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Pycnometric, viscometric and calorimetric studies of the process to release the double-stranded DNA from the Un bacteriophage

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Abstract

Knowledge of both the packaging of the linear, double-stranded (ds)DNA in bacteriophages and its subsequent release into the bacterial host is vital to our understanding of phage infection. There is now strong evidence that packaging requires a powerful rotary motor fuelled by ATP. From thermodynamic studies, however, it has been proposed that, at least for those viruses with a contractile tail, the dsDNA ejection from the phage head is a relatively simple physical process that does not require cellular energy and is facilitated by the difference in the conditions of the medium in the environments inside and outside the head. In this case, there should be no enthalpic effects associated with the dehiscence of the capsid and no destruction of it or the other structural elements of the phage. For the present study of temperature-induced phage dehiscence, we used a newly discovered phage with a contractile tail, named the Un (unknown) bacteriophage. Evidence is given of its characteristics in terms of ultrastructural morphology, serological parameters, host range and interaction with host cell. These show that, although it has similarities with the T-even phages and, in particular, the DDVI phage, it appears to be a new type. Earlier viscometric studies with it had shown that the temperature-induced release of the capsid dsDNA was completed at 70 °C. In the present investigation, a concentrated suspension of purified phage was subjected to pycnometric analysis through the temperature range of 30 to 70 °C. This showed that a significant and abrupt increase in the phage partial volume takes place, which remarkably is in the order of threefold. Viscometric measurements over time at 72 °C gave a kinetic curve from which evidence it was suggested that the temperature-induced DNA release is similar to a second order phase transition. At the same time, data from differential scanning calorimetry over the same temperature range showed no enthalpic effect. Our results indicate that the ejection of DNA from the capsid tail is driven by an entropy change.

Keywords: Bacteriophage ejection; Double-stranded DNA; Partial volume; Hydrodynamics; Thermodynamics; DSC

1. Introduction

It can be seen in simple representation of a phage in Fig. 1 that the DNA is contained within a protein shell and in contact with the protein subunits. This packaging of the genetic material in icosahedral bacteriophage capsids and its subsequent injection into the host bacteria are intriguing topics of central importance to our understanding of phage infection. From studies on the φ29 bacteriophage, it would appear that a rotary molecular motor within the portal "reels" the genomic double-stranded (ds)DNA (hereafter abbreviated to DNA) into the preformed proheads [1,2]. Although the idea is widely accepted that a translocating ATPase powers the motor at least for bacteriophage T4, evidence has been accumulating to indicate that the large portal terminase protein gp17 with ATPase activity is central to packaging the DNA [3]. The data suggests that this terminase consists of at least two functional domains, an N-terminal DNA-translocating ATPase domain and a C-terminal DNA-cutting domain. Earlier, Smith et al. [4] used optical tweezers to pull on single DNA molecules as they were packaged by the φ29 bacteriophage and thus positively

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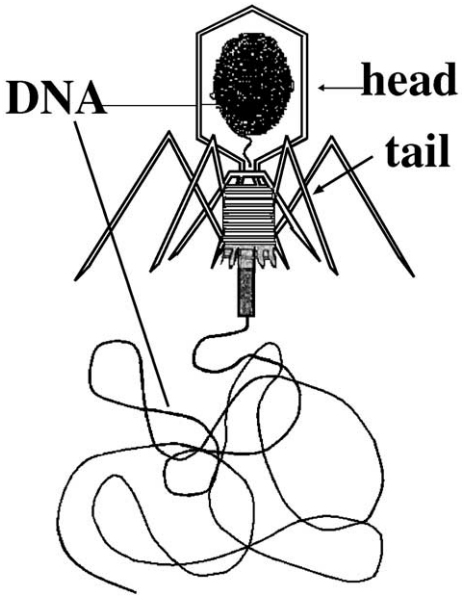

Fig. 1. Diagrammatic representation of the generalised phage of the type with a long contractile tail in the process of ejecting the double-stranded DNA that previously had been wound into the capsid by an ATP-requiring motor.

demonstrated that the portal complex with ATPase activity is the force-generating motor.

There is some evidence that the morphologically reverse process, the ejection of the DNA from the phage capsid to the host bacterial protoplasm, is closely associated with the statistical physics of the macromolecules party to it [5–9]. In contrast to the highly energetic packaging process, these experimental and theoretical studies on a model system of phage suspensions without host cells have shown that the release of the DNA into the phage medium and its subsequent hydration are relatively simple physical processes not requiring metabolic energy. For this reason, it has been called the passive "dehiscence" of the phage to distinguish it from the active cellular process. In various published studies [5,7–9], ejection appeared to be facilitated by differences in the physical–chemical properties of the DNA and the water chemical potential inside and outside the capsid. There is also evidence from studies of DNA release into the phage medium [5,6] and into the host cell [10,11] of a two-phase ejection: first, the phage particle appears to release a few hundred base pairs and, secondly, the rest of the macromolecule is released into the host protoplasm. It is important to note that the conformational changes resulting from the different environments are not accompanied by the destruction of either the capsid or the other structural elements of the phage. At the same time, it should realised that the translocation (as well as the condensation) of the interphage DNA from the phage protein shell into the host cell not only has its obvious biological importance, but it is also a matter of particular interest purely in terms of the physics of the process. This is because it is a rather curious example of the conformational transition of the particular, worm-like DNA molecule of the globule-to-coil type [5,7] along a protein channel with a diameter close to that of the macromolecule (see Fig. 1). In this regard, at least two important questions arise: To what type, in the sense of statistical physics, can this transition be referred? Does the transition include several parallel, compensatory thermal effects? We attempt to answer these matters in this paper.

The first clue to the mechanism for the remarkable phenomenon of phage dehiscence came from electron microscope studies [5,6], in which by chance it was observed that the DNA double helix undergoes a phase transition from the densely packed state of the interphage liquid crystal with the packing density more than 80% into the state of a solution of DNA coils with the persistent length of 55 nm. If this is correct, then it follows that the supposed transition should cause an increase in the phage particle volume or, more precisely, its partial volume. To test this idea, we designed and built a high-precision capillary pycnometer.

As the phage system for this investigation, we chose a type that was discovered only recently, an *Escherichia coli*-specific phage with the provisional name Un [12]. It appeared taxonomically to be in the same morphological group as the well-known T-even phages and, in particular, the bacteriophage DDVI. In the same way as those viruses, Un phage has a long contractile tail (Fig. 1—see [12]). Serological and electron microscope studies presented in this paper indicate that Un should be classified within this group. From the results of earlier viscometric and spectrophotometric analyses [13], it was discovered that the Un phage has one double-stranded DNA molecule with the molecular weight in the order of 10^8 Da, which makes up half the weight of the intact phage. Viscometric analysis also showed that the phage particle dehisces under physiological medium conditions within the temperature range 50 to 70 °C, at which point all the capsid DNA had been released into the medium [12]. At this stage, nothing was known about the kinetics of the conformational transition. In order to establish the nature of this property at constant temperature, we had to upgrade the electronics of a modified Zimm-Crothers-type temperature-scanning rotary viscometer [12].

In order for these studies to have validity, however, it was important to confirm the earlier supposition that there is no enthalpic component to the phase transition [8]. Therefore, the phage suspension was subjected to examination by differential scanning calorimetry (DSC) over the temperature range of the phase transition.

2. Experimental

2.1. Bacteriophage culture and purification

The standard *E. coli* B strain was used as the host for the bacteriophage. It was grown in a strongly aerated 6-l steel Frazer-type fermenter on the M9 synthetic medium at 37 °C and pH 7.0 [14]. Bacterial growth was monitored off-line nephelometrically at 660 nm and by counting colony forming units. Once the latter had reached 5×10^8/ml, the Un phage was inoculated into the bacterial suspension in the fermenter at the

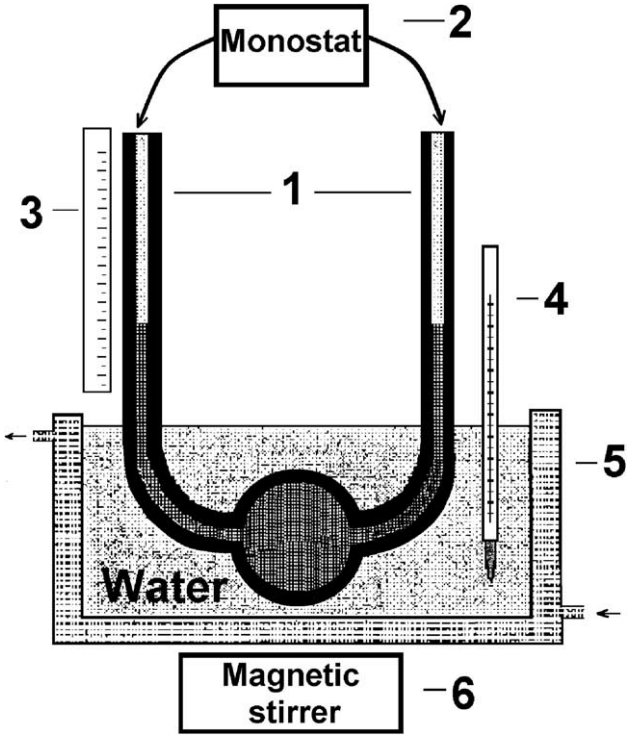

Fig. 2. Schematic representation of the pycnometer: (1) pycnometer consisting of two fine-bore capillaries connected at the base by the reservoir, (2) monostat with a pressure measure range of 1 to +1.5 atm (measurement accuracy is 0.1 atm), (3) micrometer, (4) thermometer, (5) water bath with thermostat and (6) magnetic stirrer set to approximately 60 rev/min. Considering the inaccuracies in obtaining the exact number concentration of phages in suspension, the height of the liquid in the capillaries and the temperature of measurement, the measurement error was <10%.

multiplicity of infection of 0.02. The temperature was then reduced to 32 °C and the OD of the culture monitored as before to record the decrease in the bacterial population. Once the critical point had been reached, aeration was terminated and chloroform to a final concentration of 0.5% (v/v) was added to the culture to cause bacterial lysis. After chilling for 12–16 h, the bacterial debris was removed from the phage lysate by centrifugation at 8000×*g* for 30 min. The supernatant containing the phage with the titre 1×10^{10}–1×10^{11} pfu/ml was then purified and concentrated by ion exchange chromatography on a DEAE cellulose column with elution of Un phage using 0.45 to 0.6 M NaCl. This was followed by further differential centrifugation at 40,000×*g* for 1 h and centrifugation on a CsCl gradient at 60,000×*g* for 3 h. The concentrations of DNA in solution and of the phage suspended in 0.015 M phosphate (PBS) buffer were estimated spectrophotometrically at 260 nm in which 1 μg/ml DNA = 0.023 OD. Protein was determined by the Lowry method.

2.2. Phage characterisation

Serological features of the phage were studied by means of the neutralization reaction with specific antiphage sera (APS) [15]. APS against the T4 and DDVI were prepared by immunization of rabbits [10]. The adsorption characteristics, the one-step growth characteristics of the phage and experiments to describe the characteristics of the interaction of the Un phage with the host cell with were determined as described by [16]. The morphology of the nucleocapsids was studied using an Opton M10 transmission electron microscope (Opton-Carl Zeiss, Oberköchen, Germany). Samples were placed on collodium grids and negatively contrasted by

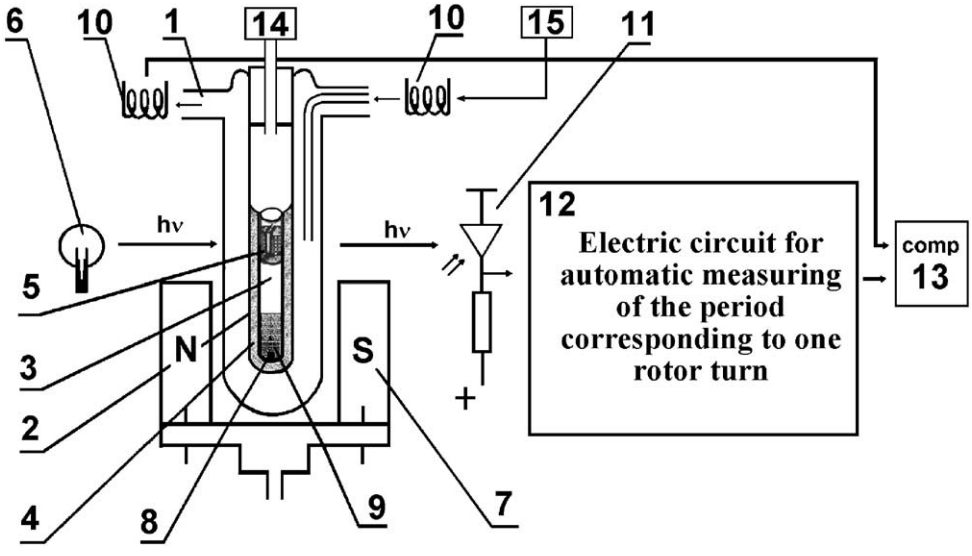

Fig. 3. Schematic diagram of the viscometer designed and built for temperature-dependent studies of the viscosity of phage suspension and subsequently adapted for the kinetic experiments principally by changes to the electrical circuit. (1) Thermostat jacket, (2) stator, (3) rotor, (4) test phage suspension, (5) cover with a slit cut for incident light, (6) light source, (7) rotating magnets, (8) bronze plate, (9) plastic plate with P_2O_5, (10) thermisters, (11) photodiode, (12) electrical circuit, (13) PC, (14) monostat (see Fig. 2 for description) and (15) temperature scanning thermostat. The main technical features of the viscometer are the following: (i) the working volume of the viscometer is 1.2 ml; (ii) the interval of the shift tension is 10^{-4}–$5\cdot10^{-2}$ din/sm^2; (iii) the accuracy of temperature monitoring is 0.1 °C; (iv) the working temperature interval is 0–100 °C; (v) the accuracy of the registration of the separate counts is 0.5%.

2% uranyl acetate [17]. For the morphological measurements, a minimum of 20 intact particles was randomly selected from three electron-micrographs.

2.3. Analytical techniques

The capillary pycnometer was constructed with calibrated capillaries of 0.308 mm diameter. The circuit diagram for the liquid flow of this instrument is shown in Fig. 2. The liquid in the capillaries was maintained at an external pressure of 0.8 Pa in order to eliminate the possibility of bubbles forming in the liquid during heating. The liquid column height in both capillaries was measured using a micrometer. The increase in liquid volume was equal to the sum of volume increases in both capillaries. The height of the liquid column in the capillaries for each temperature rise was measured at strict temperature equilibrium every 15 min up to 70 °C to take due account of the kinetic character of the phage particle destruction. It should be noted that, during calibration experiments, no significant effect of the ambient temperature on the measurement results was observed, meaning that the temperature changes did not affect the apparent diameter of the capillaries.

A diagram for the modified Zimm-Crothers viscometer is shown in Fig. 3. Measurements were made under physiological medium conditions of 0.15 M NaCl in 0.015 M PBS buffer at pH 7 after rapidly heating the phage suspension from ambient temperature to 72 °C (heat shock method) and maintaining it at that temperature for the required period. This value was chosen because the DNA ejection process is completed at this temperature. The meaning of the term “heat shock method” is that the temperature of the phage suspension immediately changes from ambient to that at which there is a full release of the DNA from the phage particle and naturally this process will accompanied by low kinetics. The apparent heat capacity (ΔC) of the phages suspended in ISSC buffer was measured using the capillary DSC instrument, DASM 4A (Puschino, Russia), the measuring vessel of which has an operational volume of 0.46 ml and a temperature range of −10 to +150 °C. The C_p measurement error did not exceed 10%.

All the analytical measurements were made a minimum of five times.

3. Results and discussion

The basic characteristics of the Un bacteriophage are displayed in Table 1. The phage has a wide spectrum of lytic activity towards strains of *E. coli* and the closely related *Shigella* sp. In terms of neutralising the phages with specific antisera, it appeared that the Un phage has surface antigens in common with those of the DDVI and the T4 phages (Table 1). In terms of its interaction with the *E. coli* host-cell, the Un phage revealed features of the what is called the One-Step Growth Cycle [16] characteristic of the virulent phages, that is there was a high adsorption rate, short latent period and a harvest over a series of three experiments of more then 100 particles per host-cell. As part of the comparison with other coliphages, electronmicrographs were taken of the Un phage preparation (Fig. 4). Measurements were taken to show that the elongated head size was an average of 900×650 Å (S.E. ±10 Å), with the tail length at 950×140 Å (S.E.±10 Å). These were compared against the other coliphages (Table 1) and Un phage was found to be in the middle of the three, smaller than DDVI and slightly larger than T4. According to the data for nucleocapsid morphology, Un phage belongs to the family Myoviridae [18], revealing a close relationship to T-even phages and DDVI (Table 1). It is relevant to the discussion about the mechanism of phage dehiscence to draw attention to the fact there appears to be an intermediate stage in the process between the intact phage and the empty capsid clearly seen in the electron micrograph (Fig. 4). On closer inspection it appears that the tail of some phages had contracted to reveal DNA.

The results of the pycnometric measurements to determine any changes in the volume of the phage suspension with increasing temperature over the range from 35 to 70 °C are presented in Fig. 5. At the initial temperature, the liquid column height in both capillaries for the buffer and the phage suspension was taken to be zero. It will be seen that, after reaching the maximum measurement temperature, it was

Table 1
Comparison of the biological properties of the Un phage and some other coliphages

Phage	Host strain	Phage nucleocapsid morphology			Host range as % of lysed strains			Parameters of neutralization (%) with antibodies				Interaction with the host cell [a]			
		Head (nm)	Tail (nm)	Type [b]	*E. coli*	Shigella sonnei	Shigella flexneri	T4-APS		DDVI-APS		Maximum adsorption rate at 37 °C		Latent period (min)	Mean burst size
								%	K^c (min^{-1})	%	K^c (min^{-1})	%	Time (min)		
DDVII	*E. coli* C	55×50	140×15	Syph	45.6	1.6	68.5	0	0	0	0	80	8	20	100
Un	*E. coli* B	90×65	95×14	Myo	72.8	56.8	8.1	8.6	25.9	96.4	79.2	85	6	18	125
DDVI	*E. coli* B	110×75	85×15	Myo	63.2	43.6	6.5	9.4	27.2	100	107.1	82	5	20	110
T4	*E. coli* B	95×65	110×20	Myo	58.1	40.5	4.9	100	114.7	9.7	11.2	86	5	22	115

[a] *E. coli.*
[b] Syph = Syphoviridae, Myo = Myoviridae.
[c] $K = \ln(P_0/P_x)D/t$ where K—neutralization constant, P_0—initial number of phage particles, P_x—number of phage particles after treatment with APS for a set time (x), D—dilution of APS and t is time (min).

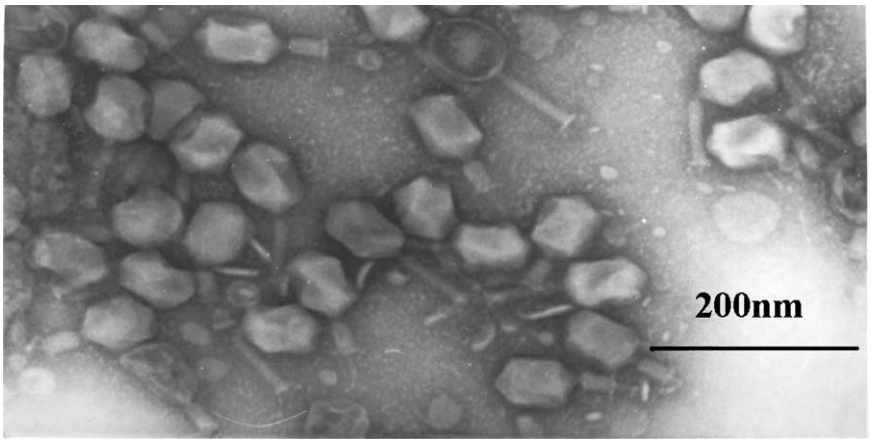

Fig. 4. Electronmicrograph of negatively contrasted Un bacteriophage on a collodium grid. It should be noted that the phages are in three morphological forms: (i) intact phage particle, (ii) phage with contracted tail but with DNA still within the phage head and (iii) phage with contracted tail and no DNA in the head (dehisced).

allowed to return to 35 °C. For the buffer, the height of the liquid column always returned to the initial value (see Fig. 5), which points to the absence of liquid evaporation in the capillary. For the phage solution, the residual column height in the capillary at 35 °C was 27 mm (Fig. 5), with a measurement error of less than 10%. The increase in the solution volume associated with the phage dehiscence can be calculated knowing the capillary diameter (d=0.308 mm) and the residual height of the liquid column for the phage solution (ΔL=27 mm), i.e.

$$\Delta V = (\pi d^2/4)\cdot\Delta L = 2.01 \text{ mm}^3$$

Considering the phage concentration (3.14 mg/ml) and the pycnometer operating volume (0.51 ml), we calculated the weight fraction of the phage that is equivalent to the observed volume change (1.6 mg). Hence, when the DNA had been completely released from the Un phage capsid, the partial volume had increased by 1.26 ml/g, compared with the value for the suspension of intact phages. Allowing for the volume of the intact phages (0.65 ml/g), we obtained the value for the partial volume of the dehisced phages equal to 1.9 ml/g (error <10%).

As mentioned in Section 1, an earlier viscometric study revealed that the viscosity of the phage suspension increased within the temperature range of 50 to 70 °C [12]. Because of the physical nature of the DNA macromolecule, this result pointed to the likelihood that the DNA had been released from the Un phage capsid into the buffer medium. In this paper, we built upon these preliminary findings [12] by using the modified Zimm-Crothers viscometer described in Section 2.3, to show the dependence of the phage suspension viscosity on the elapsed time at 72 °C (Fig. 6). It is reasonable to conclude from the shape of the viscosity curve over the above temperature range that: (i) the DNA had been ejected from the tails of the

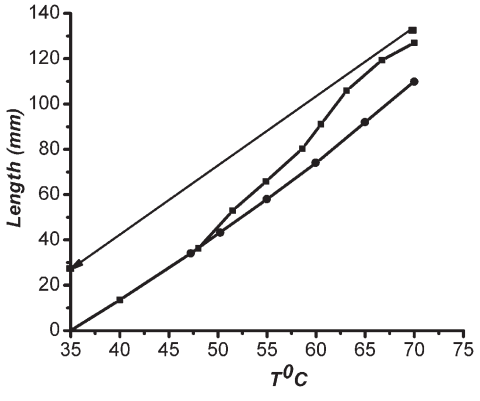

Fig. 5. Dependence of the liquid column height in the pycnometer capillary on the temperature. The control solution is ISSC buffer at pH 7 (●) and the test material is the phage suspended in the same buffer (■).

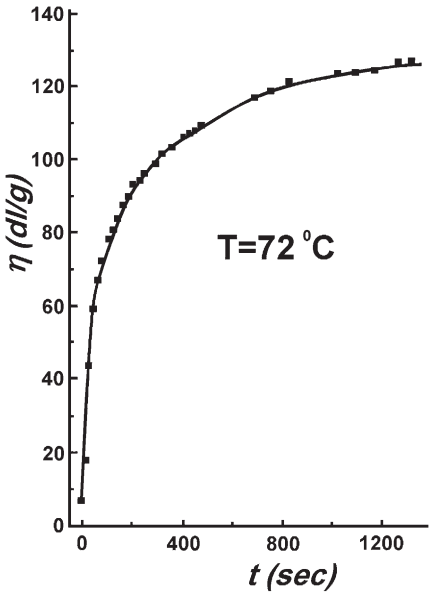

Fig. 6. Kinetic curve of the process of the DNA molecule release from the Un phage head at 72 °C.

phages because the increase of solution viscosity could only be induced by DNA ejection (as also observed in Fig. 4 and in [6]), due to the molecular weight of the dsDNA and its rigidity; and (ii) the DNA ejection is a multistage process. Thus, it is considered that at 35 °C the phage particles are native and intact so the viscosity of the suspension is small, whereas at 70 °C the DNA molecule has completely left the phage head via the narrow "pipe" of the phage tail and is now in the buffer where it causes an increase in viscosity, i.e. the phage has dehisced. This process must be kinetic in nature and thus we can use a kinetic curve to evaluate the time course of phage dehiscence. Because the results indicated that the viscosity of the solution increased to a maximum value within hundreds of seconds, it is possible to conclude that DNA ejection is a rapid and complete process with none left trailing behind to any significant degree.

When discussing these results, it is important to be clear about definitions concerning the "phase transition", sometimes known as "phase conversion". In the general sense, the phrase means the transition of the substance from one phase to another one under changing ambient conditions of temperature, pressure, etc. However, it is possible to distinguish phase transitions of two types. In the first type, an abrupt change in such substance characteristics as density, etc., takes place. It is always the case that heat is produced or absorbed during such a transition involving the mass property. In the second type, the property of a substance, e.g. density, changes continuously with the increasing distance from the transition point. During this transition, there is no release or absorption of heat [18,19]. This is why the DSC results are crucial to our understanding of the phase transition of the phage suspension. According to the DSC results for this suspension (Fig. 7), within the accuracy of the calorimeter, there were no thermal effects of any significance, i.e. no endo- or exothermic changes within the temperature range of 50 to 70 °C when it was known that the phage dehiscence takes place [12]. It should be mentioned that different factors can induce DNA ejection from a phage particle. However, temperature as one of these factors differs in a highly specific way from other factors, for example ions, denaturants, pressure, etc., because it is intensive parameter thermodynamically conjugated with enthalpy, which is extensive parameter. It means that all system changes, dependent on temperature, should be accompanied by a change in its enthalpy. The results of our investigation of the temperature induced ejection process showed that the enthalpy change for DNA ejection is practically (or more precisely "over the very wide range of the instrument") close to zero. This means that the ejection is an athermal process.

Applying the principles of phase transition stated above to the present experiments that consider the DNA ejection from the phage particle, we assume that the intact phage is in suspension and know that the head of it has the densely packed dsDNA molecule with a packing density of ~80% [9,18]. It has been reported that the DNA is in the state of an intermolecular liquid crystal [20]. Thus, the native phage suspension can be described as "one phase". Once the DNA has dehisced from the contractile tail of the protein capsid, the suspension consists of coiled DNA and the empty caspids ("phage ghosts")—this is "the other phase". From this standpoint, it must be concluded that the phage dehiscence during the heating process is similar to the phase transition of the second type. This conclusion follows from the pycnometric, viscometric and calorimetric experiments presented here, which showed that the process is accompanied by a gradual decrease in the phage particle density. In the chosen temperature region, there were no observed exo- or endothermal effects for the Un phage, a finding similar to that reported previously for other long-tailed phages [7–9].

The kinetic evidence from the viscometric experiments (see Fig. 6) indicates that the DNA translocation from the phage particle via the tail pipe with a diameter close to that of the dsDNA (2–4 nm) is rapid, but it does not occur instantaneously. This is because the small clearance would cause the "hydrodynamic deceleration" of the DNA in the phage tail. The pycnometric experiments (see Fig. 5) point to significant

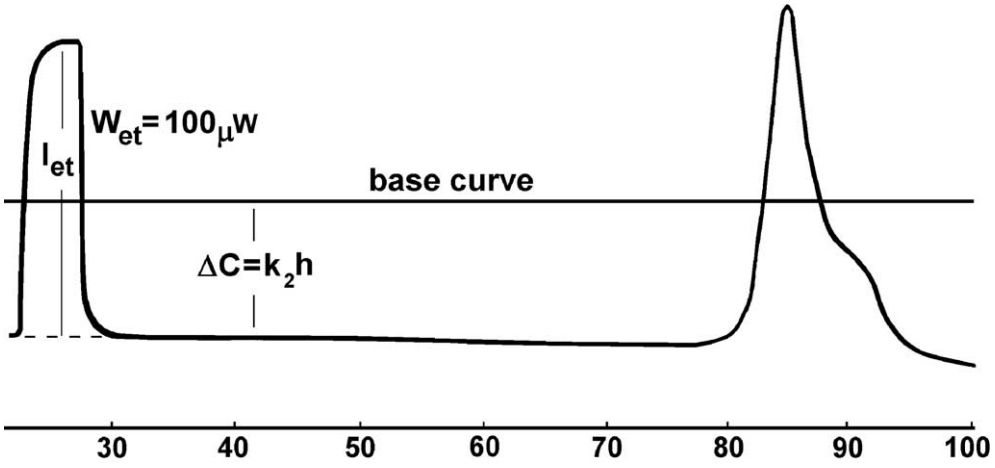

Fig. 7. DSC thermogram of the dependence of the heat capacity of a suspension of Un phages on the temperature (buffer ISSC, pH 7).

changes in density and partial (P) volume ($[V]=1/\rho$, where ρ is the density) [21]. It was calculated that the partial volume for the intact phage (I), $[V]_p^I$ was 0.65 ml/g, whereas that for the dehisced one (D), $[V]_p^D$, was considerably greater at 1.9 ml/g $[V]_p^I[V]_p^D$. A summary of above discussed results as crucial physical parameters of the Un phage is presented in Table 2.

It is known that, from the DSC thermogram showing the difference in the heat capacities between the buffer and the suspension (Fig. 7), the partial heat capacity, C_p, of the material can be calculated at any temperature [22], considering that

$$-\Delta C = C_p^p \cdot m_p - C_p^s \cdot \Delta m_s \quad (1)$$

where $\Delta C = k_2 \cdot h$ is the difference in heat capacities between the solvent and the solution of biomacromolecules; k_2, as the deviation unit value expressed in J/cm·K, is the symbol for the expression; $k_2 = W_{ref}/R - l_{ref}$ where W_{ref} is the calibration power, R is the scanning rate (K/s) and $l_{ref.}$ is the calibration mark deviation from the line; h is the point *deviation* from the base-line at any temperature (Fig. 7); C_p^s and C_p^p are the partial heat capacities of the solvent and the phage, respectively; m_s is the weight of the phages in suspension in the calorimeter vessel of operating volume is 0.46 ml (see Section 2); and Δm_s is the weight of the displaced buffer, expressed by:

$$\Delta m_s = m_p [V]_p^I [V]_p^D \quad (2)$$

where $[V]_p^s$ and $[V]_p^p$ are the partial volumes of the solvent and the phage, respectively, and

$$-\Delta C = C_p^p \cdot m_p - C_p^s \cdot m_p [V]_p^s [V]_p^p \quad (3)$$

As evident from the DSC trace (Fig. 7), within the temperature range of 50 to 70 °C, there was practically no deviation from the horizontal, straight line, which points to the invariability of the differential heat capacity (ΔC) within this temperature range. On the other hand, within this same temperature range, it was shown (see above) that there was almost a threefold increase in the phage partial volume between the value for the intact phage and the dehisced phage. As the phage weight in the calorimeter vessel was always the same in practical terms, $m_p = 0.7$ mg, the invariability of ΔC can be attributed only to the change (increase) in the partial heat capacity of the dehisced phage. Taking into account the fact that the partial heat capacity of the intact phage calculated by Eq. (1) was $C_p^I = 0.7$ J/g·K (up to 50°C, Fig. 7), it is possible to evaluate the

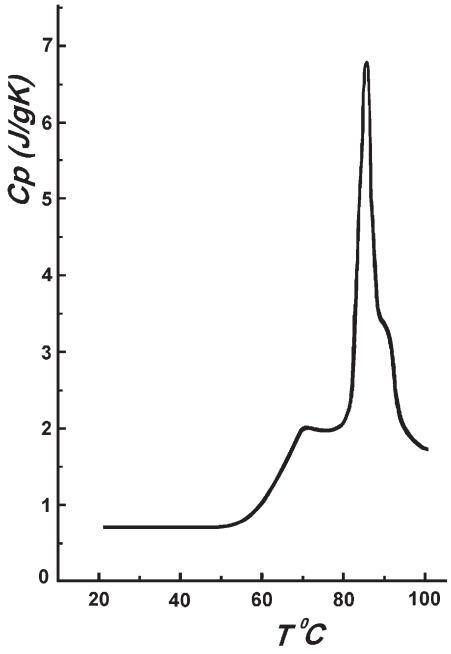

Fig. 8. Model of the dependence of the partial heat capacity of the suspension of the Un phage on the temperature.

partial heat capacity of the dehisced phage by the equation,

$$C_p^D = C_p^I([V]_p^D/[V]_p^I) = 0.7(1.9/0.65) = 2 \text{ J/g K}$$

where C_p^D and C_p^I are the partial heat capacities of dehisced and intact phage, respectively. Thus, it would appear that the temperature-induced phage dehiscence process within the temperature range of 50 to 70 °C was responsible for the threefold increase in the partial heat capacity from $C_p^I = 0.7$ J/g·K for the intact phage to $C_p^D = 2$ J/g·K for the dehisced one, a similar scale to that found for the increase in partial volume. From these results, it was possible to model the expected dependence of the heat capacity of the Un phage on the temperature. As can be seen in Fig. 8, this has the form of a second order phase transition and confirms the conclusions of the pyconometric and viscometric experiments with the phage in suspension.

As stated earlier, one of the characteristics of the second order phase transition is the absence of enthalpic effects. It is also the case that for systems in equilibrium such a phase transition must be completely reversible. Although it has been claimed that the entry of the DNA molecule into the small-diameter pore of the capsid tail is passive [23], there is now strong evidence that the packaging of dsDNA is an energetic event, requiring the activity of an ATP-driven motor [1–3]. The

Table 2
Crucial physical properties of Un phage

Specific volume, native phage (cm^3/g)	Specific volume, denatured phage (cm^3/g)	DNA specific viscosity (dl/g)	DNA molecular weight (10^6) Da	Phage molecular weight (10^6) Da	Native phage partial heat capacity (J/g K)	Specific enthalpy of phage melting (J/g)	Specific enthalpy of phage DNA melting (J/g)
0.65±0.02	1.9±0.1	281±20	100±10	2·100±0.1	0.7±0.1	27±3	34±3

fact that this is a "force-generating motor" was elegantly demonstrated recently by Smith et al. [4] who used optical tweezers to pull on the single dsDNA molecule as it was being packaged into the capsid. They estimated that the total work done to package the φ29 genetic material to near-crystalline density was 7.5×10^{-17} J. Furthermore, it was shown that the rate of packaging decreased as the prohead filled, which indicated that an internal force built up to ~50 pN owing to the confinement of the DNA. Smith et al. [4] also observed the rapid ejection of the DNA from the capsid in less than 0.014 s with no evidence of slower relaxation, meaning that there was little viscous resistance to DNA movements within the capsid. They felt it is possible that a large fraction of the work done by the motor is reversible work and that dissipation is not dominant. This supports the conclusion from the present studies that such a dissipative effect as an enthalpy change does not occur in DNA ejection (see Fig. 7). Non-thermal studies by others have also shown that phages passively, rather than by a direct energetic process, eject their DNA when exposed to the host bacterial receptor [24–26]. However, Smith et al. [4] point out that their results support a model for the φ29 phage in which the internal pressure provides the driving force for DNA injection into the bacterium for *only the first half* of the injection process. More recent studies by Gonzalez-Huici et al. [27] appear to show that injection is a two-step process in this phage in which ~65% of the genome is "pushed" into the cell at a rate slowed by bacterial protein(s) and the rest is "pulled" into the host by at least one of the viral early proteins, p17. It would seem that both steps are energy-dependent because the use of the metabolic poison, azide, overrides the whole mechanism, leading to the deregulated, passive entry of DNA [26]. In contrast, our investigation shows that, for the Un phage at least, there are no energy-dependent stages with the *full* release of the genome from the contractile tail being driven by an entropy change. This is not to deny that dehiscence is a virus-controlled process, only not an enthalpic one.

One is left to speculate on the nature of the initiator for the DNA injection. A clue to the mechanism may come from the fact that pH and/or ionic gradients have a strong influence on translocations involving phase transitions. It is known that the first part of the DNA ejection in phages such as Un with long, contractile tails is for the tail to contract, exposing a relatively small number of the genome's base pairs (see Fig. 4; also [10,11]). Perhaps, it is this contact with a different environment that triggers the complete injection of the phage DNA into the bacterial host in vivo.

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