Hypothesis

A MECHANISM FOR CONTROLLING THE STACKING AND UNSTACKING OF CHLOROPLAST THYLAKOID MEMBRANES

J. BARBER and W. S. CHOW
Department of Botany, Imperial College, London SW7 2BB, England

Received 23 May 1979

In a paper published in this journal [1] it was suggested that the stacking of chloroplast thylakoids into grana is normally controlled by the positive space charge density within a few Ångstroms of the negatively charged membrane surface. This electrical parameter was chosen since it was necessary to explain the observations of Gross and Prasher [2]. These workers had shown that when unwashed thylakoid membranes were suspended in an almost cation-free medium (0.1 M sucrose + 0.2 mM Tris base) they maintained their granal stacking. Addition of low levels of monovalent cations (~3 mM) brought about unstacking of the grana but as the monovalent cation content of the medium was raised stacking occurred again. No such unstacking/stacking phenomenon was observed when divalent cations were added to the suspension medium although in the presence of low levels of monovalent cations, divalent cations were far more effective than monovalent cations at bringing about grana formation. It was because these workers found little or no selectivity between cations of the same valency group but distinct differences between cations carrying different charges which led Barber et al. [1] to pin-point the mechanism involved as being electrical. It was shown, by employing the Gouy-Chapman theory for mixed electrolyte conditions similar to those used in the experiment, that changes in the space charge density very close to the membrane surface (proportional to $d^2\psi_x/dx^2$; where ψ_x is the electrical potential at point x in the diffuse layer adjacent to the membrane surface) had the same characteristics as the stacking/unstacking phenomenon.

Previous to the work of Gross and Prasher, several others had also noted that thylakoid stacking and unstacking were dependent on the ionic conditions of the suspension medium [3–5]. However, none of these earlier workers had noted the effect of using 'cation-free' medium. At about the time that Gross and Prasher [2] reported their observations, Vandermeulen and Govindjee [6] also reported that light scattering changes carried out with thylakoids subjected to different ionic conditions showed the same general characteristics as the unstacking/stacking changes. More recently Chow et al. [7] have extended the work of Gross and Prasher by using a method

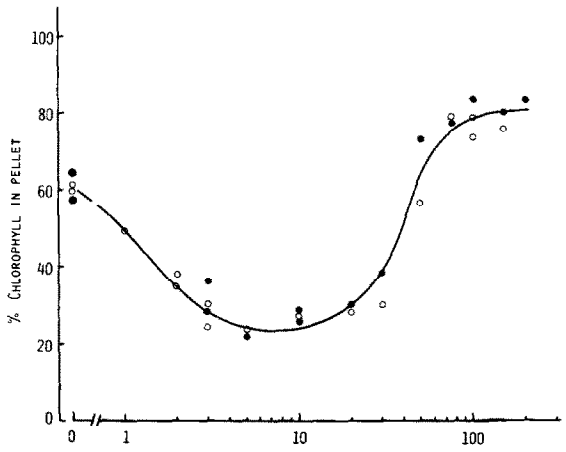

Fig.1. The degree of stacking of thylakoid membranes, as monitored by the effectiveness of digitonin action. Pea chloroplasts were suspended in 100 mM sorbitol and the cation concentrations indicated (0.1 mg chl./ml, pH 7.0). $MgCl_2$ (~10 μM) was also present. The suspensions were treated with digitonin (0.5%, w/v) for 30 min at 0°C, then diluted 7-fold with 100 mM sorbitol and centrifuged at 10 000 × *g* for 30 min at ~0°C. High and low percentages of chlorophyll appearing in the '10 K' pellets are taken to correspond respectively, to stacking and unstacking of thylakoids prior to digitonin incubation (cf. [17]). (•) NaCl; (○) lysine·HCl.

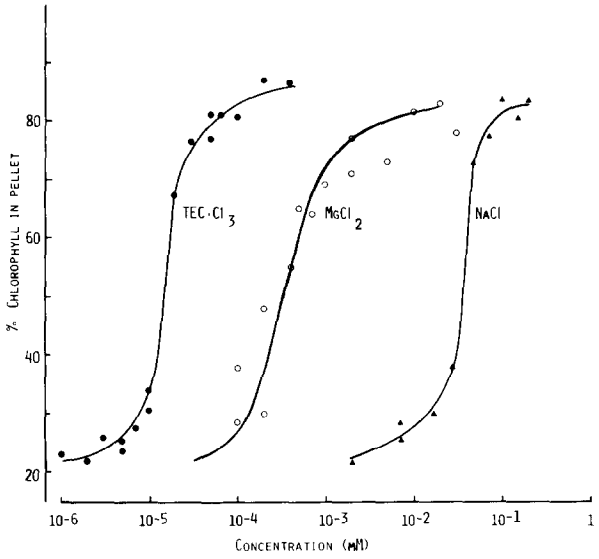

Fig.2. Effects of cations of various valencies on the degree of thylakoid stacking. Stacking/unstacking was monitored by the digitonin method as described in fig.1, except that the basic suspension and dilution medium contained 1.5 mM K_2HPO_4 (pH 7.0, HCl) in addition to sorbitol.

involving the use of digitonin treatment for determining the degree of stacking in a particular thylakoid suspension (see legend to fig.1). As fig.1 shows, their method clearly indicates that the thylakoids are stacked into grana when suspended in a 'cation-free' medium. Addition of low levels of monovalent cation (e.g., Na^+) causes unstacking to occur. After unstacking in this way, restacking can be induced by adding increasing levels of monovalent cation, but as fig.2 shows, this grana formation can be accomplished with much lower levels of divalents (e.g., Mg^{2+}). Even more effective at inducing restacking is the trivalent cation Tris(ethylenediamine)cobalt (III), (TEC^{3+}) (see [8]).

All these findings are consistent with the concept given [1] that changes in electrostatic screening (via changes in $d^2\psi/dx^2$) control grana formation with isolated chloroplast thylakoid membranes. In a very recent paper Duniec et al. [9] have given support to this idea but have also drawn to attention that changes in protonation of surface charges could also be important, especially in 'cation-free' media when substantial surface potentials (ψ_o) would exist. When ψ_o is large (and negative), protons are drawn into the diffuse layer such that the local pH at the membrane surface could be substantially different to the bulk. Such an effect could be important if the pK_a of the surface negative charges are high and bulk pH relatively low. However since a number of observations indicate that for thylakoids the surface negative charges have a pK_a of ~4.0 [10–13] the protonation effect is unlikely to be important when the bulk pH is in the region of 7.0–8.0 (see [14]).

The question arises as to how changes in the positive space charge density can control membrane stacking. The simplest explanation is already given in [1]. That is, the ability of two similarly charged surfaces to come together is controlled by the balance between two forces, attractive van der Waals forces and repulsive coulombic forces [15]. Although the van der Waals force remains constant, being dependent on the distance between the surfaces, the electrostatic repulsive force is variable depending on the degree of electrical screening (or of ionisation). Thus, when the positive space charge density is high near the surface, the repulsive coulombic force is reduced and in principle the membranes can come closer together (see fig.3). Such a notion is the basis of flocculation theory as developed by a number of people including Overbeek (see [15]). From these arguments it would seem that grana formation can be readily explained since thylakoid stacking occurs when the positive space charge density immediately adjacent to the surface is high while a lowering of this electrical parameter correlates with unstacking (see [1] for calculations).

Unfortunately this picture is too simple to explain grana formation as already mentioned in a recent publication [16]. For example, if the arguments presented above were correct then stacking would be able to occur over the whole membrane surface. In fact, in practice, stacking occurs only in patches giving rise to granal and stromal lamellae. Moreover, thylakoid stacking occurs less readily with membranes isolated from developing systems or from certain chlorophyll *b*-deficient mutants [17–20]. Thus there is a serious need to give another explanation for thylakoid stacking which still involves control by changes in electrostatic screening. Again we can turn to the established theories of aggregation of charged colloids [21] but bearing in mind that biological membranes are neither homogeneous nor rigid. In this case let us consider that the charged components of the membrane surface are associated with intrinsic protein complexes. This is

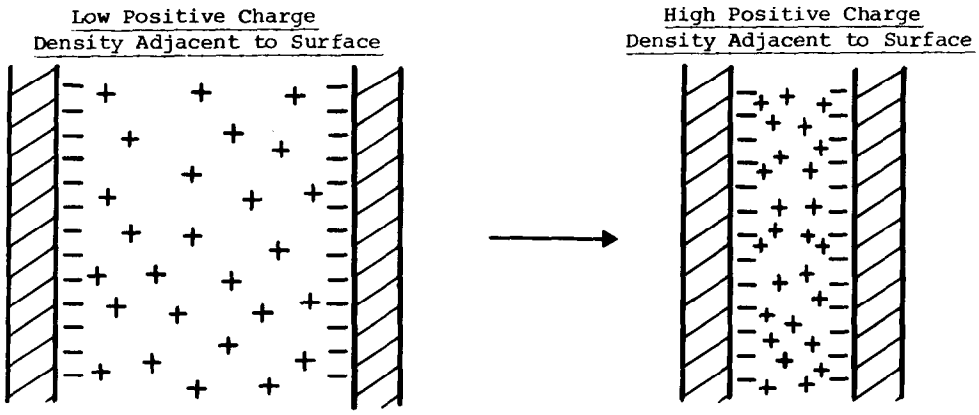

Fig.3. The stacking of negatively-charged membranes. An increase in the positive space charge density adjacent to the surfaces leads to a reduction in coulombic repulsion, resulting in net attraction due to van der Waals forces.

reasonable since it seems that on the thylakoid membrane the major contributors to the surface negative charge are the carboxyl groups of glutamic and aspartic acid residues [22]. Surface positive charges have been attributed to the guanidine group of arginine [23] and there is no clear evidence to suggest that phospholipids play any significant role in the surface charge density on the thylakoids [23]. If the lipid phase of the membrane is sufficiently fluid then changing the electrical screening of the surface charges may cause lateral reorganisation of the membrane protein complexes as indicated in fig.4 (see also [16]). Thus when the local positive space charge density is high the charged particles may aggregate so as to produce patches of high and low charge density on the membrane surface. In this way membrane stacking would be encouraged at the low-charge region due to strong van der Waals forces acting across the partition gap. Therefore, according to this model, unstacking could only occur if the charged protein complexes diffuse from the non-stacked into the stacked regions and thus peel the two surfaces apart by coulombic repulsion. Such diffusion would be expected to occur when electrostatic shielding is poor (low positive space charge density near surface). The idea of lateral diffusion of charged protein complexes controlling thylakoid membrane stacking is represented diagramatically in fig.5, although in this figure no attempt has

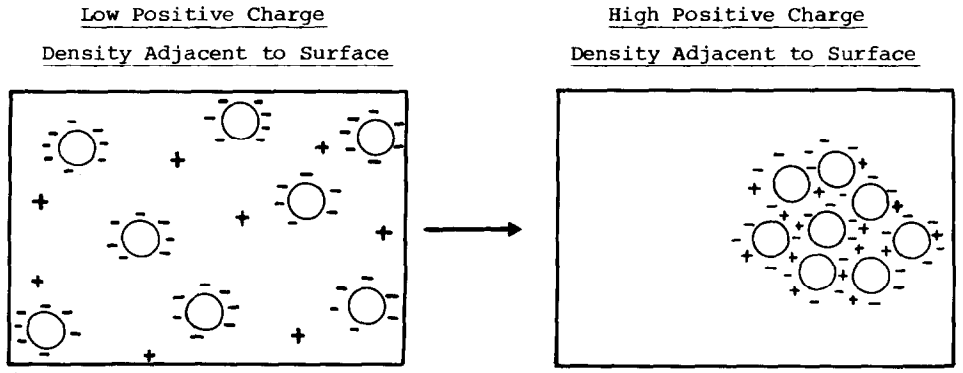

Fig.4. The lateral aggregation of negatively-charged protein complexes in a membrane, induced by cation screening. Only one type of complex is depicted. When more than one type of complex is present, aggregation would be more pronounced for the complexes which carry less charge.

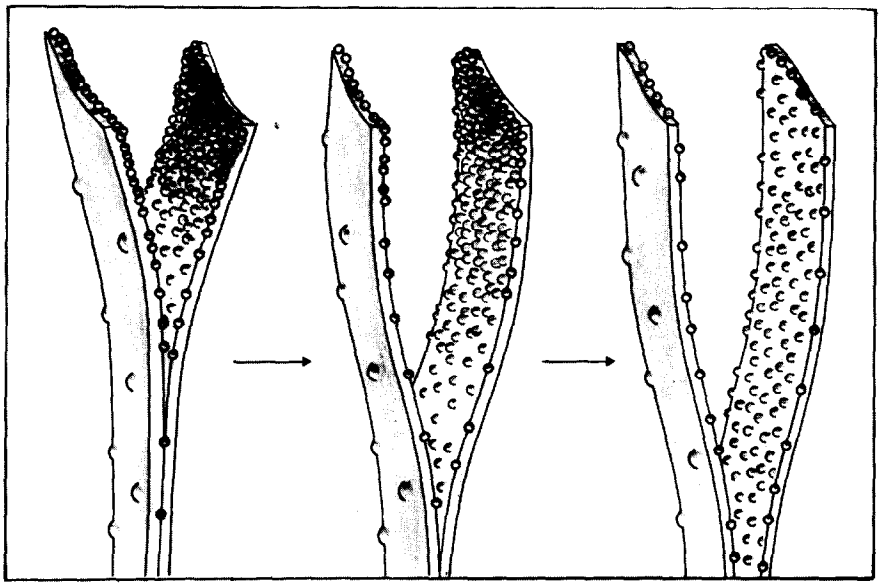

Fig.5. Unstacking induced by migration of charged protein complexes into the stacked region when screening is decreased. No attempt is made to show the various types of protein complexes seen in chloroplast membranes, or the low-charge complexes postulated to be in the stacked region.

been made to identify the various types of intrinsic protein complexes seen in chloroplast membranes (see below).

What is the evidence for such a mechanism and what is the identity of the charged proteins involved? Barber [16] has already speculated that the charged protein could be a photosystem one pigment complex observed in freeze-fracture pictures as a particle of ~8 to 11 nm diam. [24]. This idea stems from the observation that the electrophoretic mobility of chloroplasts isolated from the chlorophyll *b*-less mutant of barley is the same as the wild type [22] and also from the fact that the stromal lamellae are enriched in photosystem one and predominantly contain the 8 to 11 nm freeze-fracture particles [24]. Although membrane stacking will occur to some extent in the absence of chlorophyll *b*, it has been consistently shown that grana formation is readily induced when the chlorophyll *a*/chlorophyll *b* pigment–protein complex is present [19,20,25]. Moreover this pigment–protein complex has been identified with a larger freeze-fracture particle having a diameter in the region of 15 to 18 nm. Thus in developing chloroplasts there is a correlation between grana formation and the presence of chlorophyll *b* and these larger particles [17,26].

Although it can be argued that electrostatic screening may allow some stacking to occur in membranes devoid of the chl *a*/chl *b* protein, this complex apparently confers significant modification to the membrane surface such as to increase its adhesive nature and allow extensive granal formation. Presumably, this complex confers a stronger van der Waals attraction without introducing too much surface charge.

Freeze-fracture studies also support the general concept that stacking and unstacking of thylakoids suspended in different salt media involves lateral movements of these small and large particles [5], although it has been argued that there is some discrepancy in terms of the kinetics [27] and the cation concentration requirement [28] for the two structural changes. What is seen is that when membranes are stacked the larger particles are concentrated in the appressed regions while the smaller particles are predominantly in the stromal lamellae. When unstacking is induced by suspending the membranes in a low salt containing medium both types of particles become randomly distributed [5,24,27,28]. Staehelin [27] has shown that this type of particle migration is fully reversible in membranes of higher plant chloroplasts.

Overall the picture that emerges for thylakoid stack-

ing to occur involves an interplay between heterogeneity of charge distribution on the membrane surface and van der Waals forces. Electrostatic screening of the charged protein complexes gives rise to migration of these particles so as to allow net attraction between adjacent membranes at less polar regions and membrane repulsion at the more electrically charged regions. With poor electrostatic screening the distribution of charged complexes in the membrane becomes homogeneous (random) because coulombic repulsion is at a maximum. As a consequence, stacking is no longer possible. According to this model the surface charge density on stromal lamellae should be higher than on unstacked membranes and significantly higher than on the stacked thylakoid membranes. Moreover, the model predicts that the unstacking of granal lamellae is not possible unless the stromal lamellae are attached. This view of the stacking mechanism of thylakoids is in contrast to previous models which require some form of divalent cation (Mg^{2+}) to bridge charges on adjacent stacked membranes (see [29]). (In any case this idea was inconsistent with the ability of salts of other valency, e.g., monovalent, to induce stacking.) The model given in this paper also predicts that the appressed regions would be enriched in photosystem two while the unstacked region is enriched in photosystem one, assuming that the latter complex is more electrically charged than the former. Evidence for this type of distribution of photosynthetic activity does exist (see [20,30]). In the case of the grana, it would be anticipated that the exposed non-appressed regions are charged and contain in addition the photosystem one complexes. Thus in the simplified sketch in fig.5 the electrically charged particles shown can be identified as photosystem one complexes.

The purpose of writing this paper is to emphasise that the organisation of charged intrinsic protein complexes in a fluid lipid membrane can be controlled by electrostatic forces. Variations in these forces can allow reshuffling of the protein complexes and consequently of surface charges so that shorter-range interactions between adjacent membranes can be brought about. Such a view may have implications not only in thylakoid stacking but also in other systems where adjacent membrane interaction and fusion occur, e.g., cell adhesion, gap junctions, pinocytosis (see [31–33]). In these cases localized ion pumps could bring about the changes in electrostatic screening to allow the required lateral charge diffusion to occur.

Acknowledgements

We wish to thank the SRC and EEC (Solar Energy Programme) for financial support. Also we thank Herb Nakatani for valuable discussions and for drawing the sketch for fig.5.

References

[1] Barber, J., Mills, J. and Love, A. (1977) FEBS Lett. 74, 174–181.
[2] Gross, E. L. and Prasher, S. H. (1974) Arch. Biochem. Biophys. 164, 460–468.
[3] Izawa, S. and Good, N. E. (1966) Plant Physiol. 41, 533–543.
[4] Murakami, S. and Packer, L. (1971) Arch. Biochem. Biophys. 146, 337–347.
[5] Ojakian, G. K. and Satir, P. (1974) Proc. Natl. Acad. Sci. USA 21, 2052–2056.
[6] Vandermeulen, D. L. and Govindjee (1974) Biochim. Biophys. Acta 368, 61–70.
[7] Chow, W. S., Thorne, S. W., Duniec, J. T., Sculley, M. J. and Boardman, N. K. (1979) in preparation.
[8] Barber, J. and Searle, G. F. W. (1978) FEBS Lett. 92, 5–8.
[9] Duniec, J. T., Sculley, M. J. and Thorne, S. W. (1979) Biophys. J. in press.
[10] Mercer, F. V., Hodge, A. J., Hope, A. B. and McLean, J. D. (1955) Aust. J. Biol. Sci. 8, 1–18.
[11] Mills, J. and Barber, J. (1975) Arch. Biochem. Biophys. 170, 306–314.
[12] Searle, G. F. W., Barber, J. and Mills, J. D. (1977) Biochim. Biophys. Acta 461, 413–425.
[13] Berg, S., Dodge, S., Krogmann, D. W. and Dilley, R. A. (1974) Plant Physiol. 53, 619–627.
[14] Barber, J. and Searle, G. F. W. (1979) FEBS Lett. 103, 241–245.
[15] Overbeek, J. Th. G. (1977) J. Colloid Interface Sci. 58, 408–422.
[16] Barber, J. (1979) in: Chlorophyll Organisation and Energy Transfer in Photosynthesis. CIBA Found. Symp. new ser. 61, pp. 283–304, Elsevier/Excerpta Medica, Amsterdam, London, New York.
[17] Argyroudi-Akoyunoglou, J. H. and Tsakiris, S. (1977) Arch. Biochem. Biophys. 184, 307–315.
[18] Goodchild, D. J., Highkin, H. R. and Boardman, N. K. (1966) Exp. Cell Res. 43, 684–688.
[19] Armond, P. A., Arntzen, C. J., Briantais, J. M. and Vernotte, C. (1976) Arch. Biochem. Biophys. 175, 54–63.
[20] Boardman, N. K., Anderson, J. M. and Goodchild, D. J. (1978) Curr. Top. Bioenerg. 8, 35–109.

[21] Verwey, E. J. W. and Overbeek, J. Th. G. (1948) Theory of the Stability of Lyophobic Colloids, Elsevier, Amsterdam.
[22] Nakatani, H. Y., Barber, J. and Forrester, J. A. (1978) Biochim. Biophys. Acta 504, 215–225.
[23] Nakatani, H. Y. and Barber, J. (1979) unpublished.
[24] Arntzen, C. J. (1978) Curr. Top. Bioenerg. 8, 111–160.
[25] Remy, R., Phung Nhu Hung, S. and Moyse, A. (1972) Physiol. Veg. 10, 269–290.
[26] Arntzen, C. J., Armond, P. A., Briantais, J.-M., Burke, J. J. and Novitzky, W. P. (1977) Brookhaven Symp. Biol. 28, 316–337.
[27] Staehelin, L. A. (1976) J. Cell.Biol. 71, 136–158.
[28] Staehelin, L. A. and Arntzen, C. J. (1979) in: Chlorophyll Organisation and Energy Transfer in Photosynthesis. CIBA Found. Symp. new ser. 61, pp. 147–175, Elsevier/Excerpta Medica, Amsterdam, London, New York.
[29] Anderson, J. M. (1975) Biochim. Biophys. Acta 416, 191–235.
[30] Arntzen, C. J. and Briantais, J.-M. (1975) in: Bioenergetics of Photosynthesis (Govindjee, ed) pp. 51–113, Academic Press, New York.
[31] Ahkong, Q. F., Fisher, D., Tampion, W. and Lucy, J. A. (1975) Nature 253, 194–195.
[32] Poste, G. and Allison, A. C. (1973) Biochim. Biophys. Acta 300, 421–465.
[33] Schuler, G., Plattner, H., Aberer, W. and Winkler, H. (1978) Biochim. Biophys. Acta 513, 244–254.