GRANA FORMATION IN CHLOROPLASTS MAY PROMOTE ENERGY TRANSFER BETWEEN PHOTOSYSTEM II UNITS

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1. Introduction

The biological significance of grana formation in chloroplasts is of considerable interest. The suggestion that grana formation may be a means of achieving a high density of light harvesting assemblies [1] has not been corroborated (reviewed [2]). The quantum efficiency of reduction of electron acceptors added to isolated chloroplasts, stacked or unstacked, has been measured directly or indirectly [1–8]. However, at low light intensities and in the presence of an electron acceptor the primary electron acceptor of photosystem II, Q, is largely oxidised. Thus any effect of grana on energy transfer between photosystem II units would not have been detected [9,10].

Oxygen flash yield studies [9] demonstrated that $MgCl_2$ (3 mM) or KCl (100 mM) induces pronounced energy transfer between photosystem II units. The influence of cations on oxygen flash yield only became evident as Q became reduced. A similar conclusion was reached on the basis of fluorescence induction studies [11]. As these cation concentrations are similar to those required for grana formation [12], it seemed possible that the two phenomena may be related. Here we have utilised the observation [2,13] that lowering the medium pH to 5.4 brings about the formation of grana which are morphologically indistinguishable from those induced by metal cations at neutral pH values. These grana seem also to be biochemically similar to those produced by metal cations as indicated by the chlorophyll *a/b* ratios of digitonin fractions [14]. We now demonstrate that incubation of chloroplasts at pH 5.4 strongly favours energy transfer between photosystem II units, and suggest that this may be due to grana formation.

2. Materials and methods

Chloroplasts were extracted from freshly harvested spinach leaves in 30 mM tricine buffer (pH 8) containing 10 mM NaCl and 0.4 M sucrose. They were subsequently resuspended in the same solution and conserved at 0°C for the duration of the experiment, which was $\leqslant 2$ h from the time of chloroplast extraction.

Reactions were conducted at different pH values from 5.1–6.4. The reaction buffer was morpholine ethane sulfonic acid (25 mM) brought to pH with NaOH and NaCl was added so that final $[Na^+]$ was 25 mN in all cases.

Ferricyanide (1 mM) reduction was measured indirectly as oxygen evolution utilising a Clark-type oxygen electrode. The light used was 6000 ergs . cm^{-2} . s^{-1} intensity, filtered through a Corning 4-96 filter. Chlorophyll was 20 μg/ml and the uncoupler gramicidin (1.2 μM) was also present. Under these conditions doubling the light intensity lead to a 70–80% increase in the rate of electron transport.

The induction of chlorophyll fluorescence was measured with the instrument set-up in [15]. The exciting light was of 10 000 ergs . cm^{-2} . s^{-1} intensity, filtered through a Corning 4-96 filter. Chlorophyll was 4 μg/ml.

3. Results

The sigmoidal nature of the chlorophyll fluorescence induction curve in the presence of DCMU has been attributed to energy transfer between photosystem II units [10,16]. In fig.1 we show that in the absence of divalent cations at pH 6.4 the induction

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Table I
Ferricyanide reduction at pH 5.4 and pH 6.4 in the presence and absence of DCMU (a), and the influence of $MgCl_2$ at pH 6.4 in the presence and absence of DCMU (b)

a

−DCMU		+DCMU	
pH 5.4	pH 6.4	pH 5.4	pH 6.4
34	33	22	13

b

−DCMU		+DCMU	
$-MgCl_2$	$+MgCl_2$	$-MgCl_2$	$+MgCl_2$
48	60	20	37

Ferricyanide reduction was measured with water as electron donor. DCMU was 50 nM and $MgCl_2$ was 5 mM. Chloroplasts were incubated under the indicated conditions for 4 min in the dark and for 1 min in the light before ascertaining the rate. For further details see section 2. Data are in μequiv. mg chl^{-1} . h^{-1}

curve is exponential. The addition of $MgCl_2$ (5 mM) converts this curve into one with a sigmoidal shape. The same process is also observed, in the absence of divalent cations, as the pH is lowered. The most marked sigmoidicity is observed at pH 5.4. It should also be noticed that the fluorescence rises to a maximum at pH 5.4, in accord with [17].

The data in table 1 compare the velocity of photosystem II electron transport (low light intensity) at pH 5.4 and 6.4, in the absence and presence of DCMU, sufficient to block a little >50% of the system II traps. It can be seen that with most of the system II traps open (in the absence of DCMU) the rate of ferricyanide reduction was similar at pH 5.4 and pH 6.4, as we have shown [2]. However with a substantial proportion of the traps closed (in the presence of DCMU), chloroplasts incubated at pH 5.4 are much more photochemically active than those incubated at pH 6.4. In a control experiment conducted with $MgCl_2$ at pH 6.4 (table 1b) it can be seen that in the presence of DCMU the effect of Mg^{2+} on the photochemistry of photosystem II was much greater than in the absence of DCMU. This result confirms the observation in [9] under our experimental conditions.

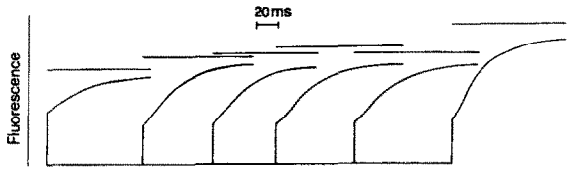

Fig.1. Chlorophyll fluorescence induction kinetics at different pH values. Chloroplasts were incubated for 5 min at room temperature in the dark before the induction kinetics were measured. DCMU(10 μM) was always present. For further details see section 2. From left to right: pH 6.4; pH 5.9; pH 5.7; pH 5.4; pH 5.1; pH 6.4 + $MgCl_2$ (5 mM). The horizontal lines above the induction curves are the final fluorescence levels.

4. Discussion

The rate of photosystem II can be expressed by the relation [9,18]:

$$v = \frac{Q}{1-(1-Q)\,.\,\alpha}$$

where Q is the probability that a trap is open and α is the probability of energy transfer between photosystem II units. Taking into account the concept introduced in [19], that closed photosystem II traps, when excited, may either disperse the energy by non-radiative means or transfer it back to the light harvesting chlorophyll, this equation becomes [20]:

$$v = \frac{Q}{1-(1-Q)\,.\,\alpha\,.\,k_t}$$

where k_t is the probability that an excited trap transfers energy back to the light harvesting chlorophyll.

It has been shown [10,16] that the sigmoidal nature of the fluorescence rise curve is due to energy

transfer between photosystem II units. The degree of sigmoidicity can also be greatly modified by changes in k_t [20]. Thus this observation that lowering the medium pH to 5.4 causes an increase in the sigmoidicity could be explained by increases in either α and/or k_t. The fact that the fluorescence yield rises to peak at pH 5.4 would perhaps favour an explanation based on an increase in k_t, as k_t increases inevitably lead to increased fluorescence yields [19].

We have also observed that under experimental conditions in which most photosystem II traps are open there is no effect of incubation at pH 5.4 on the rate of photosystem II photochemistry with respect to pH 6.4. However when a little more than half the traps are closed by DCMU addition, pH 5.4-incubated chloroplasts were much more active than pH 6.4-incubated chloroplasts. These observations are also compatible with either of the above explanations, involving increases in α and/or k_t.

Grana formation induced by protons attains the maximal level at pH 5.4 [14]. We now show that the same pH is also optimal for promoting photosystem II–photosystem II energy transfer (α and/or k_t processes). This, together with the observation that cations promote photosystem II–photosystem II interaction, strongly suggests the possibility that grana formation may be involved. It cannot be entirely excluded however, that the similar effect of metal cations and protons could be due in some way to the masking or neutralisation of negative charges on the membrane surfaces [21,22], independent of the concomitant grana formation.

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