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THE ISOLATION AND CHARACTERIZATION OF COLIPHAGE N20F', AND PROPERTIES OF THE INDUCED LYTIC ENZYME

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SUMMARY

1. The present study describes the isolation of a new coliphage, N20F' and two spontaneous mutants. The three isolates are antigenically identical but can be distinguished by their plaque morphology. The relative activity of the lytic enzymes in lysates of N20F' infected cells is correlated with the plaque morphology of the various mutants, that is, the specific activities of the induced lytic enzymes derived from infected host cells increase with increased plaque clarity.

2. The phage-induced lytic enzyme produced by the three mutants possesses the same properties. The crude enzyme exhibits two pH optima, one at pH 8.5 and the other at pH 5.5. EDTA and divalent metal ions enhance the pH 5.5 activity but inhibit at pH 8.5. Carboxylic acids at high concentrations produce the same effects as EDTA. However, in the presence of low concentrations of carboxylic acids the pH 5.5 activity is drastically reduced, while there is no inhibition of the pH 8.5 activity. Thermal inactivation studies also show that the heat stability of the two activities are not the same.

INTRODUCTION

As a result of screening experiments we have isolated a coliphage from raw sewage which produced very turbid plaques when plated on certain strains of *Escherichia coli* K12. Further investigations showed that this bacteriophage, N20F', will give rise to two spontaneous mutants, which were selected on the basis of their differing plaque morphology on the same bacterial host. The differences in the type of plaques shown by the mutant bacteriophages can be correlated with the activity of a lytic enzyme which is induced following virus infection.

The present report is concerned with properties of three bacteriophages and the lytic enzyme that they induce after infection of host bacteria.

MATERIALS AND METHODS

Isolation of bacteriophage

N20F′ was isolated from raw sewage obtained from the Nashville Sewage Plant. The bacterial host used in all experiments was *E. coli* W13a, a galactose negative, phage-sensitive mutant of *E. coli* K12. Appropriate dilutions of sewage were plated on 1% tryptone agar plates using standard bacteriophage technique[1] and incubated overnight at 37°. Phage stocks were made by the agar overlay method[1], purified by centrifugation, and freed of bacterial cells and debris by passage through millipore filters (0.45-μ membrane filters). Phage particles were concentrated by centrifuging high titered lysates (10^{11} phages/ml) for 3 h at 30 000 rev./min in a Beckman Spinco Model L-2 ultracentrifuge (rotor No. 30) at 4°.

Density gradient centrifugation

A purified phage stock was mixed with a CsCl solution (57%, w/v), both liquids having been heated to 45° prior to mixing. The mixture was centrifuged in cellulose nitrate tubes in a SW-39 rotor of the Beckman Spinco Model L ultracentrifuge. Centrifugation was carried out at 27 000 rev./min for 12 h, followed by 36 h at 18 500 rev./min at 18°. The gradients were dripped from the bottom after puncture of the tubes with a needle.

Refractive indices of every third fraction were read in a Bausch and Lomb Abbe refractometer. The buoyant density of the phage was calculated according to the method of WEIGLE AND MESELSON[2].

Electron microscopy

A drop of phage suspension was applied to parlodion-coated grids. The grids were washed with 2% ammonium acetate and then negatively stained with 2% phosphotungstic acid (pH 7.0). The procedure is essentially that of FAURE *et al.*[3]. The grids were examined in a Hitachi HU-11B-1 electron microscope.

Isolation of phage N20F′Tu DNA and the determination of its melting temperature and base composition

DNA was isolated by the method of MANDELL AND HERSHEY[4]. The melting point of DNA was determined according to MARMUR AND DOTY[5], using a Gilford Model 2000 recording spectrophotometer equipped with dual thermospacers. The method of BROCK *et al.*[6] was used to determine the kinds of bases present in phage DNA, and the absorption spectra of purines and pyrimidines were visualized using a Cary Model 15 recording spectrophotometer.

Preparation of phage-induced lytic enzyme

Bacterial cells were grown in 1% tryptone broth at 37° for the preparation of crude enzyme extracts. *E. coli* cells at a concentration of $2 \cdot 10^8$ cells/ml were exposed to a multiplicity of infection of 10 phages/cell. After 60 min, the infected cells were chilled and collected by centrifugation. The cells were then resuspended in 0.001 M phosphate buffer (pH 7.0) and sonicated for 30 sec. The sonicate was centrifuged at 15 000 rev./min in a Servall RC-2 centrifuge (SS-34 rotor) for 15 min, and the supernate was used as the crude extract.

Enzyme assay

The "substrate" used in the assay for lytic activity was prepared and assayed by a modification of the method of SEKIGUCHI AND COHEN[7]. Logarithmically growing *E. coli* cells in tryptone broth were collected by centrifugation and washed twice in 0.01 M Tris buffer (pH 8.5). The cells were then resuspended in approximately the same volume of chloroform-saturated 0.01 M Tris buffer (pH 8.5) and allowed to mix gently at room temperature for 1 h. After this period, the cells were washed three times in distilled water, lyophilized to dryness, and kept frozen.

To prepare the substrate of lyophilized cells for assay, a portion of these cells was resuspended in the appropriate buffer to an absorbance of approx. 0.500 as determined at 650 mμ.

The incubation mixture contained 1 ml substrate *plus* 0.1 ml enzyme extract. The reaction was carried out at room temperature (23°) in cuvettes with a 10-mm light path. Decrease in absorbance was followed at 30-second intervals at 650 mμ using a Gilford Model 2000 recording spectrophotometer. The reaction proceeded in a linear fashion until the turbidity decreased to approx. 60% of the original level. There was a good linear relationship between the changes in turbidity, and the amount of extract used. The enzyme activity was calculated from the rate of change in turbidity.

The unit of enzymatic activity is defined as a decrease of 0.100 absorbance unit per min. The specific activity is the number of units of activity per mg of protein present in the sample. Under similar conditions of assay, using the same substrate, egg-white lysozyme (Worthington Biochemical Corp.) has a specific activity of 330 in 0.1 M phosphate buffer (pH 7.0).

Protein was determined by the method of LOWRY *et al.*[8], as well as by the biuret method[9]. Crystalline egg-white lysozyme (Worthington Biochemical Corp.) and bovine serum albumin (Armour Pharmaceutical Co.) were used as the standards.

RESULTS

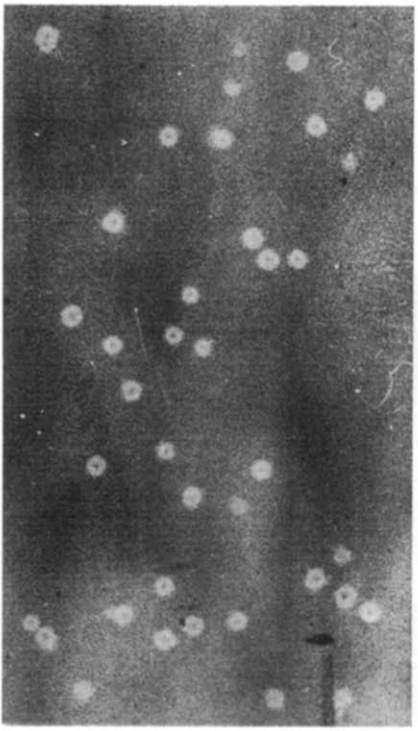
Isolation of bacteriophage N20F' Tu

Phage N20F' was isolated from Nashville sewage using a mutant of *E. coli* W13a known to be resistant to phage T4, T5, T6 and λ as the host cell. Fig. 1 illustrates the plaque morphology of bacteriophage N20F'. This virus was designated N20F' turbid (N20F' Tu) because of the very turbid plaque formed following infection of the above host. Table I lists the plaque morphology of this phage on various mutants of *E. coli*. Table I indicates that the turbid N20F' phage will infect the K and C but not the B strains of *E. coli*. It is interesting to note that clear plaques are formed at 30° while turbid plaques are present at 37° when W13a (λ + λdg) was the host bacterium. Similar results (not shown in Table I) were observed when W13a (λ) served as the host, indicating that the transducing phage λdg is not necessary for this effect. Clear plaques are also observed at both 30° and 37° when the bacterial host was W13a (424). In all other cases tested, turbid plaques were seen. These results imply that the presence of the prophage in the W13a (λ + λdg) lysogen at 30°, and W13a (424) lysogen at 30° and 37° may contribute some function after infection by N20F'Tu which permits the formation of clear plaques. Further, in the case of the λ prophage this function is in some way related to the region between genes N and Y

TABLE I

PLAQUE MORPHOLOGY OF N20F′Tu ON *E. coli*

E. coli strain	*Origin*	*Plaque type*	
		30°	*37°*
K W13aλ^s	P. Starlinger, University of Cologne	Very turbid plaques	Very turbid plaques
K W13a(λ + λdg)	H. Wiesmeyer, Vanderbilt University	Clear	Very turbid plaques
K (434)	F. Jacob, Institute Pasteur	Very turbid plaques	Very turbid plaques
K (434hy)	A. Kaiser, Stanford University	Very turbid plaques	Very turbid plaques
K (21)	F. Jacob, Institute Pasteur	Very turbid plaques	Very turbid plaques
K (82)	F. Jacob, Institute Pasteur	Very turbid plaques	Very turbid plaques
K W13a(424)	H. Wiesmeyer, Vanderbilt	Clear	Clear
B CR63	F. Womack, Vanderbilt University	No plaques	No plaques
B S/6	F. Womack, Vanderbilt University	No plaques	No plaques
C (18)	E. Six, University of Iowa	Very turbid plaques	Very turbid plaques



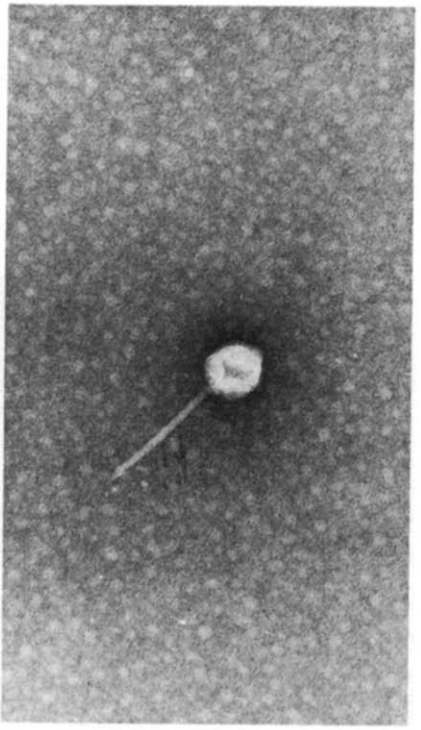


Fig. 1. The plaque morphology of N20F′Tu using *E. coli* W13a as the host bacteria. Gram's iodine was added to the plate in order to emphasize the appearance of the plaques which are completely turbid. The haloed areas represent regions of the greatest turbidity.

Fig. 2. Electron micrograph of N20F′Tu. Negatively stained with 2% phosphotungstate, magnified 200 000 ×.

of the phage genome (compare results of λ prophage with that of prophage 434 and 434 hy).

Morphology of N20F'Tu

The electron micrograph (Fig. 2) shows that N20F'Tu has a morphological structure that resembles the T-even phages. The head is approx. 1000 Å in length and 840 Å in diameter, while the tail is 1700 Å in length and 110 Å in diameter. The head is hexagonal in shape, and the tail ends in a spike without the tail fibers and other components that are characteristic of the T-even bacteriophages. Other micrographs show that cross striations are present in the tail region of the phage.

Buoyant density of N20F'Tu

The buoyant density of N20F'Tu was determined to be 1.553 from the relationship[2]: $d_{20} = 10.86\, n_D^{25} - 13\,500$, where n_D^{25} is the index of refraction of the solution for sodium light at 25°. The refractive index of the fraction where the initial increase in phage titer occurred was used in the calculation.

Composition and melting profile of DNA derived from phage N20F'Tu

Paper chromatographic and subsequent spectral analyses of formic acid hydrolysates of the DNA isolated from phage N20F'Tu by the method of MANDELL AND HERSHEY[4] indicate that guanine, cytosine, adenine, and thymine are the nitrogeneous bases present. No unusual bases were found. The purified DNA has a melting temperature (T_m) of 90.8° in a solution containing 0.15 M NaCl *plus* 0.015 M sodium citrate (pH 7.0). This T_m would correspond to a mole % of guanine + cytosine of 50% (see ref. 5).

Isolation of the less turbid (N20F'Ltu) and clear (N20F'Cl) mutants of phage N20F'Tu

When phage from a single plaque of N20F'Tu was plated on *E. coli* W13a

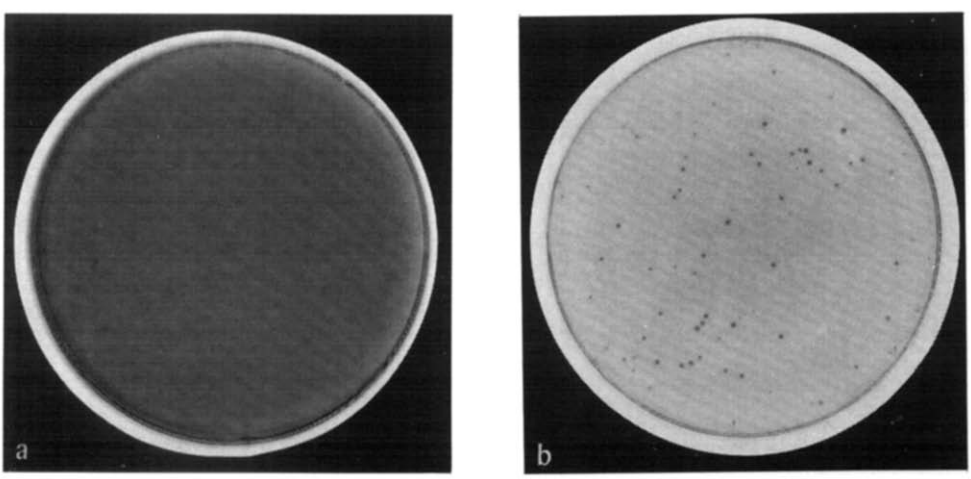


Fig. 3a. The plaque morphology of N20F'Ltu using *E. coli* W13a as the host bacteria. In the contact print the dark points represent clear areas of the plaques which are surrounded by turbid halos.

Fig. 3b. The plaque morphology of N20 F'Cl using *E. coli* W13a as the host bacteria. In the contact print the dark points represent clear plaques. No halos are present.

two mutant types arose spontaneously at a frequency of about 10^{-5} phage. These two mutants were described as less turbid (Ltu) and clear (Cl) according to their plaque morphology. Plaques produced by these phages are illustrated in Figs. 3a and 3b.

Determination of the latent period and the burst size of cells infected with N20F′

The phage strains described above were used to infect the sensitive, nonlysogenic bacterium W13a. Cells logarithmically growing in tryptone broth at 37° with aeration were infected at a multiplicity of infection of 1. Under these conditions the three N20F′ phage strains exhibit a latent period of 60 min and an approximate burst size of 320 phages per infected cell.

Antigenic properties of the Tu, Ltu, and Cl strains of phage N20F′

Antibodies directed against each of the N20F′ strains were prepared by a series of injections of 10^9 to 10^{11} phage particles into rabbits over a three-week period. The rabbits were bled and serum adsorbed with noninfected, nonlysogenized extracts of *E. coli*. Inactivation constants using antisera prepared against each phage and tested against all three phage types were determined according to the procedure described in ADAMS[1,17]. Table II indicates that antibodies made against each mutant will inactivate all three phage strains to the same extent. Anti-T4 and anti-λ serum did not inactivate the N20F′ phage. *E. coli* made resistant to N20F′Cl was also resistant to N20F′Tu and N20F′Ltu. These observations imply that the N20F′ mutants are related in that their tail components are antigenically similar and the same cell wall attachment site is shared by the three strains.

TABLE II

INACTIVATION OF BACTERIOPHAGE N20F′Tu, N20F′Ltu, AND N20F′Cl BY ANTI N20F′Tu SERUM

The experiments and calculations were done according to ADAMS[1]. Similar inactivation of all three mutant phages was also achieved by using N20 F′Cl and N20 F′Ltu antisera.

Bacteriophage mutant	*Inactivation constant K*
N20F′Tu	256
N20F′Ltu	256
N20F′Cl	256
N20F′Tu	No inactivation with anti λ
N20F′Tu	No inactivation with anti T4

Correlation of plaque morphology and specific activity of the lytic enzyme in phage-infected cells

The presence of three mutants of N20F′ which differ markedly in plaque turbidity suggested that these mutants might be defective to varying degrees in the lytic enzyme which is involved in the release of mature virus particles following the latent period of phage development. Based on this assumption it should be possible to detect differences in the activities of the lytic enzyme that is produced by the N20F′ mutants. Fig. 4 illustrates a plot of the specific activities of the enzymes in the crude lysates *versus* time after infection by the three N20F′ mutants. N20F′Cl has the highest specific activity, while N20F′Ltu has a greater specific activity than

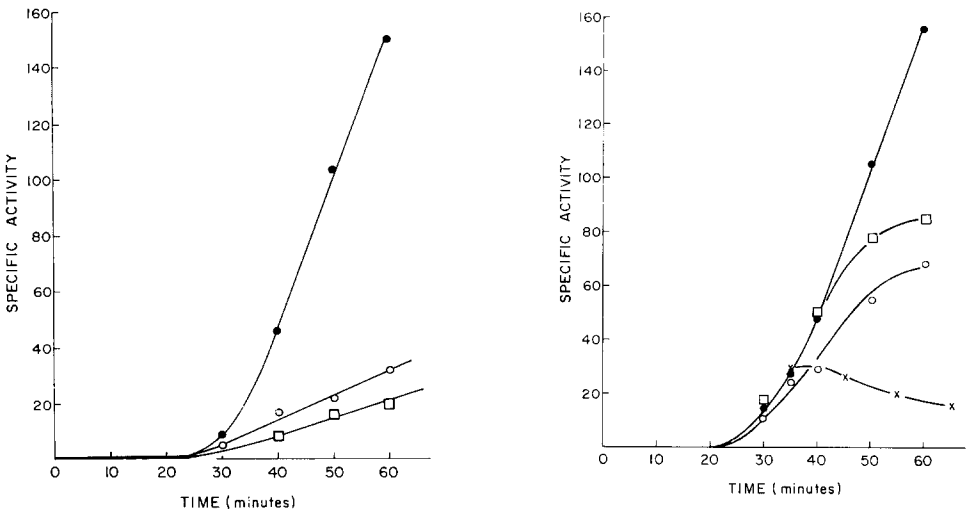


Fig. 4. The relative activities of the lytic enzymes produced by three mutants of bacteriophage N20F'. 100 ml of infected cells were removed at the times indicated, centrifuged, and sonicated. The specific activity (units of activity/mg protein of crude extract) of the lytic enzyme was then determined. The assays were carried out in 0.1 M phosphate buffer (pH 7.5). ●—●, N20F'Cl lytic activity; ○—○, N20F'Ltu lytic activity; □—□, N20F'Tu lytic activity.

Fig. 5. The effect of streptomycin (Squibb) and chloramphenicol (Park Davis Co.) on the lytic activity of N20F'Cl enzyme. Chloramphenicol was added 30 min after infection, while streptomycin was added at 35 min. 100 ml of infected cells were removed at the times indicated, and the crude enzyme extract was prepared as described in MATERIALS AND METHODS. Assays of the crude enzyme extracts were performed in 0.1 M phosphate buffer (pH 7.5). The specific activity is expressed as units of activity/mg protein of the crude extract. ●—●, Control; □—□, 40 μg/ml chloramphenicol; ○—○, 100 μg/ml chloramphenicol; ×—×, 100 μg/ml streptomycin.

N20F'Tu. These data would indicate that the plaque morphology exhibited by a particular N20F' mutant can be correlated with the activity of the lytic enzyme(s) that it produces.

The de novo synthesis of the lytic enzyme

The effect of two protein inhibitors on the production of the lytic enzyme after infection of *E. coli* W13a was examined. In this and the following experiments, the lytic enzyme induced by the N20F'Cl mutant was studied since it had the greatest activity and also its properties were representative of the other two mutant enzymes. The effect of the separate addition of chloramphenicol and streptomycin on the synthesis of the lytic enzyme in N20F'Cl phage-infected cells is shown in Fig. 5. The addition of chloramphenicol 30 min after infection leads to a gradual decrease in the rate of lytic enzyme synthesis. In contrast, the effect of the same concentration of streptomycin is much more dramatic. 100 μg/ml of streptomycin added 35 min after infection produces a sharp and immediate drop in the specific activity of the enzyme.

Inhibition of the synthesis of the lytic enzyme by known inhibitors of protein synthesis, and the absence of this enzyme in uninfected bacteria, indicate that the lytic enzyme is produced as a consequence of phage infection and probably arises by *de novo* synthesis.

In addition, subsequent studies on the purified enzymes induced after infection

by the three phages show that the lytic enzymes have different amino acid sequences. This evidence supports the assumption that the lytic enzyme activity is phage induced and arises after virus infection of host cells.

Effect of pH on the lytic enzyme from N20F' phage-infected cells

The effect of hydrogen ion concentration on the lytic enzyme produced by infection of *E. coli* by N20F'Cl is shown in Fig. 6. Two pH optima are observed, one at pH 5.5 and the other at pH 8.5. The specific activity (units of activity/mg protein crude extract) at the alkaline pH is approximately twice that at pH 5.5. Similar results were obtained with the lytic enzymes isolated following infection of *E. coli* by N20F'Tu and N20F'Ltu, with the exception that the specific activities were correspondingly lower. However, the ratio of specific activities at the two pH optima is the same as for the N20F'Cl enzyme. The best activity at pH 5.5 is obtained by using acetate and maleate buffers, while Tris proved to the most effective buffer at pH 8.5. Fig. 7 shows that both pH 5.5 and 8.5 activities appear at approximately the same time after N20F'Cl infection.

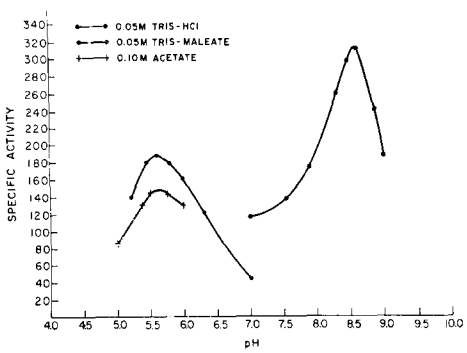


Fig. 6. The dependence of the activity of N20F'Cl lytic enzyme on pH. The assays were carried out in the buffers listed. The enzyme was a crude extract prepared from infected cells (MATERIALS AND METHODS). The specific activity is expressed as units of activity/mg protein of the crude extract.

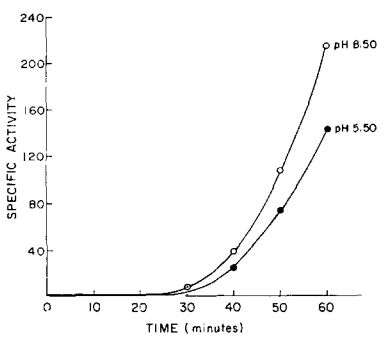
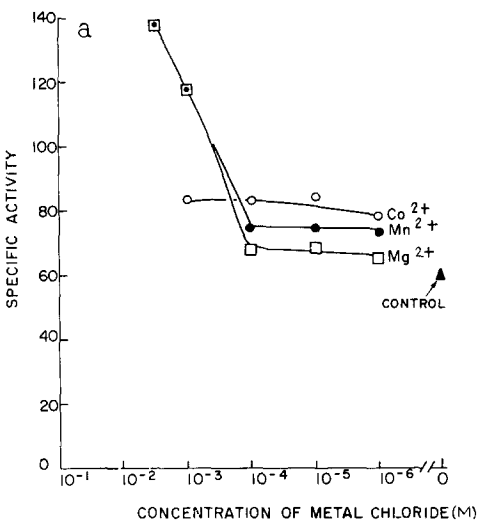
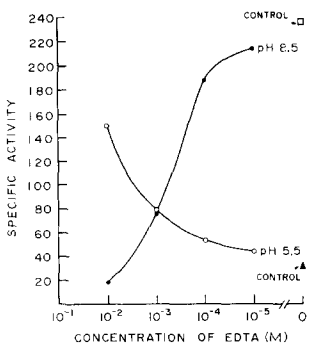


Fig. 7. Time of appearance of the pH 8.5 and 5.5 lytic activities after N20F'Cl infection of *E. coli* W13a. 100-ml samples of infected cells were removed at the times indicated, and crude cell-free enzyme extracts were made. Assays were performed in 0.05 M Tris buffer (pH 8.5) and in 0.1 M acetate buffer (pH 5.5). The specific activity is expressed as units of activity/mg protein of the crude extract.

Effect of EDTA and divalent cations on the phage-induced lytic activity

EDTA has been shown to increase the activity of egg-white lysozyme by chelating divalent metal ions[10]. Fig. 8 depicts the effect of this chemical on the pH 5.5 and 8.5 activities of the N20F'Cl enzyme. These results indicate that with increasing molar concentrations of EDTA there is an enhancement of the pH 5.5 activity, while there is a concomitant decrease in the pH 8.5 activity under these conditions. The effect of divalent metal ions on these two activities was studied in order to determine whether EDTA exerted its effect by chelating divalent metal ions or to some other function. These results are shown in Figs. 9a and 9b. At pH 5.5 (Fig. 9a) the lytic activity increases with increasing molar concentrations of Mn^{2+} and Mg^{2+}. Co^{2+} show no apparent enhancement of the activity. In contrast, at pH 8.5 (Fig. 9b)





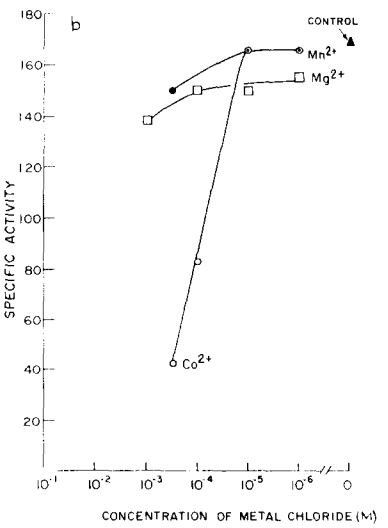


Fig. 8. The effect of EDTA concentration on the lytic activity of N20F'Cl enzyme. The appropriate amount of EDTA was added to 0.01 M Tris and the pH adjusted to 8.5 or 5.5 with NaOH or HCl. Crude enzyme was used in these assays. The specific activity is expressed as units of activity/mg protein of the crude extract.

Fig. 9. The effect of divalent metal ions on the activity of N20F'Cl lytic enzyme at pH 5.5 (a) and pH 8.5 (b). The indicated amount of metal chloride was added to 0.01 M Tris–acetate buffer. The pH was adjusted when required with NaOH or HCl. A crude enzyme preparation was used in these assays.

TABLE III

LYTIC ACTIVITY OF N20F′Cl PHAGE ENZYME AT pH 8.5 AND pH 5.5 IN THE PRESENCE OF ACETIC, PROPIONIC, BUTYRIC, AND MALEIC ACIDS

The reaction mixture contained the appropriate concentration of carboxylic acid in 0.01 M Tris buffer. The pH was adjusted when required with NaOH or HCl. At pH 8.5 in 0.01 M Tris the specific activity was 233 (specific activity is expressed as units of activity/mg protein of crude extract). This was taken as 100%. At pH 5.5 the best activity was obtained at the highest concentration of monocarboxylic acids used. In maleate the optimum activity was attained at a concentration of $5 \cdot 10^{-2}$ M at pH 5.5. These experiments were done with crude enzyme extracts (see MATERIALS AND METHODS).

Molarity	*Specific activity remaining (%)*							
	Acetic acid		*Propionic acid*		*Butyric acid*		*Maleic acid*	
	pH 8.5	*pH 5.5*	*pH 8.5*	*pH 5.5*	*pH 8.5*	*pH 5.5*	*pH 8.5*	*pH 5.5*
10^{-1}	11	100	22	100	20	100	6	29
$5 \cdot 10^{-2}$	13	47	23	42	20	37	9	100
10^{-2}	72	38	84	40	75	31	72	9
10^{-3}	93	34	96	27	79	19	75	
10^{-4}	98	29	98	18	86		89	
10^{-5}	98		99		90		90	

increasing concentrations of Mn^{2+}, Mg^{2+} and Co^{2+} lead to a decrease in the activity of the lytic enzyme. The effect of Co^{2+} is the most pronounced.

Effect of carboxylic acids on the lytic activity

The apparent paradox seen in the activation of the pH 5.5 activity by both EDTA and divalent metal ions suggested that the action of EDTA might be due to some function other than its capacity to chelate metal ions. Table III illustrates the effect of aliphatic mono and dicarboxylic acids on the lytic enzyme. At pH 8.5 there is a decrease in the activity with increasing molarity. The presence of maleate produces the sharpest inhibition. At pH 5.5 the opposite effects are observed. The activity of the enzyme increases with increasing carboxylic acid concentration. The aliphatic monocarboxylic acids at a concentration of 0.1 M promote the lytic activity best. The action of maleate differs in that the optimal enzymatic activity occurs at $5 \cdot 10^{-2}$ M. In the complete absence of carboxylic acids, the pH 5.5 activity is drastically reduced and relatively high concentrations of acids are essential at this pH in order to obtain good activity. These results suggest that EDTA may function in the previous experiments by providing carboxyl groups rather than by chelating divalent metal ions.

Heat stability of the lytic enzyme

The effect of temperature on the inactivation of the lytic enzyme is seen in Table IV. The enzyme was heated at both pH 8.5 and 5.5 and then each sample was assayed for activity at both pH optima. The top half of the table shows that incubation at pH 8.5 causes a similar inactivation of both the pH 5.5 and 8.5 activities up to 50°. At higher temperatures the activity assayed at pH 5.5 is a little lower than at pH 8.5. The rest of the table illustrates the effect of incubation at pH 5.5. The inactivation of the pH 8.5 activity under these conditions is similar to that for

TABLE IV

THE EFFECT OF TEMPERATURE ON THE INACTIVATION OF N20F'Cl LYTIC ACTIVITY AT pH 8.5 AND pH 5.5

The incubation was carried out in 0.05 M Tris buffer at pH 8.5, and at pH 5.5 in 0.1 M acetate buffer. Each sample was heated for 5 min at the specified temperatures and then chilled in ice. The assays were also done in the same buffers (the specific activity is expressed as units of activity/mg protein of crude extract). The activity at 24.5° was taken as 100% and the other activities were expressed in terms of this value. These experiments were done with crude enzyme extracts (see MATERIALS AND METHODS).

Incubation temp.	*Specific activity remaining (%)*	
	Assay at pH 8.5	*Assay at pH 5.5*
At pH 8.5		
24.5°	100	100
29.5°	92	92
39.5°	84	84
50.0°	60	60
62.0°	42	36
70.0°	35	25
At pH 5.5		
24.5°	100	100
29.5°	93	90
39.5°	87	83
50.0°	68	33
62.0°	39	<5
70.0°	31	No activity

the corresponding assay following incubation at pH 8.5. However, assay of the pH 5.5 activity after incubation at this pH indicates that there is a much more rapid decrease of enzymatic activity as compared to the pH 8.5 assays. At 50° the activity falls off sharply, and at 70° no detectable activity is observed. These results may be explained in terms of the following possibilities: (a) the presence of two separate enzyme molecules; (b) a single molecule with two enzymatic sites of different heat stabilities; (c) a single molecule in which the two activities are attributable to a single enzymatic site which is sensitive to heat. Heating may alter this site in such a way that the conditions necessary for the lytic activity at one pH is maintained while those at the other pH are destroyed.

DISCUSSION

The investigation reported here has dealt with the isolation of a new coliphage N20F'Tu and two spontaneous mutants, N20F'Ltu and N20F'Cl.

We have examined the lytic enzyme that is induced after phage infection and found that the enzyme possesses several unusual properties. The observation of two pH optima, and the striking behavior of the enzyme in the presence of EDTA, divalent metal ions, and carboxylic acids, as well as heat inactivation studies indicate that these properties might be due to any of the explanations proposed by SCHWIMMER[11] to describe the presence of enzymes with double pH optima. He considers that double pH optima may be the result of any of the following: (a) the presence of two

isozymes with different pH optima; (b) the existence of more than one ionic species of enzyme that combines uniquely with the substrate; (c) the presence of an amphoteric inhibitor, only one ionic species of which combines with one ionic species of the enzymes to form a dissociable enzyme–inhibitor complex, and (d) the presence of an amphoteric activator. Other investigators have reported double pH optima for liver β-glucuronidase[12], Ehrlich ascites ribonuclease[13], myosin ATPase[14], and bull seminal plasma 5′-nucleotidase[15].

However, further studies on the purification and physicochemical properties of the lytic enzyme show that a single protein is responsible for both pH optima. The lytic enzyme can exist in monomeric and dimeric forms, and both of these are enzymatically active. An elaboration of the properties of this enzyme will be the subject of another report[16].

ACKNOWLEDGMENTS

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