BBA 75369

EFFECT OF INFECTION WITH RNA PHAGE R23 ON MEMBRANE PERMEABILITY AND K^+ FLUXES IN *ESCHERICHIA COLI*

HIROKO WATANABE AND MAMORU WATANABE

Departments of Medicine and Biochemistry, University of Alberta, Edmonton, Alberta (Canada)

(Received June 30th, 1969)

SUMMARY

1. Infection of *Escherichia coli* by the RNA bacteriophage R23 produced a rapid, transient increase in K^+ efflux from cells preloaded with $^{42}K^+$. R23 also stimulated the influx of $^{42}K^+$ for the first 5–10 min. The enhanced efflux was terminated by a "sealing reaction" 10–15 min after infection.

2. The phage effect on K^+ flux, which was multiplicity-dependent, did not appear to require viral RNA penetration or replication but was related to phage adsorption.

3. Analysis of $^{42}K^+$ influx and efflux indicated little or no net change in intracellular K^+ content after R23 infection.

The bacterial membrane has been implicated as the site of DNA replication, protein synthesis, and transport processes[1]. Certain agents which inhibit bacterial growth and macromolecular synthesis have been postulated to do so by producing changes in surface properties. Increased membrane permeability has been demonstrated on infection of *Escherichia coli* by T-even phage[2,3] and colicins[4]. T-even phages[3], colicins[4], antibiotics[5] and steroidal diamines[6] increased K^+ efflux without increasing (or altering) influx and were considered to induce permeability changes without directly altering transport. Changes in membrane protein and phospholipid synthesis or metabolism have been reported in *E. coli* infected with T4 (ref. 7) and colicins[4].

The RNA bacteriophage R23 characteristically produces a marked inhibition of host nucleic acid and protein synthesis on infection of *E. coli*[8,9]. Initial effects on host metabolism may be related to contact of the phage with the bacterial membrane and the ensuing consequences. With the ultimate aim of elucidating the mechanism of inhibition of host macromolecular synthesis by R23, an investigation of the nature and consequences of its interaction with the membrane was initiated by focusing on its effects on K^+ influx and efflux. The most striking finding was that R23, in contrast to T-even phage and the compounds listed above, produced an increase in the rates of both influx and efflux of K^+.

The bacterial strain *E. coli* K38, the RNA phage R23, conditions for bacterial growth and phage assay, tryptone medium (which contains 0.6 mM K^+) have been

previously described[8]. Efflux and influx of $^{42}K^+$ (Charles E. Frosst & Co., Montreal, P.Q.) were determined as described by SILVER *et al.*[3].

K^+ flux was investigated in K^+-rich cells suspended in medium poor in K^+, that is, in the presence of the high concentration gradient normally present during the early logarithmic phase of growth of *E. coli*[10]. Infection of *E. coli* by R23 resulted in a rapid loss of intracellular $^{42}K^+$ (Fig. 1). The accelerated loss was detected almost

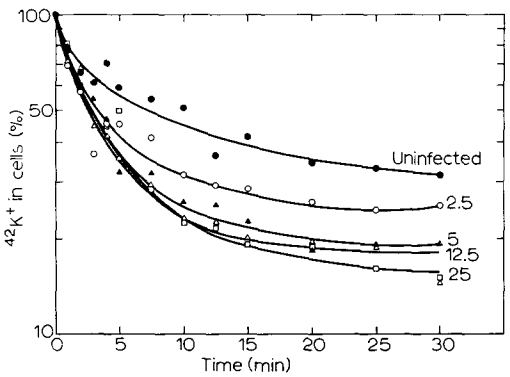

Fig. 1. K^+ efflux: effect of R23 at different multiplicity of infection. Bacteria were grown for several generations in tryptone broth containing $^{42}K^+$ (0.3 μC/ml), centrifuged, washed and resuspended in fresh broth containing 2 mM $CaCl_2$. R23 was added at time zero at multiplicities of 2.5–25 phage per cell. The culture was incubated at 37°. The radioactivity present within the cell at the time of infection is represented as 100%.

TABLE I

REQUIREMENTS FOR $^{42}K^+$ EFFLUX IN R23 INFECTED CELLS

The experiment was performed as described in Fig. 1. The value for the rate of $^{42}K^+$ loss represents the difference between the $^{42}K^+$ levels in uninfected and infected cells at 10 min and the number in parentheses represents the rate observed in the corresponding wild type R23 infection. The $^{42}K^+$ remaining in cells at 30 min represents the value at equilibrium for uninfected and infected cultures.

Infected by	*Rate of $^{42}K^+$ loss induced by infection (% lost at 10 min)*	*$^{42}K^+$ remaining in cells at 30 min (%)*	
		Uninfected	*Infected*
R23	28*	32*	17*
Ultraviolet irradiated R23	21 (24)	29	19
Enzyme mutant	29 (27)	31	13
Maturation protein mutant	29 (28)	34	15
R23 + chloramphenicol (1 mg/ml)	21 (21)	25	16
R23 + rifampicin (150 μg/ml)	39 (34)	42	14
R23 + EDTA (10 mM)	30 (31)	22	12
R23 + ribonuclease (100 μg/ml)	18 (20)	51	31
R23 + antiserum	4 (21)	33	25
Defective particles	12 (28)	34	23
Coat protein subunits	2 (26)	33	33

* Average values.

immediately after addition of phage; it was maximal for the first 10–15 min and became negligible thereafter. The average $^{42}K^+$ remaining within the cells at 10 min was 55 and 25% of the input in uninfected and infected cells, respectively. The leakage increased with increasing multiplicities to a maximum at 12.5 phage per cell (when maximum number of cells are infected).

The rapidity with which K^+ efflux was induced by phage infection suggested that intracellular expression of a phage function was not required. Ultraviolet-irradiated phage and amber mutants defective in viral RNA synthetase and maturation protein cistrons induced a K^+ leakage comparable to that produced by wild type R23 (Table I). Host or viral RNA or protein synthesis was not required for phage-induced K^+ efflux. Leakage occurred in the presence of chloramphenicol or rifampicin, an inhibitor of DNA-dependent RNA polymerase. Penetration, and probably injection, of phage RNA were not necessary since R23 enhanced efflux in the presence of EDTA or ribonuclease, although the latter does not completely prevent RNA injection[11]. It appeared that a step between adsorption and penetration was involved. Leakage was not observed on infection with defective particles lacking the maturation protein or when adsorption was prevented by R23 antiserum. Purified coat protein subunits were incapable of reproducing the effect of the complete phage. The phage effect on K^+ efflux was diminished at temperatures below 33° and essentially absent below 24° (Table II). The leakage from uninfected cells was decreased at 4° but was comparable to the efflux at 37° at all other temperatures tested. Thus, the reduction in the phage effect at lower temperatures was attributable to the inability of RNA phage to successfully infect *E. coli* at these temperatures[12].

The effect of R23 on K^+ efflux was independent of the concentration of K^+ or Na^+ in the medium. R23 infection increased $^{42}K^+$ efflux from cells suspended in media completely lacking K^+ or Na^+. Efflux of $^{42}K^+$ was enhanced by the presence of K^+ or Na^+ in the medium in both uninfected and R23-infected cultures. In each case, however, a further and similar increment in efflux was produced by R23 infection over uninfected control levels. In contrast to T-even phage-infected cells[3], high concentrations of Mg^{2+} did not eliminate the phage effect.

The accelerated K^+ efflux ceased after the first 10–15 min of infection, and in

TABLE II

EFFECT OF TEMPERATURE ON R23-INDUCED K^+ LOSS

The experiment was performed as described in Fig. 1 except that cultures were incubated at different temperatures. The $^{42}K^+$ remaining within the cell 9 min after infection was determined in uninfected and infected cultures and the difference between these values represented as the loss induced by R23 infection.

Temp.	$^{42}K^+$ *remaining in cells* (%)		*Loss induced by R23* (%)
	Uninfected	*Infected*	
4°	79.8	78.4	1.4
22°	55.0	54.9	0.1
24°	61.7	59.8	1.9
29°	57.5	39.3	18.2
33°	58.4	26.5	31.9
37°	60.3	27.8	32.5

studies continued for 60 min, a slight increase in intracellular radioactivity was noted after the first 30 min, indicating reaccumulation of $^{42}K^+$. This arrest in K^+ loss was comparable to the "sealing reaction" described in T-phage infection of *E. coli*[2,3]. Superinfection with R23 up to 30 min did not release significant additional K^+.

Infection by R23 also resulted in an increased rate of $^{42}K^+$ influx during the first 5–10 min (Fig. 2). Thereafter, there was a gradual decline so that by 25–30 min the radioactivity accumulated was the same in uninfected and infected cells. As with efflux, the effect on influx was independent of protein synthesis or intracellular functioning of phage nucleic acid and did not require viral RNA penetration.

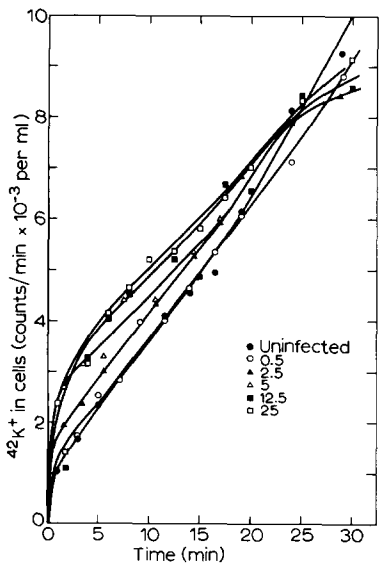

Fig. 2. K^+ influx: effect of R23 at different multiplicity of infection. Bacteria were grown in tryptone broth to a density of $2 \cdot 10^8$ cells per ml and infected with R23 at a multiplicity of 0.5–25 phage per cell. $^{42}K^+$ was added to a concentration of 0.3 μC/ml at the time of infection.

R23 infection, therefore, stimulated movement of K^+ in both directions, both along and against the initial concentration gradient. It is difficult to distinguish between a primary action of R23 on influx and an initial effect on efflux since K^+ influx and efflux are probably coupled. This initial study has illustrated that R23 is able to affect the membrane to the extent that transport and provision of a permeability barrier are significantly impaired or altered. R23 appears to produce a subtle membrane alteration without causing cell lysis or the gross leakage produced by T-even phage, colicins, antibiotics, steroidal diamines, *etc.*, which increased leakage and inhibited influx of K^+. One speculation is that contact of the phage (maturation protein) with the F pili triggers a conformational change in the membrane which leads to increased permeability and allows enhanced activity of the K^+ carrier. The determination of the exact molecular events in this effect will require extensive investigation.

This work was supported by Research Grants ME 2851 and MA 2822 and a Research Fellowship (H.W.) from the Medical Research Council of Canada.

REFERENCES

1 L. ROTHFIELD AND A. FINKELSTEIN, *Ann. Rev. Biochem.*, 37 (1968) 463.
2 T. T. PUCK AND H. H. LEE, *J. Exptl. Med.*, 99 (1954) 481.
3 S. SILVER, E. LEVINE AND P. M. SPIELMAN, *J. Virol.*, 2 (1968) 763.
4 M. NOMURA AND A. MAEDA, *Zentr. Bakteriol. Parasitenk. Abt. I. Orig.*, 196 (1965) 216.
5 F. M. HAROLD AND J. R. BAARDA, *J. Bacteriol.*, 94 (1967) 53.
6 S. SILVER AND E. LEVINE, *J. Bacteriol.*, 96 (1968) 338.
7 C. S. BULLER AND L. ASTRACHAN, *J. Virol.*, 2 (1968) 298.
8 M. WATANABE, H. WATANABE AND J. T. AUGUST, *J. Mol. Biol.*, 33 (1968) 1.
9 H. WATANABE AND M. WATANABE, *J. Virol.*, 2 (1968) 1400.
10 S. G. SCHULTZ AND A. K. SOLOMON, *J. Gen. Physiol.*, 45 (1961) 355.
11 K. A. IPPEN AND R. C. VALENTINE, *Biochem. Biophys. Res. Commun.*, 27 (1967) 674.
12 N. D. ZINDER, *Ann. Rev. Microbiol.*, 19 (1965) 455.