Review Letter

On the primary structure of amylases

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There may be a family of enzymes (such as the serine proteases) of carbohydrate hydrolases where certain amino acid residues compose the active site, and while the sequence may not be homologous, the arrangement of the catalytic site may be (as is the case for trypsin and subtilisin). This question is even more intriguing because of the nuances in specificity of action that exist for the amylases (e.g., α and β, saccharifying and liquefying, etc.).

When tested immunologically, the α-amylase secreted by *Bacillus amyloliquefaciens* does not cross-react with that produced by *Bacillus subtilis* or with that elaborated by *Aspergillus oryzae*. Nor is there a cross-reaction with the α-amylase isolated from pig pancreas [1].

In a recent publication [2] the amino acid sequence of the enzyme obtained from *A. oryzae* (taka-amylase A) was compared with that of hog pancreatic α-amylase isoenzyme I [3] and a few homologous sequences were found. Since the amino acid sequence of the *B. amyloliquefaciens* α-amylase has now been established by sequencing the DNA [4] and also by sequencing the major part of the protein [5–7] the comparison should be extended to the enzyme produced by that strain (see fig.1). In this way, the amino acid residues indispensible for enzyme action can be more finely defined.

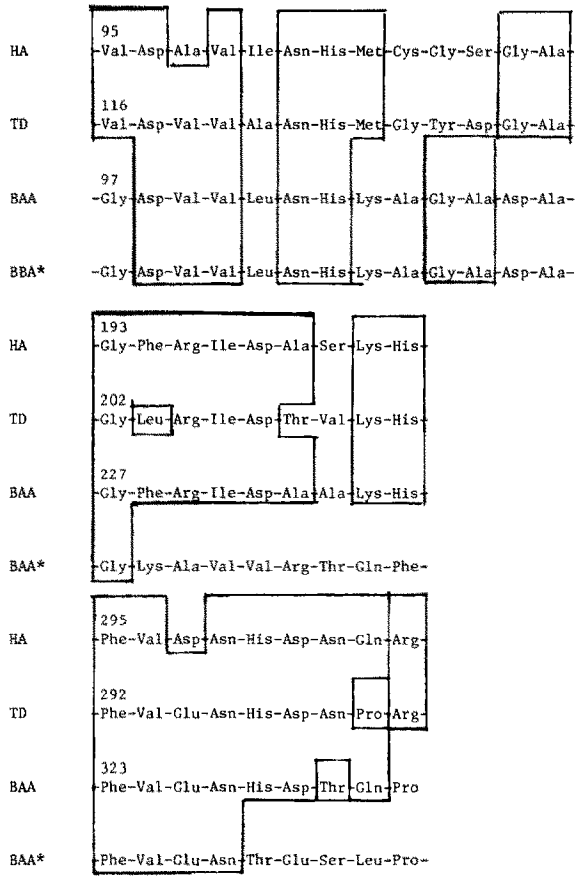

Fig.1. Comparison of the amino acid sequences of the homologous regions of hog pancreatic α-amylase, taka-diastase α-amylase, and two types of *B. amyloliquefaciens* α-amylase.

Abbreviations: HA, hog pancreatic amylase; TD, taka-amylase A, prepared from taka-diastase obtained from *Aspergillus oryzae*; BAA, *B. amyloliquefaciens* α-amylase (sequence deduced from the nucleotide sequence of the cloned gene); BAA*, *B. amyloliquefaciens* α-amylase (sequence deduced by protein sequencing studies)

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Disturbing, however, is the lower amount of homology in the depicted portions of the two types of *B. amyloliquefaciens* α-amylase when compared with each other. The workers who deduced the protein sequence from the nucleotide sequences of the cloned gene, utilized *B. amyloliquefaciens* E 18 – an industrial α-amylase producer [4]. Studies sequencing the proteins were done on the commercial crystalline enzyme supplied by Sigma (A 6380) [5–7]. Undoubtedly, in both instances extensive mutagenesis has to be expected. Still, the higher lack of homology for the sequence (BAA*) derived from protein degradation if compared to all the other enzyme sequences listed in fig.1 might require sequence verification.

NOTE

B. subtilis produces two types of α-amylase (1.4 α-D-glucan glucanohydrolase, EC 3.2.1) liquefying and saccharifying (Fukimoto and Okada (1963) Ferment. Technol. 41, 427). Although the organism producing the liquefying enzyme has been reclassified as *B. amyloliquefaciens* (Welker and Campbell (1967) J. Bacteriol. 94, 1124), the term *B. subtilis* α-amylase has been retained in many publications. Mantsala and Zalkin (1979) J. Biol. Chem. 254, 8530, found that purified *B. subtilis* α-amylase (from strain YY88) has the terminal sequence: Leu-Thr-Ala-Pro-Ser-Ile-Lys-(Ser) etc. When tested immunologically, this enzyme cross-reacts with α-amylase from several other strains of *B. subtilis*, but not with that of *B. amyloliquefaciens*. We have done structural studies on an enzyme supplied commercially as a product of *B. subtilis* (Sigma A 6380). Its N-terminal sequence differs completely from that noted by Mantsala and Zalkin (Chung and Friedberg (1980) Biochem. J. 185, 387). We also isolated α-amylase secreted into the medium by *B. amyloliquefaciens* N (A.T.C.C. 23845). It cross-reacts with the commercial preparation. Thus the commercial material produced should be labelled as *B. amyloliquefaciens* rather than as *B. subtilis* α-amylase.

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