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Differential Scanning Calorimetry Study on the Inner Membrane Lipids Prepared from Barotolerant *Pseudomonas* sp. BT1

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We investigated the properties of membrane lipids of barotolerant *Pseudomonas* sp. BT1 by differential scanning calorimetry and spectrophotometry using a system equipped with a hydrostatic pressure controller. In the case of cells grown under high pressure, an endothermic peak appeared under high-pressure measurement conditions. However, in the case of cells grown at 0.1 MPa, such a peak was not observed. It was also observed on spectrophotometry that the membrane lipids from cells grown at 30 MPa had stable properties in comparison with those grown at 0.1 MPa various hydrostatic pressures and temperatures.

Key words: barotolerant *Pseudomonas*, barotropic phase transition, differential scanning calorimetry, hydrostatic pressure, membrane lipids.

Although the deep-sea is a severe environment, the pressure at depths of about 1,000 to 11,000 m being in the range of 10 to 110 MPa, and the temperature being approximately 2 to 4°C, several types of bacteria have been isolated from deep-sea sediment since the 1980s (*1–3*). However, the reasons why these bacteria are able to grow in such environments have not been elucidated.

In this study, we determined the properties of the inner membrane lipids of an microorganism grown under several conditions by differential scanning calorimetry (DSC) using a system equipped with a hydrostatic pressure controller: this is *the first* report of a study in which the membrane properties of a barotolerant bacterium were analyzed by DSC.

Pseudomonas sp. BT1 was grown in a pressure vessel without agitation at growth temperatures of 20 and 37°C, and hydrostatic pressure levels of 0.1 (atmospheric pressure) and 30 MPa. A sample of the extracted inner membrane lipids from the microorganism (*4*) suspended in distilled water and a reference sample of distilled water were separately injected into one-terminal shielded SUS capillaries. The sample and reference capillaries were inserted into the sample and reference cells, respectively, of an MC-2 scanning calorimeter (MicroCal, MT). The capillaries were connected to a TP-500 hand pump (Teramecs, Kyoto) and then pressurized. DSC scans, from 5 to 70°C, were made at the rate of 1°C/min. The trace for distilled water, taken as the baseline, was subtracted from that for a sample. The DSC properties under the same conditions were determined in at least three independent experiments.

The results are shown in Fig. 1, a–h. The DSC properties under the same conditions were determined in at least three independent experiments and reproducible results were obtained. The DSC thermograms of the membrane lipids prepared from cells grown at 0.1 MPa were not clearly different from those in the cases of 0.1 and 40 MPa (Fig. 1, a, b, e, and f). However, the thermograms of the inner membrane lipids prepared from cells grown at 30 MPa were different from those in the cases of cells grown at 0.1 MPa. Under the high-pressure measurement conditions at 40 MPa, a wide endothermic peak clearly appeared at 35–55°C, which was much less intense on measurements under atmospheric pressure conditions (Fig. 1, c, d, g, and h). The thermogram in Fig. 1d seems to represent the superposition of the two peaks illustrated by dot lines. The appearance of the endothermic peak was a barotropic phenomenon and it was considered to occur as a result of phase transition of the lipids under high-pressure measurement conditions. The inner membrane composition of *Pseudomonas* sp. BT1 under various conditions has already been reported (*4*). With the change in growth conditions in this study, the relative concentration of cardiolipin, a negatively charged lipid known to influence the phase transition temperature and membrane fluidity, markedly changed. Additionally, a marked decrease in the concentration of unsaturated fatty acids in the inner membrane was observed with the change in growth temperature from 20 to 37°C at 30 MPa. These changes in unsaturated fatty acids as constituents of phospholipids affect the phase transition temperature of the inner membrane lipids and membrane fluidity. Transition temperature of cardiolipin is close to that of phosphatidylethanolamine (*5*). Therefore, it appeared that these compositional changes in the phospholipids and fatty acids contributed to some structural effect on the membrane properties.

The ratio of the absorbency (at 560 nm) to that at 20°C/0.1 MPa is illustrated in Fig. 2, with a constant hydrostatic pressure (a) or constant temperature (b). The absorbency of membrane lipids from the cells grown at 20°C and 0.1 MPa was very sensitive to temperature and hydrostatic pressure. The change of the absorbency was assumed to indicate phase transitions among $L_{\alpha}/P_{\beta'}/L_{\beta'}$ (*6, 7*). On the other

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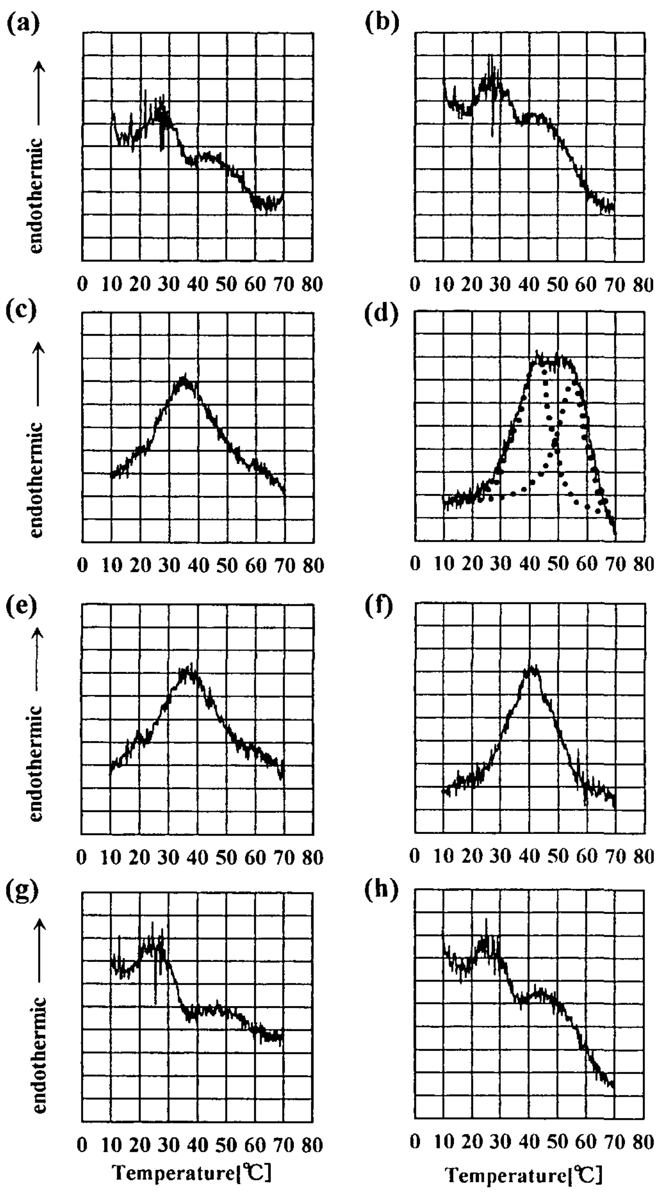

Fig. 1. **DSC of inner membrane lipids of *Pseudomonas* sp. BT1.** The growth conditions and measurement conditions were as follows: (a) growth at 20°C and 0.1 MPa, measurement at 0.1 MPa; (b) growth at 20°C and 0.1 MPa, measurement at 40 MPa; (c) growth at 20°C and 30 MPa, measurement at 0.1 MPa; (d) growth at 20°C and 30 MPa, measurement at 40 MPa; (e) growth at 37°C and 0.1 MPa, measurement at 0.1 MPa; (f) growth at 37°C and 0.1 MPa, measurement at 40 MPa; (g) growth at 37°C and 30 MPa, measurement at 0.1 MPa; (h) growth at 37°C and 30 MPa, measurement at 40 MPa. The DSC properties under the same conditions were determined in at least three independent experiments and reproducible results were obtained.

hand, that of membrane lipids from the cells grown at 20°C and 30 MPa was slightly affected by temperature and hydrostatic pressure. Although there was no significant change in the absorbency of the membrane lipids, the stable properties under high hydrostatic pressure conditions were assumed to play a role in maintenance of the proper conditions of the membrane.

These results suggest that the membrane of cells grown

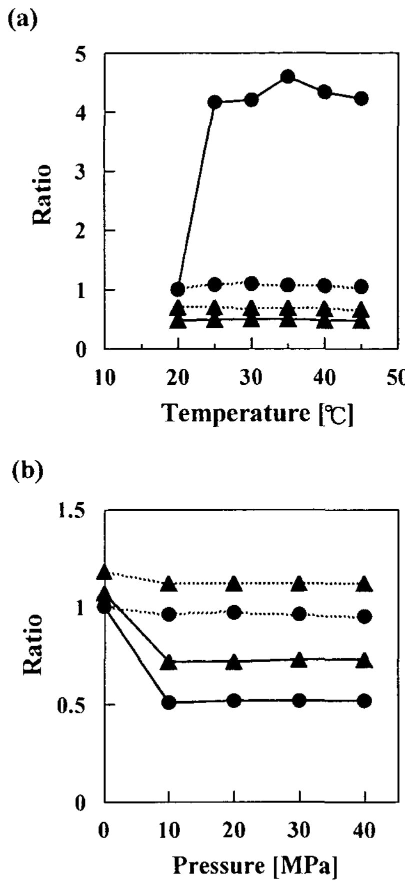

Fig. 2. **Thermotropic and barotropic phase-transitions of the membrane lipids of *Pseudomonas* sp. BT1 with an optical method (wavelength, 560 nm).** (a) Growth conditions: solid lines, 20°C/0.1 MPa; dotted lines, 20°C/30 MPa. Measurement conditions: closed circles, 0.1 MPa; closed triangles, 40 MPa. (b) Growth conditions: solid lines, 20°C/0.1 MPa; dotted lines, 20°C/30 MPa. Measurement conditions: closed circles, 20°C; closed triangles, 45°C. The values are the average of three measurements.

at high pressure has unique properties that only appear under high pressure conditions, in other words, barotropic properties. The appearance of an endothermic peak indicates that the heat capacity of the membrane was increased by high hydrostatic pressure. To maintain proper membrane conditions and properly functional life systems, the heat capacity of the membrane plays a role by compensating for thermal fluctuation in the environment in which the cells are growing.

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