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DIFFERENTIAL SCANNING CALORIMETRY AND HYDRODYNAMIC STUDY OF BACTERIAL VIRUSES
Effect of solution conditions

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Abstract

This paper deals with the study of the V-group, family T-even, *E. Coli bacteria phage* (named *unphage*). According to electron microscopic pictures, the geometrical parameters of this phage are 750×560 Å (from head) and 900 Å (from tail).

Bacterial viruses genome – ds-DNA – expulsion from the phage capsid is induced by temperature and is not accompanied by heat effects (temperature interval 45–75°C). Thus the temperature induced *ejection* of genetic material from phages is predominantly entropic. ds-DNA output from the capsid increases the viscosity of the phage suspension at least 100 times. ds-DNA output from the capsid is accompanied by a significant change of partial volume. The disruption of 1 mg of phage produces $\Delta V=1.3\times10^{-9}$ m^3 which corresponds to a volume increase of 200%. This produces exothermic heat effects in closed calorimetric cells, with free volume above the measured liquid.

The contraction of the tail of phage plays an important role in the injection, in the step where phage attaches the outer membrane of the host cell. The main factors of the DNA condensation and packaging in the virus head, and after its ejection through the hole with a diameter close to that of ds-DNA, are caused by the surrounding solution *quality*, so-called *hydration forces* between ds-DNA parallel packaged segments and more exactly by the difference of this parameter inside and outside the capsid of the phage.

Keywords: DSC, ds-DNA ejection, long-tail phage, partial volume, thermal properties, viscosity

Introduction

It is known that the bacterial viruses (bacteriophages) represent very well ordered nucleoproteic particles containing a protein shell (capsid) and phages genetic material – nucleic acid (DNA), as the internal part of the head [1]. This icosahedral geom-

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etry head has a tail, composed also from proteins, which has the function of injecting genetic material of the viruses into the host cell [1] (Fig. 1).

ds-DNA inside the bacteriophage capsid is in a tightly packed state [2–8]. Recent investigations [8] have indicated that the state of DNA compaction in the viral particles should correspond to a three-dimensional hexagonal crystalline phase of DNA, predicting the viability of such a model for other phage heads.

In agreement with the theoretical model [9, 10] ds-DNA inside the capsid is located in the spherical cavity in a globular state and expresses liquid crystal order, interacts with coat proteins and can be *ejected* in the surrounding aqueous medium through a hole with a diameter close to that of DNA. DNA remaining in the capsid keeps the globular state, whereas DNA duplex leaving the protein shell is in the coil state. Accordingly, DNA gradual ejection from the phage head can be considered as a spent globule to coil transition [11–13].

We have studied the specific phase transition in the heated phage suspension (modelling the process of injection of genetic material into the cell): the transition of interphage ds-DNA from the state of intramolecular liquid crystal to the coiled state ds-DNA [10–14]. The other transitions represent the thermal denaturation of coat proteins and helix-coil transition in the DNA double helix [10–13].

The aims of this paper are: 1. to study the cooperative conformational reorganisations taking place in long tail *unphage* (Fig. 1) phage suspensions (at different pH and ionic strength), during temperature change, including thermoinduced ds-DNA ejection from the phage capsid, using DSC; 2. detailed hydrodynamic analysis of suspensions in the temperature intervals of the detected transitions; 3. to study the process of renaturation of genomic molecule upon cooling in the presence of phage proteins, using DSC.

Experimental

Materials

The bacteriophage we have used is V-group, family T-even, *E. Coli bacteria phage*. It was grown in a glass fermenter (capacity 10 litres) as described earlier [12]. According to electron microscopic pictures, the geometrical parameters of this phage are 750×560 Å (from head) and 900 Å (from tail) (Fig. 1). Because the morphological parameters of this phage are different from other phages of this family, including DDVI phage, it was named *unknown phage – unphage*. Concentration and purification were carried out on DEAE cellulose and with use of differential centrifugation. The highly purified phage suspensions were obtained by additional ultracentrifugation in a CsCl density gradient, followed by dialysis. According to spectrophotometric and hydrodynamic results, DNA content is 50% in the form of double stranded molecule with $MW=110\times10^6$ D. Concentration of DNA in solution and phages in suspensions were determined spectrophotometrically (1 mg DNA in 1 mL=0.018 OD and 0.024 respectively; OD is optical density). Protein content was determined by the Lowry method.

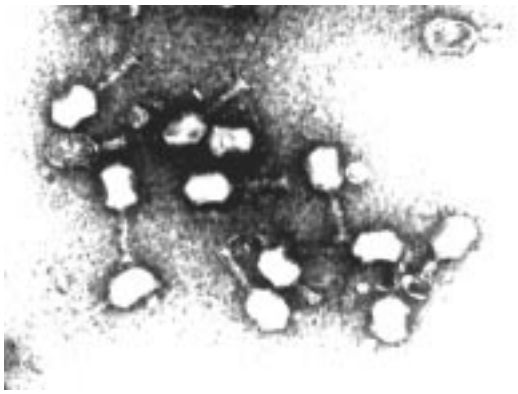

Fig. 1 Electron micrograph of *unphage* bacteriophage. Magnification 176 000×

Methods

The apparent heat capacity (C_p) measurements were carried out using the capillary differential scanning calorimeters DASM-4 and DASM-4A (Pushchino, Russia) [15] with an operational volume of the calorimetric cells of 0.6 mL and an operational temperature range of –10 to +120°C. The C_p measurement error did not exceed 1–2%. The study of the thermal effects in the process of heating and cooling of phage suspensions was carried out using SETARAM micro DSC III calorimeter. Sealed vessels with capacity of 1 mL have been used. In both cases, a scanning rate of 1 K min^{-1} has been used.

Phage suspension rheological features have been studied with a temperature scanning rotation viscometer of the Zimm–Crothers type. Continuous heating is achieved by using a circulating bath with different heating rates. A PC processes the values of viscosity in real time through a home built interface and calculates the values and temperature dependence of the specific viscosity: $\eta_{sp}=\eta(\eta-\eta_0)/c\eta_0$, where η is the viscosity of solution, η_0 is the viscosity of the solvent and c is the concentration. The working volume of the viscometer is 0.6 mL, the temperature is measured with an accuracy of ±0.2 K and the error of the specific viscosity does not exceed ±5% [13].

Results and discussion

Figure 2a shows the specific heat capacity *vs*. temperature curves for the *unphage* suspensions at different values of pH. The measurements were performed under an excess constant pressure (up to $1.5\cdot10^5$ Pa) to prevent formation of gas bubbles and boiling of aqueous solutions above 100°C. For all ranges of pH studied, only one intense heat absorption peak is observed, in the temperature range 80–100°C, corresponding to the nucleoproteic (DNA+phage proteins) structural changes. An insignificant heat absorption (~60°C) for pH=6.0 and a heat capacity increase (~64°C) for pH=7.0 are also detected (see below). The character of the temperature dependence of the heat capacity, the width of the transition and the intensity and maximum posi-

tion of heat absorption peaks strictly depend on pH. It can also be seen that heat absorption peaks are asymmetric and reveal the complicated character of the transition. This transition includes the denaturation of proteins and melting of ds-DNA. The second scan (after cooling of denatured suspensions) reveals only the helix-coil transitions of DNA in the presence of denatured proteins (Fig. 2b).

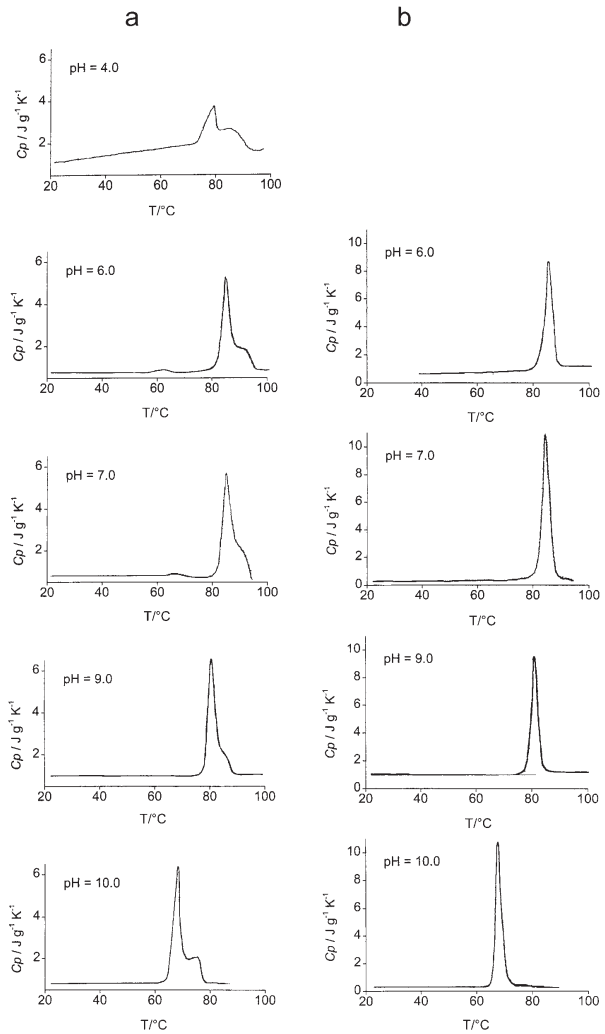

Fig. 2 Temperature dependencies of the heat capacity (C_p) for aqueous suspensions of *unphage* bacteriophage at different pH (a); Repetitive heating after cooling the denatured phage at different pH (b)

In order to demonstrate the influence of pH on the character of whole phages and DNA denaturation, Fig. 3 presents the temperature and the enthalpy of melting of

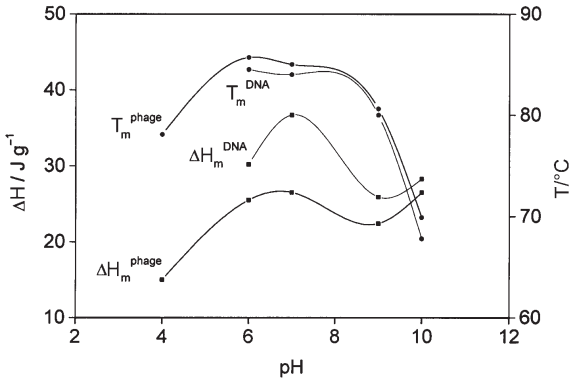

Fig. 3 Effect of pH on the enthalpy and on the temperature of transition for whole phages and for renaturated DNA

phages and DNA *vs.* pH. Analysis of the observed heat effects shows that the pH dependence of the total denaturation enthalpy and of the temperature of transition for whole phages is similar to that observed for isolated DNA in dilute solutions [16]. So the genomic molecule dictates the response of whole phages to the changes in the physicochemical state of the surrounding aqueous media.

The results obtained using the capillary DSC, performed under an excess constant pressure, show the absence of any thermal effect in the phage suspension at temperatures less than 75°C (in the interval 45–75°C). However, the phage suspension viscosity increases abruptly in the temperature interval 45–75°C (Fig. 4). The thermal effect observed above the given temperature interval corresponds to phage genome release according to [11–14]. It should also be noted that a modification of the virus particle takes place (e.g. structural changes in the basal plate) before DNA ejection process starts. Only a small endothermic heat effect for pH=6.0 (~ 60°C) and a heat capacity increase (~ 64°C) for pH=7.0 are detected. This probably corresponds to changes of the coat protein hydrophobic group – water interactions, because when part of ds-DNA is released from the phage head (~ 60°C) water diffuses to the interior of the head and occupies the free volume. So, according to capillary DSC data, ds-DNA expulsion from the capsid, induced by temperature, is not accompanied by any significant heat effect over the temperature interval 45–75°C.

Upon ds-DNA ejection from the capsid (45–75°C), the viscosity of the suspension increases at least 100 times. (See also [13] and the pioneering work of Rialdi and coworkers [14]). The helix-coil transition in DNA (85–95°C) is naturally accompanied by a sharp decrease in viscosity (Fig. 4).

The thermal properties of *unphage* suspensions have been studied using a SETARAM micro DSC III, in the same temperature region. Figure 5 presents the heat effects detected upon heating of whole *unphage* suspensions (Fig. 5a) and upon repeated heating of denatured phages–DNA melting (Fig. 5b) at different ionic strengths. Figure 5a shows the heat absorption peak corresponding to the denaturation of proteins and melting of ds-DNA, which occurs in the interval of temperature

85–95°C. Its asymmetric shape reveals the complicated character of the transition. Increasing the ionic strength, the temperature of transition increases and the separation of the processes of thermal denaturation of double helical DNA and proteins becomes more clear. The second scan (after cooling) of denaturated suspensions corresponds only to the helix-coil transitions of DNA in the presence of denaturated proteins (Fig. 5b). Increasing the ionic strength, the heat absorption peak becomes sharper and the temperature of transition also increases.

So, the heat effects detected by both calorimetric devices are identical: high temperature absorption peak for whole phages and a sharp transition peak of DNA melt-

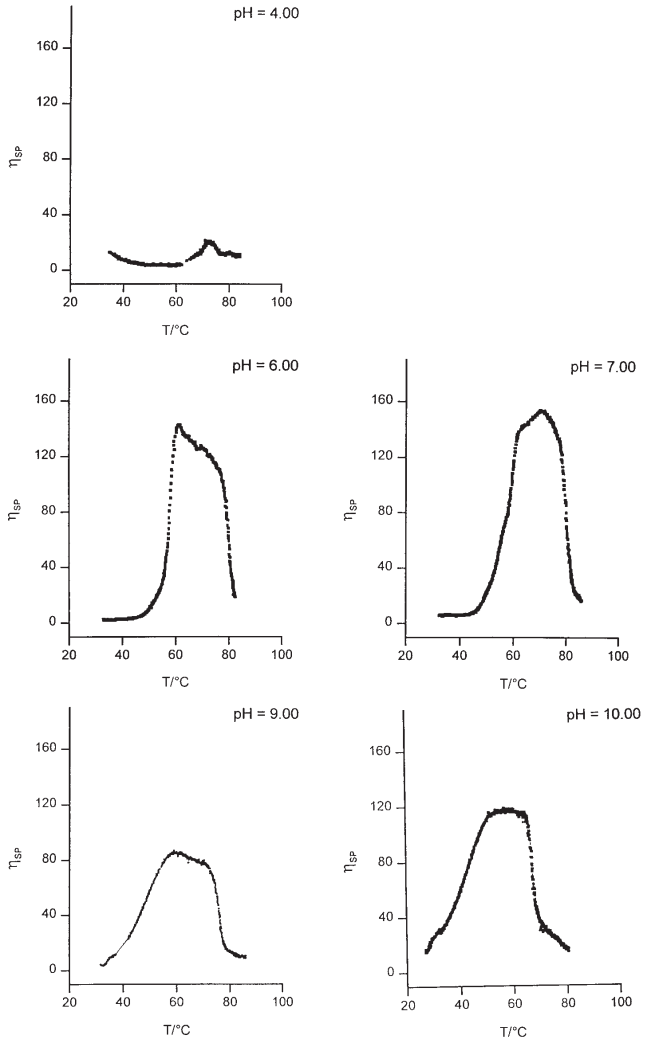

Fig. 4 Effect of pH on the specific viscosity of the phage suspensions as a function of temperature

ing upon second heating scan. However, in the sealed vessel, with free volume above the measured liquid, an exothermic heat effect is detected, exactly in the same temperature region (45–70°C) where ds-DNA release from the phage capsid takes place.

To solve this disagreement, the analysis in the accompanying paper [17] will show that ds-DNA output from the capsid is accompanied by a significant change of partial volume. Disruption of 1 mg of phages produces ΔV=1.26·10^{-9} m^3 [18] – the partial volume of the disrupted phages becomes 1.91 mL g^{-1} while it is 0.65 mL g^{-1} for the native phage, corresponding to an increase in the partial volume of phages of 200% [17, 18]. This produces exothermic heat effects, in closed calorimetric cells

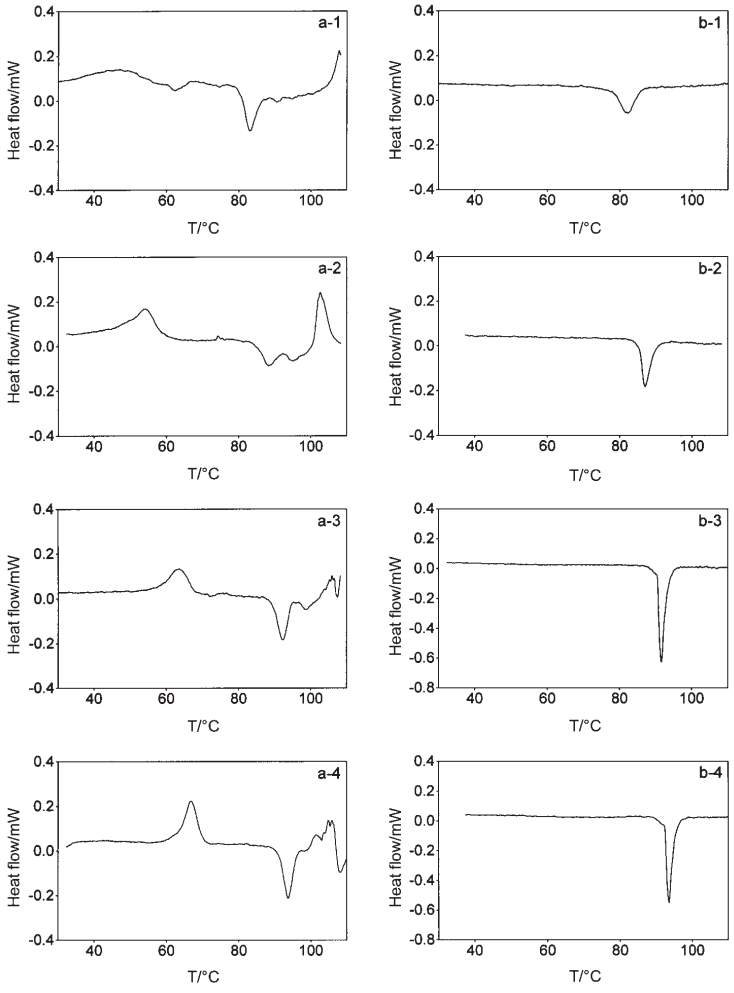

Fig. 5 Effect of the ionic strength of the suspensions of phages on the heat flow as a function of temperature for the first a – and the second b – heating scans. Scanning rate=1 K min^{-1}. Sodium citrate buffer, pH=7.0. NaCl=0.075 mol dm^{-3} (1), 0.15 mol dm^{-3} (2), 0.30 mol dm^{-3} (3), 0.45 mol dm^{-3} (4)

with free volume above the measured liquid: 1. heat effect originated by increment of volume and 2. heat effect resulting from condensation of saturating water vapor [17].

We tried to characterize this *imaginary* heat effect in two different ways: 1. changing the concentration of phages (at constant volume) and 2. changing the free volume above the suspension of phages (at constant concentration). In the latter case, the heat effect should increase with the decrease of free volume above the measured solution and vice versa: decreasing the volume of measured solution below a critical volume, the heat effect should disappear. Experimental results are in full agreement with this prediction (Fig. 6).

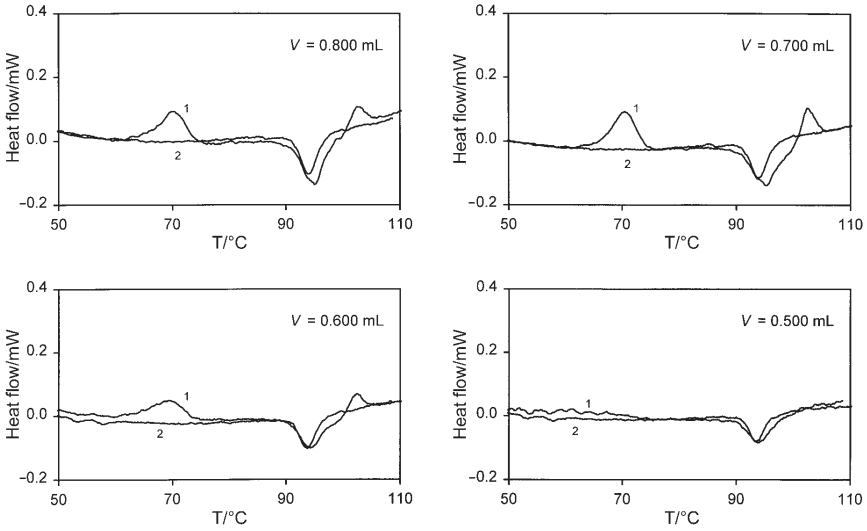

Fig. 6 Effect of the free volume above the solution of phages in the closed calorimetric cell on the heat flow as a function of temperature for the first (1) and the second (2) heating scans (scanning rate=1 K min^{-1}). The total volume of suspension in the calorimetric cell, 1 mL capacity, is indicated in the figures

Therefore, the temperature induced *ejection* of genetic material from phages is entropic. It is possible that this process consists of one or more steps which thermal effects have opposite signs. In either case, Nature selects a way with minimal energetic cost for injection of the genetic material of viruses into the cell [11–13].

The next important question is the possibility of detecting the heat of renaturation of DNA double helix upon cooling of denatured suspensions of whole phages and renaturation of DNA after the second scan, when only DNA melting occurs (proteins are irreversibly denatured). Figure 7 represents results of such experiments.

DSC results of the process of renaturation of phage DNA upon cooling show that in the presence of phage proteins DNA double helix renaturates practically completely and after the first heating of whole phages the percentage of renaturation is 70%. Denaturation of proteins do not influence this percentage of reversibility following heating and cooling. This means that there are strong interactions between

some genome sites and some proteins aminoacid sequences inside the phage capsid; these interactions do not influence the quantity of heat release in the process of renaturation of DNA upon cooling. The shift in the temperature of the maximum and the apparent broadening of the peak observed upon cooling are caused by the kinetics of rewinding of DNA duplex in the presence of phage proteins.

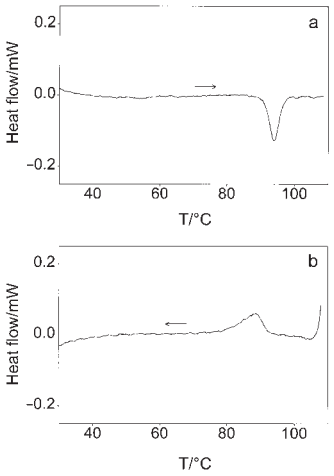

Fig. 7 Heat flow as a function of temperature for the renaturated DNA at the heating (a) and cooling (b) scans. Scanning rate=1 K min^{-1}

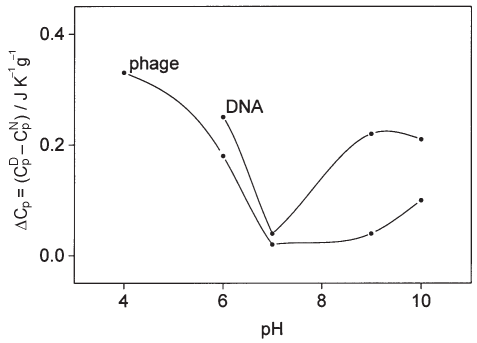

Fig. 8 Effect of pH on the denaturational heat capacity increment for the whole phages and for the renaturated DNA

It is worth mentioning that there is a heat capacity increase, ΔC_p, associated with both whole phages and DNA melting. Moreover, the dependence of ΔC_p with pH is similar for whole phages and DNA (Fig. 8), similarly to what is observed for the pH dependence of the enthalpy of transition and the temperature of transition (Fig. 3).

It has been shown [19] that a parameter that characterizes the strength of heat capacity effects on thermodynamics of melting is $\alpha=\Delta C_p/\Delta S$ – the ratio of change in heat capacity to the change in entropy of melting. The maximum experimental values

of α for phages and for DNA in the presence of proteins are 2.6–3.0 (for pure DNA α=2–4) and this value strictly depends on pH. At neutral pH, α=0 (entropy–enthalpy compensation). At the same time individual enthalpy and entropy components experience significant variation with solution condition (pH, ionic strength) which are entirely due to the shift of its melting temperature [19].

Experimental results of the temperature induced phage DNA injection and the assumed statistic-mechanical model of DNA packaging and *creeping out* from phage [9, 10] suggest that the physical state of the surrounding medium and that of the filled capsid is the dominant factor making the nucleic acid to leave the coat protein shell. The contraction of the tail of phages plays an important role in the ejection but probably only in the step where the phage attaches the outer membrane of the host cell.

Conclusions

The main factors of the DNA condensation and packaging in the virus head and after its ejection through the hole with a diameter close to DNA's are caused by the solution *quality* and more exactly by its difference between inside and outside the shell. The solution *quality* can particularly reflect such aspects as: the double helix hydration specificity expressed in hydration layer structural microheterogeneity [20], the DNA hydration change in transition processes within the double stranded conformation [20], the various ions that influence water structure, the DNA and coat protein shell interaction accompanying the reorganization of hydration layers structure [21], and, finally, the presence of so called *hydration forces* [22, 3] between interphage DNA which are parallel packaged segments. For understanding the general aspects of package (and following ejection) of phage chromosome (ds-DNA) inside the virus capsid it is also necessary to increase our knowledge about the process of passive entry of a double helix form of DNA into small pores in vesicle [23].

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