BACTERIAL MEMBRANE FRAGMENTS AS PHAGE DNA EJECTION STIMULANTS

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Abstract. The first steps of phage-bacteria interaction (phage adsorption on membrane fragments and *DNA* ejection from the phage) on the model system consisting of a phage and bacterial membrane fragments were investigated. The membrane fragments from *E.coli* with the presented active receptor system were obtained by ultrasound disintegrartion (sonication). Using the viscometric method, the *DNA* ejection from the phage capsid into the solvent 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 is demonstrated that the specific viscosity of the suspensions of of both the phage and the membrane fragments is almost zero. The specific viscosity increase with time (a kinetic process) occurs only in the case of the phage - membrane fragments complex. The kinetic process, in its turn, is the phage adsorption on the bacterial membrane fragments and the *DNA* ejection from the phage into the solvent.

Keywords: phage, bacterial membrane fragments, adsorption, DNA ejection.

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 the viral genome penetration in the host cell occurs. However, the forces promoting genome transportation from the phage to the cell cytoplasm are still a question.

The nucleic acid release from the phage head can occur because of the interaction between the virus and bacterial membrane fragments [1,2] or 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. The investigation in this field can lead to solution of the important problem for molecular biology and medicine – to affect process of bacterial infection by bacteriophages.

The present work is dedicated to the biophysical investigation of the first steps of phage-bacteria interaction (phage adsorption on membrane fragments and *DNA* ejection from the phage) on the model system consisting of a phage and bacterial membrane fragments. For realization of this study, membrane fragments with the active receptor system, on which the bacteriophage can adsorb, that by a native way promote *DNA* ejection from the phage particle were obtained from bacterial cells by the ultrasound method. Such a model system allows using different biophysical methods for investigation of the first steps of the host cell infection process by the phage.

Materials and methods

Bacteriophage *DDVI* and membrane fragments of *E. coli C* (the host cell for *DDVI*) were chosen as objects of investigation. The *DDVI* phage head size is 1100x860Å and the size of the tail is 1250Å. This phage contains *ds-DNA*, Mw 110×10^6 *Da*, which is approximately half of the mass of the whole phage [7]. The phage purification was carried out by centrifugation in the *CsCl* density gradient. The concentration of the phage by *DNA* was determined using a spectrophotometer, assuming that 0.023 *OD* under the absorption at the wavelength of 260 nm corresponds to 1 μg/ml.

Using the ultrasound disintegration, the membrane fragments from *E.coli C* bacterial cells were obtained. Correct determination of the frequency and energy of disintegration allowed us to obtain the membrane fragments with the active receptor system, which is able to adsorb the phages on its surface. To do this, 1ml of the bacterial culture (titre 10^9) was diluted with 40 ml of *PBS* (0.1M *NaCl*+0.05M phosphate buffer, *pH*7) and was disintegrated by ultrasound at 22 kHz using dispergator USDN-2T (Russia). The energy of disintegration was chosen of the order of 10W. The disruption of bacterial suspension was carried out discreetly. For this purpose, the tube with the bacterial culture was put in the ice bath and it was sonicated 7 times for 1 minute at 1 min intervals.

For purification of the bacterial membrane fragments Ultracentrifuge CP2-25 (Ukraine) was used. For separation of big parts of the bacteria and some unbroken bacteria, the cells were centrifuged for 30 min at 2800×g. For separation of cell membrane fragments from the bacterial cell, the supernatant was diluted with *PBS* up to 100 ml and then again centrifuged for 1.5 h at 25 000×g. This process was carried out 3 times.

For determination of the membrane fragment activity to phages, the hydrodynamical investigations were carried out using the Zimm-Crothers-type viscometer (designed in our lab) with automatic time registration of rotor rotations [8].

Experimental results and discussion

One of the methods with the help of which the phage *DNA* ejection in solution can be observed is viscometry. This method allows judging about the *DNA* release from the phage capsid in environment (solution) as the suspension viscosity increases or because of the kinetics of this process.

The viscometric investigations at constant temperature (37°C) and *pH*8 were carried out to determine if the phage adsorption on membrane fragments of bacteria and the next step, the *DNA* ejection from the phage capsid, had occurred. Such a *pH* value was chosen experimentally, considering the tendency of membrane fragments to form an aggregation, which decreased with the icrease in the *pH* of the solvent. At *pH*8, less aggregation was observed.

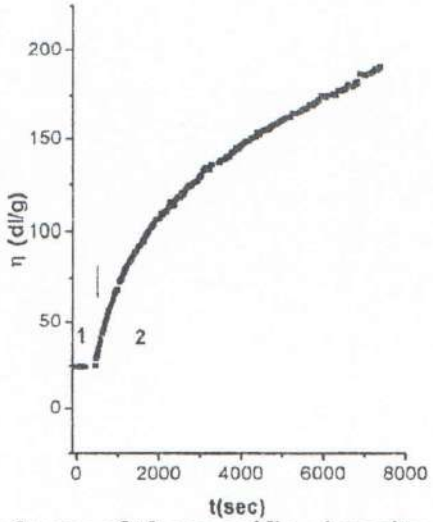

Fig.1. The dependence of the specific viscosity of the *DDVI* phage-bacterial membrane fragment complex on time, at 37°C

Solvent – *PBS* (0.38M *NaCl* + 0.03M phosphate buffer), *pH*8
Interval 1 – the dependence of the specific viscosity of membrane fragments on time
Interval 2 – the dependence of the specific viscosity of the phage-membrane fragment complex on time

This *pH* value is the most acceptable also, because we suggest that, at *pH* higher than *pH*8, denaturation of the membrane proteins-receptors can occur. The suspension of bacterial membrane fragments obtained by sonication was measured first. The value of the specific viscosity of the suspension was almost zero (Fig.1, interval 1). Then, in few minutes the *DDVI* phage (with final concentration 100 μg/ml) was added to the suspension of the membrane fragments and the final phage/membrane fragments ratio was 10:1 by absorption at 260 nm. A sharp kinetic increase in the viscosity immediately occurred, which is typical for the *DNA* ejection process from the phage capsid in to the solvent (Fig.1, interval 2).

To except the suggestion concerning the influence of the dilution effect on *DNA* release from the phage in solvent, another experiment was carried out (Fig.2, b). The *DDVI* phage was diluted and the specific viscosity of the suspension was measured. In this case the viscosity value is close to the viscosity of the solvent and there is no kinetics, which is typical for the *DNA* release (Fig.2b, interval 1). The moment of the addition of membrane fragments is pointed at by the arrow (Fig. 2b). As in the previous experiment the kinetic starts only at the moment of addition of bacterial membrane fragments (Fig.2b, interval 2). The final concentration of the phage and membrane fragments was the same as in the previous experiment.

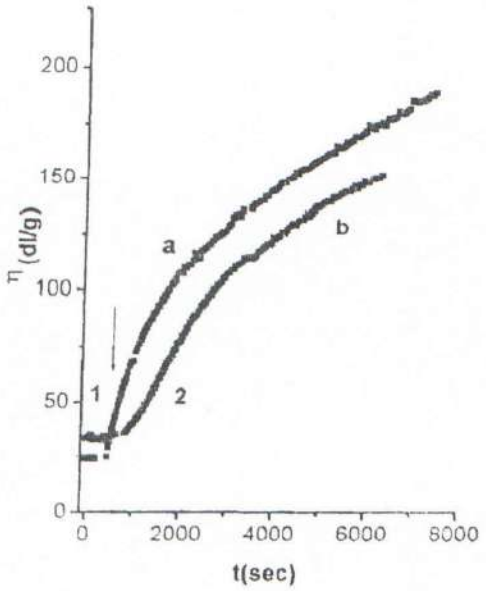

Fig. 2. The dependence of the specific viscosity of the DDVI phage-bacterial membrane fragment complex on time, at 37°C

a – the dependence of the specific viscosity of the complex on time in the case of the DDVI phage addition to the membrane fragments;

b – the dependence of the specific viscosity of the complex on time in the case of the membrane fragments addition to the DDVI phage;

Interval 1 – the dependence of the specific viscosity of the phage on time;

Interval 2 – the dependence of the specific viscosity of the phage -membrane fragment complex on time

Solvent – *PBS* (0.38M *NaCl* + 0.03M phosphate buffer), *pH*8

The curve from Fig.1 is shown in Fig.2 as *a* to be compared with curve *b*. The shapes of the curves are similar, but there is also some difference between them, which (Fig.2 a and b) can be related related to a little aggregation of membrane fragments (Fig.2 b). The aggregation to our mind could occur because in the second case the membrane fragments were used in several hours after purification, whereas in the first experiment (Fig.2 a) they were used immediately after purification. The aggregation leads to the decrease in open adsorption places and prevents phage adsorption on them. The results of these experiments show that the

phage *DNA* ejection process occurs only in the case of the phage - membrane fragment complex. The complex, in its turn, means the phage adsorption on bacterial membrane fragments.

Finally, from our experimental results we make a very interesting and important conclusion that the "start" of the *DNA* ejection process from the phage particle does not take place at the expense of additional energy, neither physical (for example temperature) nor chemical (for example *ATP* molecule). The energy that is necessary for the transfer of genetic material from the phage capsid to the host cell is "given" to the phage particle already during its assembling process in the host cell. This "spare" energy of the phage, to our mind, is in the structural organization of the phage genome inside the phage head.

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РЕЗЮМЕ

БАКТЕРИАЛЬНЫЕ МЕМБРАННЫЕ ФРАГМЕНТЫ КАК СТИМУЛЯТОРЫ ЭЖЕКЦИИ ФАГОВОЙ ДНК

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Были исследованы первые этапы взаимодействия фага и бактерии (адсорбция фага на мембранных фрагментах и эжекция ДНК из фага) на модельной системе, состоящей из фага и бактериальных мембранных фрагментов. При использовании метода ультразвуковой дезинтеграции были получены мембранные фрагменты из бактерии *E.coli*, с сохраненной активной рецепторной системой. Посредством метода вискозиметрии наблюдалась эжекция ДНК из фагового капсида в растворитель. Вискозиметрические исследования были проведены в щелочной среде, т.к. в данном случае наблюдалась наименьшая агрегация бактериальных мембранных фрагментов. Показано, что специфическая вязкость суспензии, как фагов, так и мембранных фрагментов близка к нулю. Увеличение специфической вязкости во времени (кинетический процесс) происходит только в случае комплекса фага и мембранных фрагментов. Кинетический процесс, в свою очередь, означает фаговую адсорбцию на бактериальных мембранных фрагментах и эжекцию фаговой ДНК в растворитель.

Ключевые слова: фаг, бактериальные мембранные фрагменты, адсорбция, эжекция, ДНК.