# THE ROLE OF ENERGY-YIELDING ATPase AND RESPIRATORY CHAIN AT EARLY STAGES OF BACTERIOPHAGE T4 INFECTION

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## 1. Introduction

Recently a chemiosmotic mechanism of DNA transport has been formulated [1], according to which the entry of phage DNA is considered to be dependent on the energy-yielding activity of ATPase and the respiratory chain.

Here we report an experimental examination of the role of ATPase and the respiratory chain in the early stages of bacteriophage infection.

## 2. Materials and methods

*Escherichia coli* K12 AN 180 ($F^-$, *arg*$^-$, *thi*$^-$, *mtl*$^-$, *xyl*$^-$, *str*) and an isogenic strain AN 120 (*unc A* 401) were generous gifts of Professor F. Gibson (Australian National University). Stock suspension of wild-type bacteriophage T4 containing $10^{11}$ infectious particles/ml salt solution was obtained from Dr R. Nivinskas (Institute of Biochemistry, Vilnius). The salt solution contained 7 g $Na_2HPO_4$, 3 g $KH_2PO_4$, 5 g NaCl, 1 mmol $MgCl_2$ and 0.1 mmol $CaCl_2$ in 1 liter distilled water. Before each experiment the stock solution of bacteriophage T4 was diluted 40-fold with medium I (pH 7.0) containing 75 mM $KH_2PO_4$, 1 mM $MgSO_4$, 0.1 mM $CaCl_2$, 0.4% glucose and L-tryptophan (50 μg/ml). *E. coli* was grown up to $3-5 \times 10^8$ cells/ml in a gyratory shaker (250 rev./min) at 37°C containing liquid medium 9 [2] supplemented with 1% casein hydrolysate and thiamine hydrobromide (5 μg/ml). Then the cells were sedimented by centrifugation at $5000 \times g$, resuspended in medium I and used in the experiments.

The antiserum ($K = 500\ min^{-1}$) for T4 phage inactivation was prepared and tested as in [3]. The stock solution of antiserum was diluted 10-fold with medium I for experiments at 37°C. For experiments at 10°C, undiluted antiserum was used. Phage T4 infectivity was determined by a plaque-forming method [3].

*Abbreviations:* ANS, anilinonaphthalene 8-sulfonate; ATPase, adenosine triphosphatase (EC 3.6.1.3); m.o.i., multiplicity of infection; p.f.u., plaque-forming units

## 3. Results

The use of strain AN 120 cells with genetically impaired membrane-bound ATPase and its isogenic strain AN 180 which contains an active ATPase [5] enabled us to examine the role of ATPase and the respiratory chain in the process of phage infection. It has been shown [6] that the membrane of AN 120 cells is energized solely by the respiratory chain while glycolyzing AN 180 cells maintain a high-energy state of the membrane despite the inhibition of respiration. Therefore, the experiments described below were performed in the presence of glucose.

In the experiments illustrated in fig.1 the host cells were exposed to cyanide only at the early stage of phage infection cycle. Then the infected cell suspensions were diluted and incubated under optimal conditions for plaque formation. The cyanide being diluted at least $10^4$-fold. Exposure of AN 180 cells to cyanide

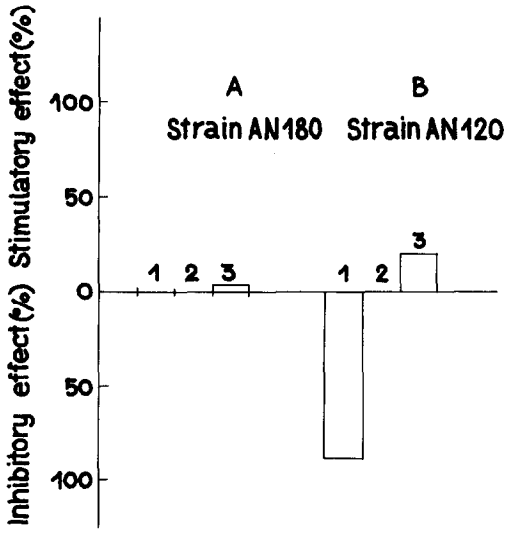


Fig.1. Effect of cyanide on p.f.u. formation and phage T4 adsorption to AN 120 (A) and AN 180 (B) cells. Strains AN 120 and AN 180 had m.o.i. 0.67 and 0.94, respectively. To determine the effect of cyanide on the ability of phage T4 to infect cells (expt. 1, figs.1A,1B), 0.9 ml aliquots of the cell suspension were exposed to 10 mM KCN for 10 min at 37°C. Then 0.1 ml portions of phage T4 suspension were added and incubation continued for 5 min at 37°C. Then 0.05 ml portions of T4 antiserum were added. The suspensions were mixed for 1 min and incubated for a further 4 min at room temperature. P.f.u. were assayed as in [3]. To determine the effect of cyanide on the adsorption of phage T4 to cells (expt. 2, fig.1A,1B), 0.9 ml aliquots of the cell suspension were exposed to 10 mM KCN for 10 min at 37°C. Then the 0.1 ml portions of phage T4 suspension were added. Incubation was continued for 5 min at 37°C and then 0.1 ml portions of chloroform were added. The suspensions were mixed vigorously for 10 s and incubated for 5 min at room temperature, p.f.u. then assayed. To determine the effect of cyanide on the plaque-forming ability of the infected cells (expt. 3, figs.1A,1B), the cells were incubated with phage for 5 min at 37°C and then exposed to 10 mM KCN and plated as above.

prior to the phage addition had no effect on p.f.u. formation, while exposure of AN 120 cells led to 88% inhibition of infection (expt. 1, fig.1A,1B). In different experiments inhibition varied from 60–90%. At the same time cyanide had no effect on phage adsorption to the host cells of both strains (expt. 2, fig.1A,1B). The exposure of the host cells to cyanide after 5 min incubation with phage caused a slight stimulation of p.f.u. formation (expt. 3, fig.1A,1B).

The take up of phage DNA by the host cell at 37°C is complete after about 40 s, while at 10°C this process takes 4–5 min [7]. In the experiments shown in fig.2 and 3 the AN 120 cells were exposed to phage for 1 min at 10°C. Then non-adsorbed phage was inactivated by addition of antiserum and incubation continued for 1 min more. Then incubation for the time intervals indicated in fig.2 started. Thereafter aliquots were withdrawn from the suspension and blendor-resistant p.f.u. were assayed. The blendor-

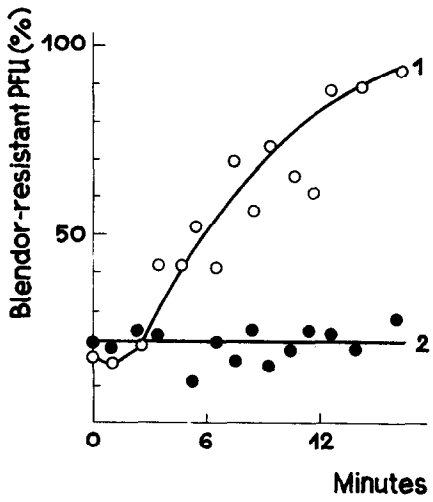


Fig.2. Effect of cyanide on blendor-resistant p.f.u. formation. AN 120 cells and phage T4 were suspended in medium I supplemented with 2% ethanol and the temperature was adjusted to 10°C. To 7.2 ml cell suspenion 0.8 ml of the phage suspension was added and incubation continued at 10°C for 1 min (m.o.i. 0.08 and 0.1 for expt. 1 and 2, respectively). Then 0.8 ml T4 antiserum was added and the mixture incubated a further 1 min at 10°C. After incubation 0.5 ml aliquots were withdrawn from the suspension and immediately diluted $10^3$-fold with the ice-cold medium [11] and this final suspension was kept in ice throughout the experiment. Portions (0.5 ml) of the suspension were diluted 20-fold with medium I and plated for p.f.u. assay. Portions (0.5 l) of the suspension were placed in the chamber of a blendor (type 324, made in Poland) which was kept in ice and the suspension was treated at 14 000 rev./min. Then p.f.u. were assayed. The p.f.u. level at the beginning of abscissa axis corresponds to the blendor-resistant phage–cell complexes that were formed during the first 2 min of infection. Expt. 1 was carried out in the absence of cyanide. In expt. 2 the phage–cell complexes formed during the first 2 min of infection were exposed to 15 mM KCN, cyanide being present throughout the rest of the experiment.

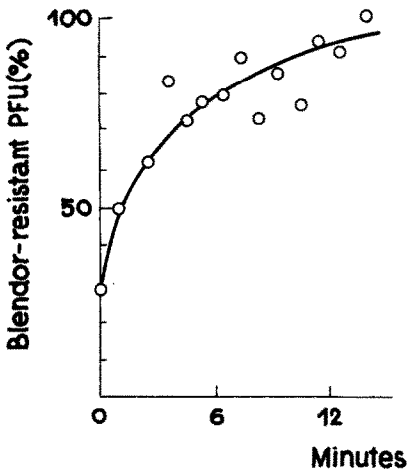


Fig.3. Development of resistance to cyanide during phage infection at 10°C. Cell–phage complexes were formed as in fig.2 (m.o.i. = 0.17). These complexes were incubated at 10°C for the time intervals indicated, then exposed to 15 mM KCN for 15 min. Blendor-resistant p.f.u. were assayed as in fig.2.

resistant p.f.u. appears as a DNA molecule carrying all the genes needed for the synthesis of an infective phage particle enters the host cell [7]. Expt. 1 (fig.2) illustrates the appearance of blendor-resistant p.f.u. in the absence of cyanide. It can be seen that after termination of phage adsorption the level of blendor-resistant p.f.u. remains unaltered for a further 3 min.

The quantity of blendor-resistant p.f.u. during this period depends on the blending conditions. Stabilization is followed by an increase in percent blendor-resistant p.f.u. (expt. 1, fig.2). After 12 min incubation 90% of p.f.u. become blendor-resistant. In expt. 2 (fig.2) the AN 120 cells were exposed to phage for 1 min at 10°C. Phage adsorption to the cells was stopped by addition of antiserum and incubation continued for 1 min more at 10°C. The resulting phage–cell complexes were exposed to cyanide. It can be seen that cyanide prevents increase in the blendor-resistant p.f.u. level.

In another set of experiments (fig.3) phage–cell complexes were exposed to cyanide at different stages of blendor-resistant p.f.u. formation. The phage–cell complexes were only sensitive to cyanide during the initial stages of blendor-resistant p.f.u. formation. Increase in the blendor-resistant p.f.u. level was accompanied by development of cyanide resistance (c.f. curve 1, fig.2, fig.2). It must be remembered that cyanide does not induce immediate de-energization of AN 120 cells. In control experiments with fluorescent indicators of the high-

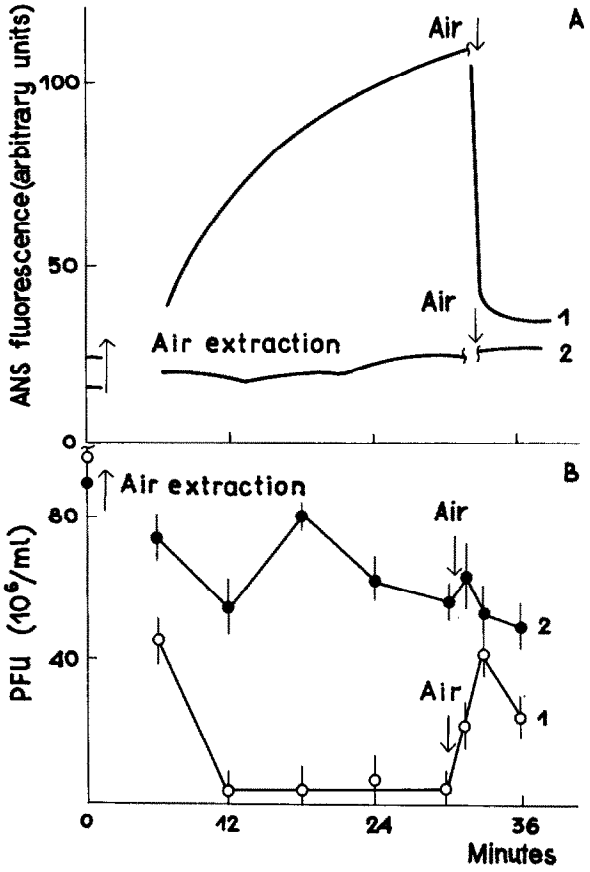


Fig.4. Time-course of ANS fluorescence changes (A) and of p.f.u. formation (B) at 10°C. Cells and phage were suspended in medium I supplemented with $5 \times 10^{-5}$ M ANS. Portions of cell and phage suspensions (3 ml and 0.1 ml, respectively) were placed in different compartments of Tunberg tubes immersed in a water bath at room temperature and then the air was extracted with a vacuum pump. After that the tubes were immersed in a water bath at 10°C and incubated for the time intervals indicated. The suspensions were mixed anaerobically and incubated for a further 10 min at 10°C. Portions (0.15 ml) of T4 antiserum were added anaerobically at the end of the experiment. The p.f.u. level at the beginning of expt. B shows infection of the cells by phages under aerobic conditions. ANS fluorescence intensity was measured in a Tunberg tube at 10°C as in [4].

energy membrane state (not shown), cyanide-induced cell de-energization was observed after a 3 min exposure of the cells to the inhibitor. Therefore it may be concluded that the kinetics of resistance development depends on the rate of DNA transfer to the cell as well as on the rate of cyanide-induced cell de-energization.

Figure 4 illustrates the time-course of ANS fluorescence intensity changes and of p.f.u. formation. To measure ANS fluoresence (fig.4A), the cell suspensions were placed in two Tunberg tubes and the initial levels of fluorescence intensity measured. Air extraction from the AN 120 cell suspension caused an increase in fluorescence intensity (curve 1, fig.4A), indicating de-energization of the cell membrane [4]. Aeration of this anaerobic cell suspension lowered fluorescence intensity (curve 1, fig.4A). Air extraction from the suspension of AN 180 cells produced only a slight effect on fluorescence intensity (curve 2, fig.4A). Aeration of this suspension had no effect on fluorescence (fig.4A).

The effect of membrane de-energization on p.f.u. formation is shown in fig.4B. The initial level of p.f.u. formation represents infection of the energized cells under aerobic conditions. To de-energize AN 120 cells, Tunberg tubes containing cells and phages in different compartments were evacuated and the incubation was carried out anaerobically at 10°C. After the time intervals shown in fig.4B cell suspensions and phage were mixed and incubation continued for a further 10 min at 10°C. Then aliquots of T4 antiserum were added anaerobically and p.f.u. were assayed. It can be seen (curve 1, fig.4B) that air extraction from the AN 120 cell suspension resulted in a decrease of p.f.u. level. Subsequent aeration of the suspension led to an increase in the p.f.u. level. Neither air extraction nor aeration of anaerobic AN 180 cell suspensions produced appreciable effects on p.f.u. formation (curve 2, fig.4B).

## 4. Discussion

The results obtained show that de-energization did not affect the cell's ability to adsorb phage T4 irreversibly. On the other hand, phage–de-energized-cell complexes manifest sensitivity to chloroform. This can be interpreted to mean that initiation of DNA release from phage envelopes occurs in phage–de-energized-cell complexes. Inhibiton of p.f.u. formation (fig.1 and 4) caused by de-energization of AN 120 cells could reflect the infection-disturbing release of phage DNA into the periplasmic space of the host cells.

Studies on the kinetics of blendor-resistant p.f.u. formation (fig.2, and 3) lend strong support to the concept of the high-energy membrane state being involved in the DNA transfer process. Energy can be needed either for joining the tip of the phage tail tube to the cytoplasmic membrane and thereafter for transmembraneous channel formation or/and for transport of the phage DNA. The de-energization-induced inhibition of blendor-resistant p.f.u. formation (fig.2) together with the findings in [7] concerning DNA transfer initiation in the cell–phage complexes under our experimental conditions (fig.2) prompt us to the conclusion that the cell membrane must be kept energized for the overall period of phage DNA transfer to the host cell. The findings in [8] indicating that the high-energy membrane state of *E. coli* cell is involved in the irreversible absorption of phages T1 and $\phi$ 80 also lend support to this idea.

Energy for bacterial cells is supplied in the form of a protonmotive force or a phosphorylation potential [9,10]. In the cells with genetically impaired membrane-bound ATPases the respiratory chain is indispensible for generation of protonmotive force while the phosphorylation potential of the mutant cell is maintained at a certain level due to glycolysis. The analysis of the experimental material presented above suggests that protonmotive force is involved in the process of phage DNA entry into the cell. The role of the phosphorylation potential in this process is still to be clarified.

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