Selective modification of cytoplasmic membrane fluidity by catalytic hydrogenation provides evidence on its primary role in chilling susceptibility of the blue-green alga, *Anacystis nidulans*

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Cytoplasmic and thylakoid membranes were isolated at different stages of catalytic hydrogenation of fatty acyl double bonds in living cells of *Anacystis nidulans*. The reaction was limited to the lipids of cell-surface membranes during the initial period of hydrogenation, thus, we were able to produce alga cells, modified exclusively in the cytoplasmic membrane. In these unique cells, neither compositional nor physical changes were detected in the lipid phase of thylakoids. Due to the rigidification of the alkyl chains of its lipids, however, the cytoplasmic membrane became leaky and phase separated at considerably higher chilling temperatures. Since an irreversible inactivation of photosynthetic electron transport was found simultaneously, it provided direct evidence for the hypothesis that the thermotropic properties of the lipids within cytoplasmic membranes, and not those of the thylakoids, control chilling susceptibility of the blue-green alga, *A.nidulans*.

Catalytic hydrogenation *Blue-green alga* *Cytoplasmic membrane* *Chilling susceptibility*

1. INTRODUCTION

Although the blue-green alga *Anacystis nidulans* is a procaryotic organism it has provided a useful model system for studies which aim to understand the mechanism of thermo-adaptation and chilling susceptibility of higher plants [1,2,12].

According to the pioneering work of Ono and Murata [3–5] an adaptive mechanism exists in the blue-green algae which is reflected in a rapid shifting of the onset of the liquid-crystalline to gel phase transition of membrane lipids simultaneously with a shifting of the growth temperature. In thylakoids, however, the lipid phase transition occurs always at a higher temperature than in cytoplasmic membranes [6,7]. Despite this fact, it has been suggested that it is primarily the occurrence of the phase separation of the cytoplasmic membrane lipids which induces irreversible chilling injury in cyanobacteria [4–6]. The primary role of the cytoplasmic membrane in thermal acclimation of *A. nidulans* has been shown in our laboratory [8] but hitherto there is no direct evidence that chilling injury relates to lipid phase changes in the cytoplasmic membrane. This report describes a basically new approach for elucidation of this problem. Recently we have utilized a specific technique for modulating the degree of membrane lipid unsaturation and thereby the 'physical state' of membranes in situ on isolated thylakoids [9,10], plant protoplasts [11] and algal cells [12]. This strategy involved the homogeneous catalytic hydrogenation of the double bonds of unsaturated fatty acids [13] within intact membranes conducted under isothermal conditions in the presence of some water-soluble catalysts. Here we have again applied this technique, using the Pd-alizarine complex as catalyst, with one considerable difference.

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By separating the cytoplasmic and thylakoid membranes at different stages of hydrogenation, we have been able to establish the time sequence for the removal of fatty acid double bonds in the different membranes. Since the saturation reaction is apparently limited to the cytoplasmic membrane during the initial stages of catalytic hydrogenation, we have been able to produce in vivo modified algal cells which provide an excellent tool to test the role of the cytoplasmic membrane in chilling sensitivity.

2. MATERIALS AND METHODS

2.1. *Chemicals*

The catalyst, $Pd(QS)_2$ (QS, sulphonated alizarine, $C_{14}H_6O_7NaS$), was prepared as described in [12]. DNase 1 and lysozyme were purchased from Sigma. All other chemicals were of commercial laboratory grade.

2.2. *Alga culture and hydrogenation procedure*

A. nidulans IU 625 (ATCC 27144) was propagated axenically in Kratz and Myers' liquid medium C at 28°C and illuminated with white light at an intensity of 3.6×10^4 mW · m^{-2}. Hydrogenation of the alga cells was carried out essentially as described in [12]. Briefly, an appropriate amount of $Pd(QS)_2$ complex in H_2-saturated distilled water was added to the algal suspension (25 ml) placed in high-pressure glass reaction vessels, evacuated and subsequently filled with hydrogen. Following the chosen period of incubation under hydrogen with rotation at 50 rpm, the gas pressure was slowly released over a period of 10 min. In control experiments, alga cells were treated with the catalyst under nitrogen.

The analysis of fatty acids was performed as in [14].

2.3. *Thylakoid and cytoplasmic membrane isolation*

Both the untreated and partially hydrogenated alga cells were treated with lysozyme and then disrupted in a French pressure cell similar to that in [8]. The procedures, for both the isolation and separation of thylakoid membranes on a linear sucrose gradient (30–90%) and the cytoplasmic membranes on a step-wise sucrose gradient, were exactly the same as reported in [6,15]. Intact and fragmented thylakoids obtained by gradient centrifugation were combined and designated 'thylakoid' for future studies. Identification of the 2 types of membranes was made according to [15].

2.4. *Effect of chilling treatment on K^+ release, photosynthetic O_2 evolution and spectral change of carotenoids*

For the chilling treatment the control and partially hydrogenated alga cells were kept at appropriate temperatures between 0 and 25°C, in the dark. After rewarming to 25°C the suspensions were recentrifuged at 5000 × *g* for 10 min, taken up in fresh medium and subjected to measurements. Determinations of K^+ release were performed as in [12]. Photosynthetic oxygen evolution was detected as described [3]. The light-absorption spectra of the algal cells were recorded at 25°C with an absorption spectrophotometer (Unicam SP 1800).

3. RESULTS AND DISCUSSION

It was previously shown that by using $Pd(QS)_2$, the sulphonated alizarine complex of Pd(II), as a water-soluble catalyst, efficient hydrogenation can be achieved in the membranes of living algal cells, which preserve their integrity and physiological activity [12]. Based on the assumption that the amphipathic and rather bulky molecules of the catalyst are unable to penetrate into the cell interior immediately, the major objective of this work was to establish appropriate experimental conditions for partial hydrogenation, confined exclusively to fatty acids esterified in the lipids of cytoplasmic membranes of algal cells. Therefore, we determined the parameters needed to optimize the rate of saturation of acyl chains within cytoplasmic and thylakoid membranes separated from cells after hydrogenation under isothermal (28°C) conditions. These were a catalyst concentration of 0.08 mM, and a hydrogen pressure of 0.3 MPa at a cell density corresponding to 10 mg chlorophyll/l algal suspension. Under these conditions, provided the reaction time had a maximum of 60 min, satisfactory hydrogenation of fatty acyl residues located in the cytoplasmic membrane was obtained, while the double bond content remained constant in thylakoid membranes. As soon as the reaction time exceeded 60 min, however, the

saturation reaction extended to the thylakoids (fig.1). Table 1 highlights the effect that a 60 min hydrogenation of intact cells can produce on the fatty acid pattern within separated cytoplasmic and thylakoid membranes. A sufficient rate of saturation of the single *cis* double bonds was observable for all 3 unsaturated fatty acyl residues, i.e. 14:1, 16:1 and 18:1, found in the cytoplasmic membrane. At the same time, there was no discernible hydrogenation of thylakoid fatty acids. It was shown that the temperature at which a maximum is found in the curve of the fluorescence yield vs temperature coincides with the main disorder-order transition of the thylakoid lipids [16] in *A. nidulans*. In fact, using the fluorescence of chlorophyll *a* as an intrinsic membrane probe for the physical state of the thylakoid lipids in living algal cells, we did not find any displacement in the maximum (11–14°C) of the fluorescence yield after 60 min hydrogenation (not shown).

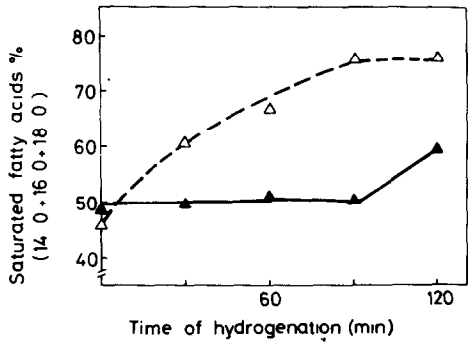

Fig.1. Changes in the content of saturated fatty acids (%) in the lipids of cytoplasmic (△) and thylakoid (▲) membranes during hydrogenation. Incubation was performed under 0.3 MPa H_2 pressure in the presence of 0.08 mM catalyst, at 28°C. In control experiments alga cells were treated with the catalyst under nitrogen pressure.

Table 1

Fatty acid composition of the cytoplasmic (C) and thylakoid (T) membranes before (1) and after (2) 60 min hydrogenation

Fatty acids	Molar %			
	C		T	
	1	2	1	2
14:0	3.2	5.4	3.6	3.6
14:1	6.9	2.8	2.6	2.3
16:0	33.4	43.5	40.0	40.4
16:1	24.0	15.5	30.5	31.1
18:0	9.5	18.3	5.5	5.0
18:1	23.0	11.5	17.8	17.6
Saturated/unsaturated ratio				
Total	0.86	2.25	0.96	0.96
14:0/14:1	0.50	1.92	1.38	1.56
16:0/16:1	1.40	2.81	1.31	1.29
18:0/18:1	0.41	1.59	0.30	0.28

Several findings support the idea that the chilling-induced absorption increase of zeaxanthin around 390 nm observed in *A. nidulans* is a sign of the phase change in cytoplasmic membranes [3,8,17]. Whether this spectral change is a result of conformational alteration or an aggregation of this pigment when the membrane lipids enter a phase-separated state is an issue of controversy. This method, however, provides a unique basis for discerning the thermotropic changes of lipids within the cytoplasmic membrane, in vivo.

Fig.2 shows the absorbance changes of zeaxanthin at 390 nm at different chilling temperatures, in cells grown and partially hydrogenated at 28°C. Parallel with the progress in saturation of fatty acids within the cytoplasmic membrane, midpoint values for the critical temperatures of lipid phase

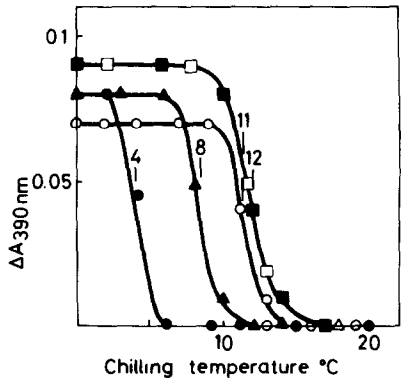

Fig.2. Dependence of A_{390nm} on chilling temperatures. Alga cells were grown at 28°C (●) and grown and hydrogenated at 28°C for 30 min (▲), 60 min (○), 90 min (■) and 120 min (□). The cells were suspended in the culture medium to give a concentration of 4.5 mg chlorophyll/l and treated at chilling temperatures for 30 min.

separation shifted gradually upwards. These values were obviously not a linear function of the double bond content, since saturation further increased by ~10% between 60 and 120 min of the reaction, while the midpoint values remained almost identical (11 and 12°C at 60 and 120 min, respectively). This phenomenon is most likely due to the selectivity of the catalyst for different lipid classes and to their fatty acid molecular species.

To investigate the effect of chilling treatment on K^+ release, cells subjected to 60 min hydrogenation were selected and a comparison was made with the non-hydrogenated population. As in our earlier studies [12] the midpoint value for the critical temperature of K^+ efflux was around 4°C in non-hydrogenated cells, grown at 28°C (fig.3A). As a result of hydrogenation of the lipids within the cytoplasmic membrane, cells became passively permeable to K^+ at much higher chilling temperatures (12 vs 4°C). Nevertheless, critical temperature regions detected by ion release coincided with alterations revealed by spectral change of zeaxanthin (fig.2).

Finally, studies were carried out to investigate the relationship between the lipid phase change in cytoplasmic membranes and the chilling susceptibility of a physiological activity of the alga cells. In control and hydrogenated cells (60 min) depression of photosynthetic oxygen evolution was observed at midpoint values of 4 and 11°C, respectively (fig.3B). These data were well correlated with the difference between the apparent transition temperatures of the lipids of cytoplasmic membranes detected in the 2 populations.

As proposed by Murata and his co-workers, phase transitioning of the thylakoid membranes reduces the photosynthetic activity only reversibly, and that of the cytoplasmic membrane induces irreversible damage [4–6]. On the basis of these results we have provided a unique experimental tool for supporting this hypothesis. Due to the membrane selectivity of the reaction, the cytoplasmic membrane became leaky and phase separated in a higher temperature region after 60 min hydrogenation, whereas neither compositional nor physical changes were detected in the lipid phase of thylakoid membranes. Irreversible inactivation of photosynthetic electron transport was simultaneously found after a considerably milder chilling treatment. In addition to its value

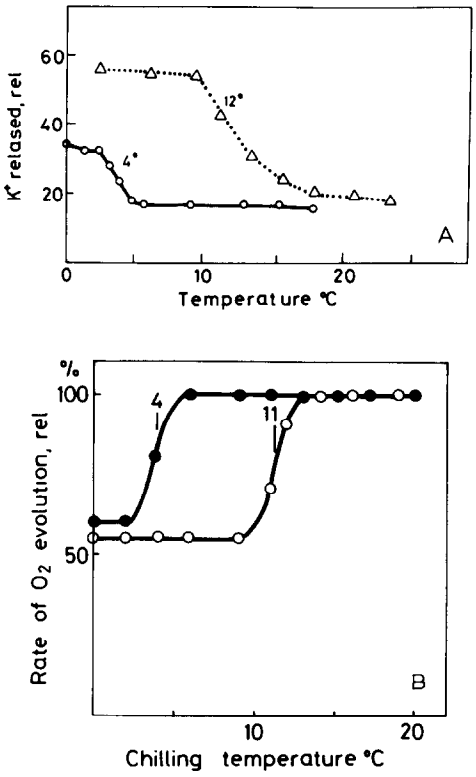

Fig.3. (A) Dependence of K^+ release on chilling temperatures in alga cells, grown at 28°C (○) and grown and hydrogenated for 60 min (△). The cells were treated for 10 min at chilling temperatures. The density of cells was adjusted to 100 mg chlorophyll/l. (B) Dependence of photosynthetic O_2 evolution on chilling temperatures in cells grown at 28°C (●) and grown and hydrogenated at 28°C for 60 min (○). Rates for 100% were 530 and 480 mol O_2 evolved · chlorophyll^{-1} · h^{-1} for control and hydrogenated cells, respectively. The cell density was 10 mg chlorophyll/l.

for the experimental proof of the primary role of fatty acid unsaturation and the physical state of the cytoplasmic membrane in chilling injury of blue-green algae, the present procedure is of importance in a wider sense. Programmed, in vivo, modification of the composition and fluidity of the cell-surface membranes of intact cells (or protoplasts) by the Pd-alizarine catalyst could provide a wealth of information about various cellular processes currently being studied. Among the potential applications, for example, are the accessibility and function of cell-surface receptors and antigens [18], signal transduction of various hormones [19],

cytolytic capacity of T lymphocytes and macrophages [20], all of which are directly related to the fluidity level of the lipid matrix.

REFERENCES

[1] Quinn, P.J. and Williams, W.P. (1983) Biochim. Biophys. Acta 737, 223–266.
[2] Minorsky, P.V. (1985) Plant Cell Environ. 8, 75–94.
[3] Ono, T. and Murata, N. (1981) Plant Physiol. 67, 176–181.
[4] Ono, T. and Murata, N. (1981) Plant Physiol. 67, 182–187.
[5] Ono, T. and Murata, N. (1982) Plant Physiol. 69, 125–129.
[6] Wada, H., Hirasawa, R., Omata, T. and Murata, N. (1984) Plant Cell Physiol. 25, 907–911.
[7] Tsukamoto, Y.T., Ueki, T., Mitsui, T., Ono, T. and Murata, N. (1980) Biochim. Biophys. Acta 602, 673–675.
[8] Gombos, Z. and Vigh, L. (1985) Plant Physiol., in press.
[9] Vigh, L., Joó, F., Van Hasselt, P.R. and Kuiper, P.J.C. (1983) J. Mol. Catal. 22, 15–19.
[10] Vigh, L., Joó, F., Droppa, M., Horváth, L.I. and Horváth, G. (1985) Eur. J. Biochem. 147, 477–481.
[11] Vigh, L., Joó, F. and Cséplö, Á. (1985) Eur. J. Biochem. 146, 241–244.
[12] Vigh, L. and Joó, F. (1983) FEBS Lett. 162, 423–427.
[13] Szalontai, B., Droppa, M., Vigh, L., Joó, F. and Horváth, G. (1985) Photobiochem. Photobiophys., in press.
[14] Vigh, L., Horváth, I., Horváth, L.I., Dudits, D. and Farkas, T. (1979) FEBS Lett. 107, 291–294.
[15] Omata, T. and Murata, N. (1983) Plant Cell Physiol. 24, 1101–1112.
[16] Murata, N. and Fork, D.C. (1975) Plant Physiol. 56, 791–796.
[17] Brand, J.J. (1979) Arch. Biochem. Biophys. 193, 385–391.
[18] Caroll, J.E., Landry, A.S., Elliot, M.E., Yatvin, M.B., Vorpahl, J. and Goodfriend, T.L. (1983) Life Sci. 32, 1573–1581.
[19] Schlessinger, J. (1980) Trends Biochem. Sci. 5, 210–214.
[20] Schlager, S.I., Meltzer, M.S. and Madden, L.D. (1983) Lipids 18, 483–488.