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SHORT COMMUNICATION

HYDROLYSIS OF OLIGOPEPTIDES BY THE STAPHYLOLYTIC ENZYMES, L-11 ENZYME AND ALE

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In investigations on the lytic actions of the L-11 enzyme, produced by a bacterium of the genus *Flavobacterium* (Kotani et al., 1959; Kato et al., 1962; Kato and Strominger, 1968; Kato et al., 1968) and ALE, from an EP-K1 strain of *Staphylococcus epidermidis* (Suginaka et al., 1967; Suginaka et al., 1968) on the cell walls of *Staphylococcus aureus*, their hydrolytic actions on various commercially available oligopeptides were studied. Some of the oligopeptides tested were partially similar structurally to the peptide moiety of *S. aureus* cell wall peptidoglycan.

Two preparations of the L-11 enzyme were used in this study. One preparation (lot 9B) was obtained from the culture supernatant of L-11 bacterium grown in 0.1 per cent casamino acid medium and was purified by chromatography on a hydroxylapatite column as described previously (Kato et al., 1962). The other preparation (lot 32-CM) was derived from a crude enzyme specimen precipitated by saturated ammonium sulfate from the supernatant of a culture of L-11 bacterium grown in 2 per cent glucose broth with aeration. This crude enzyme was submitted to gel filtration on a Sephadex G-75 column. Active fractions eluted from the column with 0.01 M potassium phosphate buffer, pH 8.0, were pooled, and fractionated further by adsorption on CM-cellulose and stepwise elution with potassium phosphate buffers (pH 8.0) of 0.01 M 0.05 M and 0.1 M. The staphylolytic enzyme eluted with 0.1 M buffer was dialyzed against 0.01 M buffer and used in this study. The ALE preparation used was obtained from the supernatant of a culture of EP-K1 strain grown in Trypticase Soy Broth with aeration. The preparation was obtained by salting out with ammonium sulfate, precipitation with acetone, chromatography on a CM-cellulose column, and reprecipitation with acetone. Details of the procedures were described in a previous paper (Suginaka et al., 1967). Penta-glycine (grade I) and glycly-L-lysine (grade II) were obtained from CYCLO Chemical Corporation, Los Angeles 1, Calf. (U.S.A.). Other oligopeptides, of guaranteed reagent grade, were purchased from Tokyo Chemical Industry Co., Tokyo.

The hydrolyzing actions of the L-11 enzyme and ALE on the test oligopeptides were examined as follows. A sample of 800 mμmoles of an oligopeptide was incubated at 37 C with 8 to 16 lytic units (against *S. aureus* cell walls) of enzyme in a total volume of 400 μ liter of an appropriate buffer (see Table 1 and 2). Control tubes with substrate but without enzyme were incubated similarly. Aliquots were removed at intervals and immediately heated at

TABLE 1. *Hydrolysis of oligopeptides by the L-11 enzyme*

Oligopeptide	Liberation of N-terminal groups[a] during incubation for						Hydrolysis products
	Exp. 1 : lot 9B		Exp. 2 : lot 32-CM				
	5	25 hrs	1	3	6	24 hrs	
Gly-gly	−2	−2	−3	−2	−2	2	
Gly-gly-gly	0	0	7	5	7	12	
Gly-gly-gly-gly	4	6	9	12	15	26	
Gly-gly-gly-gly-gly	20	41	24	44	63	116	Gly-gly, Gly-gly-gly
D, L-Ala-gly	−2	−2	—	3	2	2	
D, L-Ala-gly-gly	0	0	1	−3	3	7	
D, L-Ala-D, L-ala	—[b]	8	5	6	5	9	
Gly-L-lys	0	0	−4	4	−2	−1	
Gly-D, L-ala	—	−2	—	−2	6	3	
Gly-D, L-leu	—	0	—	−8	11	−5	

0.01 M potassium phosphate buffers of pH 6.8 and 8.0 were used in experiments 1 and 2, respectively.

[a] $\left(\frac{E_t-E_o}{E_o}\times\frac{C_t-C_o}{C_o}\right)\times 100.$ [b] Not tested.

E_o : mμmole at 0 time in test
E_t : mμmole at specified time in test
C_o : mμmole at 0 time in control
C_t : mμmole at specified time in control

TABLE 2. *Hydrolysis of oligopeptides by ALE*

Oligopeptide	Liberation of N-terminal groups[a] during incubation for				Hydrolysis products
	Exp. 1		Exp. 2		
	5	25 hrs	5	25 hrs	
Gly-gly	3	13	2	6	
Gly-gly-gly	14	53	7	29	Gly, Gly-gly
Gly-gly-gly-gly	13	18	7	8	
Gly-gly-gly-gly-gly	7	17	7	8	
D, L-Ala-gly	16	50	14	45	Ala, Gly
D, L-Ala-gly-gly	24	71	20	66	Ala, Gly-gly
D, L-Ala-D, L-ala	—[b]	54	21	65	
Gly-L-lys	4	39	6	17	
Gly-D, L-ala	—	—	9	30	
Gly-D, L-leu	—	30	5	31	

0.01 M potassium phosphate buffer, pH 6.8, containing 0.75 M sodium chloride was used.

[a] Expressed as in Table 1.
[b] Not tested.

100 C for two minutes to stop the enzyme activity. Then the N-terminal groups were estimated. If significant liberation of N-terminal groups was recognized, 100 μ liter aliquots of the reaction mixture were treated with 1-fluoro-2,4-dinitrobenzene and submitted to thin-layer chromatography to identify hydrolysis products. Determination of N-terminal groups and thin-layer chromatography of dinitrophenylated amino acids or peptides were performed as described by Ghuysen, Tipper and Strominger (1966).

The results with the L-11 enzyme are summarized in Table 1, and those with ALE in Table 2. The followoing points are seen from these tables. Both the L-11 enzyme and ALE attacked glycine peptides but in different ways. Penta-glycine was a good substrate for the L-11 enzyme, being split into di-glycine and tri-glycine, but it was hydrolyzed only to a limited extent by ALE. The reverse was true for tri-glycine. This peptide was very susceptible to ALE, giving glycine and di-glycine as hydrolysis products, but it was a poor substrate for the L-11 enzyme. Neither di-glycine nor tetra-glycine were attacked appreciably by either enzyme. A sharp difference was recognized between the actions of the L-11 enzyme and ALE on di- and tripeptides containing glycine and/or alanine. ALE liberated a significant amount N-terminal groups from these peptides, but the L-11 enzyme had no detectable hydrolyzing activities against any of them.

The finding that the L-11 enzyme can hydrolyze penta-glycine is in harmony with the previous observation that this enzyme can split glycyl-glycine linkages in a pentaglycine bridge connecting basal tetrapeptides of *S. aureus* cell wall peptidoglycan (Kato et al., 1968; Kato and Strominger, 1968). The fact that the action of L-11 enzyme on penta-glycine was markedly inhibited by the addition of sodium chloride to the sasay system, as shown in Fig. 1, is also in accord with the reported inhibition of the staphylolytic activity of this enzyme by solutions of high ionic strength (Kato et al., 1962). There are apparent discrepancies, however, between the action of the L-11 enzyme on D-alanyl-glycine linkages in the cell wall peptidoglycan of *S. aureus* and in oligopeptides. About 30% of the pentaglycine bridges in *S. aureus* cell walls were hydrolyzed at the D-alanyl-glycine linkage by the L-11 enzyme in a previous study (Kato and Strominger, 1968), but neither D,L-alanyl-glycine nor D,L-alanyl-glycyl-glycine were attacked by the enzyme in this study.

The results obtained on the action of ALE on oligopeptides are not compatible with pre-

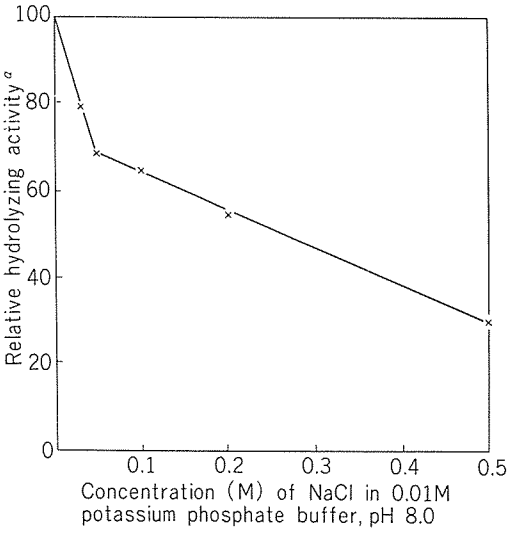

FIGURE 1. *Inhibition of hydrolysis of penta-glycine by the L-11 enzyme by addition of sodium chloride to the reaction mixture*

[a] $\dfrac{\textit{Liberation (m}\mu\textit{moles) of N-terminal groups in the presence of NaCl}}{\textit{Liberation (m}\mu\textit{moles) of N-terminal groups in the absence of NaCl}} \times 100$

vious findings on the mode of action of this enzyme on the cell walls of *S. aureus* (Suginaka et al., 1968). This is illustrated by the fact that ALE did not split D-alanyl-glycine and N^{ϵ}-glycyl-L-lysine bonds in the cell wall peptidoglycan but caused definite hydrolysis of D,L-alanyl-glycine, D,L-alanyl-glycyl-glycine and glycyl-L-lysine. The observations that ALE hydrolyzed penta-glycine interpeptide bridges in the peptidoglycan like a glycyl-glycine endopeptidase, and hydrolyzed tri-glycine but did not hydrolyze penta-glycine, also seem conflicting.

How can these discrepancies be explained? One possibility is that the size or molecular configuration of the substrates is important in determining its susceptibility to an enzyme. This explanation could apply to both the L-11 enzyme and ALE. Another possible explanation for the findings with ALE is that hydrolysis of di- and tripeptides is caused by an aminopeptidase or an aminopeptidase and a dipeptidase, present in the enzyme preparation as a different entity or entities from the endopeptidase which solubilizes *S. aureus* cell walls. It may be added here that possibly only the L-isomers of D,L-alanyl-glycine and D,L-alanyl-glycyl-glycine are hydrolyzed.

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