THE EFFECTS OF RIBONUCLEASE AND DEOXYRIBONUCLEASE ON BACTERIOPHAGE FORMATION IN PROTOPLASTS OF *BACILLUS MEGATERIUM*

by

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LESTER[1] and BELJANSKI[2] have shown that cells of *Micrococcus lysodeikticus* after digestion with lysozyme in the presence of sucrose retain the ability to incorporate amino acids into proteins. The same type of treatment converts the cells of *B. megaterium* into spherical protoplasts[3], in which amino acid incorporation has recently been demonstrated by MC QUILLEN[4]. It seems reasonable to assume that the lysozyme-sucrose lysates of *M. lysodeikticus* also contained protoplasts and that these structures were responsible for the observed incorporation of amino acids. Both LESTER[1] and BELJANSKI[2] observed distinctive effects with ribonuclease (RNase) and deoxyribonuclease (DNase); the addition of RNase suppressed amino acid incorporation completely while DNase exerted a marked stimulatory effect. From these results it appeared possible that protoplasts were permeable to high molecular weight enzymes and that ribonucleic acid was essential for protein synthesis.

Protoplasts of *B. megaterium* preserve sufficient structural and functional integrity to support the growth of bacteriophages provided that the cells are infected prior to lysozyme treatment[5,6]. The present experiments were originally undertaken to investigate the effects of DNase and RNase on bacteriophage growth in protoplasts with the object of establishing the respective roles of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) in virus replication. This expectation was not in fact realised, but since the experiments throw light on the reactions of protoplasts with enzymes they are reported in the present communication.

MATERIALS AND METHODS

Strain KM of *Bacillus megaterium* and the *megaterium* bacteriophage, strain C, were obtained from the Department of Bacteriology, University of California, Berkeley. The method of assay of bacteriophage is described in the previous paper[5].

Asparagine-phosphate medium: 1 g L-asparagine, 0.54 g NaCl, 0.3 g KCl, 0.1 m*M* Na_2SO_4, 0.01 m*M* $CaCl_2$ and 0.1 m*M* $MgCl_2$ in 100 ml 0.03 *M* phosphate buffer, pH 7.0.

Sucrose-buffer: 0.2 *M* sucrose in 0.03 *M* phosphate buffer, pH 7.0.

Enzymes: Crystalline DNase (Worthington) and crystalline RNase (Armour) were gifts from Dr. H. K. Schachman.

References p. 534.

EXPERIMENTS AND RESULTS

1. *Effects of DNase and RNase on bacteriophage formation in cells and protoplasts*

Cultures of *B. megaterium* KM were grown at 35° C with aeration in asparagine-phosphate medium. The cells were harvested when they had reached a density of $5–7 \cdot 10^7$ per ml, infected with C phage at a multiplicity of 0.01, and a portion treated with lysozyme using procedures described previously[5]. At time $t = 0$, the suspensions of infected cells and infected protoplasts were diluted tenfold into 2.5 ml of asparagine-phosphate medium made 0.2 *M* with respect to sucrose in 6" × 1" tubes. RNase and DNase were added, and the growth tubes shaken at 60 oscillations per minute in a waterbath maintained at 25° C. Samples were removed at $t = 0$ and after four hours incubation. Appropriate dilutions were assayed for their phage content and the protoplasts counted in a haemocytometer chamber.

The results of this experiment are given in Table I, from which it can be seen that the addition of either DNase or RNase to intact infected cells does not affect the growth of bacteriophage. These results may be compared with those of BELJANSKI[2] who found that incorporation of aminoacids by intact cells was not influenced by these enzymes. After treatment with lysozyme, the input infective centres decrease because the protoplasts at this stage do not contain mature phage, the original particles having entered the eclipse period of intracellular development[5]. At the end of the growth period there is a marked increase in phage concentration, the average burst size being smaller than that obtained with the intact cells. The addition of DNase decreases the burst size in the protoplasts, but this is probably not significant because of the known variability of phage production by protoplasts. On the other hand, RNase produces a thousandfold decrease in the average burst size. This effect of RNase is correlated with the low survival of the protoplasts and it appeared probable that the suppression of phage formation was due to lysis of the protoplasts induced by RNase.

TABLE I

EFFECTS OF DNASE AND RNASE ON BACTERIOPHAGE FORMATION IN CELLS AND PROTOPLASTS

Culture	*Enzyme*	*Phage per ml of growth tube*		*Average burst size**	*Protoplasts per ml of growth tube*	
		t = 0 hour	*t = 4 hours*		*t = 0 hour*	*t = 4 hours*
Cells	None	$1.4 \cdot 10^5$	$6.4 \cdot 10^6$	46		
	DNase 10 μg/ml	$1.4 \cdot 10^5$	$6.0 \cdot 10^6$	43		
	RNase 50 μg/ml	$1.4 \cdot 10^5$	$5.8 \cdot 10^6$	42		
Protoplasts	None	$3.2 \cdot 10^2$	$4.2 \cdot 10^6$	30	$1.8 \cdot 10^7$	$1.6 \cdot 10^7$
	DNase 10 μg/ml	$3.2 \cdot 10^2$	$3.4 \cdot 10^6$	24	$1.8 \cdot 10^7$	$1.5 \cdot 10^7$
	RNase 50 μg/ml	$3.2 \cdot 10^2$	$4.9 \cdot 10^3$	0.035	$1.8 \cdot 10^7$	$> 10^4$

* Average burst size is calculated in all cases on the basis of input infective centres, *i.e.* $1.4 \cdot 10^5$/ml.

References p. 534.

2. Lysis of protoplasts by RNase

A culture of *B. megaterium* KM was grown in asparagine-phosphate medium to a density of $8 \cdot 10^7$ per ml, centrifuged, and the cells resuspended in sucrose-buffer. 20 μg/ml lysozyme was added and the changes in turbidity followed at 23° C in a photoelectric colorimeter. After 30 minutes, the suspension was divided into two portions, 50 μg/ml RNase added to one portion, and the changes in turbidity recorded. The results are presented in Fig. 1. The addition of lysozyme produces a rapid drop in the optical density of the suspension as the cells are transformed into protoplasts. When this process is completed, the optical density remains constant. Subsequent addition of RNase induces a further decrease of the turbidity of the suspension resulting eventually in a viscous suspension of ghosts.

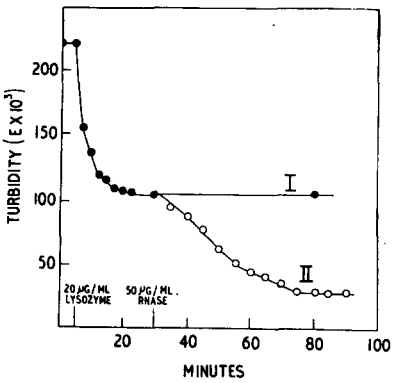

Fig. 1. Turbidity changes in a suspension of *B. megaterium* cells treated with lysozyme (I) and then with RNase (II).

DISCUSSION

When a bacteriophage infects the cell, the viral DNA enters the host carrying all the genetic information for the synthesis of new progeny phages[7]. Even if this information is transferred to another structure, replication of DNA is necessary for the formation of mature phage. The absence of any marked effect of DNase on phage development in protoplasts suggests that the phage DNA is not accessible to the enzyme; the simplest hypothesis that can be put forward is that the protoplasts are impermeable to DNase. Although BELJANSKI[2] found evidence for the degradation of DNA by DNase in protoplast suspensions, it is probable that the DNA had been released into the medium by spontaneous lysis of the protoplasts. Protoplasts are extremely fragile and unless precautions are taken spontaneous lysis commonly occurs. GALE AND FOLKES[8] have shown that purines and pyrimidines stimulate protein synthesis, and it is likely that the stimulatory effects observed with DNase are due to the enhancement of amino acid incorporation in intact protoplasts by low molecular degradation products of DNA released into the medium by spontaneous lysis.

The lysis of the protoplasts induced by RNase with consequent dispersion and dilution of the contents of the structure provides sufficient reason for the suppression by this enzyme of both phage formation and amino acid incorporation. In the light of the experiments with DNase, it is unlikely that RNase penetrates into the protoplasts. It is possible that RNA forms an integral part of the structure of the protoplast membrane and that its depolymerisation by the enzyme destroys the permeability barrier to sucrose producing osmotic lysis. This possibility is supported by WEIBULL's finding[9] that 15% of the RNA of lysed protoplasts sediments with the ghost membranes.

The present experiments indicate that protoplasts are not, in general, permeable to macromolecular substances. This is not surprising in view of the existence of a permeability barrier which prevents both the leakage of low molecular weight intra-

References p. 534.

cellular substances[3] and the entry of sucrose. The main conclusion to be drawn is that the experiments of LESTER[1] and BELJANSKI[2] do not afford any evidence as to the possible role of RNA in protein synthesis. To what extent inhibition of amino acid incorporation by RNase in microsomes[10,11] and other systems[8] is due to a similar disintegration of aggregated structures containing RNA cannot at the moment be decided. However, it is evident that this effect of RNase must be excluded in all experiments in which this enzyme is used to demonstrate the role of RNA in protein synthesis.

SUMMARY

DNase has no significant effect on bacteriophage formation by protoplasts of *Bacillus megaterium*, while RNase suppresses bacteriophage growth almost completely. This action of RNase is due to lysis of the protoplasts induced by the enzyme with consequent dispersion of the protoplast contents. It is concluded that protoplasts are not generally permeable to macromolecular substances such as enzymes.

RÉSUMÉ

La DNase n'a pas d'action significative sur la formation du bactériophage par les protoplastes de *Bacillus megaterium*, tandis que la RNase supprime presque complètement la croissance du bactériophage. Cette action de la RNase est due à une lyse des protoplastes provoquée par l'enzyme et suivie de la dispersion de leur contenu. Les auteurs concluent que les protoplastes ne sont pas, en général, perméables aux substances macromoléculaires telles que les enzymes.

ZUSAMMENFASSUNG

DNase übt keine bedeutsame Wirkung auf die Bakteriophagenbildung durch Protoplaste von *Bacillus megatherium* aus, während RNase das Wachstum der Bakteriophagen fast vollständig unterbindet. Diese Wirkung von RNase ist der Lyse der Protoplasten zuzuschreiben, welche durch das Enzym verursacht und von der Dispersion des Protoplasteninhaltes gefolgt wird. Daraus wird die Folgerung gezogen, dass Protoplaste makromolekularen Substanzen wie z.B. Enzymen gegenüber nicht immer durchlässig sind.

REFERENCES

1 R. L. LESTER, *J. Am. Chem. Soc.*, 75 (1953) 5448.
2 M. BELJANSKI, *Biochim. Biophys. Acta*, 15 (1954) 425.
3 C. WEIBULL, *J. Bacteriol.*, 66 (1953) 688.
4 K. MCQUILLEN, *Biochim. Biophys. Acta*, 17 (1955) 382.
5 S. BRENNER AND G. S. STENT, *Biochim. Biophys. Acta*, 17 (1955) 473.
6 M. R. J. SALTON AND K. MCQUILLEN, *Biochim. Biophys. Acta*, 17 (1955) 405.
7 A. D. HERSHEY AND M. CHASE, *J. Gen. Physiol.*, 36 (1952) 39.
8 E. F. GALE AND J. P. FOLKES, *Nature*, 173 (1954) 1223.
9 C. WEIBULL, *J. Bacteriol.*, 66 (1953) 696.
10 V. ALLFREY, M. M. DALY AND A. E. MIRSKY, *J. Gen. Physiol.*, 37 (1953) 157.
11 P. C. ZAMECNIK AND E. B. KELLER, *J. Biol. Chem.*, 209 (1954) 337.

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