Thermotrophic Properties of Thermophilic, Mesophilic, and Psychrophilic Blue-green Algae

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ABSTRACT

Thermotrophic properties of blue-green algae grown at high, room, and low temperatures in H2O and D2O media were studied by highly sensitive differential scanning microcalorimetry. The thermograms of these organisms contain an endothermal peak in the temperature range of 50 to 70 C with an endothermal heat ranging from 0.14 to 1.91 joules per gram organism. The temperature at which the endothermal peak occurs is comparable with the thermal denaturation temperature of phycocyanin, the major biliprotein isolated from these algae. A good correlation can be found for the relative thermal stability of various organisms with that of the isolated biliproteins. The ability of these algae to resist thermal disruption is correlated with the thermal environments in which these algae grow. The thermal stability of normal algae is in the order of thermophile > mesophile > psychrophile. It was found that the deuterated mesophilic algae were less able to resist thermal disruption than ordinary mesophilic algae.

Blue-green algae can be classified as thermophilic (high temperature), mesophilic (room temperature), or psychrophilic (low temperature), according to the temperature of the culture media, in which these organisms grow. Some of the mesophilic algae can be cultured in D2O medium. This characteristic provides opportunities for the use of blue-green algae as a probe in the studies of the adaptation of an organism to grow in various environments. Phycocyanin, a major biliprotein extracted from these algae, can be used as a probe in the investigation of the effect of variations in environmental stress on the structure and function of proteins. It has been reported that the ability of phycocyanin to resist urea denaturation is in the order of thermophile > mesophilic > psychrophile. Based on the data of apparent free energy of protein unfolding, thermophilic phycocyanin was found to be more stable than that from the mesophilic one. Moreover, it has been reported that deuterated phycocyanin undergoes thermal denaturation at a temperature 5 C lower than normal phycocyanin.

Differential scanning microcalorimetry has been applied highly sensitive differential scanning microcalorimetry to elucidate the thermograms of whole cells of blue-green algae grown at various temperatures in H2O and D2O media. This type of work is important since it can provide information on the relative thermal stability of these cells, which could give insight into their adaptation under various environmental stress. The present studies also examine the correlation of the stability of the isolated biliprotein with that of the intact blue-green algal cell.

MATERIALS AND METHODS

Growth of Algae. These studies were carried out on blue-green algae, including Mastigocladus laminosus, Plectonema calothrixoides, Phormidium luridum, and Mastigocladus vaginatus. The culture temperatures for these algae are listed in Table I. These cultures reflect optimum but not tolerances, of the species. Special algal medium (1) was used for thermophile and mesophilic, and Castenholz medium (3) was used for psychrophile. The solvents were H2O and D2O. Normal H2O was double-distilled; D2O (99.8% purity) was obtained from Bio-Rad Laboratories. In the incubators, the cultures were illuminated with fluorescent bulbs at approximately 5,000 lux and were agitated with Eberhard rotary shakers. A mixture of N2 (95%) and CO2 (5%) was bubbled into the culture medium. Algae grown in H2O medium were harvested after 4 weeks; those grown in D2O medium required more than 2 months of growth.

Preparation of Whole Cell Sample. Algae suspended in culture medium were centrifuged at 27,000g at 5 C. The medium solution was removed, and the algae were rinsed three times with 0.1 M Tris buffer (pH 7.9) to wash out the rest of the algal medium solution. Cell lysis would have been evident by the presence of the blue phycocyanin in the Tris buffer; however, none was observed. Finally, 0.75 ml algae suspended in Tris buffer was used to fill the sample cell. The reference cell contained Tris buffer in equal volume. Both cells were transferred to the microcalorimeter.

Separation of Biliproteins from Algal cells. Algal cells were ruptured by adding about 3 mg lysozyme and was stirred in the cold room overnight. The suspension then was centrifuged at 27,000g at 5 C. The supernatant contained biliproteins; the residue of the cell was in the pellet. The extracted biliproteins and the residue of the cell were used for calorimetric measurements.

The procedure for separation of biliproteins from algae was also used to determine quantitatively the amount of biliproteins in the algae. Extraction was repeated three to four times. Totally, about 10 ml buffer solution was used. The disappearance of blue color from the supernatant was an indication of completion of extraction. To determine the relative amount of biliproteins and of the residue of the cell, biliprotein extracts and cell residues were separately dialyzed in distilled H2O (to remove salts in the buffer solution), dried in the air, and weighed repeatedly until constant weights were obtained.

Instrumentation. A differential scanning microcalorimeter contains two identical cells filled with the sample solution and the solvent, respectively. During the measurement, electrical heat is
A thermal change in the sample solution can be detected by recording the difference in power required to heat the cells equally. The recorder monitors voltage as a function of temperature. If there is no thermally induced physical or chemical change in the sample solution, a smooth line with a certain slope will be found in the voltage-temperature plot. On the other hand, a thermally induced transition will result in a peak in either exothermic or endothermal direction.

The microcalorimeter used was a newly developed, heat-conduction-type differential scanning microcalorimeter, constructed according to Ross and Goldberg (20) with some modifications. It was designed for measuring heat capacities and heat effects in dilute solution.

The center block of the instrument consisted of a copper block and inner and outer cylinders. A 6115A Hewlett Packard precision power supply connected to a heater was used to raise the temperature of the block. The temperature could be determined by using a potentiometer (model 2790, Rubicon Co.) to measure the voltage of a copper constantan thermocouple with an ice point reference using an ice point cell (Omega Engineering, Inc.). The thermocouples surrounding the two separate cells were conducted in electrical opposition, and the resultant voltage was fed into a Keithley 150B microvolt ammeter, which was connected to a Leeds and Northrup model 620 recorder. Another pair of copper constantan thermocouples was used to measure the temperature difference between the copper block and the inner aluminum cylinder. The differential output of these thermocouples was fed to a Keithley 147 null detector. A 232-ohm standard resistor and a Keithley 225 current source were used for the electrical calibration.

A circulating refrigerated ethylene glycol-water bath was used to cool the microcalorimeter to 0 C or lower. The operational temperature range for the instrument was from 0 to 90 C. The scanning rates could be selected from 3 to 40 C/h. Each calorimeter cell had a volume of approximately 0.80 ml and was made of platinum.

The calibration constants were 21.49 to 21.27 w/v in the temperature range of 40 to 80 C. The sensitivity of the instrument in this range was about 48 µW/mw. The absolute temperature determination was ±0.05 C. A typical measurement of 0.36 mg/ml sonicated dipalmitoyl-L-α-lecithin solution showed a fairly sharp liquid phase transition at 41.9 C, as compared to 42.2 C in the literature (20). The enthalpy of transition was 30.4 kJ/mol, which is in agreement with the literature value of 30.8 kJ/mol (10). The precision of the measurement of heat associated with transition is about 3%.

Measurement. After both cells were installed in the microcalorimeter, the cold ethylene glycol-H2O mixture was circulated through the system overnight to bring the temperature down to 0 C or lower. The refrigeration unit was disconnected, and the system was warmed at a rate of 0.15 C/min. Since no exothermic or endothermic peak was found in the thermograms below room temperature, most of the measurements were thermally equilibrated and started from room temperature. The recorder chart speed was 20 cm/h. After measurement was completed, the algal suspension was removed from the sample cell and dried in the air for determination of the dry weight of alga used. The area under the peak of the thermogram was measured by a Science Accessories Corp. sonic digitizer.

RESULTS AND DISCUSSION

A typical thermogram of a blue-green alga (P. calothricoides) grown in H2O medium at 27 C is shown in Figure 1. The base line deflection in the thermograms is about 5 × 10⁻² µW. A smooth base line is found in the control measurement (Fig. 1a). The thermogram of the whole algal cell exhibits an endothermal peak at 58.7 C (Fig. 1b). (The thermogram starts at 56.0 C, reaches a minimum at 58.7 C, and maintains a finite slope after 60.8 C.) The thermogram below 50 C exhibits no endothermal or exothermal peak and is not shown in the figure. After the initial measurement, the whole cell sample was cooled to below room temperature and followed by a repeat measurement. The original endothermal peak disappeared in the repeat measurement (Fig. 1c). The irreversibility of the thermogram indicates that the endothermal peak is related to thermal disruption of cell components; such cell components cannot return to the original state in the cell after thermal disruption.

Thermograms of thermophilic, mesophilic, and psychrophilic algae grown in normal H2O are compared in Table I. The temperature at which the endothermal peak occurs (t41) is related to the ability of the algae to resist thermal stress. Values of t41 are in the order of thermophile > mesophile > psychrophile. These results provide a nice correlation between thermal disruption of algal cells with the thermal environments in which these cells grow. This finding is evidently related to the fact that some algae can grow at higher temperatures and some can grow only at lower temperatures.

Previous studies (5) showed that the ability of phycocyanin to

1 Abbreviation: J, joule(s).
resist the denaturant urea follows the same order of thermophile > mesophile > psychrophile, and phycocyanin extracted from the thermophile is more thermally stable than from the mesophile. Moreover, it has been reported (14, 15) that proteins such as cytoplasmic proteins, bacterial flagella, and triose-P isomerase from thermophiles are more thermostable than those from mesophiles. Triose-P isomerase from a psychrophile was also found (21) to be less resistant to heat than that from a thermophile or mesophile. These results on protein thermal denaturation as well as the present results on the thermotropic properties of whole cells suggest that the relative thermal stability of a protein extracted from various organisms is analogous to that of the whole organisms.

Thermograms of two mesophilic algae grown in H2O and D2O media are also compared in Table I. The algae grown in D2O medium (deuterated algae) contain deuterium instead of hydrogen in their molecular compositions. For the same species, the value of \( t_{a1} \) is higher for algae grown in H2O than in D2O medium. This shows that deuterated algae are less able to resist thermal disruption than ordinary ones, which can tolerate a temperature 3 to 7 C higher than deuterated algae. This difference in thermal stability is obviously associated with the fact that blue-green algae grow and survive in H2O much better than in D2O. It has been shown that deuterated phycocyanin undergoes thermal denaturation at a temperature 5 C lower than normal phycocyanin isolated from *P. calothricoides* (2, 9). This suggests that the relative thermal stability of proteins extracted from the whole cells of normal and deuterated algae is also analogous to that of the whole cells.

Table I also lists the observed values of heat associated with the endothermal peaks in the thermograms (\( \Delta Q_{a} \)). They range from 0.7 to 1.9 J/g organism for normal algae and from 0.1 to 0.4 J/g organism for deuterated algae. These \( \Delta Q_{a} \) are assumed to be the minimal thermal energies required to disrupt algal cells. For normal algae, \( \Delta Q_{a} \) of mesophilic algae is higher than that of thermophilic or psychrophilic one. Comparison of \( \Delta Q_{a} \) for the same algae grown in D2O with that in H2O shows that \( \Delta Q_{a} \) of deuterated algae is generally much lower than that of normal algae.

Blue-green algae contain significant amounts of biliproteins (4). The temperatures at which the endothermal peaks occur are in the range of 50 to 70 C, which is comparable with the range of thermal denaturation temperatures of the common proteins (16). To gain some insight from the algal thermogram in relation to the structural components of the cell, it is important to elucidate the thermograms of biliproteins extracted from the cell (Fig. 2). The endothermal peak of the whole organism from *P. luridum* (Fig. 2a) starts at 54.5 C and ends at 62.1 C. (After 62.1 C, the thermogram maintains a finite slope.) The temperature at the minimum is 57.0 C. The thermogram of biliproteins (including phycocyanin and a small amount of allophycocyanin) isolated from the algae shows an endothermal peak at 59.0 C (Fig. 2b). The cell residue after removal of biliproteins shows no endothermal peak in its thermogram (Fig. 2c). These findings strongly suggest that the endothermal peak in the thermogram of whole algal cell is qualitatively related to thermal denaturation of biliproteins in the cell. It should be noted that the other proteins in the cell are also likely to contribute to the endothermal peak since thermal denaturation of most of the proteins in general occurs in this temperature range (16).

To determine if there was a quantitative correlation between \( \Delta Q_{a} \) and the amount of biliproteins in the cell, amount of biliproteins in the cell had to be assessed. It was found that the weight percentage of biliproteins in the whole cell is 36% for *M. laminosus* (H2O), 14% for *P. calothricoides* (H2O), 2% for *P. calothricoides* (D2O), 26% for *P. luridum* (H2O), 2% for *P. luridum* (D2O), and 6% for *M. vaginatus* (H2O). The results demonstrate that the relative content of biliproteins in normal algae is in the order of thermophile > mesophile > psychrophile. The significantly smaller content in the psychrophile, *M. vaginatus*, is consistent with the fact that this psychrophile grows much slower than a thermophile and a mesophile. The higher content in the thermophile may be associated with its ability to survive at a higher temperature and with its faster growth rate. Comparison of normal biliprotein content of normal algae is much higher than that of deuterated algae. This result is in agreement with the observation that algae are much easier to grow in H2O than in D2O.

A typical quantitative comparison of thermograms of whole cell from *P. luridum* and its isolated biliproteins is shown as follows. For the whole cell, \( t_{a1} \) was 5.70 C and \( \Delta Q_{a} \) was 1.79 J/g organism. For the isolated biliproteins, its thermal denaturation temperature (\( t_{a1} \)) was 59.0 C and the heat associated with thermal denaturation (\( \Delta Q_{a} \)) was 0.73 J/g biliproteins. The biliprotein content in *P. luridum* was 26% of the dry weight of the cell. This means that the heat, which an alga would have if the endothermal peak were entirely a contribution from the thermal denaturation of the biliproteins, turns out to be 0.19 J/g alga. This number is much smaller than \( \Delta Q_{a} \). This comparison suggests that \( \Delta Q_{a} \) contains contributions not only from the thermal denaturation of biliproteins but also from other factors, such as disruption of the organized cell structure and thermal denaturation of other proteins in the cell. In addition, the aqueous environment of proteins in the cell is quite different from that of isolated proteins in Tris buffer solution. This factor should also contribute to the above discrepancy.

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**LITERATURE CITED**