Biochimica et Biophysica Acta, 602 (1980) 673—675

BBA Report

BBA 71488

RELATIONSHIP BETWEEN GROWTH TEMPERATURE OF *ANACYSTIS NIDULANS* AND PHASE TRANSITION TEMPERATURE OF ITS THYLAKOID MEMBRANES

YUJI TSUKAMOTO[a], TATZUO UEKI[a,*], TOSHIO MITSUI[a], TAKA-AKI ONO[b] and NORIO MURATA[b]

[a]*Department of Biophysical Engineering, Faculty of Engineering Science, Osaka University, Toyonaka, Osaka 560, and* [b]*Department of Biology, College of General Education, The University of Tokyo, Komaba, Meguro-ku, Tokyo 153 (Japan)*

(Received May 23rd, 1980)

Key words: Thylakoid membrane; Phase transition; X-ray diffraction; Temperature adaptation; (A. nidulans)

Summary

The temperatures of the lipid phase transition at which the solid phase disappears were determined by using the X-ray diffraction method in thylakoid membranes of the blue-green alga, *Anacystis nidulans*. The temperatures were determined as 26 and 16°C for cells grown at 38 and 28°C, respectively.

There have been several investigations on the lipid phase transition in intact cells and thylakoid membranes of the blue-green alga, *Anacystis nidulans*. In the results, the lipid phase transition is accompanied by the characteristic changes in various photosynthetic activities, such as the photosynthetic electron transport reactions, the pigment state 1—state 2 shift, O_2 evolution, the intensity of delayed fluorescence of chlorophyll *a* and the phosphorylation reactions [1—3]. These changes occur at temperatures between 21 and 24°C for 38°C-grown cells and between 12 and 15°C for 28°C-grown cells. The transition temperatures determined from spin-labelling and chlorophyll *a* fluorescence are 24 and 25°C for 38°C-grown cells and 13°C for 28°C-grown cells [1, 4]. The present paper describes X-ray diffraction studies on the lipid phase transition of thylakoid membranes isolated from *A. nidulans* grown at two different temperatures.

*To whom correspondence should be addressed.

Abbreviation: Tes, *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid.

The thylakoid membranes from *A. nidulans* grown at 38 and 28° C were prepared according to the procedure of Ono and Murata [3, 5] with the following modifications: the lysozyme-treated cells were ruptured with a French pressure cell and the thylakoid membranes were purified by sucrose gradient centrifugation. For X-ray diffraction measurement, the membranes were washed in 0.6 M sucrose, 5 mM $MgCl_2$, 10 mM NaCl, 15 mM Tes/NaOH buffer (pH 7.0) and centrifuged at 220 000 × *g* for more than 12 h. The resultant pellets were sealed into thin-walled glass capillaries, 1.5 mm in diameter.

The X-ray diffraction procedure was the same as that employed in previous studies of membrane fragments isolated from *Escherichia coli* [6].

Fig. 1 shows the X-ray diffraction patterns of the thylakoid membranes isolated from 38° C-grown cells. A sharp reflection at the Bragg spacing of 4.2 Å appears at low temperatures and disappears when the temperature reaches 27.5° C, above which only a broad peak is present at the approximate spacing of 4.5 Å. This broad peak coexists with the sharp reflection below 25° C. This fact indicates the coexistence of the solid (or ordered) and the fluid (or disordered) phases, as generally observed in membranes of a multicomponent lipid [7].

Below, we shall call the temperature at which the 4.2 Å reflection disappears the transition temperature and denote it as T_f. This is the temperature at which all the lipid bilayer in the membrane turns into the fluid state. The transition temperature, T_f, was determined as the temperature at which the integrated intensity of the sharp reflection at 4.2 Å becomes zero, as seen in Fig. 2. Details of the procedure are described in the previous paper [6]. From Fig. 2a and b, the T_f values were estimated to be 26° C for 38° C-grown cells and 16° C for 28° C-grown cells. These lipid transition temperatures are in good agreement with the values determined using other methods

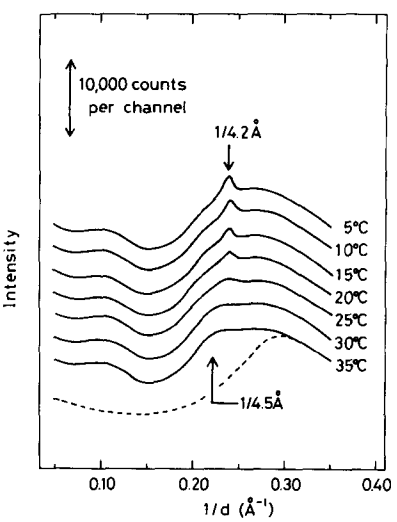

Fig. 1. X-ray diffraction patterns of the thylakoid membranes from 38° C-grown *A. nidulans* cells observed at various temperatures. Recorded with a position-sensitive proportional counter. Abscissa: inverse of spacing, $1/d = 2\sin\theta/\lambda$, where 2θ is the diffraction angle and λ is the wavelength (1.542 Å). The dashed line indicates background scattering by buffer solution at 35° C.

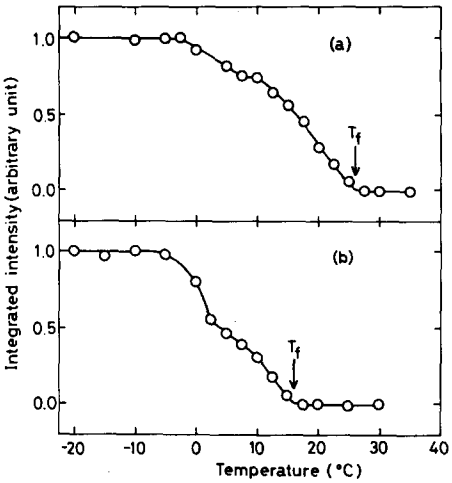

Fig. 2. Temperature dependence of the integrated intensity of the 4.2 Å sharp reflection in Fig. 1. (a) Plot for the thylakoid membranes isolated from 38°C-grown cells and (b) from 28°C-grown cells. T_f is the transition temperature of lipids. The integrated intensity is normalized for the intensity of the 4.2 Å peak at the lowest temperature of the experiment, −20°C.

[1—4]. These results indicate that the lipids in the thylakoid membranes are entirely in the fluid state at the growth temperatures. Sato et al. [8] reported that the ratio of saturation to unsaturation of the fatty acid components in the thylakoid membranes from *A. nidulans* increases as the growth temperature increases from 22°C to 28 or 38°C. Unsaturated hydrocarbon chains of lipids have the tendency to disturb the arrangement of saturated hydrocarbon chains in the lipid membranes, and to lower T_f, as demonstrated in synthetic lipid membranes [9, 10]. In this respect, our X-ray results coincide with the result of Sato et al. [8]. The biological significance of the difference of 12°C between the growth and lipid phase transition temperatures is as yet unclear.

The authors wish to thank to Dr. K. Furuya of National Institute for Physiological Science and to Mr. H. Nakayama of Department of Biophysical Engineering, Osaka University for their advice in the course of the present study.

References

1 Murata, N., Troughton, J.H. and Fork, D.C. (1975) Plant Physiol. 56, 508—517
2 Ono, T. and Murata, N. (1977) Biochim. Biophys. Acta 460, 220—229
3 Ono, T. and Murata, N. (1979) Biochim. Biophys. Acta 545, 69—76
4 Murata, N. and Fork, D.C. (1975) Plant Physiol. 56, 791—796
5 Ono, T. and Murata, N. (1978) Biochim. Biophys. Acta 502, 477—485
6 Nakayama, H., Mitsui, T., Nishihara, M. and Kito, M. (1980) Biochim. Biophys. Acta, in the press
7 Shechter, E., Letellier, L. and Gulik-Krzywicki, T. (1974) Eur. J. Biochem. 49, 61—76
8 Sato, N., Murata, N., Miura, Y. and Ueta, N. (1979) Biochim. Biophys. Acta 572, 19—28
9 Ladbroode, B.D. and Chapman, D. (1969) Chem. Phys. Lipids 3, 304—356
10 Furuya, K. and Mitsui, T. (1979) J. Phys. Soc. Jap. 46, 611—616