METABOLIC REGULATION OF THE THYLAKOID PROTEIN KINASE

J. P. MARKWELL, N. R. BAKER* and J. P. THORNBER
Department of Biology and Molecular Biology Institute, University of California, Los Angeles, CA 90024, USA

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1. Introduction

Optimization of photosynthetic energy production is likely to require precise control of the balance of excitation energy distributed to the photochemical reaction centers of photosystem I (PSI) and photosystem II (PSII). It has been established [1–4] that phosphorylation of some thylakoid proteins changes the excitation energy distribution, the so-called state 1–state 2 transition. Largely on the basis that the protein kinase catalyzing this phosphorylation is converted to a more active form by reduction of plastoquinone (PQ) [5] in the electron-transport system, a model has been presented [2] which demonstrates how the redox state of PQ could modulate energy distribution to PSI and PSII. A rapid modulation of kinase activity by changes in the redox state of PQ is predicted to effectively control steady-state photosynthesis [2]. Changes in the redox state of PQ are in the order of milliseconds [6], whereas conversion of the kinase from its active to less active form by oxidation of reduced PQ has a half-time of 5 min [7]. This suggests that PQ may not be the primary modulator of the thylakoid protein kinase under steady-state conditions.

We have identified some chloroplast metabolites which may modulate the enzyme's activity at steady-state [8,9]. We had found that ADP, but not AMP, inhibited the kinase activity with respect to phosphorylation of membrane proteins including the apoproteins of the light-harvesting chlorophyll *a/b*–protein complexes (LHCP), its major protein substrates; 25 and 100 μM ADP caused a 37% and 59% inhibition of the kinase activity [9]. Here, we continue these studies by showing that adenylate energy charge is implicated in regulation of the enzyme during steady-state photosynthesis, and that Mg^{2+} can stimulate the kinase activity. We suggest that adenylate energy charge and Mg^{2+} are more likely to be key factors in steady-state regulation of the kinase than PQ.

2. Materials and methods

Nicotiana tabacum (tobacco) was grown in a soil–vermiculite mixture in a greenhouse. Leaves were excised and homogenized twice with a Waring blender for 5 s in ice-cold 0.4 M sorbitol, 1 mM NaCl, 25 mM sodium *N*-Tris-(hydroxymethyl)-methylglycine (pH 7.6). All further steps were carried out at 0–5°C. The brie was filtered through 4 layers of Miracloth (Calbiochem, Inc.) and centrifuged at 3000 × *g* for 1 min. The chloroplasts in the pellet were disrupted in 1 mM KCl, 25 mM sodium *N*-Tris-(hydroxymethyl)-methylglycine (pH 7.5) with the aid of a glass homogenizer. The material was centrifuged at 27 000 × *g* for 5 min to separate metabolites and soluble proteins from the membranes. The pelleted thylakoid membranes were again homogenized in 1 mM KCl, 25 mM sodium *N*-Tris-(hydroxymethyl)-methylglycine (pH 7.5) and used immediately for assay of protein kinase. The protein kinase activity was somewhat unstable and a noticeable decrease in activity (>20%) could be observed over 1 h.

The assay for protein kinase activity contained in 0.1 ml the following: 1 mM KCl, 5 mM $MgSO_4$ (unless otherwise indicated), 25 mM sodium *N*-Tris-(hydroxymethyl)-methylglycine (pH 7.5), 5 μCi [γ-^{32}P]ATP (3 Ci/mol), 200 μM unlabelled ATP (unless otherwise indicated) and a pre-illuminated aliquot of membranes containing 20 μg total chlorophyll. Preillumination was used to reduce plastoquinone and activate the protein kinase. Assays were performed in triplicate. The mixture was incubated at 30°C for 2 min using

* Permanent address: Department of Biology, University of Essex, Colchester, CO4 3SQ, Essex, England

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photosynthetically active light (400–700 nm) at a photon fluence rate of 1 mmol . m^{-2} . s^{-1}. The reaction was halted by spotting 50 μl aliquots onto 23 mm circles of Whatman 3 MM filter paper which were immediately plunged into ice-cold 7.5% (w/v) trichloroacetic acid. The filters were washed 3 times, for ⩾20 min each, in cold 7.5% trichloroacetic acid (15 ml/filter paper) followed by 20 min at 90°C and an additional wash in cold 7.5% trichloroacetic acid. Washing with trichloroacetic acid removes radioactivity not covalently bound to protein. The filter papers were then bathed in absolute ethanol, absolute ethanol:petroleum ether (1:1, v/v), and finally in petroleum ether. After air drying, the amount of ^{32}P incorporated was determined by Cerenkov spectrometry [10].

Establishment of adenylate energy charge in the assays was carried out by addition of ATP and AMP in a molar ratio giving the desired energy charge, and the mixture incubated with 1.5 units of adenylate kinase for 10 min at 30°C prior to addition of the thylakoid membranes. This procedure generated ADP, and 10 min appeared to be sufficient to allow an equilibrium between ATP, ADP and AMP. Chlorophyll and protein concentrations were determined as in [11] and [12], respectively. Labelled ATP was purchased from Amersham. All other organic chemicals were purchased from Sigma Chemical Co.

3. Results

Our observations that ADP inhibited the kinase suggested to us that perhaps the enzyme was modulated by adenylate energy charge. Many enzymes are regulated by ADP or AMP and are thought to be controlled by the energy charge [13]. Adenylate energy charge is a ratio defined as [ATP] + $\frac{1}{2}$ [ADP]/[ATP] + [ADP] + [AMP]. The concept of adenylate energy charge was developed to express the amount of metabolic energy stored in the adenylate nucleotide system, and to explain regulation of cellular energy metabolism within the broad framework of competing anabolic and catabolic pathways [14].

Thylakoid protein kinase activity was determined as a function of adenylate energy charge at a total nucleotide concentration of 0.75 mM (fig.1). The activity of this enzyme was also measured for ATP concentrations equal to those used in the energy charge studies, but with ADP and AMP omitted (fig.1).

Kinase activity in the presence of 200 μM ATP was

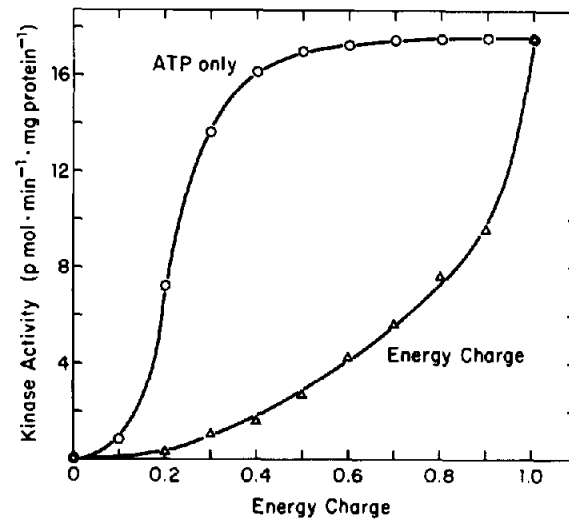

Fig.1. Effect of adenylate energy charge on the thylakoid protein kinase: (△) effect of adenylate-energy charge with a pool of 0.75 mM total adenylates; (○) activity of kinase at ATP concentrations used for the energy charge determinations, but in the absence of ADP, AMP, or adenylate kinase. The ATP concentrations in the latter plot were calculated assuming an equilibrium constant for the adenylate kinase reaction of 1.0 [14].

stimulated by increases from 0.2–20 mM $MgSO_4$ (fig.2). Magnesium acetate produced a similar stimulation. Examination of the enzyme kinetics revealed that Mg^{2+} increased the V_{max} of the reaction but not the affinity of the enzyme for ATP (fig.3). It should

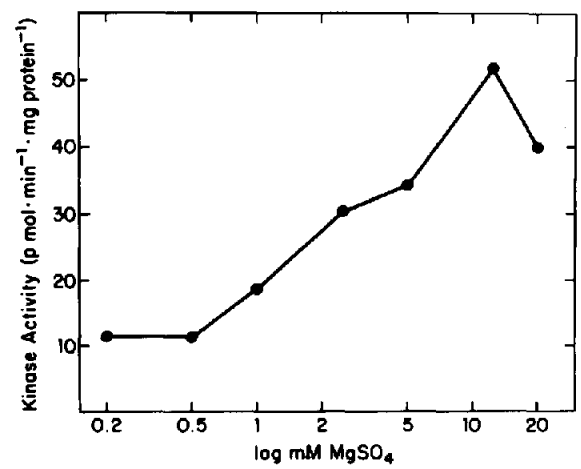

Fig.2. Effect of $MgSO_4$ on the kinase activity measured with 200 μM ATP present.

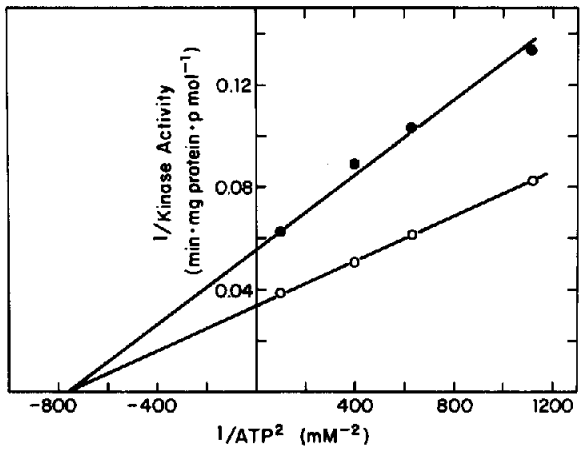

Fig.3. Lineweaver-Burk plot showing the effect of Mg^{2+} on the kinase activity: (•) 2 mM Mg^{2+}; (○) 10 mM Mg^{2+}.

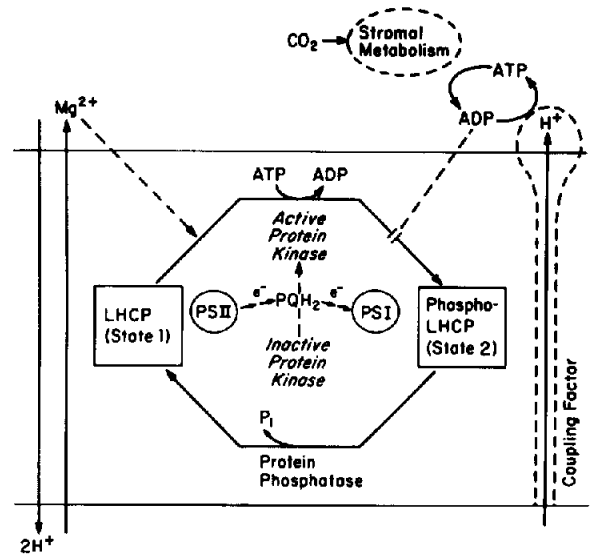

Fig.4. Proposed model for control of photosynthetic energy production through modulation of kinase activity via changes in steady-state stromal levels of ATP and ADP (adenylate energy charge) and Mg^{2+}.

be noted that V_{max} and $S_{0.5}$ were determined by plotting the reciprocal of the reaction rate *vs* $1/[ATP]^2$, rather than $1/[ATP]$, since the Hill coefficient for the enzyme is 2.0 [9].

4. Discussion

The results indicate that the adenylate energy charge must be considered to understand the activity of the thylakoid protein kinase in vivo. The enzyme activity measured when ATP is the only adenylate present simply reflects the affinity of the kinase for ATP. The decrease in activity under the equilibrium conditions defined by the adenylate energy charge represents the potential for control of this activity under steady-state conditions in vivo. Values for energy charge of 0.45–0.81 have been reported within the chloroplast [15,16]. Variations of energy charge within this range produce large changes in the activity of the thylakoid protein kinase (fig.1).

The stimulation of protein kinase activity by Mg^{2+} is not solely due to formation of a Mg^{2+} · ATP complex, the substrate for the kinase, because added Mg^{2+} (up to 20 mM) at least equalled or exceeded the [ATP] (0.2 mM). Furthermore, the stimulation did not appear to involve changes in the affinity for ATP (fig.3) which would be expected if its only effect had been formation of the Mg^{2+} · ATP complex. This stimulation by Mg^{2+} may parallel the well-established stimulation of ribulose 1,5-bisphosphate carboxylase–oxygenase by this ion [17].

These results have led us to the view that the concentrations of chloroplast metabolites bring about the steady-state regulation of the thylakoid protein kinase activity (fig.4), with adenylate energy charge within the chloroplast probably being a prime controlling factor. Additionally, the chloroplast energy charge directly reflects the cytoplasmic energy charge and vice versa [15]. Thus, changes in the rate of photosynthetic carbon fixation or other anabolic processes inside or outside the chloroplast could be effectively communicated via the adenylate energy charge to the photosynthetic membrane, thereby enabling the membrane to respond to the metabolic needs of the cell. Modulation of the kinase activity, and hence the extent of protein phosphorylation, will change the distribution of excitation energy between PSI and PSII [1–4]. This would lead to alterations in the rate of energy (ATP and NADPH) production by the thylakoid membrane. Using this hypothesis, we predict that under steady-state conditions an increased rate of carbon fixation should cause a decrease in the kinase activity, shift the photosynthetic membrane toward a state 1 condition and result in an increased production of photosynthetic energy by the thylakoid. Since Mg^{2+} efflux is directly related to light-induced proton influx into the thylakoid, the stimulation by

Mg^{2+} may act as a governor to stabilize the photophosphorylation potential of the membrane. Finally, we suggest that the function of the redox state of PQ, with respect to the kinase, is not that of a steady-state modulator of the enzyme. It may, however, function as a switch to activate the kinase on transition from dark to light. This would prevent a futile cycle of protein phosphorylation and dephosphorylation in the dark, which would result in unnecessary energy losses.

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