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SALT-INDUCED MICROSCOPIC CHANGES IN CHLOROPHYLL FLUORESCENCE DISTRIBUTION IN THE THYLAKOID MEMBRANE

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Summary

Addition of 3 mM $MgCl_2$ to isolated pea thylakoids suspended in a medium of low osmotic strength at room temperature induces an increase in chlorophyll fluorescence similar to that observed with unswollen thylakoids. Fluorescence microscopy indicates that the $MgCl_2$ induced increase in the emission intensity involves the formation of highly fluorescent patches on the swollen vesicles. The data seems to give additional support to the concept that salt induced chlorophyll fluorescence changes involves the lateral movement of pigment-proteins within the thylakoid membrane in such a way as to form discrete domains.

Based on a number of different types of observations [1—9] a model for explaining salt-induced chlorophyll fluorescence and thylakoid stacking changes has recently been presented [10]. It has been argued that the effect of changing the ionic conditions of the suspending medium is to bring about reorganisation of pigment-protein complexes by lateral diffusion within the lipid matrix of the membrane. The conformational changes seem to be controlled by variations in the balance of coulombic repulsive and van der Waals attractive forces operating between adjacent membranes and between the surfaces of exposed segments of integral proteins within the same membrane

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Abbreviations: DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethyl urea; PS I, Photosystem I; PS II, Photosystem II.

[6,11,12]. Essentially the idea is that when coulombic repulsion is at a maximum, then various types of integral protein complexes, including the pigment-proteins, are randomly distributed in the plane of the membrane and no membrane stacking occurs. However, manipulation of the salt level in the suspending medium, so as to minimise the double-layer repulsive forces, allows formation of discrete pigment-protein domains. It is suggested that it is the production of separate domains for Photosystem I (PS I) and Photosystem II (PS II) protein complexes which gives rise to the increase in chlorophyll emission due to a decrease in excitation transfer from the PS II to the PS I. It is also argued that the creation of electrical charge heterogeneity due to domain formation is responsible for the generation of granal and stromal lamellae [10].

In this communication we present results which may be indicative of this type of domain formation using the technique of fluorescence microscopy. The work was done using a microscope equipped with a sensitive image-intensified video camera, details of which have been given elsewhere [13]. This approach allowed excitation intensities to be used which did not cause significant photobleaching of chlorophyll during the time of observation. Experiments were conducted with isolated pea thylakoid membranes treated with 5 μM DCMU, to avoid fluorescence changes due to variations in the redox state of PS II, and suspended in a solution of 1 mM NaCl at about pH 7. This medium was used to initially establish the minimum fluorescence condition and to produce swollen membranes having salt dependent fluorescence properties which could be studied independently of large changes in the degree of stacking.

In Fig. 1 it can be seen that under the low osmotic conditions employed the addition of 3 mM $MgCl_2$ caused a significant increase in the yield of chlorophyll fluorescence monitored at 685 nm. This change has slower kinetics than similar changes induced at normal osmotic strength (e.g. 300 mosmoles). Inspection of the membranes in this dilute salt containing medium by phase microscopy, showed them to consist of 'blebs' budding off from a central mass of membranes as shown in Fig. 2A. The membranes which constitute these blebs contain chlorophyll as observed by fluorescence microscopy (see Fig. 2B). In some cases the blebs became detached from the parent membrane system and existed as isolated vesicles. Although the formation of these blebs and isolated vesicles occurred in response to the low osmotic strength of the medium their appearance was slow, taking at least 30 min at room temperature and considerably longer times when kept on ice. It could be that their growth requires the donation of lipid and protein from the parent membranes which would account not only for their slow formation but for the fact that they maintain ionic permeability properties similar to those of normal thylakoids even though there has been a dramatic increase in surface area [14]. Alternatively they may arise from a complicated unfolding of the parent membrane system. When 3 mM $MgCl_2$ was added to the sample it was found that the blebs still persisted but there seemed to be an increase in the number of highly fluorescent patches on their surfaces (see Fig. 2D). To demonstrate this phenomenon more convincingly, studies were conducted on vesicles which had become detached from their 'parent' membrane system. This was advantageous because it avoided the interference of the brightly fluorescing region resulting

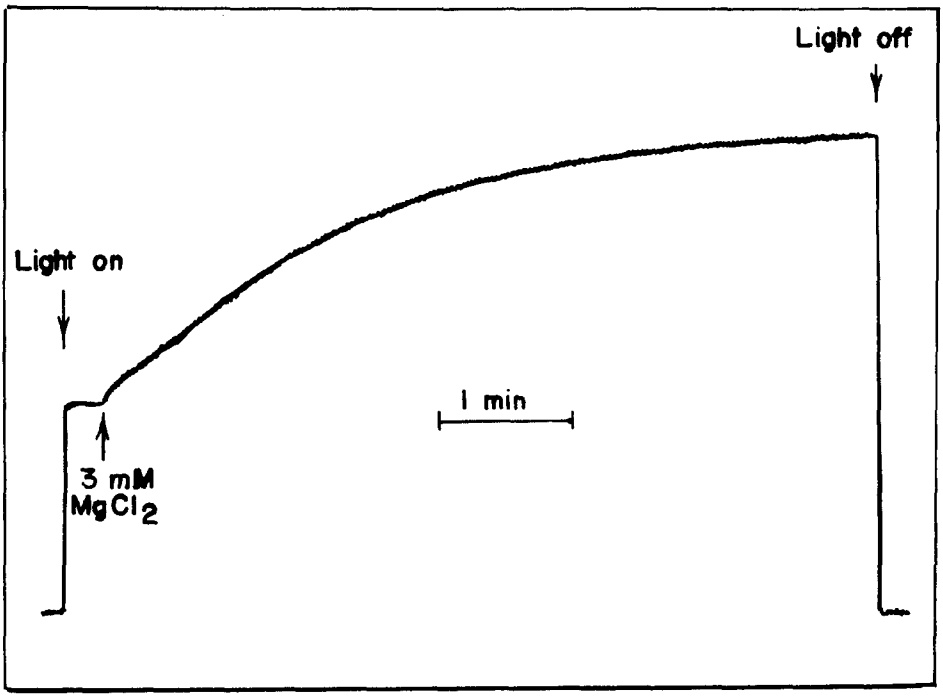

Fig. 1. Increase in chlorophyll fluorescence yield from thylakoid membranes, isolated from peas by a method given in Ref. 13 induced on addition of 3 mM $MgCl_2$. The membranes were suspended for one hour at room temperature in 1 mM NaCl plus 5 μM DCMU before carrying out the addition of $MgCl_2$. The excitation light was 460 nm (slit width 20 nm) and the emission was measured at 685 nm (slit width 2 nm). The experiment was conducted in a 1-cm cuvette using a Perkin-Elmer MPF44A spectrofluorimeter.

from the high chlorophyll concentration associated with the folded membranes which also appeared to give indications of patching depending on the plane of view (see Fig. 2B).

In the absence of Mg^{2+} it was common to find isolated vesicles having an even distribution of chlorophyll fluorescence as shown in Fig. 3 A and B. The uniformity of the fluorescence could be checked by focusing at different planes through the vesicle which also established whether it was still associated with the highly fluorescing folded membrane system from which it was derived. After adding a few mmoles of Mg^{2+} it was not possible to find the type of vesicles observed before its addition, but instead vesicles showing patches of strong fluorescence as shown in Fig. 3 C and D. These patches of intense fluorescence were shown to be widely distributed on the membrane surface by focusing at different planes through the vesicle.

These observations seem to give support to the ideas briefly given above, but presented in detail elsewhere [10], that the addition of $MgCl_2$, and other salts, bring about segregation of different types of chlorophyll protein complexes into discrete domains. Whether membrane folding occurs at the sites of increased fluorescence could not be clearly resolved but may be expected based

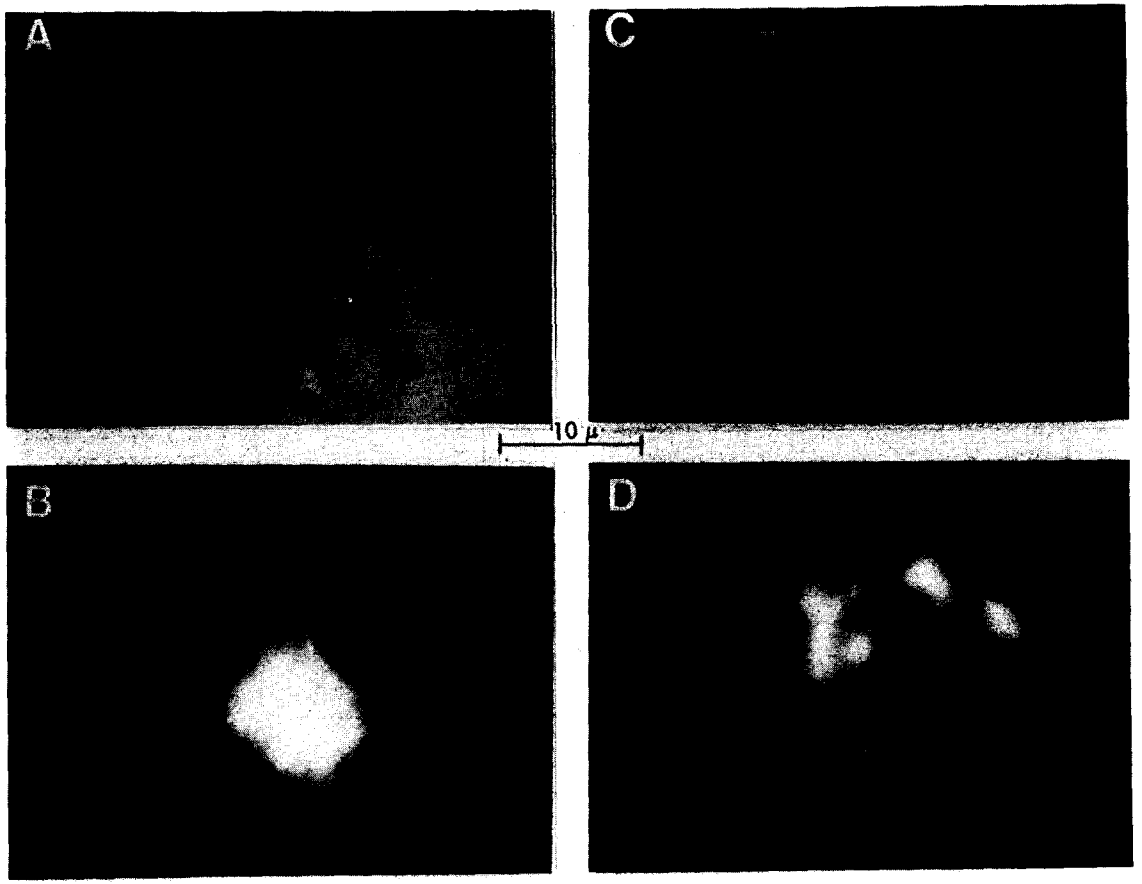

Fig. 2. (A) Phase and (B) fluorescence micrographs of the same field showing multiple blebbing from a 'parent' membrane complex. This sample was suspended in 1 mM NaCl plus 5 μM DCMU. (C) Phase and (D) fluorescence micrographs of the same field showing blebs after addition of 3 mM $MgCl_2$. In contrast to (B), plate (D) shows intense patches of chlorophyll fluorescence. The salt addition was made by mixing an equal volume of chloroplast suspension and 6 mM $MgCl_2$ and the microscope slide, and allowing to stand for 10 min before making observations. The chlorophyll fluorescence was excited by blue light and the red emission passed through a cut-off filter which did not transmit the excitation light. The pictures were taken using a polaroid camera from a television screen that projected the intensified images.

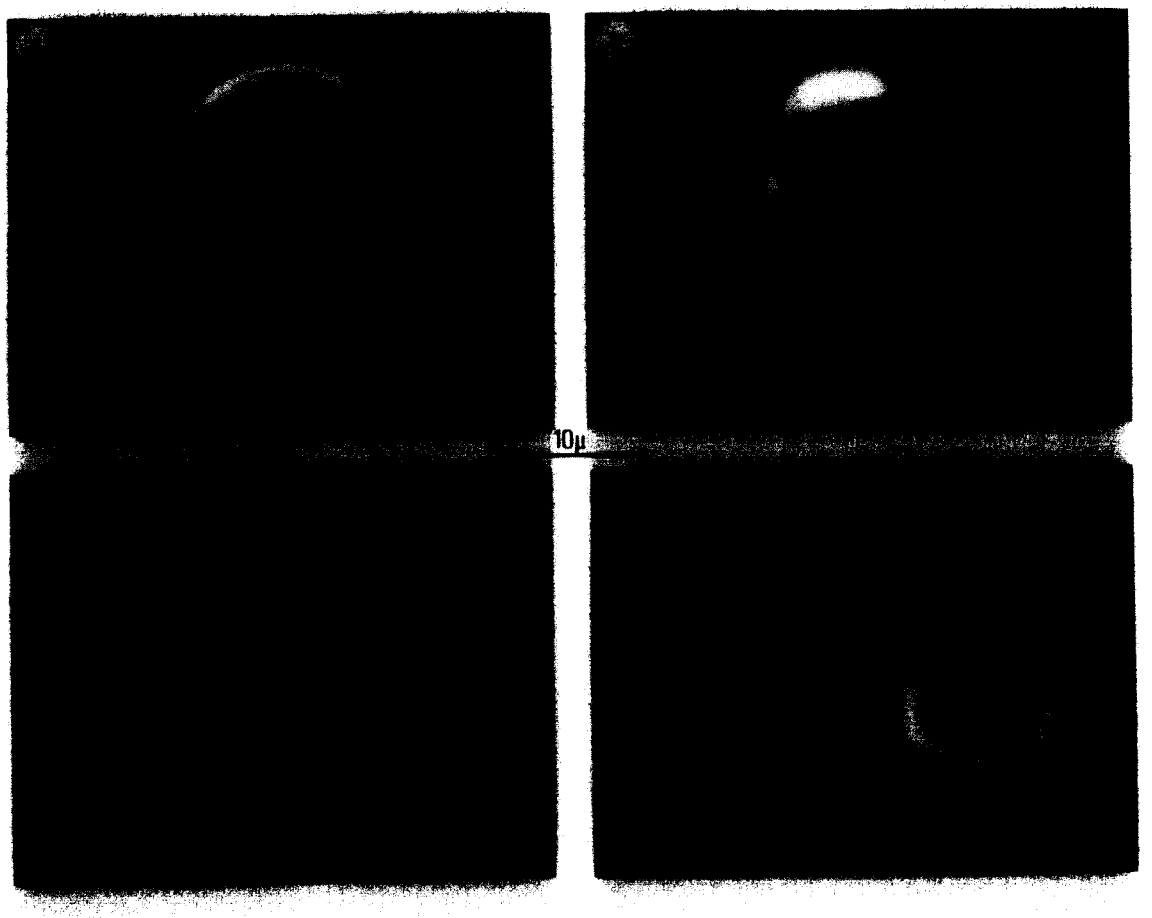

Fig. 3. **Chlorophyll fluorescence from isolated vesicles.** (A) and (B) **before addition of** $MgCl_2$ **showing even distribution** of fluorescence. (C) and (D) chlorophyll fluorescence after 3 mM $MgCl_2$ addition. Other conditions as for Fig. 2.

on the close correlation between salt induced chlorophyll fluorescence and membrane stacking changes observed with thylakoids suspended in media of high osmotic strength [10]. An indication of this effect was seen by comparing phase contrast and fluorescence images on the same sample (see for example Fig. 2C and D). It was noted that where ever a fluorescent patch occurred there was a corresponding dark region in the phase contrast image indicative of an increase of material.

The concept of clustering of integral proteins is not novel to the photosynthetic membrane and is well established for many animal systems where the process is controlled by such events as the binding of antibodies, lectins and hormones [15,16]. Although our observation could be due to other effects specifically associated with the use of a swollen membrane suspension, the possibility that lateral protein diffusion occurs to create the highly fluorescent patches would emphasise the non-rigidity of the thylakoid membrane of higher plants and focus attention on the importance of lipid fluidity in normal photosynthetic functioning, a point already emphasised from freeze-fracture microscopy [7—9].

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