Biological and chemical properties of α-amylase precursor

Previous studies[1] in this laboratory suggest that the last process of α-amylase formation in *Bacillus subtilis* occurs by a transformation of a previously formed precursor protein. It was shown that about 7 % of total cellular protein was transformed into α-amylase and excreted into the medium after the cell growth reached the stationary phase.

B. subtilis (strain K) was grown on the surface of the synthetic medium as described in a previous paper[1]. Sonic extracts of the bacterial cells were centrifuged and the supernatant was fractionated by column chromatography using DEAE-cellulose.

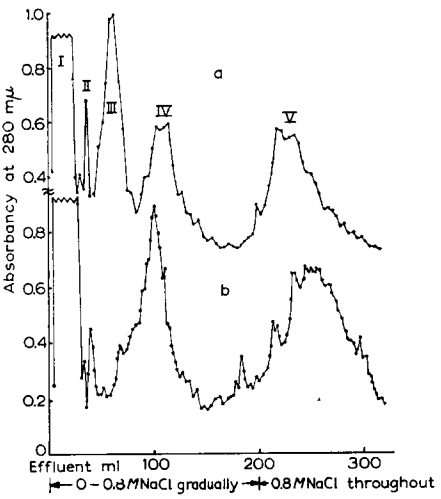

Fig. 1. Elution pattern of bacterial extract of *B. subtilis*. The bacterial cells were disintegrated by sonic oscillation (Tominaga Works, Japan, 8 kc, approx. 100 W, 30 min) in 0.02 *M* tris(hydroxymethyl)aminomethane buffer, pH 8.0, and centrifuged for 30 min at 10,000 rev./min in a Servall (SS-1) centrifuge. An aliquot of the supernatant (approx. 100 mg dry weight) was placed on a DEAE-cellulose column (0.9 × 25 cm), and eluted with a linear salt gradient at 13 ± 1°.

Typical elution patterns are shown in Figs. 1a and 1b. Five distinct protein peaks were obtained from the cells of the earlier stage at which the cell growth almost reached the stationary phase, but α-amylase activity in the medium was only 300 $D_{40°}^{30'}$/ml in Wohlgemuth's units[2] (Fig. 1a). On the other hand, the peak marked III could not be recognized in the extract of the cells at the latter stage of growth, (Fig. 1b). At this stage, α-amylase activity in the medium was 3,500–4,000 $D_{40°}^{30'}$/ml, *i.e.* α-amylase production was almost complete. The amount of the protein III which disappeared during this period was comparable to the amount of α-amylase produced in the medium. These results, together with the fact that the cells at an intermediate stage of α-amylase production contained a moderate amount of the protein III, suggest that the latter may be an α-amylase precursor. To substantiate this possibility, immunological and chemical methods were employed.

Rabbit anti-α-amylase serum was prepared by a series of intravenous injections of crystalline α-amylase, giving a total of 80 mg per rabbit. Antigenic homogeneity was established by the formation of a single line precipitation in gel diffusion agar with anti-serum and α-amylase. The ratio between α-amylase and anti-serum is about 1:3 at the equivalence point.

Antigenicity of the protein fractions obtained by column chromatography was tested by precipitation reactions and by their activity as an inhibitor of the α-amylase-anti-sera neutralization reaction under various conditions. The protein III gave

Abbreviation: DEAE-, diethylaminoethyl-.

positive results in both tests, and none of the other protein fractions had any cross reaction.

Gel-diffusion precipitation between anti-sera, α-amylase and the protein III showed a continuous line (Fig. 2), which indicates that the protein III is related antigenically to α-amylase.

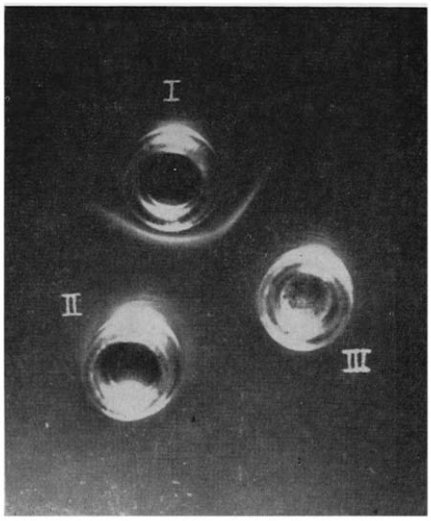

Fig. 2. Gel–diffusion–precipitation reaction between anti-serum, α-amylase and the protein III
I: anti-serum, II: α-amylase, III: protein III.

Elution patterns of the extract from an α-amylase–negative mutant of *B. subtilis* were not radically different from that of the wild strain shown in Fig. 1a. However, none of the protein classes had any cross reaction with the anti-serum.

The protein III was lyophylized after dialysis, and some of its chemical properties were investigated. It had very weak α-amylase activity, *i.e.* 1.5–2 % that of α-amylase, and after one rechromatography, about 0.6 %. This activity probably comes from a small amount of α-amylase contaminating the protein III.

Judging from the following results, the protein III is fairly homogeneous: (1) it showed a single elution peak by rechromatography, (2) it showed almost homogeneous sedimentation pattern by ultra-centrifugation, (3) it gave a single N-terminal amino acid, *i.e.* phenylalanine, by the dinitrofluorobenzene method.

From the sedimentation coefficient of the protein III ($S_{20,w} = 3.88$ S), together with the known sedimentation coefficient (4.22 S) and molecular weight (49,000) of α-amylase[3], the molecular weight of the protein III may be estimated tentatively as about 43,000.

If the protein III is an α-amylase precursor, both proteins should have common peptide chains in their molecules. "Peptide maps" of the tryptic hydrolysate of both proteins obtained by the method described by KATZ *et al.*[4] were compared. α-Amylase gave about 40 peptide spots and the protein III gave about 20. Among these spots, at least ten might be considered as common peptides. The amino acid compositions of the peptides are now being elucidated.

The protein III contains much more glutamic acid, probably in amide form, and a lesser amount of arginine than α-amylase.

The results described above and the results presented in a previous paper[1] strongly suggest that the protein III is not merely "cross-reacting material"[5–7], but it must be a real α-amylase precursor.

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The use of urea-starch-gel electrophoresis in studies of reductive cleavage of an α_2-macroglobulin

The presence of several protein components of high molecular weight in human serum is well established[1]. There are two major groups of proteins with sedimentation coefficients between 17 S–20 S. One of these groups is comprised of proteins belonging to the γ_1-globulins[2,3]; the other is composed mainly of proteins belonging to the α_2-globulins[4]. It has been postulated that these macromolecules represent polymeric proteins[5,6]. DEUTCH AND MORTON[7] have shown that the addition of mercaptoethanol and cysteine result in a fall in sedimentation rate from 18 S to 7 S and that stable monomeric units are formed in the presence of iodoacetamide.

This report is concerned with the effects of reductive cleavage with mercaptoethanol in urea on the protein of high molecular weight belonging to the α_2-globulins. This protein is variously known as 'heat-labile glycoprotein"[8], the "alpha 2 macroglobulin"[4], and as "slow alpha 2 globulin"[9,10]. SCHONENBERGER *et al.*[11] studied the physicochemical properties of this protein in great detail and described dissociation of the protein into two components (11 S and 15.7 S) in the presence of 5 *M* urea. Reaggregation to a single component of 18.1 S occurred after removal of the urea with and without the presence of iodoacetamide. Comparable results were obtained by ISLIKER[12], who measured the change in viscosity of the protein after the addition of cysteine, cysteamine and thioglycollate. Ultracentrifugal analysis revealed a 6 S component after treatment of the proteins with these components.

A method is described using starch-gel electrophoresis for the separation and