The influence of pH on thermal and hydrodynamic properties of DDVI phage and on DNA ejection from phage induced by bacterial membrane fragments

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The thermal and hydrodynamic properties of DDVI phage under the influence of pH, which was chosen as an external parameter to be varied, were studied. It was shown that over a wide pH range (pH 5–10) the phage particle conserves its structural organization. The experimental data also show that the DNA leaves the phage head completely. It was found that at pH values outside this range the DNA was not released from the phage capsid into the solvent. The first steps of phage-bacteria interaction (phage adsorption on membrane fragments and DNA ejection from the phage) in a model system consisting of DDVI phage and membrane fragments of its bacterial host cell have been studied. Membrane fragments from *E. coli* with an active receptor system were obtained by ultrasonic disintegration (sonification). Using a viscometric method, DNA ejection from the phage capsid induced by membrane fragments was observed. The viscometric investigations were carried out under alkaline conditions, because in this case less aggregation of the bacterial membrane fragments was observed. It was demonstrated that the specific viscosity of both phage and the membrane fragments is almost zero. The specific viscosity increases with time (i.e. a kinetic process) only in the case of the phage–membrane fragments complex. The kinetic process in turn results in phage adsorption on the bacterial membrane fragments and DNA ejection from the phage into the solvent.

Keywords: adsorption, bacterial membrane fragments, calorimetry, DNA ejection, pH, phage, viscometry

1. INTRODUCTION

It is known that there are specific receptors on the bacterial cell surface on which bacteriophages can adsorb. However, the information about this interaction is rather restricted. The membrane receptors promote only the first reversible stage—adsorption—after which viral genome penetration into the host cell occurs. But which forces transport the genome from the phage into the cell cytoplasm?

Nucleic acid release from the phage head can occur because of the interaction between the virus and the bacterial membrane fragments [1, 2] or with isolated bacterial membrane receptors [3–6]. However, the mechanism of this process, i.e. the basic factors that promote the acceleration or deceleration of this process, has not been adequately studied. Investigations in the field can lead to a solution of this important problem for molecular biology and medicine, and may have an influence on understanding the process of bacterial infection by bacteriophage.

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The present work is dedicated to the biophysical investigation of thermal and hydrodynamic properties of the DDVI phage and the first steps of phage-bacteria interaction (phage adsorption on membrane fragments and DNA ejection from the phage) in a model system consisting of DDVI phage and bacterial membrane fragments. In this study, the membrane fragments with an active receptor system on which the bacteriophage can adsorb and which naturally promote DNA ejection from phage particles were obtained from bacterial cells by an ultrasonic method. Such a model system allows the use of different biophysical methods for the investigation of the first steps of host cell infection by phage.

2. MATERIALS AND METHODS

The bacteriophage DDVI and membrane fragments of *E. coli C* (the host cell for DDVI) were chosen as objects of the investigation. The DDVI phage head size is 1100×860 Å and the size of the tail is 1250 Å. This phage contains ds-DNA, $M_r = 110 \times 10^6$, which is approximately half of the mass of whole phage [7]**.** Phage purification was carried out by centrifugation in a CsCl density gradient. The concentration of phage *qua* DNA was determined

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using a spectrophotometer, noting that an optical density of 0.023 OD at 260 nm corresponds to 1 μg/mL of DNA.

By means of ultrasonic disintegration, membrane fragments from *E. coli C* bacterial cells were obtained. Correct determination of the frequency and energy of disintegration allowed us to obtain membrane fragments with an active receptor system, which is able to adsorb the phage on its surface. For this, 1 mL of bacterial culture (titre 10^9), was diluted by 40 mL of PBS (0.1 M NaCl + 0.05 M phosphate buffer, pH 7) and was disintegrated by ultrasound at 22 kHz, using an USDN-2T disperser (Biological Instrument-Making Office, Pushchino, Russia). The energy of disintegration was chosen to be about 10 W. Disruption of the bacterial suspension was carried out intermittently; the tube of bacterial culture was placed in an ice bath and sonicated 7 times for 1 minute at 1 minute intervals.

For the purification of bacterial membrane fragments, an CP2-25 ultracentrifuge (Factory of Biological Equipment, Lvov, Ukraine) was used**.** To separate the big parts of bacteria and some unbroken bacteria, the cells were centrifuged for 30 min at 2800 *g*. In order to separate the cell membrane fragments from the other contents of the bacterial cell, the supernatant was diluted by PBS up to 100 mL and then again centrifuged for 1 h 30 min at 25 000 *g*. This process was carried out three times.

Determination of the hydrodynamic properties of the DDVI phage and the interaction of the membrane fragments with DDVI phage were carried out using a Zimm-Crothers type viscometer (constructed in our laboratory) with automatic recording of rotation [8].

The thermal properties of DDVI phage were studied by means of microcalorimetry using a DASM 4A microcalorimeter (Biological Instrument-Making Office, Pushchino, Russia).

3. RESULTS AND DISCUSSION

Calorimetry is the best method for evaluation of the power of the very complicated, and not fully understood, thermally induced DNA ejection process from the phage particle. The ability of denaturated phage particles to renaturate their structure or their components (DNA, protein) was also studied.

The dependence of the specific heat capacity on temperature at pH 8 is given in Fig. 1(a). As can be seen from the Figure, the curve is characterized by a sharp heat absorption peak in the temperature interval (66–90 °C). It is well known that one of the properties of ds-DNA is that thermally denaturated DNA (under so-called soft physiological conditions) is able to renaturate its structure upon cooling the solution. Exactly this feature of DNA can be used in analysing the complicated calorimetric curve to determine the temperature interval in which DNA melts. To establish that this peak (Fig. 1(a)) indeed corresponds to DNA denaturation, the experiment was carried out in a cyclic régime (heating-cooling-heating). In comparison with the results of the viscometric experiment (Fig. 1(b)) carried out for DDVI phage under the same conditions, there is no heat absorbance peak in the calorimetric curve in the temperature region in which the DNA is released from the phage capsid into the solvent.

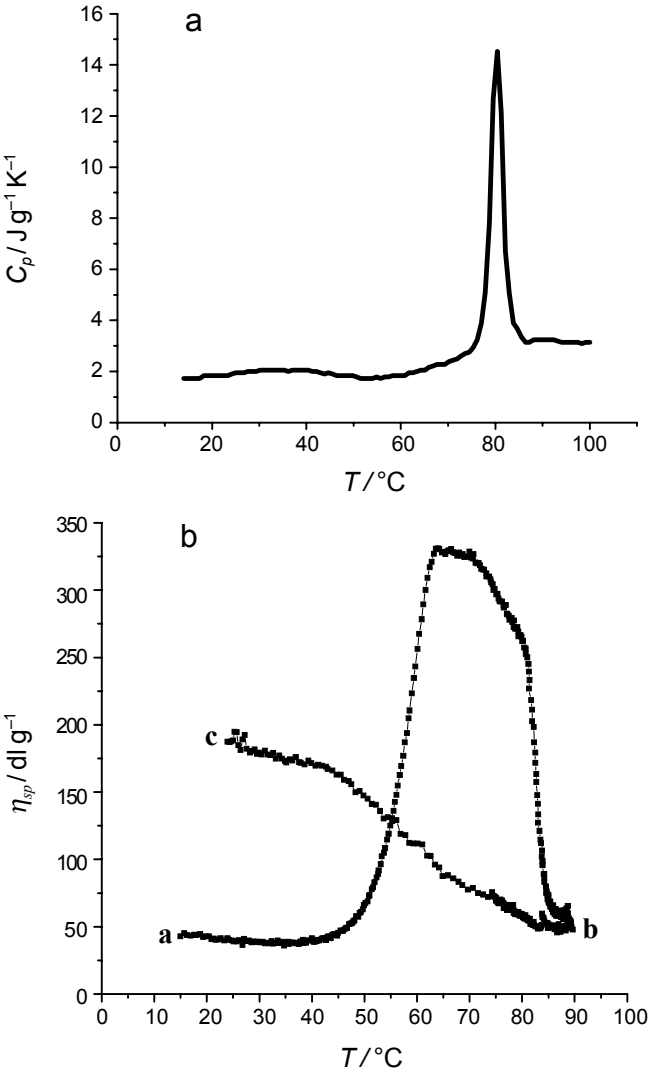

Figure 1. (a): dependence of the specific heat capacity of the DDVI phage on temperature at pH 8. (b): curve ab, dependence of the specific viscosity of the DDVI phage on temperature at pH 8; curve bc, renaturation of denaturated phage DNA during cooling the DDVI phage solution. The phage DNA concentration in the calorimetric experiment was 1.6 mg/mL and in the viscometric experiment was 0.5 mg/mL.

One of the methods by which phage DNA ejection in solution can be observed is viscometry. This method indicates DNA release from the phage capsid into the environment (solution) as a function of the suspension viscosity and time.

The dependence of the specific viscosity of a DDVI phage suspension on temperature at pH 8 is given in Fig. 1(b). The specific viscosity sharply increases in the temperature interval 45–63 °C. Thus the DNA molecule is released from the phage into the solvent (the DNA molecule is transfrmed from its compact state inside the phage head to the coiled state in the solvent). This process is accompanied by energy release. The decrease of specific viscosity in the temperature interval 63–90 °C (Fig.1(b)) indicates DNA denaturation. The subsequent cooling of the solution in the cycling régime leads to an insignificant increase of specific viscosity, which indicates that the phage DNA partially renaturates its structure.

Similar calorimetric and viscometric experiments were carried out at other pH values, acidic (pH 3–7) and alkaline medium (pH 7–12).

The dependences of the specific heat capacity of DDVI phage suspension on temperature at pH 3–8 are given in Fig. 2. As is evident from the Figure, the maximum temperatures of the heat absorption peaks increase with increasing pH. It should be noted that under acidic conditions, i.e. at pH 3 and at pH 4, the heat absorption peak is not observed.

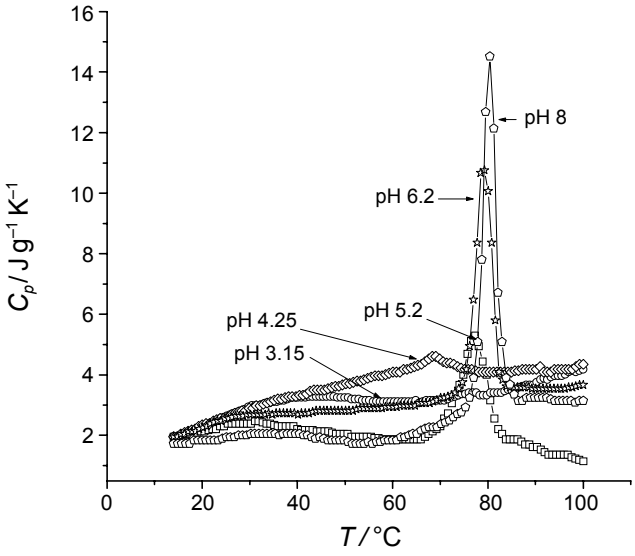

Figure 2. Dependence of the specific heat capacity of the DDVI phage on temperature at various pH values. The phage concentration varied from 1 to 1.6 mg/mL.

The dependence of the specific heat capacity of DDVI phage suspension on temperature at pH 7–12 is given in Fig. 3. In this case, the maximum temperatures of the heat absorption peaks decrease with increasing pH values (Fig. 3). At pH 12, i.e. in a highly alkaline medium, no heat absorption peak is observed. The dependences of pH values on specific enthalpy (a) and on the maximum temperature of the heat absorption peaks (b) are given in Fig. 4. These results indicate that in the region of pH 8, the

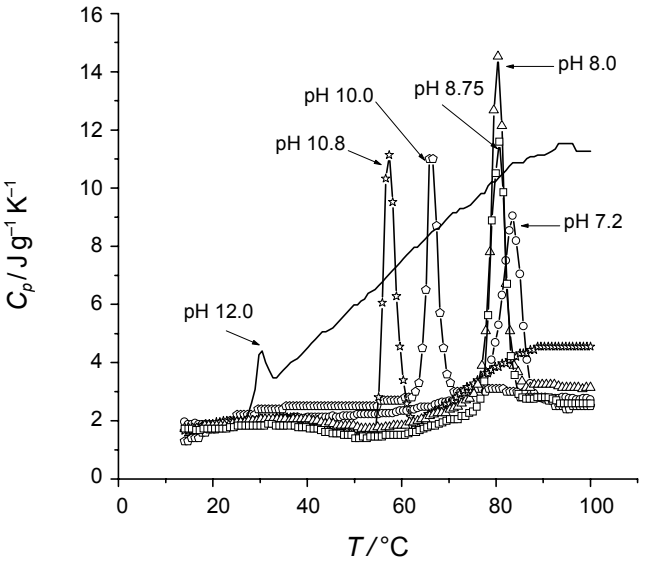

Figure 3. Dependence of the specific heat capacity of the DDVI phage on temperature from pH 8 to pH 12. The phage concentration varied from 1 to 1.6 mg/mL.

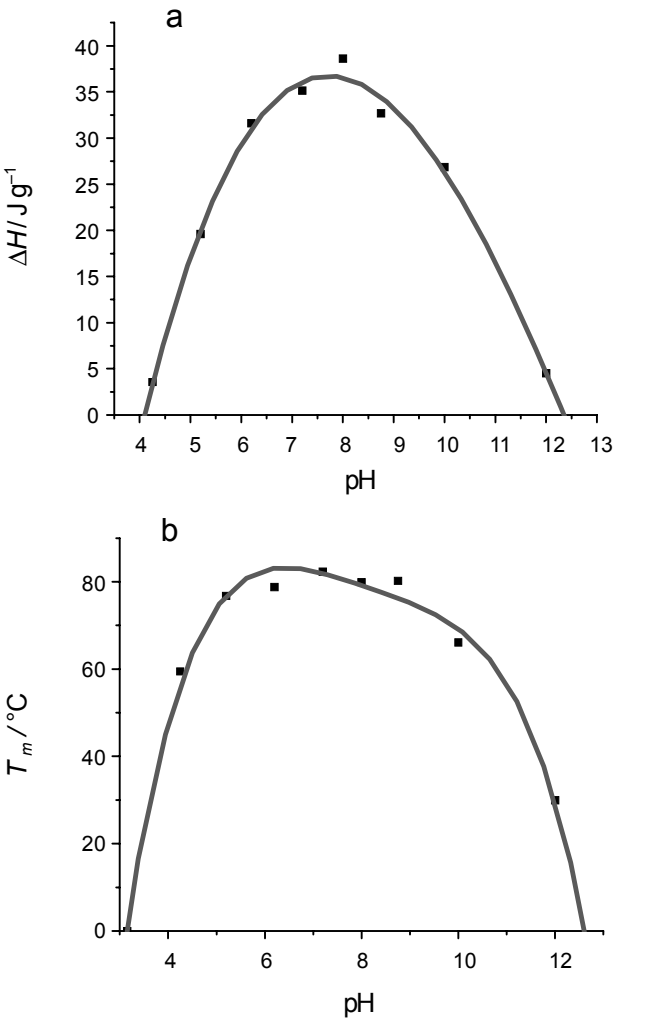

Figure 4. (a): dependence of specific enthalpy of phage denaturation on pH. (b): dependence of maximum temperature of heat absorption peaks on pH.

phage maximally conserves thermal stability. Thus the results of these calorimetric experiments show that pH as an external parameter has a significant influence on the thermal properties of DDVI phage.

The results of the viscometric study of the influence of pH on the hydrodynamic properties of DDVI phage also show that the pH of the environment plays a significant rôle. Plots of the specific viscosity of the DDVI phage suspension versus temperature at pH 5–10 are given in Fig. 5. We assume that both in an alkaline and in a slightly acidic medium (pH 5.6) the DNA completely leaves the phage capsid. The correlation between the high values of maximum specific viscosity at pH 5.6 (in comparison with other pH values) and rapid heat absorption is observed. As is evident from the figure, the specific viscosity of the phage suspension at pH 10 is shifted toward low temperatures. Let us consider this result in a greater detail. We suppose that it is related to features of the isoelectric points of the phage proteins, which are at low pH for almost all phages. In a highly alkaline medium (pH 10) the heads are negatively charged, and a strong repulsive force can be created between these proteins and and phosphate groups of DNA. However, we have one more assumption, namely that the effect of a highly alkaline medium on the structure of the basal plate of the phage is to lead to the opening of the tail channel at lower temperatures and to DNA release from the phage head.

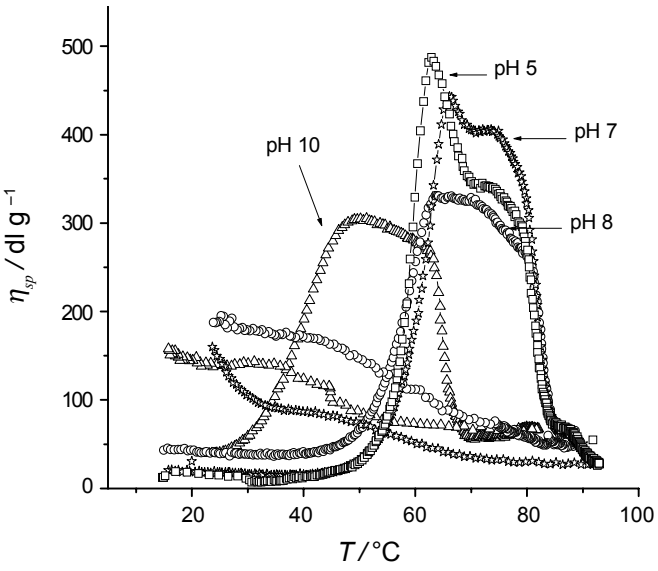

Figure 5. Dependence of the specific viscosity of DDVI phage on temperature at pH 5.6, pH 8 and pH 10. Renaturation of denaturated phage DNA occurs for above mentioned cases. The phage DNA concentration in all cases was 0.5 mg/mL.

Viscometric investigations at constant temperature (37 °C) and pH 8 were carried out to determine whether phage adsorbs on membrane fragments of bacteria and the next step, DNA ejection from the phage capsid, occurres. This pH was chosen in consideration of the tendency of membrane fragments to aggregate, which decreases with an increase of pH of the solvent. At pH 8 least aggregation was observed. This pH value is the most acceptable because we suggest that at pH higher than 8 the membrane protein receptors can be denaturated. The dependence of the specific viscosity of the phage-bacterial membrane fragments complex on time is given in Fig. 6. The difference between the curves (a and b) is that in (a), the bacterial membrane fragments, obtained by sonication, were measured first, and then after a few minutes the DDVI phage was added to the suspension of membrane fragments; and in the other case (b), the phage suspension was measured first and then the bacterial membrane fragments were added. The final concentration of phage was 100 μg/mL and the final ratio of phage/membrane fragments was 10 : 1 (by absorption at 260 nm). The value of the specific viscosity of a suspension of both bacterial membrane fragments and phage suspension separately is almost zero (Fig. 6). A sharp increase in viscosity occurs, which is typical of the DNA ejection process from the phage capsid in solvent (Fig. 6), the increase is more pronounced in the case of the phage-bacterial membrane fragments complex.

The shape of the curves is very similar, but the difference between them is probably due to a slight aggregation of membrane fragments (Fig. 6, curve b). The aggregation occurs because the membrane fragments in the second case were used several hours after purification, whereas in the first experiment (Fig. 6, curve a) they were used immediately. The aggregation leads to a decrease of open adsorption receptors and reduces phage attachment.

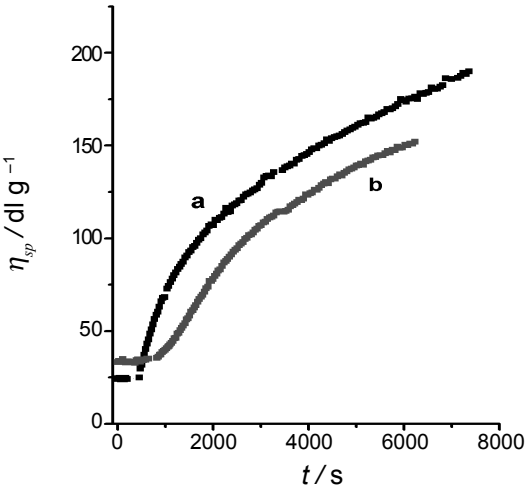

Figure 6. Dependence of the specific viscosity of DDVI phage-bacterial membrane fragments complex on time at 37 °C. The solvent was PBS (0.38 M NaCl + 0.03 M phosphate buffer), pH 8. (a): for DDVI phage added to bacterial membrane fragments; (b): for bacterial membrane fragments added to DDVI phage.

4. CONCLUSION

The results of these experiments show that the phage DNA ejection process occurs only for the phage membrane fragments complex, which implies phage adsorption on the bacterial membrane fragments.

The results of our research show that pH as an external parameter has a significant influence both on the thermal and hydrodynamic properties of DDVI phage. The results of calorimetric investigations show that at pH 8, the phage DNA maximally conserves thermal stability. We assume that both in alkaline and in slightly acidic media (pH 5.6) the DNA completely leaves the phage capsid. In a more acidic medium (pH 4), the phage DNA remains in the phage capsid.

Finally, from our experimental results we conclude that the initiation of the DNA ejection process from the phage particle requires no additional energy, neither in physical (for example temperature) nor chemical (for example an ATP molecule) form. The energy necessary for the transfer of genetic material from the phage capsid to the host cell is transferred to the phage particle during its assembly process in the host cell. It is conjectured that this "surplus" energy of the phage arises as part of the structural organization of the phage genome inside the phage head.

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