A CHANGE IN THE PROPERTIES OF DEOXYGUANYLATE KINASE OF E. COLI CAUSED BY VIRAL INFECTION

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The deoxynucleotide kinases of Escherichia coli are strikingly influenced by infecting the host with members of the T series of coliphages, (Bessman, 1959; Kornberg et al, 1959). Within 20 minutes after infection with T2, T4, or T6, deoxyguanylate (dGMP) and deoxythymidylate kinases increase 10 to 20 fold. A fundamental question is whether these increased activities represent activation of pre-existing proteins, increased synthesis of the bacterial enzyme, or synthesis of a new protein initiated by the infecting coliphage particle. An investigation of this question has lead to the observation that (1) the deoxyguanylate kinase in crude extracts of normal cells requires potassium ions for its activity, (2) the deoxyguanylate kinase in crude extracts of cells infected with coliphage T2 does not require added potassium.

The purpose of this report is to present data demonstrating these points.

Experimental

Materials and Methods. Growth of cells, infection with coliphage and preparation of extracts have been described previously (Bessman, 1959). Dialyzed crude extracts, or dialyzed "streptomycin supernatant fractions " (Lehman et al, 1958), gave identical results, and no differences were evident between extracts prepared from cells grown and infected in nutrient broth or synthetic C medium (Roberts et al, 1957). The extracts from infected cells were prepared 20 minutes after infection and were assayed for dGMP kinase as described for deoxynucleotide kinase by

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Lehman et al, (1958). All data presented represent rates.

Results and Discussion

Effect of potassium on dGMP kinase activity. The addition of KCl to the incubation medium markedly stimulated the dGMP kinase from normal cells, but it had an opposite effect on the dGMP kinase extracted from cells infected with coliphage T2. The curves in Figure 1 show that the dGMP kinase of normal cells increases in activity with increasing KCl concentration and levels off between 0.20 and 0.28 M. In marked contrast, the activity of extracts prepared from cells infected with coliphage T2 falls off with increasing KCl concentration. At concentrations where the normal extract was stimulated maximally, the enzyme prepared from infected cells was inhibited 50 to 65 percent.

Comparison of alkali metals in respect to dGMP kinase activation. The activation of dGMP kinase by KCl reported in Figure 1 could be interpreted

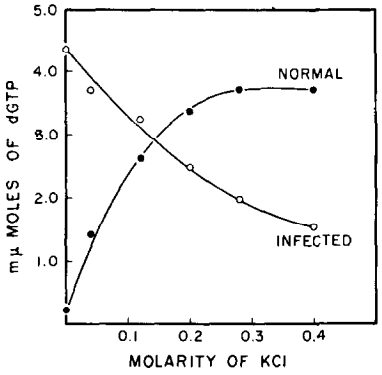

Figure 1 - Effect of potassium on dGMP kinase activity. Each tube contained 18 μg of protein (normal) or 2 μg of protein (infected).

as a specific effect of KCl or as a non specific activation due to increased ionic strength. The data in Table I point to a specific activation by certain members of the group 1 metals and NH_4^+ ion. Potassium is most active followed by NH_4^+ and Rb^+ which are about one half as active. Li^+, Na^+, and Cs^+ exert no stimulatory effect. These specific effects are contrasted to the inhibition observed when these same salts were tested with the dGMP kinase prepared from cells infected with coliphage T2. Here the inhibition appears to be a consequence of the ionic strength rather than of a

particular ionic species since all the metals tested showed a similar inhibition.

Table I

Effect of Alkali Metals on dGTP Kinase Activity

Source of Extract	Protein	mμmoles of dGTP formed in presence of						
	μg	-	K^+	Rb^+	NH_4^+	Na^+	Cs^+	Li^+
Normal cells	11	0.1	2.1	1.1	1.0	0.2	0.2	0.1
Infected cells	1	1.8	1.2	1.2	1.0	1.0	1.2	1.2

The alkali metals were added to the incubation medium as chlorides to a final concentration of 0.2M.

Table II

Effect of Mixed Extracts on Potassium Requirement

Source of Extract	Protein	dGTP formed	
	μg.	-KCl	+KCl
		mμmoles	mμmoles
Normal cells	30	0.1	3.2
Infected cells	3	2.6	1.2
Normal cells + Infected cells	30 + 3	2.8	4.1

Potassium chloride was present at a final concentration of 0.2M where indicated.

The observed inhibition was not due to chloride ions specifically since potassium chloride, phosphate, and sulfate inhibited 59, 56 and 70 per cent respectively when present at equimolar concentrations.

Although the dGMP kinase prepared from normal cells is markedly stimulated by K^+ it still retains its requirement for Mg^{++}. A crude preparation incubated in the presence of 0.2M K^+ gave values of 2.76 and 0.27 mμmoles of dGTP formed in the presence and absence of Mg^{++} respectively. The dGMP kinase from infected cells also has a requirement for Mg^{++}.

Effect of mixed extracts on dGMP kinase activity. It was of interest to determine whether the apparent difference in K^+ requirement of the dGMP kinase from normal and infected cells was due to actual differences in the enzymes or was the consequence of secondary differences in the ex-

tracts from normal and infected cells. The results of mixing the two extracts are shown in Table II.

It is evident that there is no interaction of the two extracts in the presence or absence of KCl. Thus it seems unlikely that the differences observed are due to secondary changes resulting from viral infection. It is still not clear, however, whether the dGMP kinase of infected cells is actually a new and different protein. For example the data would also support the idea of an alteration in the K^+ binding strength of the enzyme from infected cells.

The loss in the K^+ requirement of the dGMP kinase after viral infection may be of importance in the process of infection. It is known that materials rapidly leak out of E. coli infected with coliphage T2 (Puck and Lee, 1954). A leakage of K^+ would be expected to inhibit dGMP kinase with a concomitant inhibition of DNA synthesis by the host. The formation of a new enzyme capable of acting in the absence of K^+ would then be advantageous to the invading virus.

The ubiquity of K^+ as a major ionic constituent of the intracellular environment makes it of especial interest to investigate enzymatic activities influenced by this ion. It will be informative to examine the dGMP kinases from different sources.

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