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THERMAL ANALYSIS OF BACTERIOPHAGE T4

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SUMMARY The process of bacteriophage T4 morphogenesis was studied using a heat leakage scanning calorimeter. Thermograms of defective mutant 49(am NG727) in permissive and non-permissive cells of Escherichia coli showed a difference in thermal properties between packaged and non-packaged DNA molecules. In vivo, non-packaged DNA carried out their thermal transition at 85°C, the same temperature as that of T4 DNA melting measured in the standard saline citrate buffer, while the packaged DNA gave a sharper peak at 87°C due to some interaction with the head shell structure. Empty head shells showed a sharp heat absorption peak at 89°C both in vivo and in vitro, indicating the high degree of cooperativity in their conformational changes.

INTRODUCTION Recently it has been revealed that P23, the major structural protein of the head shell of bacteriophage T4, can self-assemble into polyheads in vitro (1) and that the expansion of the head shell is not necessarily linked to the intramolecular cleavage of the structural proteins (2). In this connection it is interesting to study thermal properties of each component involved in the process of phage morphogenesis in vivo as well as in vitro. The information thus obtained may offer a clue to elucidate the role of gene products in vivo or the way of phage reconstruction in vitro.

One way to obtain such information is to use the scanning calorimetry. A scanning calorimeter can show not only the existence of some higher structures in biopolymers but also the degree of stability of those structures (3), because usually the thermal denaturation of a biopolymer gives an endothermic peak and the renaturation process gives an exothermic peak in the thermogram obtained with the scanning calorimeter. One advantage of this technique is found in the fact that it can display in a single run the whole thermal features of a complex system such as intact bacterial cells without necessitating separation into each constituent fraction.

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This paper will describe some results of calorimetric studies on bacteriophage T4 with special regard to DNA packaging and the structure of empty head shells.

MATERIALS AND METHODS E. coli B^b and B40S were used as non-permissive and permissive host strains, respectively, for amber mutants of bacteriophage T4D. E. coli B^b was used also as a host strain for wild type T4D. The phage strains used were: T4D, 23(am B17), 31(am N54), 49(am NG727). The medium used throughout the present experiment contained 0.4% glucose, 1mM $MgSO_4$, M9 salts(5.8g Na_2HPO_4, 3g KH_2PO_4, 0.5g NaCl, 1g NH_4Cl per liter) and 1% casamino-acids. The buffer used for the preparation of the phage stocks and their empty head shells contained 1mM $MgSO_4$ and M9 salts. Growth of bacteria and bacteriophages was done at 37°C with aeration and phage-infected bacterial cells were collected as follows: a 2l culture of E. coli was grown to about $2x10^8$ cells/ml. Four hundred milli-liter was taken therefrom and rapidly chilled for harvesting the uninfected cells. The rest of the culture was infected with respective phage at a multiplicity of 5. At 10min, 20min, 35min and 50min after infection, 400ml of culture was gathered and rapidly chilled each time, followed by centrifugation to collect phage-infected cells of E. coli. Empty head shells were prepared from non-permissive cells of E. coli infected with 49(am NG727). E. coli B^b was grown to about $2x10^8$ cells/ml and infected with 49(am NG727) at a multiplicity of 5. At 55min after infection, cells were harvested by centrifugation. The pellet was resuspended in the buffer mentioned above added with DNase, lysozyme and several drops of $CHCl_3$. After stirring for 90min at room temperature, the lysate was centrifuged at 6000 rev/min for 15min. The precipitate was washed with the same buffer. The supernatants were mixed and centrifuged at 30000 rev/min for 60min and the pellets thus obtained were resuspended in the buffer of a desirable volume. The suspension was overlayered on a sucrose step gradient(3ml of 30%, 3ml of 20% and 2ml of 10%) in a 10ml tube and centrifuged using an angle rotor at 30000 rev/min for 90min. The empty heads sedimented at the bottom were resuspended and dialyzed overnight against the buffer. The purity of the head specimen is higher than 100:5 as estimated by the head-to-tail ratio in number counted under the electron microscope. Calorimetric studies were done using a heat leakage scanning calorimeter Model SSC-544(Daini-Seikosha Co. Ltd., Tokyo). After samples were packed in a silver sample vessel having a working volume of 70µl, the vessel was tightly sealed to prevent further water evaporation. The temperature scanning was carried out at a constant heating or cooling rate of 0.6°C/min throughout the experiment.

RESULTS AND DISCUSSION Fig.1 shows thermograms obtained with T4-infected cells of E. coli. The thermogram of non-infected cells is given as curve(a), the profile of which varies slightly in the temperature range 50°C to 70°C depending upon the growth conditions used. The main peak at 55°C through 65°C is mainly attributed to ribosomes as will be shown elsewhere in details. The peak at 91°C reflects DNA melting and the two peaks at 100°C and 110°C are supposed to be due to the decomposition of the cell wall components, since these two peaks at higher temperatures were not observed in thermograms of gram-positive bacteria. All these peaks were irreversible in the temperature range from 20°C to 120°C under the scanning conditions used as opposed to the results of Stein (4).

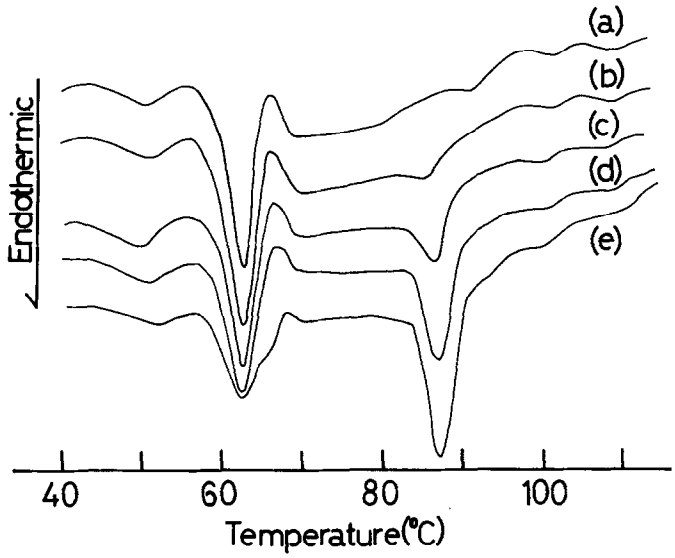

Fig.1 Thermograms of bacteriophage T4-infected permissive E. coli. E. coli B40S(su^+) was infected with an amber mutant 49(am NG727) at a multiplicity of 5 and harvested at 10min(b), 20min(c), 35min(d) and 50min(e), respectively, after infection, while (a) was taken just before infection.

The endothermic peak at 91°C due to melting of the host DNA disappeared in 10min after infection and instead a new broad endothermic peak was observed at 85°C (Fig.1(b)), corresponding to the breakdown of the host DNA and the synthesis of the phage DNA and early proteins.

The next few steps in Fig.1((c) through (e)) show how each component is assembled into normal phage particles to result in the appearance of phage progenies. The endothermic peak at 85°C shifted to 87°C and increased in its sharpness as well as the amount of heat absorbed. It may be concluded T4 phage particles carry out their thermal transition at 87°C in vivo, giving rise to a single endothermic peak on their thermogram. The results shown in Fig.1(b)-(e) were all obtained from the experiment in which a permissive strain of E. coli(su^+) was infected with phage T4 mutant 49(am NG727) which lacks the product of gene 49 required for DNA packaging. Similar results were obtained when a non-permissive strain(su^-) was infected with wild type T4D.

Fig.1 suggests that there exists a difference in the melting mode between non-packaged and packaged DNA molecules in their host bacteria. When the non-

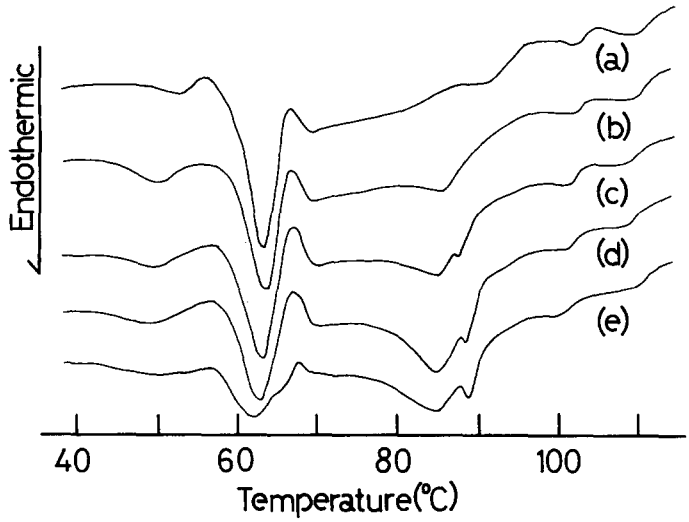

Fig.2 Thermograms of bacteriophage T4-infected non-permissive E. coli. E. coli $B^b(su^-)$ was infected with an amber mutant 49(am NG727) at a multiplicity of 5 and harvested at 10min(b), 20min(c), 35min(d) and 50min(e), respectively, after infection, while (a) was taken just before infection.

permissive strain was infected with 49(am NG727), the endothermic peak at 85°C was kept broad and remained at the same position as expected (Fig.2), indicating the fact that the melting profile of packaged DNA is different from that of non-packaged DNA. The melting of packaged DNA at 87°C seems to take place in a more cooperative way as compared to that of non-packaged one which was observed to occur at 85°C giving rise to a broader profile. The latter temperature is the same as measured by Marmur with DNA of the same origin in SSC buffer (5).

In Fig.2, another small but sharp endothermic peak was found at 89°C ((c), (d) and (e)) which was not observed in the thermograms shown in Fig.1. Preliminary experiments suggested that this endothermic peak was caused by destruction in the head shell structure, which was confirmed by comparing with the thermogram of the isolated empty head shells of T4 phage shown in Fig.3(a) and also by the experiment using a defective mutant 23(am B17) which lacks the major structural protein of the head shell (Fig.3(b)).

Fig.3(c) was obtained using mutant 31(am N54), which made clear that the heat absorbed at 89°C mentioned above is mainly due to the destruction of the head shell structure rather than the denaturation of the structural protein,

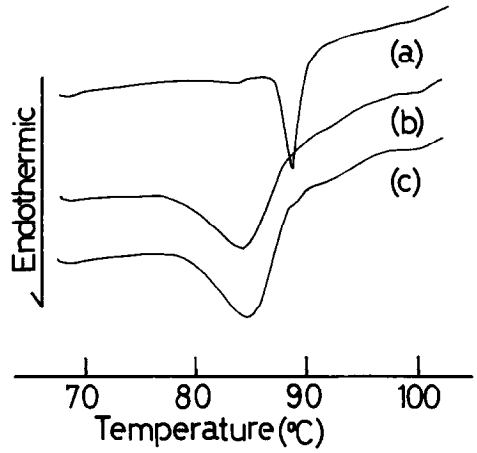

Fig.3 Thermograms related to the head of bacteriophage T4.
(a) The isolated empty head shells of bacteriophage T4.
(b) Amber mutant 23(am B17)-infected E. coli B^b harvested at 50min after infection.
(c) Amber mutant 31(am N54)-infected E. coli B^b harvested at 50min after infection.

because the mutant 31(am N54) lacks the product of gene 31 required for modifying or activating P23 for ordered assembly.

It must be mentioned that T4 DNA is endowed with high reversibility in its thermal transition profile while the head shell is thermally irreversible as to its transition profile. Furthermore, the thermograms described above were all obtained using a constant scanning rate of 0.6°C/min and their profiles depend slightly upon the scanning rate used. It would be possible in the future to obtain some kinetic parameters by studying thermographic data at various different rates of scanning in details.

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