REVERSIBILITY OF INHIBITION OF NUCLEIC ACIDS AND PROTEIN SYNTHESIS BY COLICIN K*

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Studies in this laboratory on the inhibition of host bacterial protein synthesis by phage T4 suggested that the functional capacity of the host chromosome to make specific enzymes still remains intact in the infected cells (Nomura et al, 1962). The primary inhibitory process appears to be reversible in its inherent nature. This led us to test the reversibility of inhibition of macromolecule synthesis by colicin, a bactericidal substance which resembles bacteriophage, or more specifically phage ghosts, in its mode of action (Jacob et al, 1952; Fredericq, 1958).

Purified colicin K (Goebel and Barry, 1958) was kindly supplied by Dr. T. Amano.

E. coli B grown in tris-glycerol-casamino acids medium was treated with colicin K (10 μg/ml) at 37° C, either in a growing condition or in a "resting" condition. The cells were washed and resuspended in the same medium except that glycerol and casamino acids were omitted from the resting cell culture. Ten minutes after the treatment with colicin, the number of colony formers decreased by the factor of 10^{-4}, both in the growing and in the resting cultures. Synthesis of β-galactosidase, RNA and DNA in the growing culture was immediately stopped. This finding is in agreement with the results obtained by previous workers (Jacob et al, 1952).

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Trypsin, a proteolytic enzyme which is known to inactivate colicin (Fredericq, 1948), was added (250 µg/ml) to such an inhibited culture. Both β-galactosidase synthesis (Fig. 1 (a)) and nucleic acid synthesis (Fig. 1 (b)) resumed after about 30 minutes and soon reached the rate of an uninhibited culture. Turbidity of the culture also started to increase rapidly around the same time indicating the resumption of multiplication of a majority of the "killed" cells.

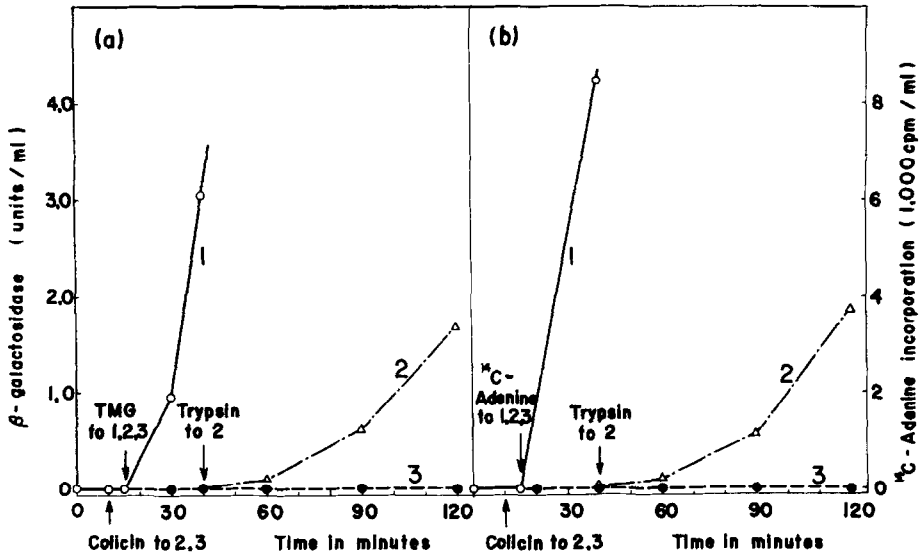

Fig. 1. Inhibition of the synthesis of β-galactosidase (a) and nucleic acids (b) in growing E. coli by colicin K and its reversal by trypsin. β-Galactosidase was assayed according to the method similar to that described by Pardee et al (1959), and was expressed in optical density units at 420 mµ after 30 minutes of incubation of assay mixture using 1 ml of toluenized culture. Synthesis of nucleic acids was followed by the incorporation of C^{14}-adenine into acid insoluble fraction.

The reversibility of the inhibition was studied further by following the β-galactosidase synthesis in the resting cells. The resting culture was treated with colicin (10 µg/ml) for 10 minutes. This treatment was sufficient to reduce the viable count by the factor of 10^{-4} and to cause the complete inhibition of β-galactosidase synthesis. Both the colicin treated culture and the control culture were then treated with trypsin (250 µg/ml) for 50 minutes. Cells were centrifuged and washed. An inducer, methyl β-

D-thiogalactoside (TMG), was then added to both resting cultures (Fig. 2). The rate of enzyme synthesis by the colicin treated culture (Curve 2) was nearly the same as that by the control culture (Curve 1), although a slight lag was sometimes observed in a colicin treated culture. It is evident that, although the cells were "killed" and could not synthesize β-galactosidase, the potential capacity for enzyme synthesis remained intact, and moreover, the recovery process from the "killed state" was completed within one hour in the resting condition, i.e., in the absence of an energy source. In order to rule out the possible participation of some endogenous metabolic processes or utilization of small amount of substances derived from the trypsin digested colicin, the experiment was also performed in the presence of cyanide (10^{-3} M) during the trypsin treatment. After the removal of cyanide by centrifugation, the synthesis of β-galactosidase was followed in the resting condition. Essentially the same initial rate of enzyme synthesis was observed in both the control (Fig. 2, Curve 3) and the colicin killed (Fig. 2, Curve 4) cultures. These results suggest that the recovery process does not involve any energy-requiring metabolic process and hence, little, if any, direct cellular damage is involved in the killing action of colicin K.

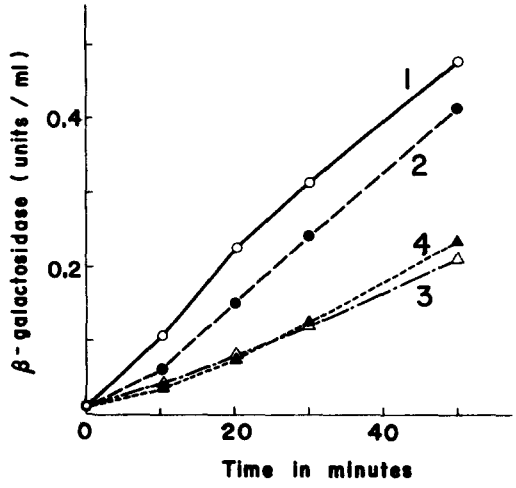

Fig. 2. Recovery of a capacity to synthesize β-galactosidase after the treatment with trypsin of colicin-killed resting culture of E. coli. The details are described in the text.

The reversal of the "killing" action of colicin with trypsin was also demonstrated by following the number of colony formers and the ability of the cells to yield infective centers when infected with phage T_4. In these experiments, the recovery process before phage challenge or before plating was found not to be affected by the presence of following inhibitors: 10^{-3} M cyanide, 10^{-3} M 2,4-dinitrophenol, 20 μg/ml chloromycetin, and 300 μg/ml azauracil. Besides, leakage of cellular substances due to the possible damage in the permeability barrier accompanied by the killing action of colicin was looked for with β-galactosidase as an indicator. No leakage was observed. The breakdown of DNA during colicin treatment was not found either. All these results are consistent with the conclusion derived from the experiments on β-galactosidase synthesis. It should be added here that Amano also has observed some increase in the number of survivors after the incubation of colicin treated culture with antiserum against colicin (personal communication).

It appears, therefore, that colicin K inhibits nucleic acids and protein synthesis of *E. coli* completely and reversibly with little direct cellular damage. The inhibition of host bacterial protein synthesis by virulent phage may be due to a similar reversible inhibitory mechanism (Nomura et al, 1962). In this connection, French and Siminovitch (1955) already reported that some preparations of T_2 phage ghosts inhibit macromolecule synthesis in *E. coli* only temporarily. The existence of a similar mechanism may also be presumed in the lysogenization process of λ in *E. coli* K12 (Fry and Gros, 1959). The possible role of such mechanisms in the regulation of normal bacterial cells and their elucidation in detail are the subjects of future study.

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