EFFECT OF A BACTERIOCIN PREPARATION (MEGACIN C) ON DNA SYNTHESIS IN BACILLUS MEGATERIUM

I. B. HOLLAND*

Microbiology Unit, Department of Biochemistry, University of Oxford

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During infection by a virulent phage (T2), the host DNA is degraded and the synthesis of both RNA and of many bacterial enzymes ceases (cf. Adams, 1959); formation of phage protein and nucleic acid then commences as the control of metabolic processes is assumed by the genome of the invading virus. Jacob, Siminovitch & Wollman (1952) have shown that a bacteriocin (cf. Fredericq, 1957), colicin ML prevents the synthesis by treated organisms of DNA, RNA and protein. Furthermore it has been reported (Nomura & Nakamura, 1962; Reynolds & Reeves, 1963) that the addition of trypsin to the inhibited bacteria overcomes the effect of colicin and leads to rapid resumption of the synthesis of macromolecules. Colicins and phages are both adsorbed by specific cell surface receptors of sensitive bacteria, but as colicins contain protein but no nucleic acid, they cannot take-over control of synthetic processes in the affected organism.

A new bacteriocin, megacin C, has been found to be present in several strains of Bacillus megaterium (I.B.

*I.C.I. Biochemistry Research Fellow.

Holland & C.F. Roberts, in preparation). It is specific to certain strains of the organism and is a bacteriocin of the colicin type and therefore distinct from the megacin first reported for this organism, which has quite different properties (Ivánovics & Alföldi, 1954; Ivánovics, 1962). Megacin C is adsorbed by sensitive organisms which are rapidly killed; net synthesis of RNA and protein ceases and a major part of the DNA is apparently degraded.

Methods. Megacin C is liberated into the medium by young cultures of some strains of B. megaterium growing in a peptone glucose broth. The organisms continue growing and do not lyse and a crude megacin preparation was obtained simply by centrifuging to remove organisms. As megacin is stable to irradiation with U.V. light the preparation was sterilized by this means and kept overnight at 4° before use. The methods used for the growth of organisms, assay of megacin and determination of viable counts have been described previously (Holland, 1961, 1962). The lysogenic strain (B. megaterium 899) and the strain (Mut.), sensitive both to megacin C and to phage, were both kindly supplied by Prof. A. Lwoff. Protein, RNA and DNA were extracted from culture samples by methods similar to those of Fry & Gros (1959); DNA was estimated with diphenylamine, RNA with orcinol and protein with Folin reagent.

Results and Discussion. Action of megacin C on growing cultures of B. megaterium Mut. A preparation of megacin C (final concentration 100 units/ml.) was added to a growing culture of strain Mut.; the viable count decreased immediately and after 30 min. was 10^{-5} of the initial count. The turbidity of the treated culture increased for a short time (Fig. 1a) but the organisms did not lyse even after several

hours. The DNA content of the culture decreased rapidly to about 25% of the initial value while net synthesis of RNA and protein ceased immediately (Fig. 1a and b). Treatment of the culture with a concentration of azide (5 mM) sufficient to stop growth and DNA synthesis prevented the loss of DNA on subsequent addition of megacin C. Furthermore with non-aerated suspensions of stationary phase organisms in peptone broth, although megacin C is adsorbed, no breakdown of DNA occurs unless the bacteria are subsequently aerated in peptone broth plus glucose, i.e. conditions permitting growth in absence of megacin.

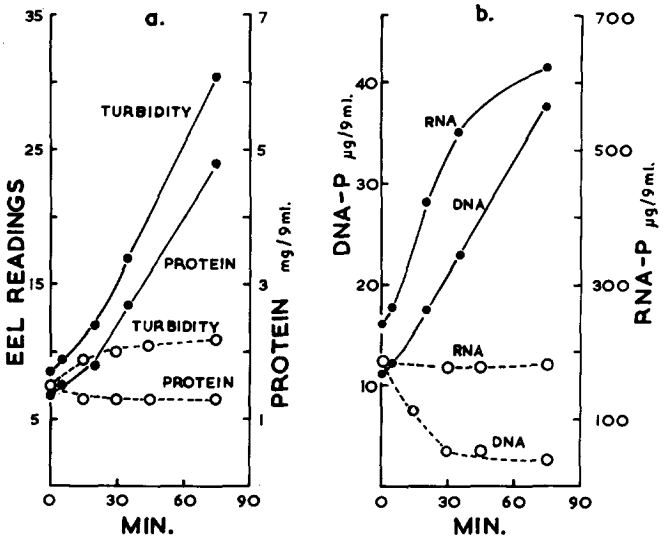

Fig. 1. Effect on DNA, RNA and protein synthesis of megacin C (final concentration 100 units/ml.) added at time 0 to growing cultures of strain Mut., 9 ml. samples were taken at intervals, mixed with 1 ml. ice-cold TCA and rapidly cooled to 0° to precipitate nucleic acids and protein.

Induction of lysogenic strain 899 with preparations of megacin C. Agents which have been previously reported to induce prophage all appear to have in common the ability

to affect DNA synthesis. Addition of a preparation of megacin C to young cultures of strain 899 induced both lysis and a massive increase in the titre of free bacteriophage (Fig. 2) when assayed with B. megaterium strain Mut. as indicator. The optimal latent period for induction with both megacin C and U.V. was 45 min. and the phage yield for the latter was 6 x 10^9 particles/ml. (cf. Fig. 2). Thus both U.V. induction and treatment with megacin C gave comparable results. These experiments suggest that although bacterial DNA synthesis is affected by megacin C preparations (with consequent cessation of RNA and protein synthesis) the systems for RNA and protein synthesis remain functional

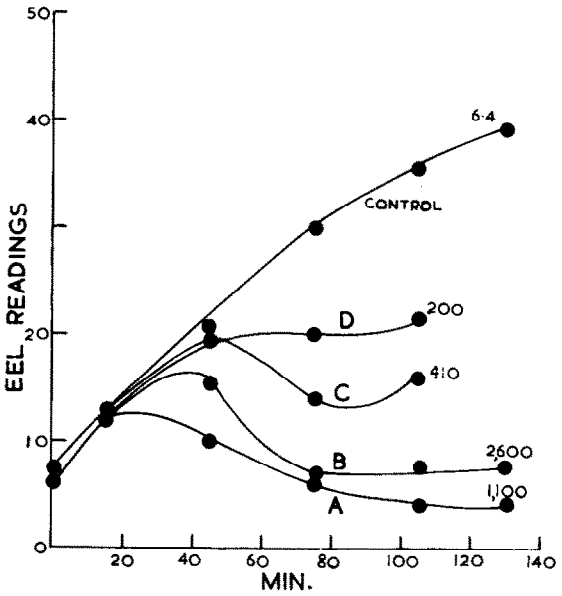

Fig. 2. Induction of lysogenic strain 899 with megacin C. Extracellular phage was determined with strain Mut. as indicator and the titres (x 10^{-6}) appear beside the curves. Control, no megacin; A, 100 units/ml. megacin; B, 50 units/ml.; C, 25 units/ml.; D, 10 units/ml.

and can be directed by the induced prophage. Until megacin C has been purified the possibility cannot be excluded that phage induction is caused by the presence of a

second factor distinct from megacin C; it may be noted however that colicin E2 also induces phage production in a lysogenic bacterium (Endo, Kamiya & Ishizawa, 1963). The fact that bacteriophages and some bacteriocins can affect the synthesis of DNA by virtue of adsorption to specific cell surface receptors lends support to the suggestion of Jacob & Monod (1962) that DNA replication in the normal organism is controlled through the mediation of components in the cell surface. The possibility that megacin C either activates or provokes the formation of a deoxyribonuclease will be investigated with purified preparations of megacin C. An enzyme has recently been described in Escherichia coli (Richardson, Schildkraut & Kornberg, 1963) whose function appears to be a preliminary degradation of DNA necessary before the latter can act as primer in the polymerase system. Uncontrolled production of such an enzyme might lead to lethal effect similar to those induced by megacin C.

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