Hypothesis

AN ION-EXCHANGE MODEL FOR THYLAKOID STACKING IN CHLOROPLASTS

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1. Introduction

Chloroplasts of higher plants contain stacks of internal membranes (thylakoids). These stacks (grana) can be reversibly unstacked in vitro by changing the salt composition in the chloroplast suspension [1]. Attempts have been made to explain the mechanism of stacking in terms of the classical theory of colloidal particle interactions which involve the interplay of electrostatic and van der Waals' forces [2]. In a [3] a quantitative analysis of these forces led to the conclusion that an additional short range force [4] was required to explain stacking. It was also estimated that for the stacking to occur the net surface charge density on the grana membranes should be <1 electronic charge (1 e)/3000 Å^2.

Such a density is low when compared with the density expected from the full dissociation of surface acidic groups of proteins and lipids [3]. The required low net surface charge density in the stacked regions of the membranes can arise from one of two possible mechanisms [3]: (a) cation binding [5–8]; or (b) redistribution of charges between grana (stacked) and stroma (unstacked) portions of the thylakoid membrane [9–11].

Here, we present a model involving binding of cations which explains the mechanism of stacking. The model is inspired by recent studies on the nature of short range repulsive forces between lipid bilayers [4,12,13] and mica surfaces [13–16].

2. Balance of forces

For phospholipid or lecithin bilayers in water an additional repulsive force arises at distances below ~30 Å [4,12]. This so called 'hydration' force dominates the interactions between the bilayers at the short distances. The origin of this additional force between mica surfaces has been studied in detail [15,16] measuring forces in a range of electrolyte solutions. It was found that hydration forces arise only when adsorbed metal ions remain bound to the mica surfaces as they are brought to distances below some tens of Ångstroms. No hydration repulsion was observed when hydrogen ions displaced the hydrated metal ions from the surface (i.e., in acid solutions or at low pH). It is essential to note that the results are applicable to a number of very different surfaces, indicating the general nature of these effects [16]. Two important conclusions which bear on the interpretation of the thylakoid stacking stem from these studies.

(1) The electrostatic repulsive force, as calculated from the theory fits the experimental results more closely when a surface charge regulation model is used where there is competitive adsorption between metal and hydrogen ions for sites on the mica surface. Under typical solution conditions the net apparent surface charge density is only a small fraction of the negative site density of mica.

(2) Since no hydration force arises between protonated surfaces [15,16] thus the surfaces can come into 'primary minimum' (true molecular) contact only when the hydrated metal ions desorb from the surface by exchange with hydrogen ions.

With these results established we return to the point of issue: The van der Waals' force between thylakoid membranes can overcome the barrier of the electrostatic repulsion only when their net surface charge density is lower than 1 electronic charge/3000 Å^2 [3]. Since the value of 1 electronic charge/3000 Å^2 is lower than expected from the density of acidic groups on the thylakoid surface [3], we must consider the possibility of a lowering of the surface charge by either a redistribution of highly charged groups away from the grana regions, or by charge neutralization due to adsorption of cations.

As calculated in [11] the redistribution of charges does not lead to the expected lowering of the calculated electrostatic repulsion. On the other hand, the upper bound on the van der Waals' force in [3] is correct for the conditions considered. Thus we must admit binding of cations to the membrane surface. We propose therefore that the cation exchange properties of the thylakoid membrane are at least partially responsible for the phenomenon of grana stacking. Similar properties of surface ion exchange may also be responsible for the active light-induced H^+ uptake in chloroplast thylakoid membranes.

3. Cation exchange model

The lowering of pH from 7.7–4.7 of chloroplasts incubated in 100 mM NaCl (i.e., with stabilized grana structures) resulted in a decrease in spacing between membranes in grana stacks [17]. The 'flattening' of grana causes increase in their refractive index. This manifests itself in an increase in 90° light scattering of chloroplast suspension. In fig.1 we plot the 90° light scattering intensity of chloroplasts containing 5 mM $MgCl_2$ (i.e., chloroplasts which at physiological pH contain grana stacks) as a function of pH of the suspension. At pH > 5.0, the variations of monovalent salt concentrations in the chloroplast suspension where only trace amounts of divalent cations are present, bring about reversible unstacking and stacking of the thylakoid membranes [1]. The changes in stacking are also reflected in the 90° light scattering (fig.2).

The above data lead us to the conclusion that between the membranes stacked by salts there exists a short range repulsive force blocking their close contact and keeping the membranes apart at the distance >40 Å [18]. By protonation of the thylakoid membrane surface at low pH it is possible to weaken this force so that the membranes can come closer. The

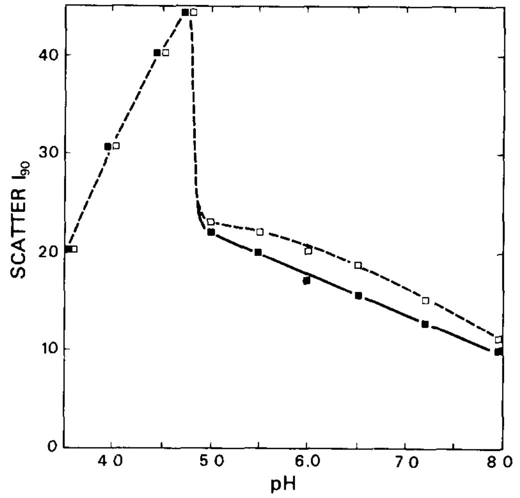

Fig.1. I_{90}, 90° light scattering of broken spinach chloroplasts at 520 nm, excitation intensity 20 μW/cm² as a function of buffer pH. Chloroplast concentration was 1.5 μg chl/ml in 100 mM sorbitol, 20 mM NaCl, 5 mM $MgCl_2$ · 2 mM EDTA with 50 mM Hepes for pH 6.5–8.0 or 50 mM succinate for pH 3.5–6.5 with a direct comparison measurement at pH 6.5. Chloroplasts were prepared as in [20] stored at 0°C, then diluted ⩾100-fold into the above buffers immediately before measurement at 20°C. 90° light scattering signals were measured in a corrected spectra fluorescence spectrometer as in [20]: (■–■) measurements in the absence of actinic light; (□– – –□) measurements in the presence of actinic red light (Filter Corning 2-64) at 20 mW/cm² incident with the photomultiplier protected by blue filter (Corning 4-96) with excitation and fluorescence monochromators set at the common wavelength 520 nm and bandwidth ±1.0 nm. Average of 3 independent measurements at each point, with corrections for cell and buffer blanks.

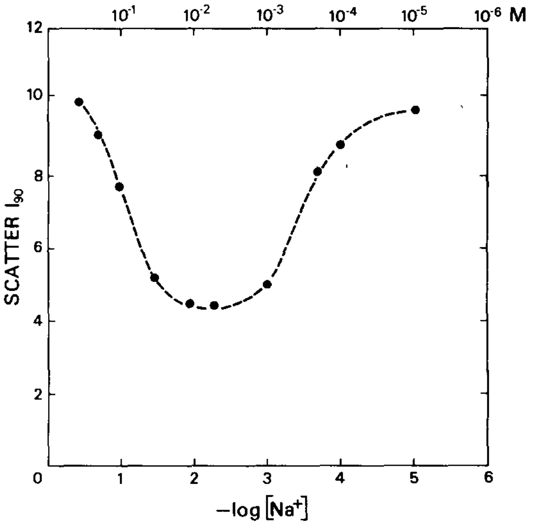

Fig.2. Relative 90° light scattering of spinach chloroplasts at 520 nm with excitation intensity of 20 μW/cm² as a function of added [NaCl] ($-\log[Na^+]$ at pH 6.5). Buffer 300 mM sorbitol 1 mM MES with pH adjusted to 6.5 with the organic ion $(CH_3)_4$–NOH. Measurements as for fig.1 at 1.5 μg chl/ml at 20°C. Average of 3 independent measurements at each point.

analogy between chloroplast thylakoid membrane stacking and other well established surface phenomena is now apparent and this prompts us to propose the following model:

1. There are binding sites for cations and hydrogen ions at the thylakoid surface. These are associated with the negative charge carrying surface groups of proteins and/or lipids.
2. The dissociation constants at the binding sites are more specific to the valence of cation than to the species within the same valence. A dependence of stacking on the species within a given valence has been observed [19] but the difference between the valences is more important.
3. Over pH 5–8 the binding sites may be either empty or occupied by metal ions. At sufficiently high concentrations of cations near the membrane surface, their adsorption results in an effective reduction of the net surface charge. The electrostatic repulsion between the membranes becomes weak enough for the van der Waals' force to bring the membranes together into a 'secondary minimum'.
4. In this secondary minimum the van der Waals' force between adjacent membranes is balanced by the hydration repulsion, and not by an electrostatic repulsion. The hydration forces may result from the interaction of hydration shells of adsorbed (exchangeable) ions on protein surfaces, as is the case for mica surfaces [15,16], or from the hydration shells of polar lipids as has been found with pure lipid bilayers [4].
5. Lowering of the pH in the chloroplast suspension causes the metal ions to be replaced by hydrogen ions. This results in a decrease of the hydration repulsion, and closer apposition of membranes. This manifests itself in an increase in the light scattering of the suspension (fig.1). On further decrease in the pH the membrane surface takes on positive charge and the electrostatic repulsion between the membranes rises, which, in turn, causes unstacking of the thylakoids and a decrease in the light scattering (fig.1).

4. Conclusion

The phenomenon of metal–hydrogen ion exchange at various surfaces and its recent correlation with short range hydration forces [15,16] provides insight into the mechanisms which underlie the stacking of chloroplast thylakoids. While the redistribution of membrane proteins between the stacked and unstacked portions of the thylakoid membrane may contribute to stacking [11], it seems probable that this is only a secondary role compared with binding of cations to specific sites on the membrane surface. The adsorption of hydrated cations brings about an effective decrease in the surface charge density. The electrostatic repulsion between thylakoid membranes is thus reduced and the van der Waals' force brings the membranes into a potential minimum where the van der Waals' attraction is balanced by the hydration repulsion arising from interactions between hydrated metal ions adsorbed to the opposing membrane surfaces.

Decrease in pH of the chloroplast suspension results in an exchange of bound cations for hydrogen ions at the binding sites. The repulsive hydration force becomes smaller and the van der Waals' forces may cause the membranes to come into tight contact at the protonated sites. We remark that for dark equilibrated chloroplasts under exposure to light a similar metal ion–hydrogen ion exchange may take place such that the light induced decrease in the spacing between the membranes in grana stacks [17] may be due to a decrease in the hydration repulsion.

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