ROLE OF S GENE OF BACTERIOPHAGE LAMBDA IN HOST LYSIS

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Received February 13,1976

SUMMARY:- Using E. coli lysogens carrying heat inducible λ defective in R or S or both R and S genes (called R, S and RS cells respectively), it has been found that induced β-galactosidase synthesis by host continued in S cells beyond the normal time of lysis. S function affected the permeability of host cell membrane. When radioactive labelled host membrane was incubated in vitro with extracts prepared from phage induced R and RS cells, R cell extract solubilized labelled lipid mostly in the undegraded form. This indicated that S gene product may be a lipophilic protein rather than a phospholipase.

INTRODUCTION

At least two late genes of bacteriophage λ, namely R and S are involved in the lysis of the host E. coli (1,2). The gene R codes for the phage specific endolysin (3,4) which acts on the cell wall. But the role of S gene is not clearly understood yet. The discovering of temperature-sensitive, supressible and nonsense mutations in gene S of λ (5) suggested that the S gene product is a protein. From the pleiotropic effects of S gene product, Adhya et al. (6) indicated it to be a phospholipase. However, from the detailed study of Reader and Siminovitch (7,8), the interference with respiratory metabolism and hydrolysis of

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Abbreviations used: EDTA, ethylenediamine tetraacetic acid; FFA, free fatty acid; LPE, lysophosphatidyl ethanolamine; MTG, methyl-β-D-thiogalactoside; ONPG, orthonitrophenyl-β-D-galactoside; PE, phosphatidyl ethanolamine; SDS, sodium dodecyl sulphate; TLC, thin layer chromatography.

phospholipid appeared to be secondary events related to some change in the membrane occurring at the normal time of lysis. The present study indicates that S gene product brings about alteration in the host cell membrane by solubilization of phospholipid without apparent degradation.

METHODS

The three lysogens, namely E. coli SA 500 (λ cI857 sus R5), SA 500 (λ cI857 sus S7) and Sa 500 (λcI857 sus R5 sus S7) were obtained from S. Adhya. These are designated as R, S and RS cells respectively. Media, culture methods and heat induction of the phage were as described by Adhya et al. (6). In short, logphase cultures of the lysogens in M9-glycerol medium at 32° were transferred to 42°, kept there for 10 min with shaking and then transferred to 37° shaker bath. The biochemical properties of these phage-induced cells were studied at different periods after heat induction.

Induction and assay of β-galactosidase were carried out according to Kepes (9). Glucose permease and β-galactoside permease were assayed by determining the uptake of ^{14}C-2-deoxyglucose and ^{14}C-MTG by intact cells for 2 min followed by rapid washing on Millipore filters . Membranes were isolated from labelled nonlysogen and heat induced lysogens by EDTA-lysozyme treatment followed by lysis with Brij 58 (ref. 10). Other methods are described in the text.

RESULTS

It has been previously observed that the rates of DNA, RNA and protein synthesis continued beyond the normal time of lysis in S cells, while R cells, these rates dropped to zero around 60 min after phage induction (11). To see whether the transcription of host DNA still continued in S cells, synthesis of induble β-galactosidase was studied (the strains used did not contain any β-galactosidase gene). Fig. 1 shows the synthesis of β-galactosidase in heat induced R and S cells in constant presence of MTG. The level of β-galactosidase increased at the same rate initially in both R and S cells. But while it reached a plateau in R cells at 60 min, it continued to increase in S cells. That the continued synthesis of β-galactosidase in S

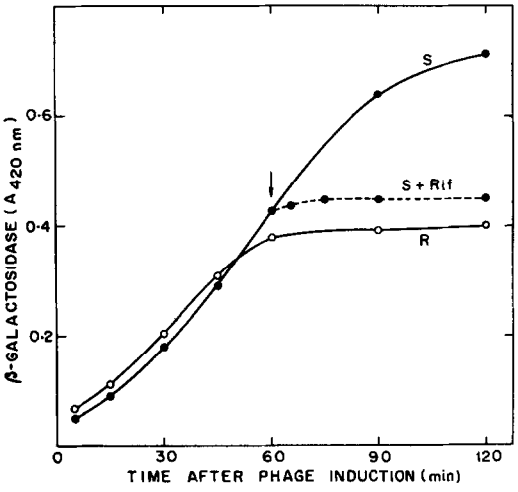

Fig. 1.

Synthesis of β-galactosidase in induced R and S cells. Cultures containing 1 x 10^9 cells/ml growing in M9-glycerol medium were heat induced at 42° and shifted to 37° shaker bath. Thiomethyl-β-D-galactoside was added to a final concentration of 3 x 10^{-3}M. At different periods, 1 ml aliquots were withdrawn, shaken with 0.02 ml of toluene for 20 min and then incubated with 0.2 ml of 0.4% ONPG for 30 min more. The reaction was stopped by addition of sodium carbonate (0.25 M final) and the color was read in Beckman DU spectrophotometer at 420 nm. Rifampicin was added (final conc. 50 μg/ml) to an aliquot of S cells at the time indicated by the arrow. -O-, R cells; -●-, S cells; --●--, S cells plus rifampicin.

cells required continued transcription of host DNA was shown by rifampicin sensitivity of the process. The addition of rifampicin to the S cells at any time after induction stopped further increase of β-galactosidase activity within minutes (Fig. 1).

Phage induced S cells have been found to be more fragile than R cells with respect to treatment with detergents and freezing-thawing (12). On the other hand, release of 260 nm UV-absorbing material was more in R cells than S cells. This indicated a change in the permeability barrier in R cells

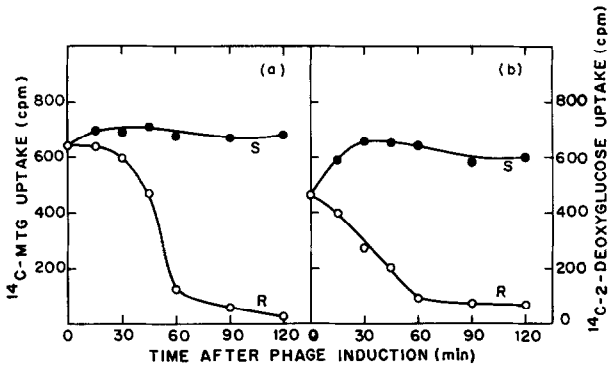

Fig.2.

Uptake of ^{14}C-TMG and ^{14}C-2-deoxyglucose in induced R and S cells. Cultures growing in M9-glycerol medium were heat induced as described under Fig. 1. To 0.5 ml aliquots (5×10^8 cells), 0.1 µCi ^{14}C-TMG (Sp. Ac. 34 mCi/mmole) or 0.1 µCi ^{14}C-deoxyglucose (Sp. Ac. 63 mCi/mmole) was added. After 1 min, the cells were filtered on Millipore filters, washed with medium, dried and counted. (a) Uptake of ^{14}C-TMG, (b) Uptake of ^{14}C-2-deoxyglucose.

○, R cells; ●, S cells.

caused by S gene product which was confirmed by following the uptake of MTG and deoxyglucose by intact cells. The results presented in Fig. 2 showed that the uptake of both ^{14}C-TMG and ^{14}C-2-deoxyglucose remained practically unchanged in S cells, but in R cells it dropped to very low level at 60 min after heat induction. The initial rise of deoxyglucose uptake in S cells can be explained by the fact that appreciable growth of these cells even after induction, provided additional membrane sites for glucose permeation.

Since S function alters the cytoplasmic membrane, it might be expected to do so by degrading either the phospholipids or the proteins of the membrane. We therefore, checked any loss of radioactivity from prelabelled membrane proteins and lipid *in vivo* after phage induction. From table 1, it is seen that the yield of membrane decreased by about 35% at 50 min after

Table 1

Loss of Radioactivity from Protein from ^{14}C-valine labelled and from Lipid of ^{14}C-acetate labelled membrane in R and RS cells by phage induction

Cells	Membrane yield (mg protein)	^{14}C-valine label		^{14}C-acetate label	
		Radioactivity in protein (cpm)	Sp. Activity (cpm/mg protein)	Radioactivity in lipid (cpm)	Sp. Activity (cpm/mg membrane protein)
R control	0.28	3,820	11,900	42,000	150,000
R 50 min p.i.+	0.18	2,600	10,810	27,000	150,000
RS control	0.28	3,620	12,930	37,000	132,000
RS 50 min p.i.+	0.30	3,560	11,860	39,000	130,000

+ p.i. = post induction

Twentyfive ml of each of the R and RS cells were grown in M9-glycerol (casamino acid -0.1%) medium for several generations at 32° in presence of ^{14}C-acetate (23.4 mCi/mmole) or ^{14}C-valine (41.4 mCi/mmole) so that the final concentration of the tracers was 0.4 μCi/ml. The cells were washed, suspended in fresh prewarmed nonradioactive medium, induced at 42° for 10 min and then quickly transferred to 37° shaker bath. After another 50 min at 37°, cells were harvested, washed and membrane was isolated as usual. Equal aliquots of the cultures were preserved without heat induction as controls. The radioactivity of the labelled protein (acid-insoluble) for ^{14}C-valine label and lipid (chloroform-methanol soluble) in the case of ^{14}C-acetate labelled membrane was determined by a Beckman LS-100 scintillation counter.

phage induction in R cells only, but in RS cells the same was slightly higher. Simultaneously, there was a loss, to the same extents, of radioactivity from proteins as well as lipids of the membranes. The results may mean that in R cells, the S gene product had solubilized membrane by degrading proteins or phospholipids or both. Thus the S gene product may act as a proteinase or phospholipase or their activator.

When membranes isolated from the nonlysogenic host E.coli SA 500 labelled for several generations with ^{14}C-valine or ^{14}C-acetate were incubated with cell-free extracts of induced R and RS cells *in vitro*, the protein radioactivity solubilized was the same for both the extracts, but the lipid radioactivity solubilized by R extract was more than double of that for RS cell

Table 2

Release of radioactivity from labelled E. coli membrane by incubation with induced R and RS extracts in vitro.

Extract	^{14}C-valine label solubilized (cpm/mg extract)	^{14}C-acetate label solubilized	
		Total cpm/mg extract	Lipid cpm/mg extract
R extract	700	14,880	13,380
RS extract	660	6,660	6,430

Membranes were isolated from E. coli SA500 cells labelled for several generations with ^{14}C-valine or ^{14}C-acetate. Aliquots of the membrane preparation equivalent to 0.3 mg protein (containing about 10,000 cpm) were incubated with suitable amounts of extracts (0.2-0.3 mg protein) isolated from R and RS cells 50 min after induction. After 30 min incubation at 37°, the mixtures were filtered through Millipore filters. The radioactivity retained on the filters, total solubilized radioactivity and lipid radioactivity solubilized as extractable by chloroform-methanol were determined.

extract (Table 2). The results were the same whether detergent (SDS or Triton X-100) was included in the incubation or not, or whether Brij-lysozyme lysis or alumina grinding was used to prepare the cell extract. Thus S gene product is most probably not a proteinase, but may be a phospholipase.

Assay of proteolytic activity by the hydrolysis of casein (13) revealed no difference in the R and RS cell extracts. Similarly, phospholipase assay using purified ^{14}C-acetate-labelled PE as the substrate showed LPE and PFA release by both R and RS extracts at the same rates (results not presented). The phospholipase activity in R and RS extracts, therefore, probably originated from the host cells. We subsequently studied the

nature of the lipid products solubilized from membranes by incubation with induced R and RS extracts in vitro as described in table 2. For this purpose, the solubilized lipids were extracted with chloroform-methanol and separated by thin layer chromatography (14). It was found that though there was a slight increase in the proportion of LPE and FFA solubilized by R extract compared to RS extract, the major fraction (>90%) of the solubilized lipid was still undegraded PE. For example, out of 4,600 cpm of total solubilized lipid spotted on TLC plate, 3900 cpm was in PE, 342 in LPE and 85 only in FFA.

DISCUSSION

Previous studies revealed three effects of expression of the λ S cistron on host cells: cessation of respiration, alteration in the permeability of the cytoplasmic membrane and phospholipid hydrolysis (6-8). Since normal lysis by λ dependent on S function can occur under conditions when phospholipid hydrolysis is greatly inhibited, this does not seem to be a cause of lysis, rather than an effect of it (8). This interpretation also finds support from the observation of Sakakibara et al. (15). They found that E. coli mutant defective in both detergent-sensitive and detergent-resistant phospholipases supports the normal growth of lambda with lysis. However, any significant release of FFA from prelabelled phospholipid was not observed at the time of lysis. Conversely, even in absence of S function, lysis results in phospholipid hydrolysis by host enzyme. This would imply that S function, by altering the membrane, makes it available for attack by host phospholipases.

Adhya et al. (6) observed that cessation of INA synthesis and O_2 uptake were not instantaneous after the expression of S

gene. Moreover, amber mutants of S could be supressed by a strong amber supressor suIII$^+$ but not by a weak amber suppressor su II$^+$ (5). An interesting feature of S mutant complementation is its inefficiency as compared to complementation of R mutants (7). These features indicate a highly dosage dependent or "stoichiometric" rather than catalytic mechanism of action for the S function. The present finding that S gene product simply acts as a lipophilic complexing agent and not as a degradative enzyme explains well all the above observations. Further studies on the nature of the S gene product are in progress.

Acknowledgements - The authors wish to thank Dr. S. Mitra and Dr. S. Adhya for generous gifts of experimental materials and for initiating them to the techniques of virology. Acknowledgements are also due to Prof. B.B. Biswas, Prof. A. Sen and Mr. J. Dutta for their suggestions and interest in this work. This research was supported in part by a grant from the Department of Atomic Energy, Govt. of India.

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