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# PARTICLE AGGREGATION IN PHOTOSYNTHETIC MEMBRANES OF THE BLUE-GREEN ALGA *ANACYSTIS NIDULANS*

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## Summary

Freeze-fracture electron microscopy demonstrates that in photosynthetic membranes of the blue-green alga *Anacystis nidulans* quenched from a temperature below growth temperature, areas devoid of membrane particles occur. We suggest that this phenomenon is related to phase transitions in the photosynthetic membrane.

In the blue-green alga *Anacystis nidulans*, grown at 38°C, spin-label ESR experiments demonstrated that photosynthetic membranes undergo a lipid phase transition at 24°C [1]. The transition temperature coincides with the temperature at which a maximum is found in the curve for the fluorescence yield versus temperature [2]. Similar results are obtained in the study of delayed fluorescence of chlorophyll *a* in blue-green algae [3]. Here we report the consequences of such transitions on the freeze-fracture morphology of the photosynthetic membranes of *A. nidulans*. In general, a modification of the freeze-fracture morphology, caused by previous cooling, has been reported for many bacterial membranes [4—10], nuclear and alveolar membranes [11,12] and mitochondrial membranes (although at very low temperatures) [13,14]. The alteration is characteristic in that intramembrane particles progressively aggregate upon lowering the quenching temperature over a transition temperature range and subsequent visualization by freeze-fracture electron microscopy. This study indicates such aggregation in the photosynthetic system of *A. nidulans* when cells are quenched from below the phase transition temperature.

*A. nidulans* was grown as described previously [15]. After two days

of culturing, the cells were harvested and equilibrated for half an hour at about 5°C and 30°C respectively. Thereafter they were fixed for 30 min in a 0.08 M phosphate buffer solution, pH 7.4, containing 3% glutaric aldehyde, 1% acrolein, 2% formaldehyde and 2.5% dimethyl sulfoxide (trialdehyde solution). Subsequently they were frozen in liquid propane at −180°C. The frozen samples were fractured in a Denton apparatus, Pt/C replicated and cleaned first in concentrated sulphuric acid and then for 17 h at 60°C in a hypochlorite solution. The replicas were washed in distilled water and transferred on to copper grids and studied and photographed in a Philips EM 301 electron microscope. More than 50 fracture faces of photosynthetic membranes were investigated. In samples quenched from 30°C (Fig. 1A) intramembrane particles are always randomly distributed. In contrast, extended smooth areas, devoid of particles are always seen in membrane samples quenched from 5°C (Fig. 1B). In the fracture faces studied, the diameter of the smooth areas varied between 100 and 150 nm. Similar phenomena have been observed for the cytoplasmic and the outer membrane of this Gram-negative bacterium as found for *Escherichia coli* [6,7,8,16].

Photosynthetic membranes can easily be distinguished by the occurrence of particles that are substantially larger than those found in inner and outer membranes of *A. nidulans*.

Exoplasmic and protoplasmic faces are inducated by EF and PF respectively (Branton et al. [17]). The aggregation phenomenon is especially prominent in PF because of the higher density of particles in this face. We did consider counting the particles so as to acquire a measure of particle density. However the results scattered widely, probably because of the curvature of the surfaces and the fact that sometimes it is very difficult to observe separate particles. The smooth area phenomenon we want to focus attention on here is very characteristic because these areas are always roughly circular and sharply limited in an area of high particle density. These areas cannot be confused with regions of incidentally lower particle density. Several control experiments were carried out to check possible side effects of glycerol, as it is known that glycerol can induce smooth areas [18]. The best results were obtained when the cells were fixed in a buffered trialdehyde solution for half an hour at the desired temperature and thereafter immersed in 25% glycerol.

The roughly circular areas, which can be seen amidst the particles are proposed to represent islets of solidified lipids [4,5]. In earlier studies the conclusion was reached that a gradual solidification of the membrane lipids causes the aggregation of the intramembrane particles that are partially or completely composed of proteins [19].

It has been shown that the phenomenon is not confined to microorganisms, but that mitochondrial membranes are also liable to such structural modification. The phase transition in mitochondrial membranes was detected at a temperature considerably lower than the culturing temperature [14]. It was therefore argued that the functions of these organelles at physiological temperatures depend on the fluidity of the inner and outer membranes. Whether photosynthesis in chloroplasts, which resemble mito-

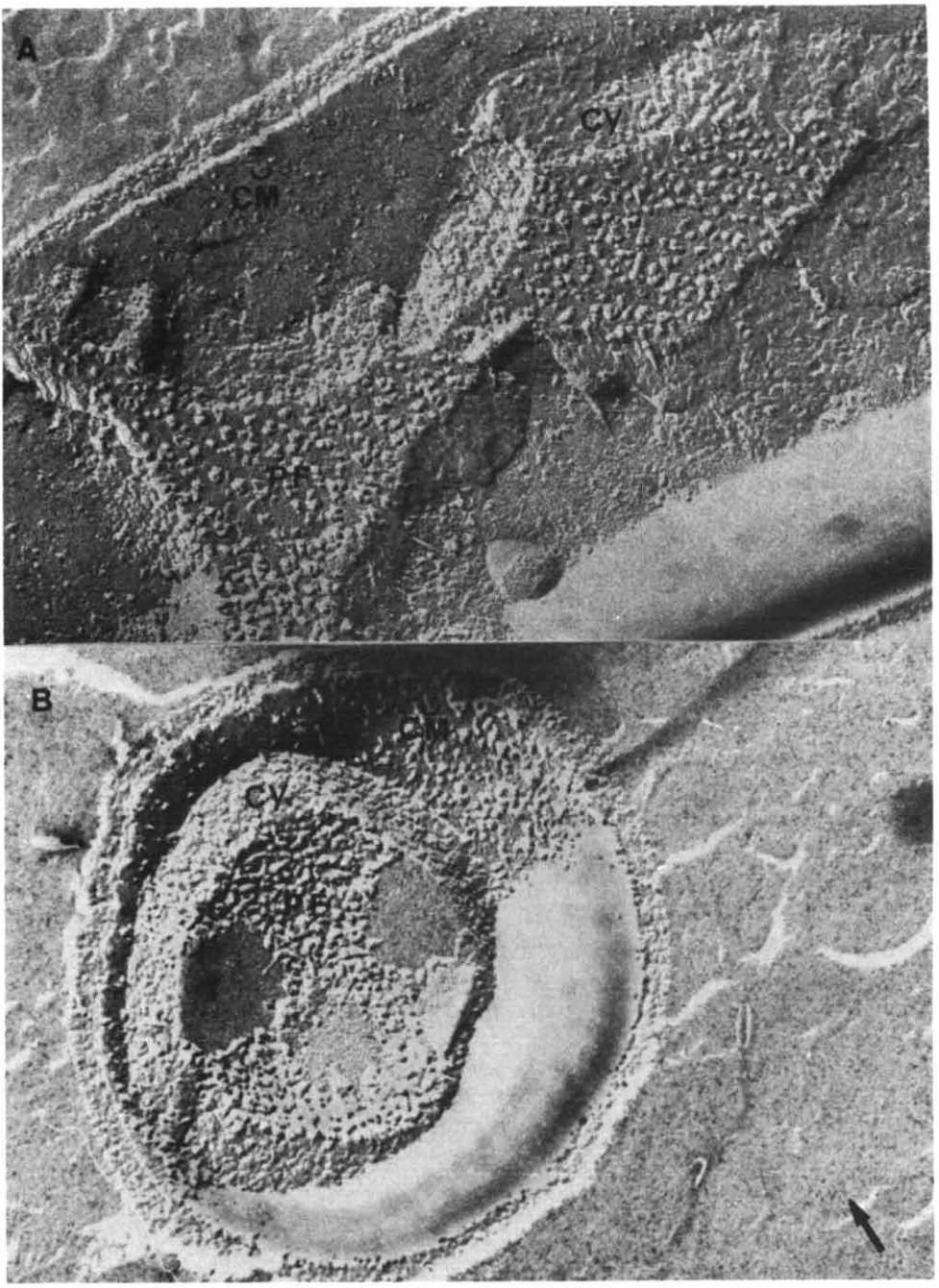


**Fig.1. (A) Fracture faces of membranes of *Anacystis nidulans* quenched from 30° C. On the protoplasmic face of photosynthetic membranes intramembrane particles are randomly distributed, CM: concave exoplasmic fracture face of cytoplasmic membrane. Cy: fracture through the cytoplasma. (B) Fracture faces of membranes of *Anacystis nidulans* quenched from 5° C. On the PF areas of presumably aggregated particles are separated from regions devoid of particles. Magnification about 100 000 ×. Arrow indicates Pt/C shadowing direction.**

chondria in their specialization on redox processes, is dependent on membrane fluidity is not yet established. Fluidity in the intact photosynthesis apparatus has been demonstrated in a freeze-etch study of stacked and unstacked chloroplasts of *Chlamydomonas reinhardii* [20]. However, the cooling of these cells to 4°C failed to produce aggregation of particles, which is found in photosynthetic membranes of *A. nidulans.* According to Hirayama [21] there is a high degree of saturation of the major fatty acids in *A. nidulans.* This can explain that the membrane alteration of this organism occurs at temperatures close to the growing temperature. In that respect the behaviour of the photosynthetic membranes of *A. nidulans* parallels the modulation in the outer and inner membranes. The aggregation of intramembrane particles occurs at the temperature at which a fluorescence emission maximum is found [2] and at which also a maximum is found in the initial rise level and a change in the steady state level of delayed fluorescence [3]. This supports the conclusion by Arntzen et al. [22] that there is a relationship between the particles and the activity of Photosystem II of photosynthesis.

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