Modulation of membrane fluidity by catalytic hydrogenation affects the chilling susceptibility of the blue-green alga, *Anacystis nidulans*

L. Vigh and F. Joó*

Institute of Biochemistry, Biological Research Center, Hungarian Academy of Sciences, PO Box 521, 6701 Szeged and **Institute of Physical Chemistry, Kossuth Lajos University, PO Box 7, 4010 Debrecen, Hungary*

Received 11 August 1983

All the changes, i.e. the phase separation temperature of thylakoid lipids, shift in the chilling induced increase of K^+ permeability and decline in photosynthetic O_2-production, respectively, brought about by temperature acclimation in *Anacystis nidulans*, can be accomplished by homogeneous catalytic hydrogenation of the fatty acids, as well, using a new water-soluble Pd(II) complex, hitherto unknown in biological applications. Since the thermo-adaptation replaced by proper hydrogenation conducted under isothermal condition results in a similar modification of chilling susceptibility, it affords direct evidence that chilling response is mediated by changing the degree of fatty acid unsaturation in *Anacystis nidulans*.

Membrane fluidity *Chilling susceptibility* *Catalytic hydrogenation* Anacystis nidulans

1. INTRODUCTION

Changes of fatty acid unsaturation with growth temperature is well documented for many organisms [1–3]. Generally, such changes lead to an increase in the amount of more unsaturated fatty acids on lowering the growth temperature. Several attempts were made to relate these alterations to the growth temperature-dependent shift of the thermotropic phase transition temperature of membrane lipids and to the accompanying characteristic changes in various membrane-bound processes.

One of the systems for which reliable evidence has been collated for the correlation between the thermotropic phase change of membrane lipids and the functional parameters, is that of the blue-green alga, *Anacystis nidulans*.

Although the blue-green algae are prokaryotic organisms, they are characterized by their photoautotrophy, moreover they can be regarded as model systems in the study on the temperature effect on plants [4].

Authors in [5,6] have demonstrated that it is the phase-separation state of the cytoplasmic membrane lipids which induces chilling susceptibility of this thermophilic alga. While the growth temperature did not affect significantly the relative composition of various lipid classes [7], the changes in the composition of fatty acids and temperatures for the phase separation of the lipids of both the thylakoid and cytoplasmic membranes were shifted in parallel with the growth temperature. The effect of growth temperature on the phase behaviour of the membrane lipids could be explained mainly by the variation of the degree of fatty acid unsaturation [6]. The direct relevance of fatty acid unsaturation to chilling susceptibility in *A. nidulans* strongly suggests that any manipulation under isothermal conditions leading to changes in the fatty acid composition should also result in changes in the chilling response.

We have recently reported on the modulation of the fatty acid composition of intact thylakoids isolated from wheat leaves by homogeneous phosphine catalysts [8]. Here we describe the utilization of this method to modify the fatty acid composition of living algal cells, and its effect on the chilling susceptibility of *A. nidulans*. Here another water-soluble catalyst, $Pd(QS)_2$ (QS,

Published by Elsevier Science Publishers B.V.
00145793/83/$3.00

sulphonated alizarine; $C_{14}H_6O_7NaS$) was introduced, being superior to the previously used phosphine complexes as regards catalytic activity and stability.

2. MATERIALS AND METHODS

2.1. *Chemicals*

The catalyst, $Pd(QS)_2$ was prepared as in [9]. All the other chemicals were of commercial laboratory use.

2.2. *Alga culture*

A. nidulans was cultured in Kratz and Myers medium C, bubbling with air enriched with 1% CO_2. The cells were grown at 28°C and 38°C, respectively, under an incident light intensity of about 5000 lux. The cultures were collected by centrifugation at 3000 × *g* for 5 min late in the logarithmic phase.

2.3. *Hydrogenation procedure*

The algal cells grown at 28°C and harvested by centrifugation were suspended in fresh culture medium and the cell density was adjusted to 10 mg chlorophyll/l. Aliquots (20 ml) of this suspension were placed into high-pressure glass reaction vessels and transferred to a thermostated (28°C) water bath. The reactors, connected to a vacuum-gas manifold, were evacuated and subsequently filled with hydrogen at a pressure of 5 atmospheres. The appropriate amount of the catalyst solution (prepared with degassed water) was injected to the algal suspension through a silicone rubber septum.

The hydrogen pressure was then increased, maximally to 7 atmospheres, and the reaction tubes were rotated at 50 rev./min, for the desired reaction time. At the end of the incubation the pressure was slowly released in about 5 min. To remove the catalyst the samples were washed 3-times with culture medium. The Pd(II) content of the cell suspension was determined by atomic absorption spectroscopy. Extraction and analysis of fatty acids were performed as in [10].

2.4. *Temperature dependence of Chl-a fluorescence*

Chl-a fluorescence in *Anacystis* cells was measured at various temperatures using a Hitachi MPF-2A spectrophotometer fitted with a temperature regulation unit. By changing the sample temperature continuously, the temperature–fluorescence yield curve could be recorded automatically. Fluorescence was excited at 430 nm and measured at 684 nm. Rates of temperature decrease and increase were 1°C/min. In order to eliminate the influence of the photochemical reactions of photosynthesis on fluorescence yield, 10 μM DCMU was added to the medium. The density of cells was adjusted to 2 mg Chl/l.

2.5. *Chilling treatment, measurement of K^+ release and photosynthetic oxygen evolution*

To investigate the release of K^+ the algal cells were washed 3-times by suspension/centrifugation with 40 mM Na-phosphate buffer, pH 7.0. For the chilling treatment the thermo-adapted or hydrogenated algal cell suspensions were kept at appropriate temperatures betwen 0°C and 25°C, in the dark. They were then warmed to the growth or hydrogenation temperature and left for 10 min. The suspensions were centrifuged at 5000 × *g* for 10 min and the amount of K^+ in the supernatant was determined by atomic absorption spectrometry.

The photosynthetic oxygen evolution was measured as in [4], following the changes in the O_2-concentration in the medium at 28°C with a calibrated Clark-type O_2-electrode.

The cell density was 100 mg Chl/l and 10 mg Chl/l, respectively, for the measurements of K^+-release and of photosynthetic O_2-evolution.

3. RESULTS AND DISCUSSION

Within the membranes of algal cells only monoenoic unsaturated fatty acids are found. Previous studies on the pattern of hydrogenation of liposomes and thylakoid membranes by water-soluble rhodium and ruthenium phosphine complexes have shown [8], that, unfortunately, these catalysts are markedly effective only in the saturation of polyenoic alkyl chains, being much less active on monoenoic ones. By comparing a number of different water-soluble homogeneous hydrogenation catalysts, however, we found a Pd(II) complex, hitherto unknown in biological applications, which seems to be suitable in providing a

sufficient rate of saturation of the single *cis* double bonds.

The effects of varying catalyst concentration, hydrogen pressure and reaction time on the rate of hydrogenation were studied under isothermal (28°C) conditions. For most of our purposes the optimal parameters were: c_{cat}, 0.1 mM, p_{H_2}, 5 atmospheres, t = 90 min, at a cell density of 10 mg Chl/l. Under these conditions the *Anacystis* cells totally preserved their integrity and biological activity. By 3 stepwise washings with the medium the catalyst could be substantially removed from the cell suspension: we observed by AAS $\leqslant 10\,\mu$g Pd/mg Chl, which means $\geqslant$ 99% removal.

In agreement with earlier studies we observed that the proportion of saturated fatty acid residues in the total lipid composition of *A. nidulans* increased with the increase of the growth temperature from 28°C to 38°C (table 1). By hydrogenation under the mentioned optimal conditions of the cells grown at 28°C, an almost identical shift could be achieved as expressed by the saturated/unsaturated acids ratio. It is remarkable that the algal cells in control experiments, treated by the catalyst under nitrogen, did not differ in any respect from the untreated population.

It is noted, that the chain length of the fatty acids was slightly influenced by shifting the growth temperature, similar to those reported in [11]. As was to be expected, there was no alteration in this respect during hydrogenation.

Although the lipid analyses were performed for whole cells, the detected alterations in the level of unsaturation predominantly reflect the changes in the fatty acid composition of the thylakoid membranes, since the cytoplasmic membranes occupy only a very small (about 10%) part of the cell membrane system [12]. Using the fluorescence of Chl-a as an intrinsic probe for the state of the thylakoid lipids in living algal cells, we found that the catalytic hydrogenation shifted the maximum of the fluorescence yield by 11°C, related to the control cells, grown at 28°C (fig.1). On the other hand, maxima (11–14°C and 16–25°C), measured for the non-hydrogenated cells cultured at 28°C and 38°C, respectively, were in good agreement with the values determined using the same method [13], and close to those for the onset of phase separation detected by the X-ray diffraction (16°C and 26°C, respectively) in [14].

Our results clearly indicate that the nearly identical alterations in the degree of saturation, irrespective of their origin (isothermic modulation or thermo-adaptation), resulted in the same shift (about 10°C) of the gel–liquid crystal phase transition temperatures of the membrane lipids.

Table 1

Fatty acid composition of total lipids of *Anacystis nidulans* grown at 28°C (1), grown and hydrogenated at 28°C (2) and grown at 38°C (3)

Fatty acids	Molar %		
	1	2	3
14:0	2.1	3.7	1.5
14:1	4.3	2.5	1.1
16:0	44.9	50.5	48.5
16:1	43.5	38.2	37.8
18:0	1.8	2.2	4.8
18:1	3.4	2.5	6.3
Saturated/unsaturated ratio			
Total	0.95	1.29	1.21
14:0/14:1	0.49	1.48	1.36
16:0/16:1	1.03	1.32	1.28
18:0/18:1	0.53	0.76	0.76

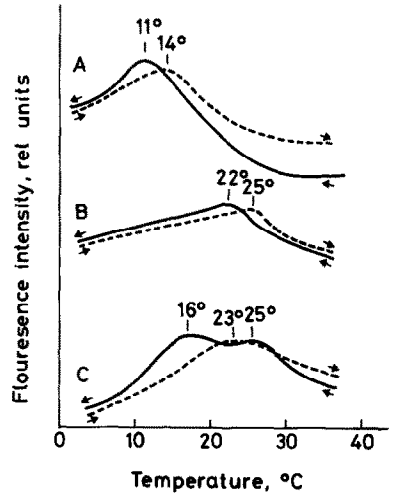

Fig.1. Temperature dependence of Chl-a fluorescence in *Anacystis nidulans* grown at 28°C (A), grown and hydrogenated at 28°C (B) and grown at 38°C (C). Solid and dashed lines were obtained by decreasing and increasing the temperature, respectively.

It has been suggested [15] that a considerable fraction of the membrane lipids contributing to the phase transition observed in the lipid extracts are not involved in the transition associated with the intact cells, but may be involved in interactions; e.g., with proteins. In addition, it has been shown [14] that an appreciable fraction of the thylakoid lipids isolated from the *Anacystis* cells are in a disordered state at 5°C, which is far below the temperature of the main phase transition. Whether the shift of the phase transition temperature induced by hydrogenation and temperature-acclimation, respectively, can be attributed to the appearance of the same discrete pool of 'high melting point lipids' of the thylakoid or not, cannot be answered yet. Further studies are needed to reveal a possible site-selectivity of the hydrogenation concerning the positional distribution of fatty acids in the glycerol moiety of lipids.

Based on the fact that to reach the thylakoid lipids the catalyst has to pass through the cell envelope of the algae, and since a great similarity of the lipid and fatty acid composition exists between the thylakoid and cytoplasmic membranes [12], it is reasonable to assume that the whole membrane system becomes hydrogenated simultaneously. As has been pointed out [6], the fatty acid composition of cytoplasmic membranes is crucial from the point of chilling susceptibility; the formation of gel-phase domains in the cytoplasmic membrane manifests itself in an efflux of small ions (e.g., K^+) to the outer medium, which in turn irreversibly destroys the physiological activities of the cell; e.g., photosynthesis. In our experiments, the midpoint value for the temperature region, critical for K^+ release, was around 4°C in cells grown at 28°C, and 15°C in cells adapted to high (38°C) temperature. Again, the effect of high-temperature acclimation could be triggered by hydrogenation; namely, the midpoint value was 14°C in case of hydrogenated algal cells, grown at 28°C (fig.2). It is of interest that in the region below the midpoint temperature, less K^+ leaked out of the hydrogenated cells than from those adapted to high temperature.

Fig.3 shows the dependence of photosynthetic O_2 evolution on chilling temperatures in hydrogenated and temperature-acclimated cells. Hydrogenation did not result in any change of the amount of O_2 evolved at room temperature (rates for

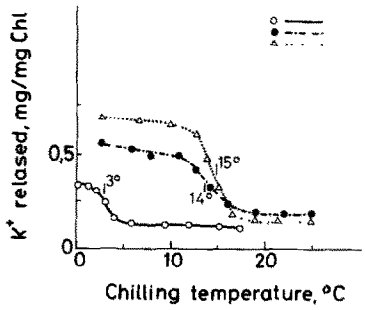

Fig.2. Dependence of the K^+ release on chilling temperatures in algal cells, grown at 28°C (○—○), grown and hydrogenated at 28°C (●- - - -●) and grown at 38°C (△···△). The cells were treated for 10 min at chilling temperatures.

100%). O_2-production, however, declined at much higher chilling temperatures in both hydrogenated and 38°C grown cells, respectively, than in cells adapted to 28°C. (Midpoint values were: 4°C, 15°C and 12°C for the 28°C grown, hydrogenated and 38°C grown cells, respectively.) These temperatures coincided not only with those for K^+ release, as was seen above, but also were equal, or very close, to those for the onset of phase separation in the cytoplasmic membranes of *Anacystis* cells grown at 28°C and 38°C, respectively [6].

In conclusion, it can be stated that all the changes (i.e., the phase separation temperature of

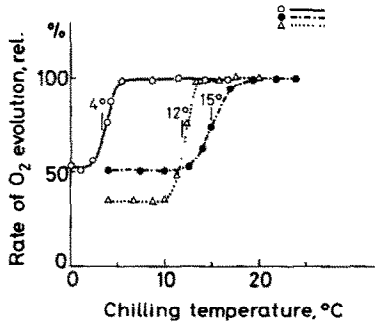

Fig.3. Dependence of the photosynthetic O_2 evolution in cells grown at 28°C (○—○), grown and hydrogenated at 28°C (●- - - -●) and grown at 38°C (△···△). The cells were treated at chilling temperatures for 60 min. Rates for 100% were 550, 510 and 400 μmol O_2 evolved.mg $Chl^{-1}.h^{-1}$ for cells grown at 28°C, grown and hydrogenated at 28°C and grown at 38°C, respectively.

thylakoid lipids, shift in the chilling induced increase of K^+ permeability and decline in photosynthetic O_2-production) brought about by temperature-acclimation in *Anacystis nidulans*, can also be accomplished by homogeneous catalytic hydrogenation. It was hitherto questionable, whether growth temperature-dependent alteration in the fatty acid unsaturation of the membranes predicts the degree of chilling-susceptibility or merely is part of a complex thermoadaptive response. Since the thermo-adaptation replaced by hydrogenation resulted in the same shift in chilling susceptibility it affords direct evidence that chilling response was mediated by changing the degree of fatty acid unsaturation in *Anacystis nidulans*.

REFERENCES

[1] Fulco, A.J. (1972) J. Biol. Chem. 247, 3511–3519.
[2] Umeki, S., Maruyama, H. and Nozawa, Y. (1983) Biochim. Biophys. Acta 752, 30–37.
[3] Farkas, T., Vigh, L., Horváth, I. and Belea, A. (1978) Cryobiology 15, 569–577.
[4] Ono, T. and Murata, N. (1981) Plant Physiol. 67, 176–181.
[5] Murata, N., Wada, H., Omata, T. and Ono, T. (1983) in: Effects of Stress on Photosynthesis (Marcelle, R., Clijters, H. and Van Poucke, M. eds) pp.193–199, Martinus Nijhoff–Dr W. Junk Publishers, The Hague, London, Boston.
[6] Ono, T. and Murata, N. (1982) Plant Physiol. 69, 125–129.
[7] Murata, N., Ono, T. and Sato, N. (1979) in: Low Temperature Stress in Crop Plants: The Role of The Membrane (Lyons, M., Graham, D. and Raison, J.K. eds) pp.337–345, Academic Press.
[8] Vigh, L., Joó, F., Van Hasselt, P.R. and Kuiper, P.J.C. (1983) J. Molec. Catal., in press.
[9] Bulatov, A.V., Izakovich, E.N., Karklin, L.N. and Khidekel, M.L. (1981) Izv. AN SSSR Ser. Khim. 9, 2032–2035.
[10] Vigh, L., Horváth, I., Horváth, L.I., Dudits, D. and Farkas, T. (1979) FEBS Lett. 107, 291–294.
[11] Sato, N., Murata, N., Miura, Y. and Ueta, N. (1979) Biochim. Biophys. Acta 572, 19–28.
[12] Murata, N., Sato, N., Omata, T. and Kuwabara, T. (1981) Plant Cell Physiol. 22, 855–866.
[13] Murata, N. and Fork, D.C. (1975) Plant Physiol. 56, 791–796.
[14] Tsukamoto, Y., Ueki, T., Mitsui, T., Ono, T. and Murata, N. (1980) Biochim. Biophys. Acta 602, 673–675.
[15] Quinn, P.J. and Williams, W.P. (1983) Biochim. Biophys. Acta 737, 223–266.