STUDIES ON THE ROLE OF DEOXYRIBONUCLEASE IN T2 BACTERIOPHAGE DEVELOPMENT*

by

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INTRODUCTION

Among the observed changes in the metabolism of *Escherichia coli* after infection with bacteriophage T2 is an increase in deoxyribonuclease (DNase) activity[1,2]. Further investigations of this increase, which has been shown to be brought about by "activation" of DNase already present in uninfected cells[3], are presented here.

The activity of DNase was examined in both uninfected and infected cells with the object of determining whether activation is specifically brought about by infection and whether phage production is always preceded by DNase activation. Attempts were made to correlate DNase activation with various other occurrences in infected cells.

MATERIALS AND METHODS

Bacteriophage T2r$^+$ and its host *Escherichia coli*, strain B, were used throughout this study. Standard phage techniques, as described by ADAMS[4], were employed. The following media were used: glycerol-Casamino acids medium (D) as described previously, but lacking $Fe_2(SO_4)_3$[5]; ammonium lactate medium (F)[6]; tryptose broth (LESLEY, *et al.*)[7]; and glucose ammonia medium (H) used by HERRIOTT AND BARLOW[8]. Unless otherwise stated, D medium was used.

Osmotically shocked phages (ghosts)[9] were prepared by rapid dilution of a 3 *M* NaCl solution of phage. Because the best ghost preparations still contained 1 % plaque formers, centrifugation at 10,000 × *g* for 60 minutes was used to remove many of the viable phages. The multiplicity of infection with ghosts was such as to give 96 % loss of cell viability, and the number of infectious centers at 0 time was 0.1 % of the bacterial concentration.

Ultraviolet-inactivated phages (u.v.-phages) were prepared by irradiating a suspension of T2 with a Westinghouse Germicidal Lamp WL-15 for 3.5 minutes at 60 cm. This gave an average survival of $5 \cdot 10^{-5}$. With a multiplicity of infection of u.v.-phages to bacteria of 6, there was negligible "multiplicity reactivation"[10].

The following procedure was used for the preparation of uninfected and infected cell samples. Two hundred ml of medium were inoculated with 10^9 cells from an 18 hour seed culture. The bacteria were grown to $2–4 \cdot 10^8$ cells/ml with strong aeration at 37° C. To serve as a control, 40 ml of uninfected culture were removed and centrifuged. An additional 60 ml of freshly grown cells were centrifuged and resuspended in 6 ml of non-nutrient medium (H medium lacking glucose and ammonia) and containing enough phages (or ghosts or u.v.-phages) to give a multiplicity of infection of about 6. After 5 minutes incubation at 37° C, without aeration, 54 ml of complete medium (or complete medium containing an inhibitor) were added and aeration was begun (0 time). At 17 minutes, 40 ml of the infected culture were removed and centrifuged. Intracellular

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phages were determined at 35 minutes[11]. Whenever synthetic medium was employed, twice the above volumes of cultures were used.

Extracts of the cell samples were prepared by treatment in the Raytheon 9 KC or 10 KC sonic oscillator. The centrifuged cells were resuspended in 2–6 ml of water, and transferred to new plastic centrifuge tubes. These tubes were placed in water in the cup of the sonic oscillator and treated for 10 minutes. This technique is essentially as efficient in breaking *E. coli* as is the usual procedure involving the sonic oscillator[12], and at present, it seems to be the best method for disrupting cells suspended in small volumes. Several samples of cells can be treated simultaneously, using this technique.

Synthetic (H) medium was used whenever the inhibitors Chloromycetin[13] or thienylalanine[14] were employed because of the antagonistic effect of amino acids. Protein concentrations were determined by the Folin method of LOWRY, *et al.*[15].

The concentration of deoxyribonucleic acid (DNA) was determined by the method of CERIOTTI[16]. After heating, Merck reagent chloroform was used for extraction. The resulting blank values and extinction coefficients, in the presence and absence of ribonucleic acid (RNA), were comparable to those reported by CERIOTTI[16]. A standard curve was prepared using salmon sperm DNA (California Foundation for Biochemical Research). DNA determinations were made on trichloroacetic acid (TCA) precipitates of extracts or of the whole cultures. Tubes containing the extract or whole culture in 5% TCA and 10 mg ovalbumin were chilled for 1 hour. After centrifugation the precipitates were resuspended with 3.0 ml of 5% TCA and heated at 95° C for 15 min, and then deoxyribose was determined in the extract. When determinations were made on whole cultures, the TCA precipitates were washed twice with 2 ml of cold 5% TCA before the DNA was determined.

Bases of DNA were isolated and identified chromatographically by the methods described by HERSHEY, DIXON AND CHASE[17]. The absorption peaks and concentrations of each of the bases were determined using the Beckman Spectrophotometer.

DNase assay

KOZLOFF has reported that most of the DNase of uninfected *E. coli* is inactive, owing to the presence of a specific inhibitor of the enzyme[3]. Much of this inhibitor, which consists, at least in part, of ribonucleic acid (RNA), is destroyed upon infection by T6, and thus an increase in DNase activity ensues. Two experiments confirming KOZLOFF's conclusions were performed. First, the DNase of extracts of infected cells (using T2) could be inhibited by the addition of heated extract of uninfected cells. Second, incubation of extract of uninfected cells with crystalline pancreatic ribonuclease (RNase) resulted in the several fold increase in DNase activity.

The DNase assay described below was designed to employ a shorter incubation period than that used previously[2]. In the earlier assay the lengthy incubation period (5 hours) could result in endogenous destruction of the RNA inhibitor of DNase and lead to erroneously high activities. In that assay, the DNase activity was determined by measuring the 260 mμ adsorption by solubilized nucleic acid, and it required subtraction of blank values resulting from endogenous RNA hydrolysis. By measuring deoxyribose, rather than absorption at 260 mμ, the high blank values could be avoided and thus larger amounts of extract and shorter incubation times could be used. Endogenous RNase was minimized by running the reaction at pH 8.5[2,3].

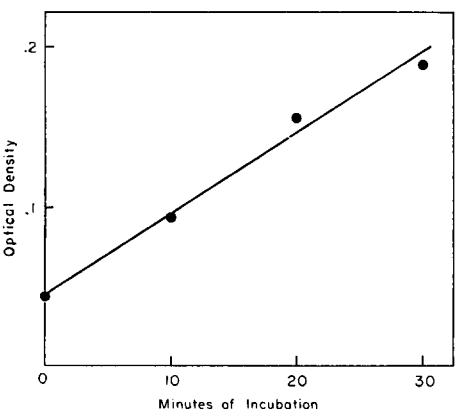

Fig. 1. Colorimetric DNase assay. The relation of color developed (representing solubilized deoxyribose) to duration of incubation of an uninfected cell extract.

The DNase assay was as follows: 0.2 ml of 0.13 *M* 2-amino-2-hydroxymethyl-1,3-propanediol buffer at pH 8.5 in 0.15 *M* $MgSO_4$, 0.2 ml of 0.3% DNA, and 0.6 ml extract (about 300 μg uninfected bacterial protein) were mixed and incubated for 30 minutes at 37° C. The reaction was stopped and the unhydrolyzed DNA was precipitated using 0.5 ml of 0.17 *M* trisodium citrate at pH 4.6, and 2.5 ml of 95% ethanol. The tubes were stored at room temperature overnight. After centrifugation, 3.0 ml aliquots of the supernatant fluid were transferred to calibrated centrifuge tubes. The solutions were then evaporated, to remove the alcohol, on a boiling water bath until the liquid volumes were about 0.3 ml. The solutions were returned to the 3.0 ml mark with water and the CERIOTTI technique was applied[16]. Assay controls were run in the same way except that they received no incubation.

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To insure the validity of this assay it was necessary to show that there is no significant endogenous destruction of the enzyme inhibitor during the incubation. A plot of optical density against the duration of incubation was linear. The lack of any upward curvature with time indicates that there is no appreciable DNase activation during this period (Fig. 1).

In all experiments to be reported, commercial salmon sperm DNA (California Foundation for Biochemical Research) was used as substrate. This material contains no detectable RNA and less than 2% protein. While the viscosity of this DNA is low, it is sufficiently highly polymerized to be completely insoluble in 60% ethanol, and thus was satisfactory for the DNase assay. A plot of the final color developed against the amount of enzyme was linear but intersected the optical density axis slightly below the origin, so a calibration curve, using an extract of uninfected cells, was prepared. One unit of DNase activity was taken as the DNase activity of 1 μg of protein of uninfected bacterial extract. One thousand units of DNase hydrolyzed approximately 1.5 μg DNA/minute. DNase results are given as specific activities (units of activity/μg protein). The results reported are averages of triplicate determinations; deviations from the average were less than 10%. Several experiments of each sort were performed, although only one is reported. In spite of efforts to completely standardize conditions of growth, harvest, and extraction, the specific activity of DNase of uninfected cells varied in experiment to experiment from 0.9 to 1.3 and the specific activity of infected cells varied correspondingly. Thus, quantitative interpretations of DNase activation ought to be discouraged.

Occasionally, experiments were performed using highly viscous calf thymus DNA[18] as substrate (η_{sp} = 1.9 for a 0.1% solution). These results were always qualitatively consistent with those obtained using the commercial DNA.

EXPERIMENTS AND RESULTS

Activation of DNase in uninfected cells

Two alternative hypotheses with regard to DNase activation may be entertained: 1) the activation is a specific consequence of infection; or 2) it is non-specific and many other environmental changes, either external or internal, result in DNase activation. The first alternative would be difficult to prove. Lack of activation after various kinds of treatment of uninfected cells would serve to indicate, not prove, that activation and infection are mutually related. *Prima facie*, the second postulate might seem the more reasonable, since the enzyme is always present and is, perhaps, in some sort of dynamic balance with the inhibitor. Any conditions which might upset this balance, such as stresses upon metabolism, would bring about activation. A study of the similarities of such agents could, in addition, lead to insight as to how this activation is effected. Below are given the effects of several different kinds of metabolic stresses upon the DNase of uninfected cells (Table I).

TABLE I

EFFECT OF VARIOUS KINDS OF METABOLIC INHIBITION ON DNASE ACTIVITY OF UNINFECTED *E. coli*

Inhibitor	*DNase (Specific activity)*	
	Treated cells	*Control*
Sodium cyanide	1.5	1.3
β-2-Thienylalanine	0.9	1.0
Proflavin	0.8	0.7
Phage ghosts	0.9	1.0

Experimental details are given in the text.

The activity of DNase was determined in cells aerated for 20 minutes in the presence of the following inhibitors: 0.05 *M* NaCN (pH 7.0), 6 μg/ml DL-β-2-thienylalanine (Arapahoe Chemical Co.), and 4 μg/ml proflavin (Sigma Chemical Co.).

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Cyanide was employed as an example of a general metabolic inhibitor, proflavin for its capacity to combine with nucleic acid[19,20], and thienylalanine as a "differential inhibitor" (inhibiting protein and RNA synthesis, while not greatly affecting DNA synthesis[21]). This inhibitory effect of thienylalanine was demonstrable within 20 minutes.

Activation of DNase in infected inhibited cells

Whether or not DNase activation can result from other processes besides infection, the question of the necessity of this activation for phage production still must be answered. To determine that the activation is not an essential process, conditions must be sought under which phage production can occur without any DNase activation.

A more specific criterion than the yield of phage with which to judge the necessity of DNase activation would be the occurrence of some essential process during infection, such as the synthesis of phage DNA. The significance of a condition where DNase was not activated, but where phage DNA synthesis was occurring—indicating that DNase activation is not essential for this part of phage production— could be overlooked if phage production alone were taken as a measure of successful infection. Furthermore, DNA synthesis would be a more precise measure of the extent of the infection processes than would be the burst size; burst sizes can be extremely variable in concentrated cultures owing to lysis inhibition and readsorption of released phages. Thus, in studying the significance of the DNase activation, conditions were sought where at least some essential process of infection is occurring, namely net DNA synthesis, without DNase activation.

The activation of DNase was studied after infection in the presence of the following inhibitors: 4 μg/ml Chloromycetin (Parke, Davis, and Co.), 4 μg/ml thienylalanine, 4 μg/ml proflavin, $5 \cdot 10^{-4}$ *M* $CoCl_2$, 0.01 *M* $MgSO_4$, and pH 8.4. In the experiments presented here, thienylalanine and proflavin completely inhibited phage production, but Chloromycetin in all cases allowed some phage production at this low concentration. Co^{++}, Mg^{++}, and increased pH have been shown to be *in vitro* inhibitors of bacterial RNase[22,23]; infection in the presence of these inhibitors might prevent *in vivo* destruction of the inhibitory RNA, and thus permit phage production without DNase

TABLE II

EFFECT OF VARIOUS INHIBITORS UPON THE ACTIVATION OF DNASE IN INFECTED *E. coli*

Inhibitor	*DNase (Specific activity)*		
	Infected in presence of inhibitor	*Controls*	
		Uninfected	*Infected*
Chloromycetin	1.4 (64)	0.9 (59)	2.4 (95)
β-2-Thienylalanine	1.1 (34)	1.1 (34)	4.1 (65)
Proflavin	5.8 (94)	0.9 (54)	5.6 (104)
$CoCl_2$	4.6 (72)	1.3 (59)	7.0 (101)
$MgSO_4$	4.2 (100)	0.9 (54)	4.2 (100)
pH 8.5	2.0 (78)	0.9 (54)	4.2 (100)
Cyanide	1.5	1.3	6.5

Numbers in parentheses are DNA concentrations (μg DNA/mg protein). Experimental details are given in the text.

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activation. The results (Table II) indicate a close connection between DNase activation and infection. In no case studied could DNA increase be shown to occur where DNase was not activated. Treatment with some inhibitors (Chloromycetin, Co^{++}, and increased pH) resulted in a partial inhibition of both DNA synthesis and DNase activation in infected cells.

Protein synthesis in infected cells

It has been reported that protein synthesis is uninterrupted after infection[21,3,25]. Thus in nutrient medium (where the generation time of the bacteria is 25 minutes) there ought to be a 1.6-fold increase of protein 17 minutes after infection. However, under the conditions used here, only a 1.2-fold increase in TCA insoluble protein of the complete culture was found at 17 minutes. In spite of this increase of TCA insoluble protein, the concentration of protein in the infected cell extracts was about the same as that of the uninfected cell extracts taken at 0 minutes, indicating incomplete recovery of protein in infected cell samples. Either some lysis, even under conditions of lysis inhibition, is occurring before the end of the latent period[26], or the infected cells are so fragile at this time[27] that cellular material is released either during the latent period[28] or during the centrifugation. Since the DNase activation after infection is of the order of five-fold, this loss of about 20% protein should not be of great importance.

Time of activation of DNase

To correlate the activation of DNase with other processes, one first ought to discover just when during the latent period the activation occurs. Previous work shows that the activation begins approximately 5 minutes after infection and the enzyme is maximally activated at about 15 minutes[2,3]; however, this time does not allow for the lag of adsorption (*i.e.* the time required for infection in all cells). One can start infection simultaneously in all cells and at a definite time by carrying out the adsorption of phage in the absence of nutrients.

Fig. 2 shows that DNase starts to increase in less than 3 minutes after addition

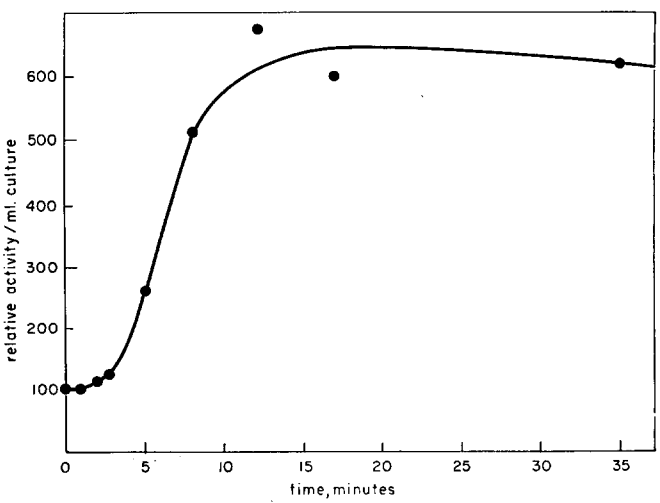

Fig. 2. Activation of DNase. The relation of DNase activity to time after infection of ***E. coli*** with T2.

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of nutrients, and becomes highly activated by 10 minutes. Because of this early appearance of the activation, one must assume that the activated DNase can have its effect at any time after the first few minutes of the latent period. There was, however, a definite lag period.

Activation of DNase a metabolic process

Since DNase activation commences soon after infection, one might expect the activation to be the consequence of "invasion" (injection of the parental phage contents[29]) and not to depend upon any subsequent processes. It was found, however, that infection in non-nutrient medium, which probably permits invasion[29], will not bring about activation of DNase.

E. coli and phage (multiplicity of 7) were incubated in H salts at 37°C, without aeration or nutrients, for 25 minutes. DNase assay of an extract of this material showed no increase of specific activity over the extract of uninfected cells—both were 0.9 units. Thus, some metabolism, following phage attachment, is required for DNase activation. This is consistent with the lack of activation of infected cells in the presence of the metabolic inhibitor, thienylalanine.

Activation of DNase in synthetic medium

COHEN has reported that DNase activity can be detected in lysates when infection is carried out in nutrient broth, but not when using F medium[30]. This might be interpreted as meaning that there is no activation of DNase when bacteria are infected in synthetic medium. In testing this, however, it was found that while the amount of DNase activation was not always consistent, a 3- to 4-fold increase of activity was generally found when synthetic media (F or H) were employed. In nutrient media (D or tryptose broth) usually about twice (5- to 7-fold) this activation was obtained. Since the activation is a metabolic process, the lesser activation in synthetic media is probably a reflection of slower metabolic processes.

Infection with inactivated phage

Osmotically shocked phage. The effect upon DNase after infection with osmotically shocked phages (ghosts)[9] was determined. That DNase might be activated by phage ghosts seemed likely in that these agents both kill and lyse bacteria[31], although it has been suggested that these actions may have little resemblance to cell death and lysis resulting from infection with viable phages[32]. Infection with phage ghosts, in the place of viable phages, brought about no activation of DNase (Table I).

Ultraviolet irradiated phage. Infection of *E. coli* with T2 inactivated with ultraviolet irradiation (uv-phages) results in both death of the bacteria and also a disorganization of the bacterial nuclear material[33]. This latter effect, evidenced by the use of chromogenic (Giemsa) stain, is similar to that seen in the early stages of the latent period of cells infected with viable T2[33,34]. Experiments were performed to determine if infection with uv-phages also brings about activation of DNase.

The results (Table III) show that DNase is activated after infection with uv-phages. This activation is not as rapid as in the control, indicating that the metabolism bringing about the increase in DNase activity is either slower in uv-phage

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infected cells, or starts later after infection. At 35 minutes, the DNase activity in the uv-phage infected cells is 90% of that of the control.

TABLE III

ACTIVATION OF DNASE BY INFECTION WITH UV-PHAGE

Infected with	*DNase (Specific activity)*		
	0 min	*17 min*	*35 min*
Phage	0.7	6.7	6.0
U.v.-phage	0.7	2.9	5.4

Times are minutes after infection (0 time sample is uninfected).

DNA of uv-phage infected cells

Experiments have confirmed the reports that there is no net change in concentration of DNA for at least two hours in cells infected with uv-phages[35,36]. It remained to be shown that this constant concentration of DNA reflects a true static condition, and not concomitant catabolism of host DNA and synthesis of phage DNA. Analysis for all bases of cells infected with uv-phages for 17 minutes showed that the DNA of the cells contained no 5-hydroxymethylcytosine (5-HMC)- a base shown to be present in DNA of T2, but not found in DNA of uninfected *E. coli*[37]. In Table IV is seen the composition of the various DNA's with respect to cytosine and 5-HMC. For comparison, the results of HERSHEY *et al.*[17] for *E. coli* are given. One sees that in the infected cell control, nearly all the cytosine has been replaced by 5-HMC, while in the uv-phage infected cells, none of this base was detected.

TABLE IV

COMPOSITION OF DNA WITH RESPECT TO CYTOSINE AND 5-HMC

	Mole %	
	Cytosine	*5-HMC*
Uninfected cells	24	0
Infected cells	4	14
U.v.-phage infected cells	27	0
Bacteria*	24	0
Phage*	0	16

Results are given as % moles of cytosine and of 5-hydroxymethylcytosine to total moles of DNA bases.

* Data from HERSHEY *et al.*[16].

Superinfecting breakdown and DNase activation

LESLEY and co-workers have shown that there is an extensive (50%) degradation of DNA of phages attacking a previously infected bacterium[38]. The similarities between this so-called "superinfecting breakdown" and activation of DNase are striking. Neither "superinfecting breakdown" nor DNase activation is initiated by phage ghosts[39], or by T3[2,40] (by which DNase is only slightly activated). Both phenomena commence during the initial stages of the latent period[39], and both apparently require more than just the adsorption of the virus[40]. Also, uv-phages will

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initiate both effects[39]. Thus it would appear likely that the activation of DNase is a necessary condition for "superinfecting breakdown". Indeed, GRAHAM has suggested that superinfecting breakdown "might result from the action of an intracellular enzyme activated by the initial infection"[39]. "Superinfecting breakdown" has been shown not to occur in *E. coli* resistant to, and in the presence of, 5 mg/ml streptomycin[41]. This result has been confirmed in the present system. In cyanide-poisoned cells (0.05 *M* NaCN, pH 7.0, added five minutes before infection) there is no formation of the superinfecting breakdown mechanism[39]. If DNase activation and superinfecting breakdown are related, one might expect to find *no* activation of DNase after infection in the presence of streptomycin or in cyanide-poisoned cells.

Cyanide. Infection in cyanide-poisoned cells brought no significant activation of DNase, see Table II. The effect of cyanide upon DNase activity *in vitro* was also tested. The presence of 0.05 *M* NaCN (pH 8.5) is the assay mixture resulted in a 1.3- to 1.4-fold *increase* of activity.

Streptomycin. The usual activation of DNase (6-fold) was found in streptomycin resistant *E. coli* infected in a medium containing 5 mg/ml streptomycin sulfate (Eli Lilly, and Co.). This shows that DNase *is* activated under conditions where superinfecting breakdown does not occur. However, as GRAHAM has suggested[39], streptomycin may be inhibiting the activated enzyme, rather than inhibiting the *activation* of the enzyme. It was found that streptomycin did inhibit DNase activity *in vitro*. Assay of DNase in the presence of streptomycin showed it to have one-fifth the activity of the control. This inhibition apparently results from direct action on the *substrate*; a visible precipitate forms in a mixture of DNA and streptomycin, in the concentrations used for the DNase assay. (Streptomycin has previously been shown to complex nucleic acid[30].) Thus the lack of superinfecting breakdown under conditions where DNase is activated—in the presence of streptomycin or a low concentration of Mg^{++}[25] —can be explained by the inhibitory effect upon the DNase *activity* itself. Since phage yields are normal in the presence of streptomycin[41], any role of DNase activation in the "superinfecting breakdown" phenomenon is not a required part of successful infection.

DISCUSSION AND CONCLUSIONS

The effects of various metabolic inhibitors upon bacterial DNase indicate that activation of DNase is closely related to phage infection. Since phage ghosts bring about cell death but will not activate the DNase, the killing process of infection is not the mechanism bringing about DNase activation. Probably the injection of the phage contents is required. This is consistent with the results showing that infection with uv-phages will activate the DNase—assuming injection does occur in this case. Since infection in the absence of an energy supply (nutrients and aeration) does not cause activation, some metabolic steps must be required. This metabolism probably involves amino acids; the addition of thienylalanine (an amino acid analog with little effect on DNA synthesis[21]) to the infected cells prevented the increase in DNase activity.

The results presented show that the activated DNase may not be involved in the breakdown of host cell DNA. At least this DNA is not catabolized by DNase activated by uv-phage infection. One cannot state conclusively that activated DNase does not

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break down host DNA during normal infection since other requirements for this degradation, perhaps transport of the enzyme to the site of the DNA, may be lacking in the cells infected with uv-phages. Host DNA may not be degraded after infection with uv-phages because the end-products of DNA catabolism, which are not removed in this case, may be inhibitory to the DNase action. (It can be pointed out that the pool of vegetative phage DNA[26] is apparently not attacked by the activated DNase during intracellular phage growth, although the enzyme will catabolize phage DNA *in vitro*[3].)

Numerous occurrences of specific and non-specific natural inhibitors of DNase have been reported[42–48]. In at least one system, besides that studied in this work the inhibitor contains RNA[44]. At the present time, the function in cellular metabolism of these inhibitors is unknown. The destruction of the DNase inhibitor, which accompanies phage production, may be a secondary effect of infection, magnifying some natural cellular process. The present studies do not tell one whether the DNase *action* subsequent to the *activation*, bears a functional relationship to the production of phage. Any conclusions regarding DNase action on parental phage[49], in the disruption of nuclear material[33, 34], or in phage DNA synthesis[17] or the cross-over phenomenon[50] seem premature. Probably correlation with these effects will require further knowledge regarding their biochemical nature.

It would seem pertinent to determine if DNase is activated in other viral systems—especially in the more general case of lysogenic phage development. An investigation of this type is being undertaken in this laboratory.

SUMMARY

Various metabolic agents (cyanide, β-2-thienylalanine, and proflavin) did not bring about increase of DNase activity in uninfected *E. coli*. Metabolic inhibitors (Chloromycetin, thienylalanine, proflavin, cobalt, increased pH, and cyanide), were applied to cells infected with T2. The results showed that DNase activation results only from phage infection, and also that DNase activation is correlated with phage DNA formation. DNase activation was shown to occur in synthetic media, as well as in nutrient broth.

The activation of DNase by T2 seems to be specifically caused by the injection of some part of the phage contents since ultraviolet-irradiated phages (u.v.-phages) bring about the activation, but osmotically shocked phages (ghosts) do not. Furthermore, some sort of metabolism requiring an energy source must be occurring to activate the DNase, since infection of cyanide-poisoned cells or infection in non-nutrient medium brings about no activation.

Activated DNase may not function in the catabolism of the host cell DNA since there was no breakdown of this material after infection with u.v.-phages, although DNase was activated. It was shown that there is no formation of phage DNA (containing 5-hydroxymethylcytosine) after infection with u.v.-phages.

A comparison of the conditions for activation of DNase and for occurrence of the breakdown of superinfecting phage indicate that DNase participates in the "superinfecting breakdown" phenomenon.

Other possible roles of the activated DNase were discussed.

RÉSUMÉ

Différents agents métaboliques (cyanure, β-2-thiénylalanine et proflavine) ne provoquent pas d'augmentation de l'activité DNasique chez *E. coli* non infecté. Des inhibiteurs métaboliques (chloromycétine, thiénylalanine, proflavine, cobalt, élévation de pH et cyanure) ont été appliqués à des cellules infectées par T2. Les résultats montrent que l'activation de la DNase résulte seulement de l'infection par le phage, et également que cette activation est liée à la formation du DNA du phage. L'activation de la DNase a lieu sur des milieux synthétiques, aussi bien que sur des bouillons de culture.

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L'activation de la DNase par T2 semble être provoquée spécifiquement par l'infection d'une partie du contenu du phage, puisque des phages irradiés par l'ultraviolet (phages u.v.) produisent l'activation mais que des phages ayant subi un choc osmotique (fantômes) ne la produisent pas. En outre, un certain métabolisme nécessitant une source d'énergie doit avoir lieu pour activer la DNase, puisque l'infection de cellules empoisonnées par le cyanure ou l'infection dans un milieu non-nutritif ne sont pas suivies d'activation.

La DNase activée ne peut pas prendre part au catabolisme du DNA de la cellule hôte, puisque ce DNA n'est pas dégradé après infection par des phages u.v., quoique la DNase soit activée. Les auteurs ont montré qu'il n'y a pas formation de DNA du phage (renfermant de la 5-hydroxyméthylcytosine) après infection par des phages u.v.

Une comparaison des conditions qui permettent l'activation de la DNase et la dégradation d'un phage surinfectant montre que la DNase prend part au phénomène de "dégradation surinfectante".

D'autres rôles possibles de la DNase activée sont discutés.

ZUSAMMENFASSUNG

Verschiedene metabolische Wirkstoffe (Cyanid, β-2-Thienylalanin und Proflavin) verursachten keine Erhöhung der DNase-Aktivität in uninfizierten *E. coli*. Mit T2 infizierte Zellen wurden mit metabolischen Hemmstoffen (Chloromyzetin, Thienylalanin, Proflavin, Kobalt, erhöhte pH-Werte und Cyanid) behandelt. Die Ergebnisse bewiesen, dass die DNase-Aktivation nur von Phageninfektion verursacht wird und dass die DNase-Aktivation mit Phagen-DNS-Bildung im Zusammenhang steht. Es wurde ferner bewiesen, dass DNase-Aktivation in synthetischen Medien, sowie in Nährlösungen vorkommen kann.

Die Aktivation von DNase durch T2 scheint spezifisch durch die Injektion irgendeines Teiles des Phageninhaltes verursacht zu werden, da die Aktivation wohl durch mit Ultraviolett bestrahlte Phagen (UV-Phagen), jedoch nicht durch Phagen verursacht wird, welche einen osmotischen Schock erlitten hatten (Schemen). Ferner muss bei der DNase-Aktivierung irgendein Metabolismus involviert sein, welcher eine Energiequelle benötigt, da durch mit Cyanid vergiftete Zellen oder in Nicht-Nährmedien keine Aktivierung verursacht wird.

Aktivierte DNase scheint im DNS-Katabolismus der infizierten Zelle keine Rolle zu spielen da nach Infektion mit UV-Phagen kein Abbau dieses Materials stattfand, obgleich die DNase aktiviert war. Es wurde bewiesen, dass nach Infektion mit UV-Phagen keine Phagen-DNS-Bildung (5-Hydroxymethylcytosin enthaltend) stattfand.

Ein Vergleich zwischen den Bedingungen, die für die DNase-Aktivierung und für den Abbau des superinfizierenden Phagen notwendig sind, wies darauf hin, dass DNase im "Superinfektions-Abbau"-Phänomen eine Rolle spielt.

Andere mögliche Rollen der aktivierten DNase wurden erörtert.

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