OUKA
The University of Osaka
Institutional Knowledge Archive

Title	Isolation of D-Alaniyl-Meso-a, a'-Diaminopimelic Acid Endopeptidase from a Crude Preparation in Streptomyces "L-3 Enzyme"
Author(s)	Matsuda, Tetsuo; Kotani, Shozo; Kato, Keijiro et al.
Citation	Biken journal : journal of Research Institute for Microbial Diseases. 1968, 11(2), p. 145-148
Version Type	VoR
URL	https://doi.org/10.18910/82874
rights	
Note	

The University of Osaka Institutional Knowledge Archive : OUKA

https://ir.library.osaka-u.ac.jp/

The University of Osaka

BIKEN JOURNAL Vol. 11, 145-148, 1968

SHORT COMMUNICATION

ISOLATION OF D-ALANYL-MESO-α,α'-DIAMINOPIMELIC ACID ENDOPEPTIDASE FROM A CRUDE PREPARATION IN *STREPTOMYCES* "L-3 ENZYME"

TETSUO MATSUDA, SHOZO KOTANI, KEIJIRO KATO and TSUYOSHI KATAYAMA

Department of Microbiology, Osaka University Dental School, Osaka

TAKAFUMI MORIYAMA[1] and MASAHIKO YONEDA

Department of Tuberculosis Research I, Research Institute for Microbial Diseases, Osaka University, Osaka

(Received February 20, 1968)

Previous works on the mode of lytic action of the L-3 enzyme from *Streptomyces* (KATO, STROMINGER and KOTANI, 1968; MATSUDA, KOTANI and KATO, 1968a, 1968b) have demonstrated that the crude enzyme preparation catalyses the hydrolysis of the following linkages in the cell wall peptidoglycans of *Corynebacterium diphtheriae* (strain Park-Williams No. 8) and of *Lactobacillus plantarum* (ATCC 8014): N-acetylmuramyl-L-alanine linkages, D-alanyl-meso-α,α'-diaminopimelic acid (DAP) cross bridges and the carbamyl (-$CONH_2$) groups of DAP amide residues. The present investigation was undertaken to see whether separate enzyme entities are responsible for these three activities.

Concentrated solution of L-3 enzyme was obtained by salting out with ammonium sulfate (30–80 per cent saturation) from filtrates of cultures of *Streptomyces sp.* (L-3 bacterium) in modified McCarty's medium, as described previously (MORI *et al.*, 1960).

The lytic activity of the enzyme against diphtheria cells or cell walls was determined by measuring the decrease of optical density of the cell- or cell wall-suspension when incubated at 37°C with enzyme preparation. The mixture contained 0.02 M phosphate buffer, pH 7.8, in a total volume of 4.0 ml. The initial optical density of the cell suspension on the reaction mixture was adjusted to 0.5 at 550 mμ in a Bausch and Lomb spectrometer. The initial concentration of the cell wall suspension was 0.5 mg/ml.

Concentrated enzyme preparation was first decolorized by negative adsorption on a DEAE-cellulose column equilibrated with 0.5 M NaCl-0.005 M phosphate buffer, pH 6.0. The lytic activity was almost quantitatively recovered in the unadsorbed fractions. The fractions with lytic activity were combined and solid ammonium sulfate was added to 80 per cent saturation. The precipitate was recovered by centrifugation, dissolved in 0.05 M phosphate buffer, pH 6.0, and dialyzed against the same buffer. An aliquot of the dialyzed sample (900 whole cell lytic units and 33.6 mg protein) was subjected to zone electrophoresis in 0.05 M phosphate buffer, pH 6.0, using starch as the

1 Present address: Department of Microbiology, School of Dentistry, Hiroshima University, Hiroshima.

supporting medium, as described by KUNKEL (1954). A starch block of 1.5×10×30 cm in size was used. After electrophoresis at 2 mA/cm² for 18 hours in the cold, the block was cut into 1 cm wide segments. These were each extracted three times with a total of 20 ml of 0.001 M phosphate buffer, pH 7.0. The lytic activities against whole cells and cell walls of *C. diphtheriae* and the protein content (by the method of LOWRY *et al.*, 1951) of the fractions were measured.

Fig. 1 shows that two fractions (F-1 and F-2) migrating toward the cathode exhibited cell wall lytic activity. F-2 lyzed both whole cells and cell walls efficiently. But, the whole cell lytic activity of F-1 was too weak to measure accurately without concentrating the solution. An aliquot of F-2 containing 3135 whole cell lytic units and 9 mg protein was further purified by column chromatography on CM-cellulose equilibrated with 0.005 M phosphate buffer, pH 6.0. The sample was dialyzed against 0.005 M phosphate buffer, pH 6.0 before chromatography. Elution was carried out with 0.001 M phosphate buffer, pH 8.0 and then with a linear gradient of increasing NaCl (0 to 1 M) and phosphate buffer (pH 8.0, 0.001 M to 0.005 M) concentrations (Fig. 2). Fractions were collected at a rate of 20 ml/tube/20 minutes, and assayed for whole cell lytic activity and protein content. The contents of tubes from No. 13 to 18 were pooled (F-2′), and concentrated by precipitation with ammonium sulfate (80 per cent saturation).

Cell walls from *C. diphtheriae* and *L. plantarum*, respectively, were incubated at 37°C with F-2′ fraction exhibiting both cell wall and whole cell lytic activity. The F-2′ fraction was used at a ratio of 0.3 cell wall lytic units per mg of substrate. Decrease in optical density and increase in peptide terminal groups during lysis of the cell walls were determined

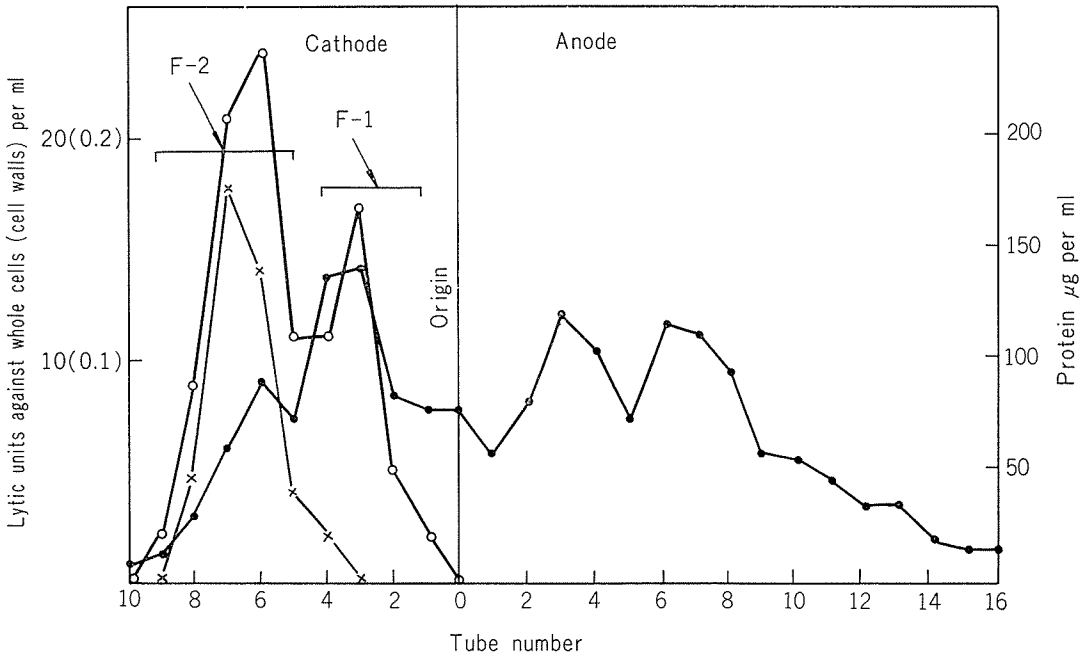

FIGURE 1 Fractionation of L-3 enzyme preparation by starch block electrophoresis.
Buffer: 0.05 M phosphate buffer, pH 6.0; Current: 2 mA/cm²; Time: 18 hours.
Symbols: ● protein concentration, × lytic activity against whole cells, ○ lytic activity against cell walls.

by the methods described by GHUYSEN, TIPPER and STROMINGER (1966). The results in Table 1 shows that the F-2′ fraction released α'-NH_2-terminal DAP and COOH-terminal alanine from both *C. diphtheriae* and *L. plantarum* cell walls but not NH_2-terminal alanine or COOH-terminal DAP. These data indicate that the F-2′ fraction catalyses only the hydrolysis of D-alanyl-meso-DAP linkages. It is concluded that the three enzymatic activities present in crude L-3 enzyme preparation are not due to a single enzyme, but that more than two enzyme entities are present in the crude preparation, and that one of these, D-alanyl-meso-DAP endopeptidase, was separated and recovered in the F-2′ fraction. Similar analyses were attempted for end groups liberated by digestion of both cell wall preparations with the F-1 fraction. Results suggested that N-acetylmuramyl-L-alanine amidase may be separated in this fraction. However, this fraction may be contaminated with D-alanyl-meso-DAP endopeptidase, since in addition to NH_2-terminal alanine residues, a small, but significant amount of α'-NH_2-groups of DAP residues was released during digestion of *L. plantarum* cell walls. Further work is neccesary on isolation of N-acetylmuramyl-L-alanine amidase as a separate enzyme entity.

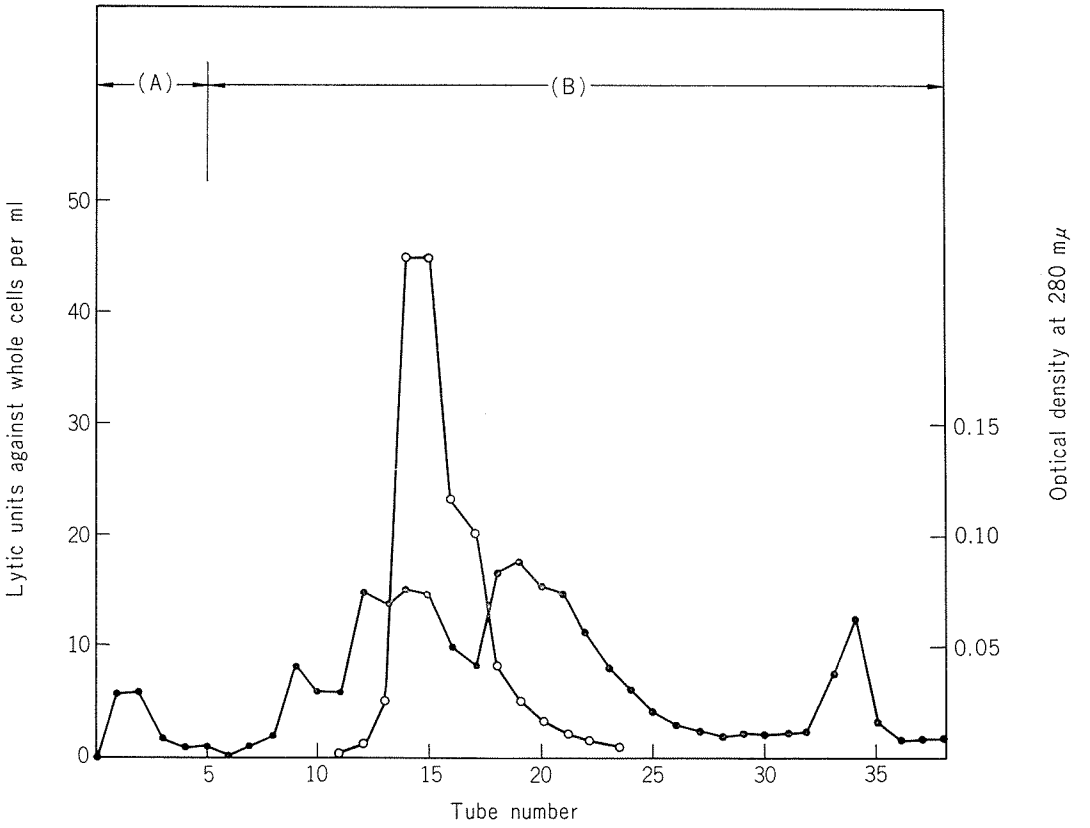

FIGURE 2 Column chromatography of F-2 fraction on CM-cellulose.
Column size: 12×150 mm; Elution: (A) 0.001 M phosphate buffer; pH 8.0, (B) linear gradient of increasing NaCl (0→1 M) and phosphate buffer (0.001 M→0.005 M, pH 8.0) concentration.
Symbols: ○ lytic activity against *C. diphtheriae* whole cells, ● optical density at 280 mμ.

TABLE 1 *Analyses of peptide terminal groups liberated by digestion of C. diphtheriae and L. plantarum cell walls with F-2′ fraction from Streptomyces L-3 bacteria*

Determination	Increase (mμmoles per mg) on 72 hours incubation	
	C. diphtheriae cell walls	*L. plantarum* cell walls
NH_2-terminal groups		
Alanine	0	0
α'-NH_2-DAP	130	50
COOH-terminal groups		
Alanine	102	70
DAP	trace	0
	Decrease (per cent) in optical density	
Optical density		
after 8 hours incubation	43	45
after 72 hours incubation	90	75

REFERENCES

GHUYSEN, J.-M., TIPPER, D. J. and STROMINGER, J. L. (1966). Enzymes that degrade bacterial cell walls. In *Methods in Enzymology* (Ed. by Neufeld, E. F. and Ginsburg, V.), Vol. VIII: Complex Carbohydrates, pp. 685–699. Academic Press, New York.

KATO, K., STROMINGER, J. L. and KOTANI, S. (1968). Structure of the cell wall of *Corynebacterium diphtheriae*. I. Mechanism of hydrolysis by the L-3 enzyme and the structure of the peptide. *Biochemistry*, in press.

KUNKEL, H. G. (1954). Zone electrophoresis. in *Methods of Biochemical Analysis* (Ed. by Glick, D.), Vol. I, pp. 141–170. Interscience Publishers, INC., New York.

LOWRY, O. H., ROSEBROUGH, H. J., FARR, A. L. and RANDALL, R. J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265–275.

MATSUDA, T., KOTANI, S. and KATO, K. (1968a). Structure of the cell walls of *Lactobacillus plantarum*, ATCC 8014. 1. Isolation and identification of the peptides released from cell wall peptidoglycans by *Streptomyces* L-3 enzyme. *Biken J.* **11**, 111–126.

MATSUDA, T., KOTANI, S. and KATO, K. (1968b). Structure of the cell walls of *Lactobacillus plantarum*, ATCC 8014. 2. Cross linkage between D-alanine and α,α'-diaminopimelic acid in the cell wall peptidoglycans studied with an L-11 enzyme from *Flavobacterium sp. Biken J.* **11**, 127–138.