Biochimica et Biophysica Acta, 555 (1979) 352—357

BBA Report

BBA 71397

PHASE SEPARATIONS IN MEMBRANES OF *ANACYSTIS NIDULANS* GROWN AT DIFFERENT TEMPERATURES

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(Received February 16th, 1979)

Key words: Blue-green alga; Photosynthesis; Phase separation; Membrane lipid; (Electron microscopy)

Summary

Freeze fracture electron microscopy studies were performed on samples of *Anacystis nidulans* quenched from different temperatures. Membrane lipid phase separations were observed to take place over the ranges 15—30°C, 5—25°C and —5—15°C for cultures grown at 38, 28 and 18°C, respectively. Differential scanning calorimetry heating curves showed endotherms which coincided with these temperature ranges. Variations of phase separation temperatures with growth temperature, and hysteresis effects in the calorimetric measurements, were related to changes in the fatty acid composition of membrane lipids.

A considerable literature exists relating reports of the existence of discontinuities in Arrhenius plots of photosynthetic processes of higher plants and algae with those of parameters thought to reflect physical changes in their photosynthetic membranes [1]. It is often suggested that these changes involve a transition of the membrane lipids between gel and liquid-crystalline states. The fact that X-ray diffraction measurements show that the phase transitions of the major lipid classes present in such membranes normally take place well below 0°C [2], suggests, however, that this may not always be a satisfactory explanation.

The only systems for which there appears to be reliable evidence for a correlation between functional parameters and thermotropic phase changes of membrane lipids are the blue-green algae *Anacystis nidulans* [3—5],

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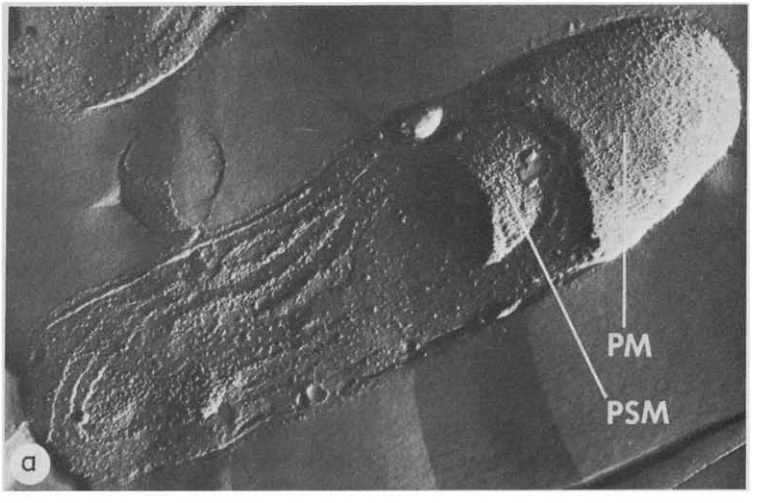
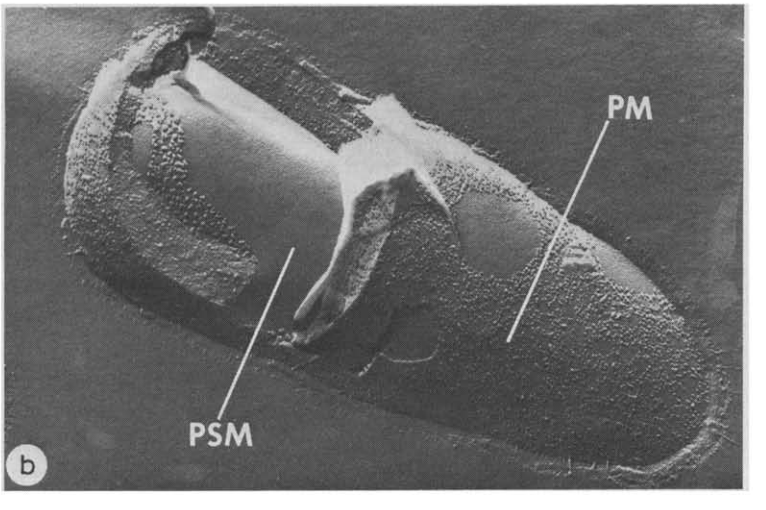
Anabaena variabilis [6], and the thermophilic alga *Cyanidium caldarium* [7]. These algae are characterised by membrane lipids containing a high proportion of fully saturated or monoenoic fatty acids. As such their lipid phase transitions, unlike those of most higher plants and algae, are likely to lie within the physiological range. Murata and Fork and their co-workers, working mainly with *Anacystis*, have demonstrated a correlation between the temperatures of Arrhenius plot discontinuities for several photosynthetic processes including O_2 evolution, state I — state II transitions, *P*-700 reduction and Hill activity [3, 5] and the temperature dependencies of physical parameters such as spin label mobility [3], chlorophyll *a* fluorescence [3, 4], delayed light [6] and electrochromic shifts [7].

Unfortunately, most of these physical parameters yield indirect rather than direct evidence of lipid phase transitions. More direct evidence for the existence of such transitions has recently been provided by Quinn and Williams [1] and Verwer et al. [8] using freeze-fracture electron microscopy. This technique allows a direct visualisation of the segregation of smooth regions of gel-phase lipids within fracture faces containing membrane-associated particles [9]. In this report, we extend these earlier observations by comparing the temperatures at which such phase separations are induced with phase transition data obtained by differential scanning calorimetry.

Anacystis nidulans was cultured in Kratz and Myers medium C [10] aerated with 5% CO_2. The cells were grown as batch cultures at 18, 28 and 38° C under an incident light intensity of about 6000 lux. Freeze fracture studies were carried out on cells pre-equilibrated to different temperatures before quenching. The cells were first cooled from the growth temperature to the desired equilibration temperature at a rate of about $0.2° C \cdot min^{-1}$ and then held at this temperature for 15 min. Glycerol was then added, to a final concentration of 25% (v/v) and the sample held for a further 15 min at the equilibration temperature before quenching in a slurry of liquid and solid N_2 to —210° C. The samples were fractured using a Polaron E7500 module and the replicas examined in a Philips EM 301G electron microscope. Heating and cooling thermograms of the cells suspended in the same media, using pre-soaked Sephadex G-25 as a reference, were obtained using a Perkin-Elmer DSC-2 differential scanning calorimeter. The fatty acid composition of the membrane lipids was estimated by gas-liquid chromatography [11].

Typical freeze-fracture electron micrographs of *Anacystis* cells grown at 38° C quenched from temperatures above and below that required to produce lipid phase segregation are shown in Figs. 1a and 1b. Exposed areas of the convex protoplasmic faces of the plasma and photosynthetic membranes are visible in both preparations. Similar replicas showing exposed exoplasmic faces can be obtained but the cleavage process tends to expose smaller areas of the concave faces of the photosynthetic membranes. Cells quenched from temperatures above the phase transitions temperature show, as previously reported [1, 8], a random distribution of membrane-associated particles whilst those quenched from temperatures below this temperature show large particle-free areas. These areas are thought to correspond to domains of gel-phase lipids that have crystallised and segregated from the lower melting point lipids: the membrane-associated particles, believed to represent the


PM
PSM
a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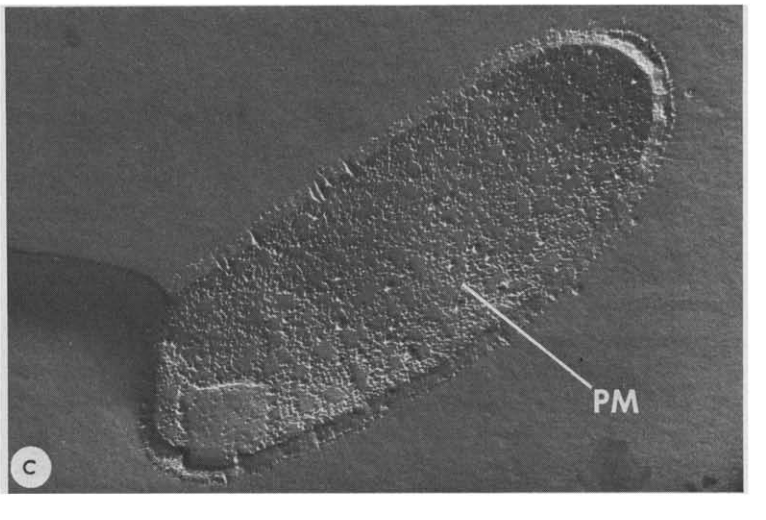
PM
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TABLE I

FATTY ACID COMPOSITION OF MEMBRANE LIPIDS OF *ANACYSTIS* CULTURED AT DIFFERENT TEMPERATURES

Fatty acids	Fatty acid composition (mol %)		
Growth temperature (°C)	18	28	38
16:1	38	46	53
16:1	45	43	36
18:1	1.6	0.8	1.6
18:1	15.4	10.2	8.9
Unsaturated	1.5	1.14	0.82
Saturated			

hydrophobic domains of interpolated membrane proteins, being excluded during the crystallisation process. An intermediate stage in the separation process (shown in Fig. 1c) in which the distribution, whilst clearly non-random, lacks the large particle-free areas typical of the fully phase-separated state, can also be identified. This intermediate stage has also been observed in freeze-fracture studies of *Escherichi Coli* [9] and is often referred to as a netted distribution.

The three types of distribution random, netted and phase separated, are most easily identified in the plasma membrane of *Anacystis*. All our observations lead us to conclude, however, that the phase separation temperature of the plasma membrane coincides with that of the photosynthetic membrane. Taking the lower limit of the phase separation temperature range to be the highest temperature at which most of the membranes show particle-free areas and the upper limit to be the lowest temperature at which the particle distribution still appears to be random, we estimated the ranges for 38, 28 and 18°C grown algae to be 15—30°C, 5—25°C and —5—15°C, respectively. Approximately 30—40 cells were examined on each electron microscope grid and on average four grids were examined for each sample. Whilst there was considerable overlap between the different types of distribution, particularly between the netted and random distributions, one type tended to predominate at each quench temperature. No attempt was made, however, to make a statistical analysis of the frequency of the different distributions.

Fatty acid analyses of the membrane lipids (Table I) show, in agreement with earlier studies [12], that the proportion of unsaturated residues tends to increase as the growth temperature is decreased. This, as pointed out by Murata et al. [3], would be expected to lead to increases in membrane fluidity and a lowering of lipid phase transition temperatures.

Thermograms obtained for cells grown at 18, 28 and 38°C are presented in Fig. 2. Each sample was subjected to two cooling and heating cycles between 35°C and —15°C at a rate of change of temperature of $5°C \cdot min^{-1}$. The two cycles were essentially identical and only the second cy-

Fig. 1. Freeze-fracture electron micrographs of *Anacystis* cells, cultured at 38°C, pre-equilibrated at different temperatures before quenching, showing freeze-fracture particle distributions in the plasma membrane (PM) and photosynthetic membrane (PSM). (a) Quenched from 35°C, shows the random distribution of freeze-fracture particles typical of samples quenched from above the phase separation temperature, (b) quenched from 15°C, shows the large particle-free areas typical of samples quenched from below the phase separation temperature and (c) quenched from 25°C, shows the intermediate partially phase separated or netted distribution. Magnification about 36 000×.

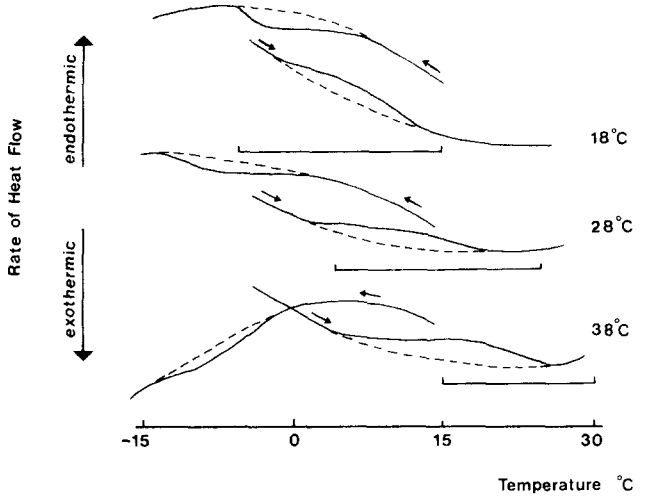

Fig. 2. Differential scanning calorimetry endotherms for *Anacystis* cultured at 18, 28 and 38°C obtained on heating pre-cooled cells at a rate of $5°C \cdot min^{-1}$ and the corresponding exotherms obtained on re-cooling the samples at the same rate. The dashed lines show the estimated position of the baseline and the bars indicate the temperature ranges for phase separation estimated from freeze-fracture studies.

cyle is shown in the figure. The curves are characterised by marked hysteresis effects. Similar but less pronounced effects have also been noted in chlorophyll *a* fluorescence studies [4]. The origin of this hysteresis can probably best be explained by considering the processes involved in lipid phase segregation. As the cells are cooled from temperatures at which the membrane components are randomly distributed, the higher melting point lipids begin to crystallise. These first form small domains of gel-phase lipid of the type seen in the netted distribution which subsequently coalesce to yield the large particle-free areas of the fully phase-separated state. The formation of these initial domains, which is probably the main event reflected in the cooling exotherms, requires a lateral diffusion of the higher melting point lipids to the developing domain. At the same time the low melting point lipids and the proteins are excluded from these regions. If the rate of cooling is higher than the rate of these diffusion processes, the exotherms are displaced to lower temperatures as seen in Fig. 2. The endotherms of the heating cycle, in contrast, correspond to the melting of pre-formed lipid pools; a process which is not subject to these limitations. This is reflected in the good agreement between the temperature ranges of the endotherms and those obtained for phase segregation under equilibrium conditions in the freeze-fracture preparations. It is noteworthy that the extent of hysteresis increases with growth temperature suggesting that the lateral diffusion processes are slower in the membranes of cells grown at higher temperatures.

Murata and Fork [3] and Ono and Murata [5] have reported discontinuities in the temperature dependence of photosynthetic parameters in *Anacystis* cultured at 38 and 28°C at temperatures of about 24 and 13°C, respectively. In both cases these temperatures lie towards the upper end of the phase transition/separation range where re-arrangements in membrane

organisation associated with the onset of phase segregation might be expected. Our observations thus add further support to the idea that such discontinuities, in *Anacystis* at least, are directly related to changes associated with membrane transitions.

The assistance of Professor D. Chapman and M. Turner with the differential scanning calorimetry measurements is gratefully acknowledged.

References

1 Quinn, P.J. and Williams, W.P. (1978) Prog. Biophys. Mol. Biol. 34, 109—173
2 Shipley, G.G., Green, J.P. and Nichols, B.W. (1973) Biochim. Biophys. Acta 311, 531—544
3 Murata, N., Troughton, J. and Fork, D.C. (1975) Plant Physiol. 56, 508—518
4 Murata, N. and Fork, D.C. (1975) Plant Physiol. 56, 791—796
5 Ono, T. and Murata, N. (1979) Biochim. Biophys. Acta 545, 69—76
6 Fork, D.C. and Murata, N. (1977) in Photosynthetic Organelles (Miyachi S., Katch, S., Fujita, Y. and Shibata, K., eds.), Special Issue of Plant and Cell Physiol., No. 3, pp. 427—436
7 Ono, T. and Murata, N. (1977) Biochim. Biophys. Acta 460, 220—229
8 Verwer, W., Ververgaert, P.H.J.T., Leuinissen-Bijvelt, J. and Verkleij, A.J. (1978) Biochim. Biophys. Acta 504, 231—234
9 Verkleij, A.J., Ververgaert, P.H.J., Van Deenen, L.L.M. and Elbers, P.F. (1972) Biochim. Biophys. Acta 288, 326—332
10 Kratz, W.A. and Myers, J. (1955) Am. J. Bot. 42, 282—287
11 Restall, C., Williams, W.P., Percival, M., Quinn, P.J. and Chapman, D. (1979) Biochim. Biophys. Acta 555, 119—130
12 Holton, R.W., Blecker, H.H. and Onore, M. (1964) Phytochemistry 3, 595—602