

# The Effect of Proteolytic Enzymes on *E. coli* Phages and on Native Proteins

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**ABSTRACT** Lambda *coli* phage is not inactivated by chymotrypsin, trypsin, or ficin. T<sub>2</sub> phage is slowly inactivated by high concentrations of  $\alpha$ -,  $\beta$ -,  $\gamma$ -, or  $\Delta$ -chymotrypsin, but not by trypsin or ficin. P<sub>1</sub> phage is slowly inactivated by  $\alpha$ -,  $\beta$ -, or  $\gamma$ -chymotrypsin, or ficin, more rapidly by  $\Delta$ -chymotrypsin, and much more rapidly by trypsin. Crystalline egg albumin, crystalline serum albumin, *E. coli* nucleoprotein, and yeast nucleoprotein are hydrolyzed slowly by  $\alpha$ -chymotrypsin. Yeast nucleoprotein, like P<sub>1</sub> phage, is hydrolyzed more rapidly by  $\Delta$ -chymotrypsin than by  $\alpha$ -chymotrypsin, but not by trypsin or ficin. Neither phages nor native proteins were attacked by papain, carboxypeptidase, deoxyribonuclease, or ribonuclease.

Native proteins and viruses are usually considered to be resistant to proteolytic enzymes. There are early reports, however, on the inactivation of bacteriophages by "trypsin" (Wollman, 1924; Schultz, 1928).

High concentrations of  $\alpha$ -chymotrypsin inactivate staphylococcus phage (Northrop, 1938), and both chymotrypsin and trypsin inactivate *B. megatherium* phages (Northrop, 1955). Group B arthropod-borne viruses are rapidly inactivated by chymotrypsin or trypsin, but group A viruses are not (Cheng, 1958).

In the present experiments the effects of the various chymotrypsins (Kunitz and Northrop, 1935; Kunitz, 1938), trypsin (Kunitz and Northrop, 1936), ficin (Walti, 1937), papain (Balls, Lineweaver, and Thompson, 1939), and carboxypeptidase (Anson, 1935) have been determined on P<sub>1</sub> phage (Bertani, 1951),  $\lambda$  phage (Lederberg and Lederberg, 1953), and T<sub>2</sub> phage and on crystalline egg albumin, serum albumin, and yeast and *E. coli* nucleoproteins.

The results of these experiments are summarized in Table I. *K* is the monomolecular velocity constant expressed as fraction per day per milligram enzyme per milliliter. The figures are the means of 5 to 30 experiments, each with different enzyme and substrate preparations. Lambda *coli* phage is not inactivated by any of the enzymes. T<sub>2</sub> phage is slowly inactivated by high

concentrations of  $\alpha$ ,  $\beta$ -,  $\gamma$ -, or  $\Delta$ -chymotrypsin (Jacobsen, 1947), but not by trypsin. P<sub>1</sub> phage is slowly inactivated by  $\alpha$ -,  $\beta$ -, or  $\gamma$ -chymotrypsin or ficin, more rapidly by  $\Delta$ -chymotrypsin, and much more rapidly by trypsin. The higher activity of  $\Delta$ -chymotrypsin compared to that of  $\alpha$ -chymotrypsin also occurs with yeast nucleoprotein. Carboxypeptidase does not inactivate any of the phages.

Crystalline, native serum albumin or egg albumin is hydrolyzed by  $\alpha$ -chymotrypsin at approximately the same rate as P<sub>1</sub> or T<sub>2</sub> phages. This is about one-ten thousandth the rate of hydrolysis of casein by the same enzyme.

TABLE I  
RATE OF INACTIVATION OR HYDROLYSIS OF VARIOUS  
SUBSTRATES BY VARIOUS ENZYMES

Substrate	Enzyme					
	Chymotrypsin				Trypsin	Ficin
	$\alpha$ -	$\beta$ -	$\gamma$ -	$\Delta$ -		
	<i>K (day-mg enzyme/ml)<sup>-1</sup> at 25°</i>					
$\lambda$ phage	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
T <sub>2</sub> phage	0.9	0.9	0.9	0.9	<0.1	<0.1
P <sub>1</sub> phage	0.8	0.15	0.2	3	40	0.5
Staphylococcus phage	10				<0.1	
<i>Megatherium</i> phage T	0.15				0.2	
<i>Megatherium</i> phage C	0.3				0.6	
Arthropod-borne animal virus group B	>200				>200	
Arthropod-borne animal virus group A	<1				<1	
Crystalline egg albumin	0.1					
Crystalline serum albumin	0.25					
<i>E. coli</i> nucleoprotein	0.7					
Yeast nucleoprotein	0.2	0.1	0.1	1.5	<0.1	
Casein	5000	5000	5000	6000	6000	
<i>E. coli</i> , living	0				0	
<i>E. coli</i> , heat-killed	1000				1000	

*Kinetics of the Reaction between  $\alpha$ -Chymotrypsin and T<sub>2</sub> Phage*

Preliminary experiments showed that the results depend markedly on the nature of the solution. In solutions in which the virus is rapidly inactivated, the enzyme decreases the rate of inactivation due to a non-specific protein effect. Inactivated enzyme, chymotrypsinogen, or most native proteins show the same effect. In concentrated peptone solutions, or others in which the virus is very stable, the enzyme has no effect, although such solutions do not inhibit the hydrolysis of casein by the enzyme. The concentration and chemical nature of the salts present and the pH of the solution also affect the

reaction. Systematic investigation of the effect of the solvent showed that low concentrations of phosphate or citrate ions accelerated the inactivation of  $T_2$  by chymotrypsin, while higher concentrations inhibited it. The results of these experiments are shown in Fig. 1. The results show that inactivation is

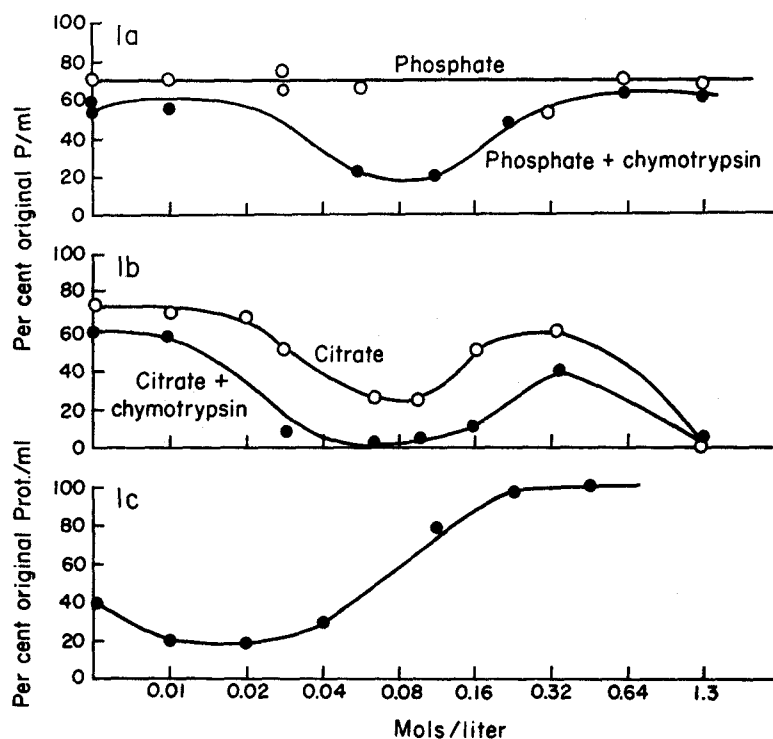


FIGURE 1 *a*. Inactivation of  $T_2$  in various concentrations of pH 7 phosphate buffer, 4 hours at  $25^\circ \pm 3$  mg chymotrypsin/ml.

FIGURE 1 *b*. Inactivation of  $T_2$  in various concentrations of pH 7 citrate buffer. Average of 2 experiments. 4 hours at  $25^\circ \pm 3$  mg chymotrypsin/ml.

FIGURE 1 *c*. Hydrolysis of yeast nucleoprotein by chymotrypsin in various concentrations of pH 7 phosphate buffer. 2 mg chymotrypsin/ml, 48 hours at  $25^\circ$ .

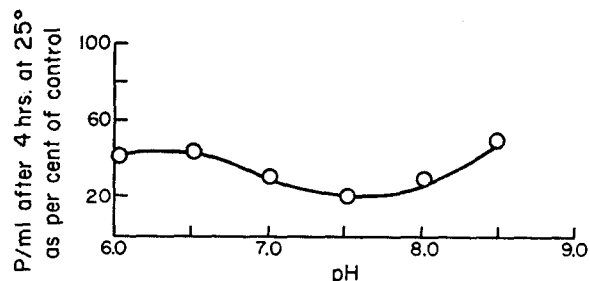


FIGURE 2. Inactivation of  $T_2$  in M/10 phosphate buffer of varying pH. Three mg chymotrypsin/ml, 4 hours at  $25^\circ$ . Control, 3 mg heat-inactivated chymotrypsin/ml. Average of 6 experiments.

most rapid in 0.05 to 0.10 M, pH 7 phosphate buffer. The hydrolysis of yeast nucleoprotein is affected in a similar way. Some T<sub>2</sub> phage preparations were found to be too unstable in this solvent and it was necessary to add 5 mg

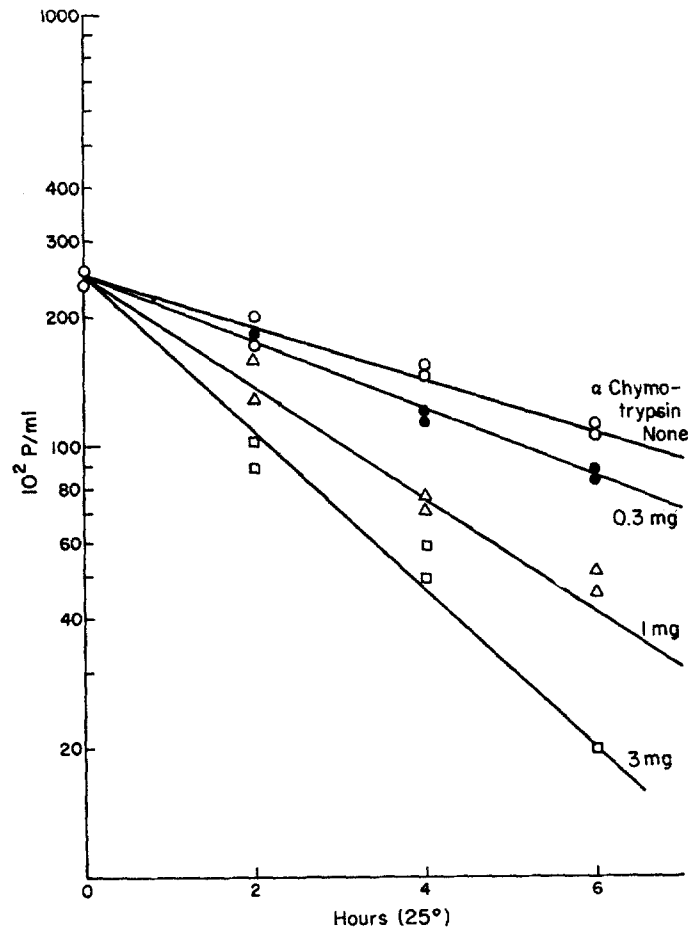


FIGURE 3. Inactivation of T<sub>2</sub> phage in 0.1 M, pH 7 phosphate buffer by varying concentrations of  $\alpha$ -chymotrypsin at 25°.

$\alpha$ -chymotrypsin/ml, mg	0	0.3	1.0	3.0
Inactivation rate day <sup>-1</sup>	0.14	0.18	0.30	0.43
Corrected for control	0	0.04	0.16	0.29
$K$ (day-mg enzyme/ml) <sup>-1</sup>		0.13	0.16	0.10

peptone/ml. All other salts tested inhibited the reaction in high concentrations.

**EFFECT OF PH** The effect of the pH of the phosphate buffer is shown in Fig. 2. The optimum pH is about 7.5.

**EFFECT OF THE ENZYME CONCENTRATION** The effect of varying en-

zyme concentration is shown in Fig. 3. The inactivation curves are all approximately logarithmic and the slope (after correcting for the "spontaneous" inactivation of the control) is approximately proportional to the enzyme concentration.

**THE EFFECT OF VARYING THE PHAGE CONCENTRATION** The inactivation of varying concentrations of the virus was determined in the presence of the same concentration of enzyme. The virus concentration varied from  $1 \times 10^4$  to  $1 \times 10^7$  particles/ml. All were about 80 per cent inactivated by 3 mg chymotrypsin/ml after 5 hours at  $25^\circ$ .

The preceding experiments indicate that the inactivation of the virus by  $\alpha$ -chymotrypsin is a true enzymatic reaction, and this conclusion is confirmed by the following experiments.

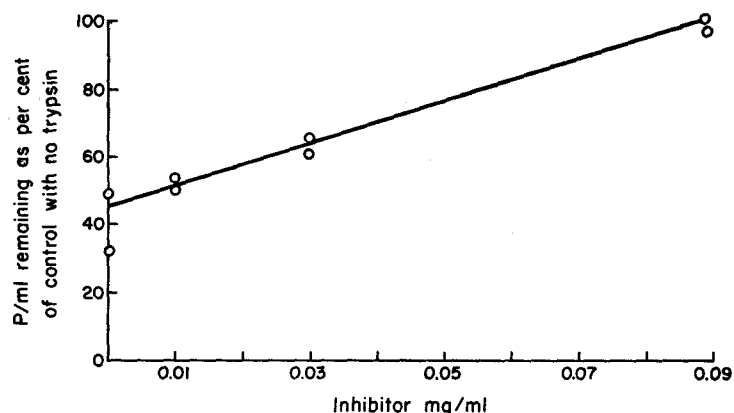


FIGURE 4. Effect of varying concentrations of soybean trypsin inhibitor on the inactivation of  $P_1$  phage by trypsin. 0.1 mg trypsin/ml, 4 hours at  $25^\circ$ .

1. Five different samples of the enzyme, including one which was 5 times recrystallized in this laboratory, all reacted alike. This virtually precludes the presence of a contaminating enzyme, since it is very unlikely that different preparations would all contain the same concentration of a contaminant.

2. Closely related proteins, chymotrypsinogen (Kunitz and Northrop, 1935) and diisopropyl-phosphoryl- $\alpha$ -chymotrypsin (Jansen, Nutting, Jang, and Balls, 1949), which differs in one group from the active enzyme, are inactive.

3. Incubation of the solution with the enzyme before the addition of the virus has no effect. It is unlikely, therefore, that the enzyme acts by destroying some stabilizing agent in the solution.

4. The inactivation of  $P_1$  phage by trypsin is prevented by the presence of soybean trypsin inhibitor (Kunitz, 1946) (Fig. 4).

## EXPERIMENTAL PROCEDURE

Lambda phage and P<sub>1</sub> phage were plated on K12S and T<sub>2</sub> phage was plated on *coli* B, using Gratia's (1936) double layer technique as modified by Adams (1950).

Hydrolysis of the proteins was followed by precipitation with hot 5 per cent trichloroacetic acid and the turbidity determined in a Klett photoelectric colorimeter.

*Preparation of Yeast or E. coli Nucleoprotein* Cells extracted with 0.2 M NaOH for 24 hours. Filtered. Filtrate adjusted to pH 4.7 with HCl. Filtered. Precipitate suspended in water and adjusted to pH 8 with NaOH. Some preparations contain 10 to 20 per cent of a protein (probably denatured) which is very rapidly hydrolyzed by chymotrypsin. This may be removed by the addition of 2 micrograms of chymotrypsin/ml. The solution is kept at 25° for 2 days and the nucleoprotein reprecipitated at pH 4.7.

*Assay* 0.5 ml nucleoprotein solution containing 2 to 5 mg/ml added to 10 ml M/10, pH 4.7 acetate buffer and the turbidity read in a Klett colorimeter.

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