Stepwise degradation of fraction *A* yielded, beside unspecified amounts of glutamic acid[5], chiefly alanine as the first, and glycine as the second, amino-acid. In the third step valine and in the fourth step aspartic acid made their appearance. The chromatograms also showed spots due to glycine and valine, and particularly large spots due to alanine in the third and fourth steps. Analysis of the water phase following the second acid treatment revealed that alanylalanine had been liberated during this part of the procedure. By more drastic acid treatment most of, if not all, the remaining alanine could be split off as alanylalanine. Incubation of the original *A* fraction with carboxypeptidase resulted in the liberation of approximately one-third of the total alanine as free alanine. These observations indicate the presence of alanylalanine at the carboxyl end of fraction *A*, in all likelihood linked to the rest of the fraction through aspartic acid. In summary, analysis of fraction *A* to date indicates that it contains as a chief constituent the hexapeptide, alanylglycylvalylaspartylalanylalanine. It may also contain other peptides which may be products of the 'unspecific' breakdown of ovalbumin.

A detailed account of these experiments will appear in the *Comptes Rendus des Travaux du Laboratoire Carlsberg* (Série Chimique). We wish to express our gratitude to Prof. K. Linderstrøm-Lang for encouragement and suggestions throughout the course of this work.

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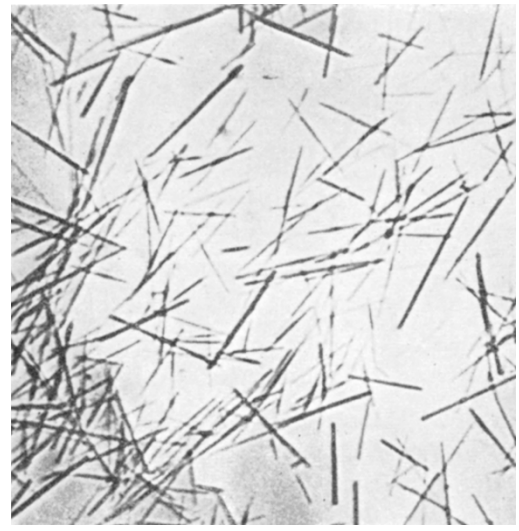
Preparation of Crystals containing the Plakalbumin-forming Enzyme from *Bacillus subtilis*

Ovalbumin is transformed by a proteolytic enzyme preparation from *Bacillus subtilis* into another protein, plakalbumin[1]. During the transformation a small amount of peptides is liberated[2]. An attempt has been made to purify the enzyme responsible for this transformation.

The bacteria were grown in submerged culture with vigorous aeration in a medium containing peptone, glucose and a salt solution containing trace metals. When after *c.* 30 hr. the enzyme concentration had reached a maximum, the bacteria were removed by centrifugation, the proteins in the culture liquid precipitated by addition of sodium sulphate, and the crude enzyme dried in a vacuum. A 10 per cent solution of the crude enzyme was purified by addition of calcium chloride in excess to precipitate the sulphate ions and of one volume of acetone to precipitate the mucins. The enzyme was precipitated by increasing the acetone concentration to 75 vol. per cent, and the precipitate dissolved in a small amount of water. After evaporation of the acetone,



Plakalbumin-forming enzyme, recrystallized. × 300

*p*H was adjusted to *c.* 5·5. The solution was made *c.* 5 per cent with respect to sodium sulphate, kept at *c.* 30° C. with mechanical stirring, and eventually seeded with crystals of the enzyme. When crystallization had started, the concentration of sodium sulphate was slowly raised to about 12 per cent. The enzyme crystallized out as long, very thin needles (see photograph). It was dissolved in water and recrystallized by addition of sodium sulphate. The activity of the enzyme was followed during the purification by measuring its ability to clot milk.

	Per cent of original nitrogen	Per cent of original activity	Activity per mgm. nitrogen	Activity per mgm. protein nitrogen
Solution of crude enzyme	(100)	(100)	5,550	
Precipitate 50–75 vol. per cent acetone	72	95	7,300	7,550
Dissolved crystals after :				
1. crystallization	64	84	7,300	7,450
2. ,,	59	76	7,100	7,300
3. ,,	55	71	7,130	7,350
4. ,,	49	64	7,200	7,450

During the purification, the plakalbumin-forming ability of the enzyme follows the milk-clotting activity within experimental error. The enzyme breaks down casein at almost the same rate as trypsin and about three times as fast as chymotrypsin. It has no amylase activity. Preliminary investigations in the electrophoresis apparatus show that about 95 per cent of the material moves as a symmetrical peak in an acetate buffer with sodium chloride, *p*H 5·3, ionic strength 0·1. In the ultracentrifuge, the material appears homogeneous with a sedimentation constant of *c.* 2·7 Svedberg units.

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