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A MEMBRANE POTENTIAL THRESHOLD FOR PHAGE T4 DNA INJECTION

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SUMMARY

DNA penetration from T4 phage adsorbed to Escherichia coli was measured at different membrane potentials. There was a precipitous reduction in DNA penetration between 110 mV and 60 mV. This threshold of membrane potential for DNA penetration is independent of ΔpH and rather insensitive to external pH between 6 and 8.

The penetration of DNA into bacteria via conjugation (1), phage injection (2, 3), or transformation (4, 5) appears to require an energized cytoplasmic membrane. The injection of phage DNA has many stages which require sequential interactions and alterations of phage and host organelles (6). With a recently developed method it was shown (3) that a membrane potential ($\Delta\psi$) of the host is both necessary and sufficient for the injection of T4 phage DNA. It was suggested that in the absence of $\Delta\psi$ DNA cannot enter the appropriate "pore" in the host membrane. In this paper we report experiments which show that the T4 DNA injection process depends on a minimum $\Delta\psi$ below which there is no penetration. This demonstration of a threshold potential reinforces the idea that the ability of the host membrane pore to admit T4 DNA is regulated by $\Delta\psi$.

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Abbreviations: $\Delta\psi$, membrane potential; pmf, protonmotive force; CCCP, carbonylcyanide-m-chlorophenylhydrazone; ΔpH, pH gradient.

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In order to evaluate the effects of the protonmotive force (pmf) on the ability of E. coli to permit injection of phage T4 DNA, we adjusted the pmf of the host in a graded manner by adding increasing amounts of the

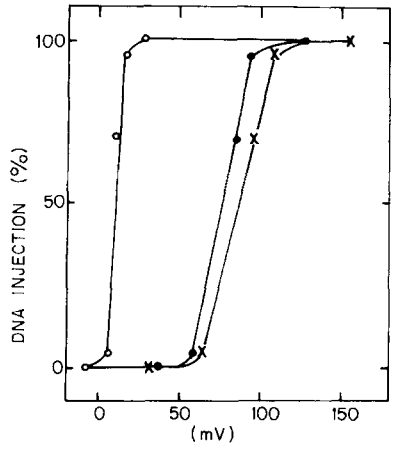

Fig. 1. Dependence of T4 DNA injection on pmf, $\Delta\psi$ and ΔpH. E. coli K-12 was grown at 37°C to 2 x 10^8 cells/ml in phage broth (3), concentrated to 10 fold, washed once at room temperature with 0.1 M potassium phosphate buffer, pH 7.0, and exposed for 10 min at room temperature to 10 mM EDTA in 0.1 M Tris-HCl, pH 7 to make the cells permeable to valinomycin. After washing twice in 0.1 M phosphate buffer, pH 7.0, at room temperature, cells were resuspended in 1/100 original volume of phage broth containing 0.1 M sodium phosphate and stored on ice. Valinomycin was added to a final concentration of 10 µM, and 0.2 ml aliquots of cells were added to 1 ml of 150 mM NaCl - 0.1 M Tris-HCl pH 7 with 0, 5, 10, 20 and 50 µM CCCP and incubated for 10 min at 37°C with shaking. Three samples were used for each CCCP concentration; one to measure $\Delta\psi$, one for ΔpH and one for DNA injection. The membrane potential was measured by the distribution of $^{86}Rb^+$ (60 µM final concentration). This ion has a high affinity for valinomycin and from the concentration gradient across the membrane of $^{86}Rb^+$ one can calculate the membrane potential by the Nernst equation. The pH gradient across the membrane was estimated from the distribution of ^{14}C-benzoic acid (final concentration 5 µg/ml), a lipophilic acid which distributes itself according to the pH gradient. The accumulation of the two probes was estimated after separating the cells from incubation medium by centrifugation through silicone oil (microfuge method) (11); the total volume of the cell pellet was monitored with ^3H-H_2O, ^{14}C-inulin was used to correct for the extracellular water space. The intracellular K^+ was 180 mM and the extracellular K^+ was 1.3 mM. Phage T4 DNA injection was estimated using a ^{32}P-labeled phage T4 gene 2 mutant in which the phage DNA is not protected against degradation by the host exonuclease V, and calculated as the amount of T4 DNA made acid soluble after adsorption of phage to the treated E. coli cells (3). 0.1 ml of ^{32}P-T4 2^- phage (4 x 10^9/ml; final multiplicity 1 phage/cell) was added to the treated cells and incubated at 37°C with shaking for 5 min, and then an equal volume of cold 10% trichloroacetic acid was added. Phage adsorption was greater than 98%. DNA injection is defined as the amount of DNA which can be made acid soluble. The maximum amount (which we define as 100% DNA injection) is the maximum amount of acid solubilized DNA (45%) minus the minimum amount (15%, obtained for CCCP above 20 µM). In unpublished experiments we found that DNA breakdown reached its final level within 5 minutes. O = ΔpH, ● = $\Delta\psi$, X = pmf.

proton ionophore carbonylcyanide-m-chlorophenylhydrazone (CCCP) to media buffered at pH 6, 7 and 8. Phage T4 DNA injection, $\Delta\psi$ and ΔpH were determined in parallel on a culture of cells after treatment with EDTA, valinomycin and various concentrations of CCCP. DNA injection was determined by measuring *in vivo*, the exonuclease V dependent degradation of ^{32}P-T4 2^- DNA to acid soluble material as described previously (3). $\Delta\psi$ and ΔpH were assayed by the distribution of $^{86}Rb^+$ and ^{14}C-benzoic acid respectively. Figure 1 shows that as the pmf was progressively reduced by increasing concentrations of CCCP, DNA injection was not reduced proportionally; rather DNA injection was inhibited precipitously between 110 mV and 60 mV (a threshold effect). When the pmf was resolved into its components we observed that the threshold value, defined as 50% of the DNA injection, of 86 mV was actually the sum of two thresholds: 76 mV for $\Delta\psi$ and 10 mV for ΔpH.

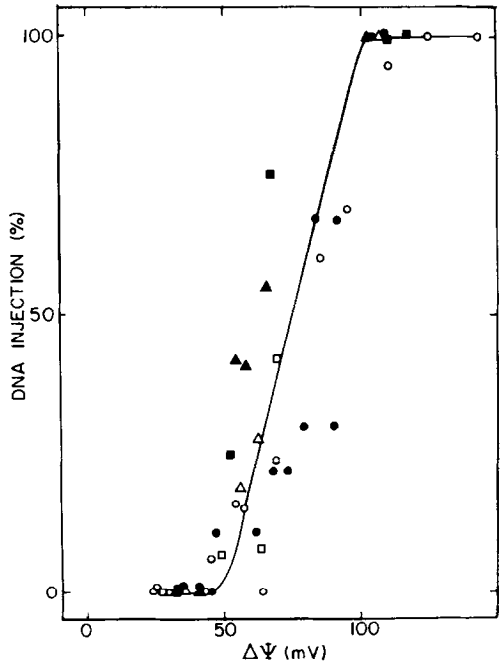

Fig. 2. Membrane potential dependent phage T4 DNA injection: Titration of $\Delta\psi$ by CCCP. *E. coli* K-12 and B cells were grown and pretreated as described for Figure 1. The cells were diluted into 0.1 M Tris-HCl, pH 7.0 or 8.0 or 0.1 M Tris-Maleate, pH 6.0 with various concentrations of CCCP. Calculations were made as in Figure 1. Open symbols refer to *E. coli* K-12, closed symbols to *E. coli* B. Δ, ▲ = pH 6.0; 0, ● = pH 7.0; □, ■ = pH 8.0.

Figure 2 shows the relationship between $\Delta\psi$ and DNA injection into CCCP-treated E. coli K-12 and B at pH 6, 7 and 8. By increasing the external pH in the absence of CCCP the contribution of ΔpH to pmf was reduced from about 90 mV to 5 mV. All data are collected in Figure 2 and show that there was no significant dependence of the threshold value (approximately 75 mV) on either external pH or cell type. These results support the preceding finding (3) about the requirement of $\Delta\psi$ for T4 DNA injection, and show furthermore that the presence or absence of ΔpH has no direct effect on the $\Delta\psi$ threshold.

Figure 3 shows the results obtained by incubating valinomycin-treated E. coli K-12 and B with concentrations of K^+ from 1.3 to 160 mM at pH 6, 7 and 8. This decreases $\Delta\psi$ at different levels of external pH. A definite threshold of $\Delta\psi$ was observed under all conditions. The threshold value decreased slightly as the external pH was reduced. The external pH did not affect the maximum DNA injection; at each pH, 45% of the added ^{32}P-T4 DNA was degraded. At pH 8 where the ΔpH was negligible (5 mV) DNA injection occurs normally with a $\Delta\psi$ threshold of 60 mV. At pH 6 where ΔpH was very large (90 mV) the injection occurred at a $\Delta\psi$

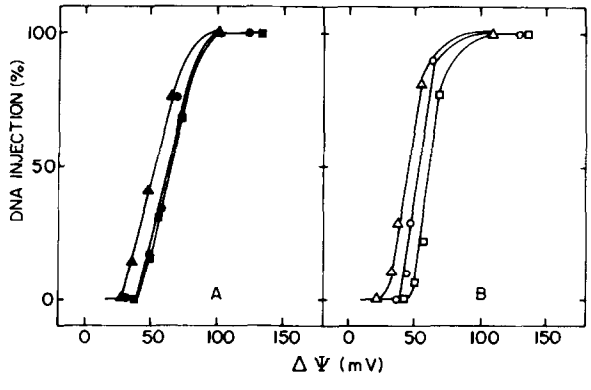

Fig. 3. Membrane potential dependent phage T4 DNA injection: Titration of $\Delta\psi$ by K^+. E. coli K-12 and B cells were pretreated and all calculations were made as given in the legend to Figure 1. For the final incubation 0.2 ml aliquots of valinomycin treated cells were diluted into 1 ml of 0.1 M Tris-HCl (pH 7 or 8) or Tris-Maleate (pH 6) containing 150 mM Cl^- with different ratios of K^+/Na^+ to give final K^+ concentrations of 1.3, 30, 60, 90 and 150 mM. Open symbols refer to E. coli K-12, closed symbols to E. coli B. Δ, $\blacktriangle$ = pH 6.0; $\overline{O}$, $\bullet$ = pH 7.0; $\square$, $\blacksquare$ = pH 8.0

threshold of 44 mV which differed from the $\Delta\psi$ threshold at pH 8 by only 16 mV. This difference is much less than the amount expected if ΔpH could be utilized directly as part of the pmf to permit DNA injection. It was shown in the CCCP experiments (Fig. 2) that external pH does not drastically alter the threshold and therefore we assume that the external pH per se will also not affect the threshold value greatly under the conditions described in Figure 3.

In bacteria, chemiosmotic-dependent solute transport is usually proportional to the protonmotive force, as in proton-galactoside co-transport (7) and the proton-cation (Na^+ or Ca^{2+}) exchange mechanisms (8). On the other hand, two cases have been reported for pmf-dependent processes which show a threshold: ATP synthesis via the proton-ATPase (210 mV) (9), and flagellar motion in chemotaxis (10). DNA injection is the first example of a macromolecular transport with a $\Delta\psi$-dependent threshold.

In summary, we propose that the ability of the membrane to permit DNA injection depends mainly on $\Delta\psi$. There is a minimum $\Delta\psi$ required to permit DNA injection. The requirement for a threshold of $\Delta\psi$ can be explained as a minimal configurational requirement to permit proper interaction between the membrane pore and the DNA or its pilot protein (12) (or complex). Thus the opening or closing of the "gate" to initiate DNA transport across the cytoplasmic membrane would be an all or nothing phenomenon. Further experiments are required to decide if there are other chemiosmotic dependent processes involved in DNA transport.

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REFERENCES

1. Grinius, L. and Berzinskiene, J. (1976) FEBS Lett. 72, 151-154.
2. Kalasauskaite, E. and Grinius, L. (1979) FEBS Lett. 99, 287-291.

3. Labedan, B. and Goldberg, E. B. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 4669-4673.
4. Griniuviene, B. B., Grinius, L. L., Kiasinyte, R. J., Khasutova, L. P. and Jasaitis, A. A. (1979) Biokhimya (USSR) 43, 1539-1548.
5. Jasaitis, A. A., Bershinskiene, J. B., Griniuviene, B. B., Grinius, L. L. and Khaustova, L. P. (1977) 11th FEBS Meeting, Copenhagen.
6. Goldberg, E. B. Recognition and Receptors (eds. Randall, L. and Philipson, L.) Chapman and Hall, London (in press).
7. Wilson, D. M., Alderete, J. F., Maloney, P. C. and Wilson, T. H. (1976) J. Bacteriol. 126,327-337.
8. Tsuchiya, T. and Takeda, K. (1979) J. Biochem. 85, 943-951.
9. Wilson, T. H., Flagg, J. L. and Wilson, D. M. (1978) Acta physiol. scand. Suppl. 389-397.
10. Taylor, B. L., Miller, J. B., Warrick, H. M. and Koshland, D. E. (1979) J. Bacteriol. 140, 567-573.
11. Heller, K. B. and Wilson, T. H. (1979) J. Bacteriol. 140, 395-399.
12. Kornberg, A. (1974) DNA Synthesis. Freeman, San Francisco.