ELECTROCHEMICAL H^+ GRADIENT BUT NOT PHOSPHATE POTENTIAL IS REQUIRED FOR *ESCHERICHIA COLI* INFECTION BY PHAGE T4

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1. Introduction

The mechanism of phage nucleic acid penetration into bacterial cell is a problem still to be solved. The chemiosmotic hypothesis of nucleic acid transport has been formulated according to which the electrochemical H^+ gradient ($\Delta\mu_{H^+}$) across the cell membrane has been considered as the driving force for the uptake of phage nucleic acid [1]. The involvement of $\Delta\mu_{H^+}$ in the process of DNA uptake has been shown in studies on energy supply for the early stages of genetic transformation [2,3], phage infection [4–6] and bacterial conjugation [7,8]. The dependence on membrane potential of phage T4 DNA penetration into *Escherichia coli* has been confirmed [9]. In quantitative studies of the energy supply for phage T4 infection the threshold value of membrane potential required for the phage DNA entrance into the cell has been revealed [10]. Yet the role of intracellular phosphate potential in the process of nucleic acid transport remains unsolved. In [3], DNA uptake by *Bacillus subtilis* proceeded despite the considerable decrease in the intracelular ATP level. However the chromosome transfer from donor to recipient cell was revealed to be dependent on both $\Delta\mu_{H^+}$ and phosphate potential [8].

Here we report a further investigation of the role of both $\Delta\mu_{H^+}$ and phosphate potential on early stages of phage infection. These data indicate that the infection of *E. coli* by phage T4 does not depend on the intracellular phosphate potential.

2. Materials and methods

Escherichia coli K-12 strain AN 180 (*F⁻*, *arg*, *thi*, *xyl*, *str-r*) and isogenic strain AN 120 (*uncA* 401) with genetically impaired H^+-ATPase were a generous gift of Professor F. Gibson (Australian National University, Canberra). Wild strain of phage T4 was obtained from Dr R. Nivinskas (Institute of Biochemistry, Vilnius).

The cultivation of cells was done as in [5]. To reveal the effect of arsenate on phage infection, the stock suspension containing $2–5 \times 10^8$ cell in 1 ml medium 9 was sedimented and concentrated 50-fold in the ice-cold medium containing 0.85% NaCl, 1 mM $MgSO_4$, 0.1 mM $CaCl_2$, 0.4% glucose and L-tryptophane (100 μg/ml). The cell suspension was kept on ice throughout the experiment. Aliquots (0.4 ml) of the cell suspension were diluted 2-fold either with the room-temperature solution of 0.158 M Na_2HPO_4 (pH 7.0), 1 mM $MgSO_4$, 0.1 mM $CaCl_2$, 0.4% glucose and L-tryptophane (100 μg/ml) or with the same solution containing 0.158 M Na_2HAsO_4 (pH 7.0) instead of Na_2HPO_4. Diluted aliquots were incubated at 37°C for 15 min and then used either for determination of plaque-forming units (p.f.u.) [5] or for extraction of adenine nucleotides [3]. ATP and ADP were determined in the medium supplemented with 2 mM phosphoenolpyruvate and pyruvate kinase as in [3].

The suspension of phage T4 with [^{3}H]methyl thymidine labelled DNA was a generous gift of Dr S. K. Zavryev (Institute of Physiology, Tbilisi). The phage suspension contained 1×10^{12} infectious particles and had radioactivity of 7.5×10^6 cpm/ml. To infect *E. coli* by phage T4 the cells were grown and harvested as in [5]. After the incubation of the cell suspension (8 ml) at 37°C for 20 min on gyratory shaker (120 rev./min) the cells were treated for further 10 min with chloramphenicol (40 μg/ml) and infected with the phage (m.o.i. = 2). After further 20 min

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incubation the cell suspension was placed for 1 min into ice bath and then centrifuged at 4°C and 5000 × *g* for 10 min. Adsorbed phages were removed from cells as in [11] with slight modifications. The sedimented cells were resuspended in 7.5 ml ice-cold solution containing 2 mM $MgSO_4$ and 0.2 mM $CaCl_2$, and placed in the ice bath and then sonicated for 20 min in the UZDN-I disintegrator at 44 kHz and 7 A in the absence of resonance. The sonicated suspension was centrifuged as above and sedimented cells were resuspended in 5 ml solution [12] and converted to spheroplasts as in [12]. The spheroplasts were sedimented as above and suspended in 0.3 ml distilled water. The spheroplast suspension or 0.4 ml cell suspension were placed on Whatman GF/A glass filters and then the filters were dried and counted for radioactivity. Where indicated either 15 mM of KCN or 20 μM carbonyl-cyanide *m*-chlorophenylhydrazone (CCCP) were added to cell suspension together with chloramphenicol, and incubation at 37°C continued for 10 min. Then phage T4 was added as above. In a parallel set of experiments the cells where incubated with phage T4 at 37°C for 10 min and then treated with inhibitors. Sonication-resistant phage [^{3}H]DNA in the cells and the spheroplasts were determined as above. ANS fluorescence was measured as in [13].

3. Results

The data of a typical experiment presented in table 1 show that the incubation of AN 180 cells in the medium containing arsenate drastically decreases the intracellular concentration of ATP and ADP. This, however, has little, if any, effect on the efficiency of phage infection. Concomitant ANS fluorescence measurement have revealed the arsenate treatment of AN 180 cells to be highly inhibitory to the ATP-dependent generation of $\Delta\mu_{H^+}$ (detailed in [13]). At the same time, arsenate treatment drasticaly increased the sensitivity of phage infection to cyanide (fig.1A) without any effect on the irreversible phage adsorbtion (fig.1B).

In the next set of experiments the effect of uncoupler CCCP and KCN on the binding of phage [^{3}H]DNA to *E. coli* cells and spheroplasts was investigated (fig.2). The concentrations of CCCP (20 μM) and KCN (15 mM) causing maximal inhibitory effect on *E. coli* infection by phage T4 were chosen as in [5]. The cells possessing intact H^+-ATPase (strain AN 180) as well as the AN 120 cells devoid of H^+-ATPase activity were used throughout [14]. Before the exposure to the phages the cells were treated with inhibitors or left intact for the control. Then sonication procedure in [11] was used to remove ⩾70% of adsorbed phage. Sonicated cells were sedimented and radioactivity of the cell pellet was counted. As one

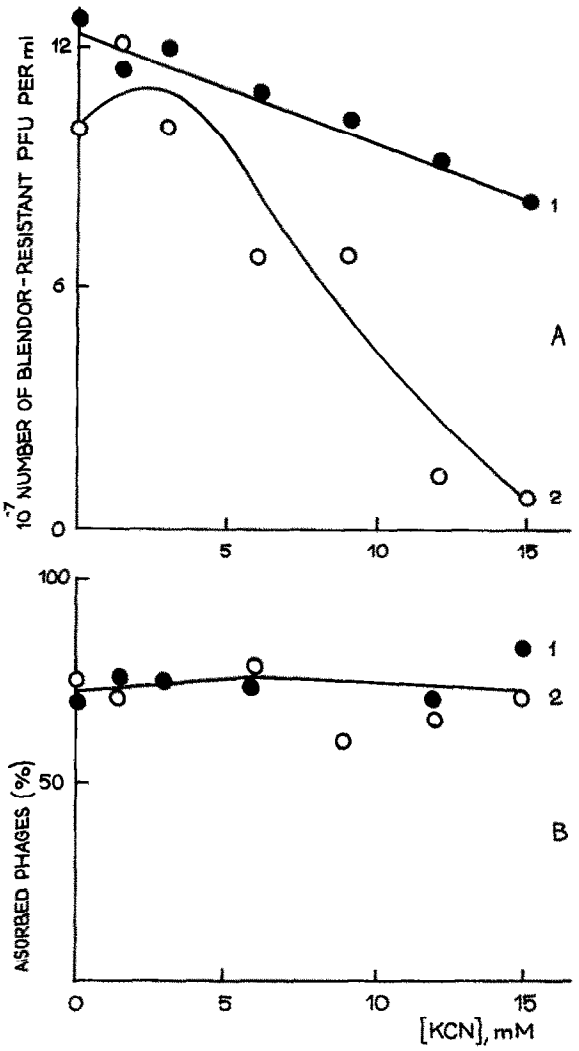

Fig.1. Effect of cyanide on the *E. coli* infection by phage T4 (A) and on the irreversible phage adsorbtion (B). AN 180 cells were pretreated in phosphate (1) or arsenate (2) medium at 37°C for 5 min. Afterwards aliquots were supplemented with indicated concentrations of cyanide and incubation at 37°C continued for 10 min. Then phages T4 were added (m.o.i. = 0.05) and incubation at 37°C continued for additional 5 min. Blendor-resistant p.f.u. and irreversible adsorbed phages T4 were determined as in [5].

Table 1
Effect of arsenate on intracellular ATP and ADP, and on *E. coli* infection by phage T4

Incubation medium	Intracellular conc. (mM)		10^6 × no. blendor-resistant p.f.u./ml
	ATP	ADP	
Arsenate	<0.18	<0.09	99 ± 38
Phosphate	0.79	0.28	127 ± 9

Concentrations of AN 180 cells and phage T4 in experiments on phage infection were 4×10^9 and 1.8×10^8/ml, respectively; cell concentration in experiments for ATP and ADP determination was 25×10^9/ml. Deviations from the mean values were calculated from triplicate counts of p.f.u.

can see from the results of typical experiment (fig.2), de-energization of AN 180 cells with CCCP before the addition of phage T4 caused 19% decrease in the level of phage [^{3}H]DNA which remained bound to the cells after sonication while the treatment of AN 120 cells with KCN under the same conditions had no effect.

Thus, both energized and de-energized cells bind practically the same amount of phage [^{3}H]DNA in the sonication-resistant form. The phage DNA could be bound on both the cell wall receptors and the cytoplasmic membrane. On the other hand, the DNA can enter the periplasmic space and the cytoplasm.

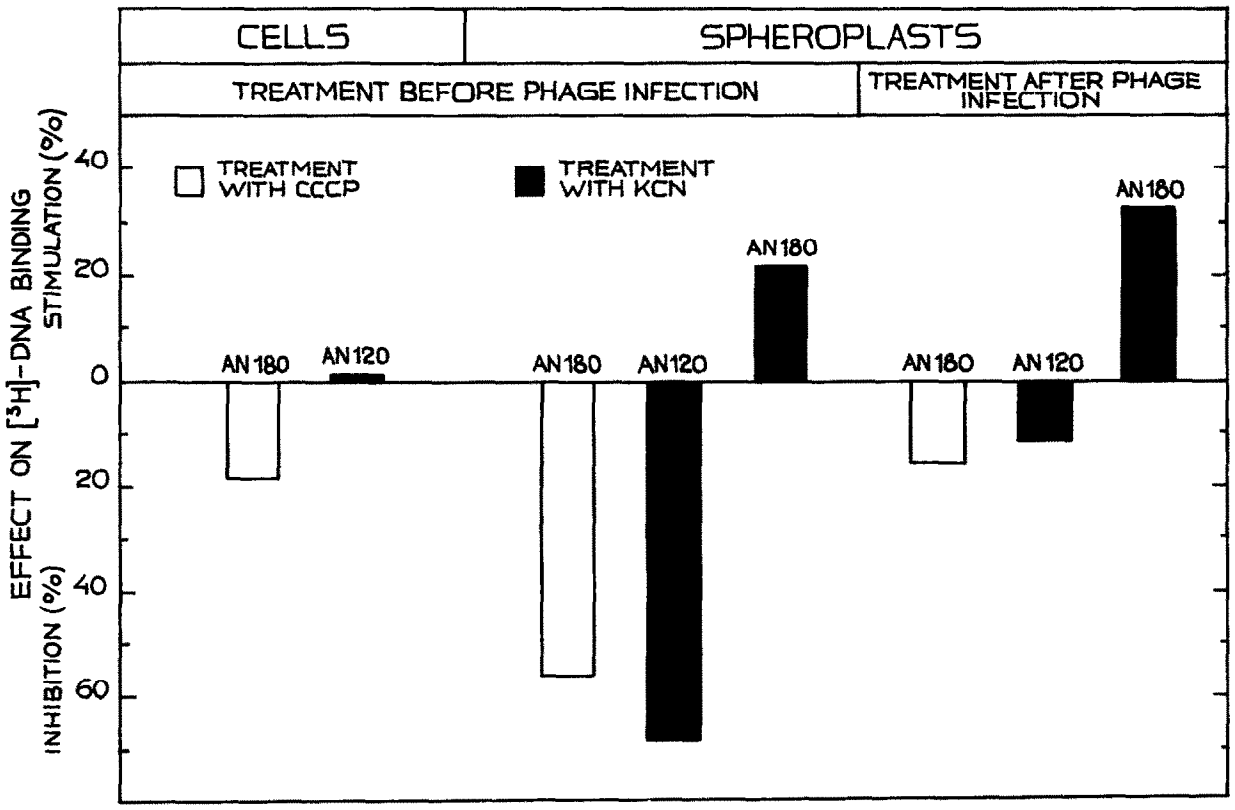

Fig.2. Effect of cyanide and carbonylcyanide *m*-chlorophenylhydrazone (CCCP) on the irreversible binding of phage T4 [^{3}H]-DNA by *E. coli* cells and spheroplasts. Experimental details are in section 2.

To remove the phage [^{3}H]DNA from both the cell wall receptors and the periplasmic space the cells containing sonication-resistant [^{3}H]DNA were converted to spheroplasts by the treatment with lysozyme and EDTA. The de-energization of cells with either CCCP (strain AN 180) or KCN (strain AN 120) during their exposure to phage T4 caused 56–69% decrease in the level of spheroplast-bound phage DNA. At the same time, the treatment of AN 180 cells with KCN led to 21% increase in the level of the spheroplast-bound phage DNA. The stimulatory effect of KCN on the binding of [^{3}H]DNA varies from 0–21% for different stocks of bacterial culture.

To investigate the reversibility of phage DNA binding the cells were infected with phage T4 then exposed either to CCCP or to KCN. The infected cells were thereafter converted to spheroplasts. It could be seen (fig.2) that only insignificant decrease occured in the level of spheroplast-bound DNA. In the experiment with AN 180 cells the stimulatory effect of KCN was observed (fig.2). It seems that KCN prevents phage DNA penetration into the cytoplasm of AN 120 cells as well as stimulates DNA binding to the membrane. One can suggest that the stimulatory effect of KCN prevails in the AN 180 cells.

Control experiments (not shown) have indicated that de-energization of cells with either CCCP or cyanide prior to the addition of lysozyme and EDTA had no effect on the light scattering properties of the spheroplast suspension.

4. Discussion

The phosphate potential value depends on [ATP]/[ADP] · [P_i] ratio [15]. The results in table 1 indicate that *E. coli* treatment with arsenate causes drastic decrease in the intracellular concentration of ATP and ADP presumably due to the conversion of both ATP and ADP to AMP. As shown above (fig.1) the treatment of *E. coli* with arsenate drastically increased the sensitivity of phage infection to cyanide. This fact indicates the requirement for at least one generator of $\Delta\mu_{H^+}$ operating in the cells during their infection by phage T4. The above data, when confronted with almost equal efficiency of *E. coli* infection by phage T4 either in the arsenate or in the phosphate-containing medium (table 1) lead to the conclusion that the phage infection does not depend on the value of intracellular phosphate potential.

One can speculate that despite the presence of arsenate the cells possess low, but sufficient, amount of ATP to drive DNA transport. On the other hand, it is well known [13,16] that arsenate-treated cells are incapable of the ATP-dependent accumulation of sugars and amino acids as well as ATP-driven generation of $\Delta\mu_{H^+}$. Thus one must postulate the presence of ATP-dependent DNA translocase which is insensitive to the changes both in the intracellular ATP concentration and phosphorylation potential. Such a suggestion seems to be incredible.

Studies on the requirement for $\Delta\mu_{H^+}$ in *E. coli* infection by phage T4 indicate (fig.2) that phage T4 [^{3}H]DNA which was bound to the de-energized cell can be released efficiently into the solution during the cell conversion to the spheroplast. On the other hand, little inhibitory effect of both CCCP and cyanide on the amount of spheroplast-bound DNA was observed if *E. coli* was exposed to the inhibitors after the cell infection by phage (fig.2). Thus it seems possible to exclude from consideration the effect of the inhibitor-induced spheroplast damage on the phage DNA binding. The accumulated experimental evidence indicates the presence of $\Delta\mu_{H^+}$ in *E. coli* during phage T4 infection to be obligatory for retention of the phage DNA by the spheroplasts. One may suggest that $\Delta\mu_{H^+}$ is involved in the $\Delta\mu_{H^+}$-dependent DNA binding on the membrane. Contrary to this, our observations [4–6] indicate the $\Delta\mu_{H^+}$ requirement for the *E. coli* infection by phage T4. These data, with the above evidence, lead us to conclude that the $\Delta\mu_{H^+}$, but not the intracellular phosphate potential, is obligatory for the phage transport across the cytoplasmic membrane of *E. coli*.

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