, only the phosphoglycerate reduction occurs which is independent of stromal pH [24].

Recently, the isolation of several kinases from chloroplasts was reported [25,26]. The 2 kinases purified from spinach had pH optima at pH 6.5 and 7.5–8.0, respectively [27]. In another study it was found that the maximal ^{32}P labeling in phosphorylated polypeptides from *Chlamydomonas* chloroplasts had double maxima at pH 6.5 and 8.0–8.5 [28]. The pH profiles of the activities of the 2 isolated spinach kinases [27] as well as polypeptide phosphorylation in *Chlamydomonas* chloroplasts [28] are very similar to the pH dependence of the balance of energy distribution in intact leaves and in isolated chloroplasts shown here (figs 2,3). It is therefore possible that these two spinach kinases [27] participate in the transition to state 2. The 2 kinases may operate simultaneously in the regulation of energy distribution. For example, one kinase could regulate the rate of electron transport by reversible diffusion of phosphorylated LHC [9]. The second kinase could phosphorylate other polypeptide(s) closely associated with PS II. Indeed, protein phosphorylation was reported to modify PS II activity and decrease the rate of PS II electron transfer [17,18]. PS II modification due to phosphorylation [17,18] could possibly result in the reported diminution in the rate of PS II excitation which is not associated with increased energy distribution to PS I [14]. Such a loss in excitation fraction (β) distributed to PS II was observed in [8]. An alternative explanation for the role of the 2 kinases in energy distribution in vivo would involve their specific activation in response to differences in stromal pH, arising at different metabolic requirements [22]. In this case, each particular kinase would be switched on in response to a specific stromal pH according to its optimal pH activity.

ACKNOWLEDGEMENT

Thanks are due to Professor S. Malkin for helpful suggestions.

REFERENCES

[1] Allen, J.F. (1983) CRC Crit. Rev. Plant Sci. 1, 1–22.
[2] Bennett, J. (1983) Biochem. J. 212, 1–13.
[3] Horton, P. (1983) FEBS Lett. 152, 47–52.
[4] Bonaventura, C. and Myers, J. (1969) Biochim. Biophys. Acta 189, 366–386.
[5] Murata, N. (1969) Biochim. Biophys. Acta 172, 242–251.
[6] Chow, W.S., Telfer, A., Chapman, D.J. and Barber, J. (1981) Biochim. Biophys. Acta 638, 60–68.
[7] Canaani, O., Cahen, D. and Malkin, S. (1982) FEBS Lett. 150(1), 142–146.
[8] Canaani, O. and Malkin, S. (1984) Biochim. Biophys. Acta 766, 513–524.
[9] Allen, J.F., Bennett, J., Steinback, K.E. and Arntzen, C.J. (1981) Nature 291, 25–29.
[10] Bennett, J., Steinback, K.E. and Arntzen, C.J. (1980) Proc. Natl. Acad. Sci. USA 77, 5253–5257.
[11] Canaani, O., Barber, J. and Malkin, S. (1984) Proc. Natl. Acad. Sci. USA 81, 1614–1618.
[12] Staehelin, L.A. and Arntzen, C.J. (1983) J. Cell Biol. 97, 1327–1337.
[13] Horton, P. and Black, M.T. (1980) FEBS Lett. 119, 141–144.
[14] Horton, P. and Black, M.T. (1982) Biochim. Biophys. Acta 680, 22–27.
[15] Barber, J. (1983) Photobiochem. Photobiophys. 5, 181–190.
[16] Telfer, A., Bottin, H., Barber, J. and Mathis, P. (1984) Biochim. Biophys. Acta 764, 324–330.
[17] Horton, P. and Lee, P. (1984) Biochim. Biophys. Acta 767, 563–567.
[18] Hodges, M., Packham, N.K. and Barber, J. (1985) FEBS Lett. 181, 83–87.
[19] Avron, M. (1960) Biochim. Biophys. Acta 40, 257–272.
[20] Poulet, P., Malkin, S. and Cahen, D. (1983) Biochim. Biophys. Acta 724, 433–446.
[21] Malkin, S. and Kok, B. (1966) Biochim. Biophys. Acta 126, 413–432.
[22] Heber, U. and Heldt, H.W. (1981) Annu. Rev. Plant Physiol. 32, 139–168.
[23] Heldt, H.W., Werdan, K., Milovancev, M. and Geller, G. (1973) Biochim. Biophys. Acta 314, 224–241.
[24] Heldt, H.W., Fliege, R., Lehner, K., Milovancev, M. and Werdan, K. (1974) Proc. 3rd Int. Congr. Photosynth., vol. 2, 1369–79, Elsevier/North-Holland, Amsterdam, New York.
[25] Lin, Z.F., Lucerno, H.A. and Racker, E. (1982) J. Biol. Chem. 257, 12153–12156.
[26] Markwell, J.P., Baker, N.R. and Thornber, J.P. (1983) Photobiochem. Photobiophys. 5, 201–207.
[27] Lucerno, H.A., Lin, Z.F. and Racker, E. (1982) J. Biol. Chem. 257, 12157–12160.
[28] Owens, J. (1982) PhD thesis, Hebrew University of Jerusalem, Israel.