THE INFLUENCE OF PROTON-INDUCED GRANA FORMATION ON PARTIAL ELECTRON-TRANSPORT REACTIONS IN CHLOROPLASTS

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1. Introduction

The bulk of the photosynthetic membranes of higher plants forms closely appressed stacks called grana. Despite the considerable effort expended in recent years to find the function of this characteristic structure, no general consensus of opinion has emerged. Based on studies with 'sun' and 'shade' plants in which large differences in grana formation were observed, it was suggested [1] that grana formation 'may be a means of achieving a high density of light harvesting assemblies.' The same idea was subsequently put forward [2] based on experiments with *Atriplex* plants grown under different light intensity regimes in which different levels of grana formation were observed. However, these workers were unable to demonstrate any difference in the quantum efficiency of dichlorophenolindophenol reduction of chloroplasts with high or low levels of grana formation. It was also suggested [3,4] that membrane stacking should lead to a higher photochemical efficiency due to a higher light-harvesting pigment density, though no new evidence was presented to support this view. Agranal maize bundle-sheath chloroplasts were reported [5] to have a lower quantum efficiency of NADP reduction with water as the reductant than the granal mesophyll chloroplasts from the same plant. An agranal mutant of *Chlamydomonas* was shown [6] to reduce benzoquinone with a quantum efficiency similar to that of cells containing granal chloroplasts. All the above-mentioned experiments suffer from the defect that the degree of grana formation is not the only experimental variable. Almost certainly, and in some cases this was demonstrated, substantial differences in membrane composition occur along with different degrees of grana formation.

More recently, a different experimental approach has been used, based on the original observation [7], that reversible grana formation can be induced in isolated chloroplasts by adding cations to the incubation medium. The importance of the light-harvesting chlorophyll *a*/*b* protein complex in cation-induced grana formation has been demonstrated [8–11]. They have shown a close correlation between membrane stacking and the regulation of energy distribution between the two photosystems, leading to an increase in the photochemical efficiency of PS II and a decrease of PS I. This idea is in accord with an earlier suggestion [12] where, on the basis of freeze fracture studies, that grana formation was proposed to permit a greater degree of interaction between the two photosystems. The major experimental problem with work based on the in vitro induction of membrane stacking with cations is that the cations themselves seem to exert a number of effects at the level of the photosystems, which may not be related to membrane stacking [13–16]. In an attempt to circumvent this problem we have utilized the recent observation [17] that lowering the suspension medium to pH 5.4 leads to the formation of grana which are indistinguishable morphologically from those induced by cations at higher pH values and the formation of which seems to involve the same membrane site(s). We show that low pH-induced grana formation does not influence the photochemical efficiency of PS II, though PS I operates at a decreased efficiency.

Abbreviations: DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea; MES, 2-(*N*-morpholino)ethane sulphonic acid; PS I, photosystem I; PS II, photosystem II

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, as in [16]. Unless otherwise stated, they were resuspended and conserved for the duration of the experiment in the same buffer at 0°C.

Reactions were conducted at two different pH values: At pH 6.2, the medium was 25 mM MES, 14 mM NaCl (total Na^+ 26 mN); at pH 5.4, the medium was 25 mM MES, 24 mM NaCl (total Na^+ 26 mN).

The photochemical activities measured were potassium ferricyanide (1 mM) and methylviologen (0.1 mM) reduction. The latter reaction was carried out in the presence of 5 mM ascorbate, 20 μM 2,6-dichlorophenolindophenol, 2 μM DCMU and 2 mM sodium azide. Both reactions were followed with a Clark-type oxygen electrode at 22°C. White light was employed, and the intensity was varied by means of Balzers neutral filters. Chlorophyll was 30 μg $.ml^{-1}$.

The preparation of samples for electron microscopy was performed essentially as in [16].

3. Results and discussion

In fig.1 the typical aspect of chloroplasts suspended for 4 min at pH 5.4 and pH 6.2 prior to fixation can be seen. At the lower pH, well-developed grana stacks are visible, demonstrated [17] to be morphologically indistinguishable from those formed in the presence

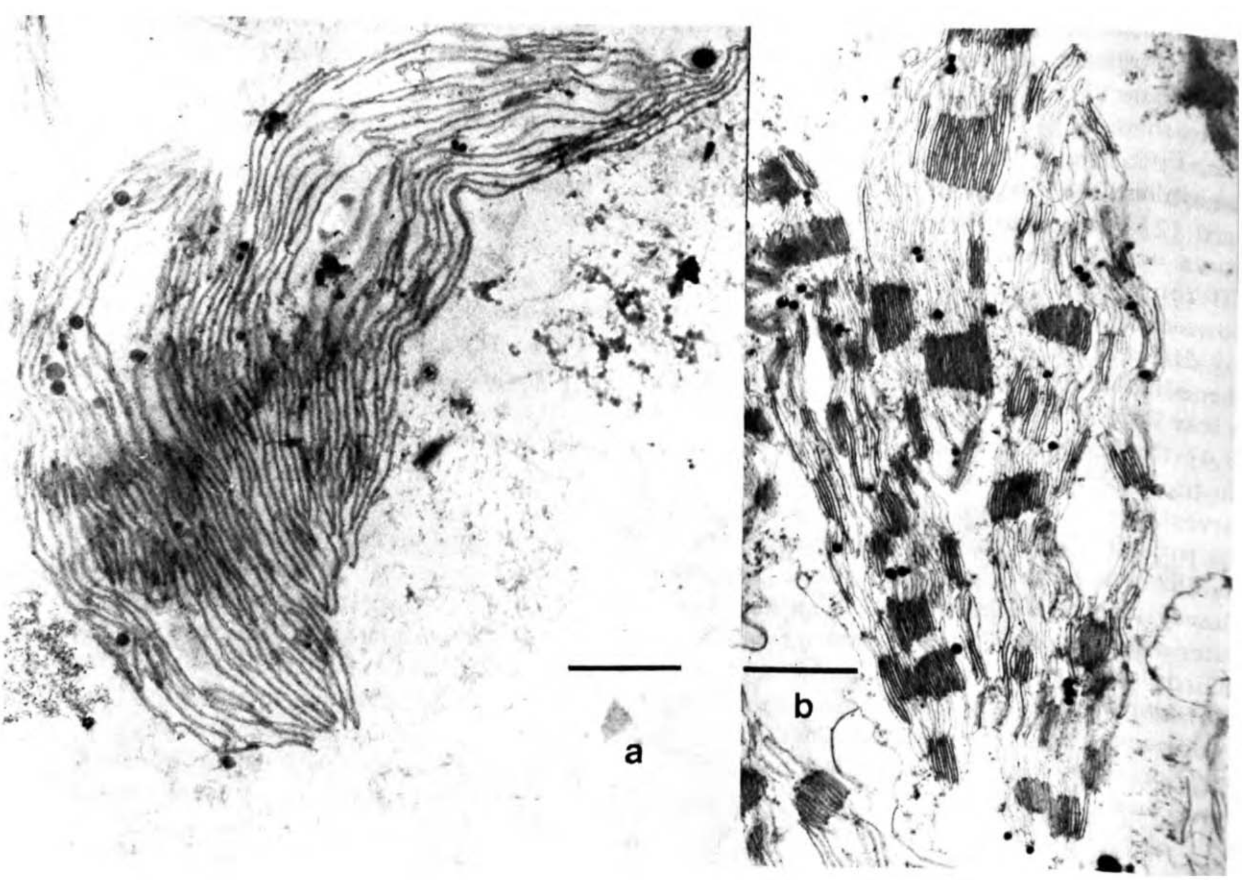

Fig.1. Electron micrographs of chloroplasts suspended at 20 μg $chl.ml^{-1}$ in: MES, 40 mM; sucrose, 0.1 M; Na^+, 20 mN. (a) pH 6.2; (b) pH 5.4. Scale bar: 1 μm.

Table 1
Partial electron transport reactions measured at pH 6.2 and pH 5.4 at different light intensities

Light intensity (ergs.cm^{-2}.s^{-1})	Methylviologen		Ferricyanide	
	pH 6.2	pH 5.4	pH 6.2	pH 5.4
43 000	–	–	43	46
18 500	42	35	35	34
5000	34	27	21	20
1250	17	10	–	–

Ferricyanide reduction was measured with water as the electron donor, methylviologen reduction was measured with reduced dichlorophenolindophenol as electron donor. For further details see section 2. Data are nequiv.μg^{-1}.chl.h^{-1}

of divalent cations at higher pH values. At pH 6.2, on the other hand, almost no membrane stacking is apparent.

In table 1 data are presented which indicate that lowering the pH from 6.2–5.4 causes a substantial decrease in the PS I-mediated reduction of methylviologen. This effect increased with decreasing light intensity and so probably reflects a decreased PS I photochemical efficiency. However, the reduction of ferricyanide, reduced exclusively by PS II at these pH values [18], was not influenced over a wide range of light intensities. Similar results were obtained with chloroplasts which, after extraction, were resuspended at either pH 6.2 or 5.4 and maintained at 0°C for 60 min before performing the experiment (data not shown). These results indicate that although grana formation induced by low pH may correlate with a decrease in PS I photochemical efficiency, no similar

Table 2
Effect of $MgCl_2$ on methylviologen reduction at pH 6.2 and 5.4

Additions	Methylviologen reduction	
	pH 6.2	pH 5.4
None	12.8	8.9
$MgCl_2$ 5 mM	8.6	8.3

Reduced dichlorophenolindophenol was the electron donor (see section 2). The light intensity was ~540 ergs.cm^{-2}.s^{-1}. Figures are nequiv .μg^{-1} chl .h^{-1}

relation exists between grana formation and PS II efficiency.

Since the addition of cations at higher pH values (pH 7–8) has been shown to induce grana formation [7] and to decrease PS I photochemical efficiency [19], we investigated the effect of magnesium at pH 5.4 and 6.2 on the PS I efficiency. The data presented in table 2 show that in the presence of Mg^{2+}, sufficient to induce grana information at pH 6.2, the effect of low pH on PS I efficiency is completely eliminated. The data are consistent with the interpretation that the reduced photochemical efficiency measured upon lowering the pH from 6.2–5.4 is caused in some manner by membrane stacking, though other interpretations are possible.

The data presented here indicate that membrane stacking per se is not sufficient to bring about an increase in the quantum efficiency of either photosystem; nor does it permit the regulation of energy distribution between the two photosystems, since PS II activity remains unaffected by pH-induced grana formation. However, we cannot exclude the possibility that low pH might itself prevent a possible stimulatory effect on PS II consequent on thylakoid stacking.

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