STUDIES ON THE RATE OF SYNTHESIS OF PHAGE-INDUCED ENZYMES IN RELATION TO THE MULTIPLICITY OF INFECTION *

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Since the first report by Flaks and Cohen (1959) of the phage-induced formation of hydroxymethylase, a number of other phage-induced enzymes have been reported (Kornberg et al., 1959, Somerville et al., 1959, Koerner, et al., 1960 and Keck et al., 1960). Kinetic studies revealed that in E. coli infected with 4-5 phage particles per bacterium, these enzymatic activities increase during the first minutes after infection and then level off at a constant value approximately fifteen minutes after infection. If the phage were irradiated with an appropriate dose of u. v. light, the enzymatic activities continue to rise even after fifteen minutes of infection (Dirksen et al., 1960). The factors that govern the synthesis of these enzymes, both in terms of the rate and the final level, are not known. We wish to report in this communication some experimental results which show that the rate of synthesis of these enzymes in infected bacteria is not directly related to the multiplicity of infection.

Experimental

E. coli B were grown and infected with T2 phage essentially as described by Flaks and Cohen (1959). The only variation was the ratio of phage to bacterium which was such as to give either one or more than five phage particles per infected bacterium. The average number of phage particles absorbed per infected bacterium was determined five minutes after mixing according to Fredericq (1952). The bacteria harvested at different intervals of incubation at 25°C. after infection were broken in cold 0.02M. Tris-acetate buffer pH 7.5 in a micro-VirTis homogenizer according to Lamanna and Mallet (1954). This gave virtually complete breakage of the bacteria and reproducible recovery

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of protein. The activity of the dCTP pyrophosphatase (dCTPase) in the extracts clarified by centrifuging for 1 hour at 13,000 xg, was determined according to Koerner et al., (1960) but in the presence of mercaptoethanol (Zimmerman and Kornberg, 1961).

Results and Discussion

The results of two separate experiments are shown in Table I. It can be readily seen that in the cases of high multiplicity of infection where there are 6 or 9 sets of viral genes per infected bacterium, the rate of synthesis of the dCTPase is not higher than in those bacteria with only one set of viral genes. Indeed, at high multiplicity of infection, both the rate of synthesis and the final level of the dCTPase appeared to be lower than at low multiplicity of infection. Similar results have been obtained with two other phage-induced enzymes, hydroxymethylase and dCMP deaminase. Since the multiplicity of infection was determined 5 minutes after mixing the phage with bacteria, there remained at that time both an excess of uninfected bacteria and unabsorbed phage in the

TABLE I

Formation of dCTPase in Infected Bacteria: mμ moles of dCMP/10^9 Infected Bacteria

Minutes after Infection	Experiment A		Experiment B	
	1 T2/coli	9 T2/coli	1 T2/coli	6 T2/coli
2.5	73	28.5		
5.0	70	37.8		
7.5			153	44
10.0	244	145.0		
15.0	297	132.0	340	120
17.5	270	165.5		
20.0	305	230	575	263
22.5	402	230		
25.0	424	262.3		
30.0	362	209.0	635	311
40.0	262	209.0		

The reactions were carried out in a total volume of 1 ml. with 2-10 μl. of extract, 10 μ moles of mercaptoethanol, 0.9 μ moles of dCTP, buffer and magnesium as described by Koerner, et al., (1960) at the end of five minutes of incubation at 37°, the enzyme was inactivated by boiling.

case of low multiplicity (more than 60% with both bacterium and phage). The number of infected bacteria at a later time would be larger than the value which was determined at 5 minutes after mixing and used for calculations. It is therefore not warrented to compare the final level of induced enzyme per infected bacterium for the multiplicities of infection. However, it can be concluded that the rate of synthesis of the new enzymes in those bacteria with several sets of phage gene is essentially the same as, if not lower than, that in bacterium with only one set of phage gene.

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