precursor proteins in the periplasm. Immaturation of the precursor indicated a loss of the catalytic activity in the mutants. The enzyme activity (k_{cat}/K_m) of D225E for Boc-Val-Leu-MCA was significantly decreased (1/400), mainly due to the increase of K_m (100-fold). These results suggest that the disposition of a given negative charge of the side chain at Asp225 is essential for the binding of the substrate.

Recently, the three-dimensional structure of API has been elucidated by X-ray crystallography (Sashi *et al.*, 1990). The tertiary fold and the structure of the active site are very similar between API and bovine trypsin. The critical structural difference in the S1 substrate binding subsite is the shallow binding pocket in addition to the presence of His210 in place of Ser in trypsin. The Asp225 is located at the wall of the pocket. Its side chain sticks out inside the pocket, making the bottom of the pocket 2 Å shallower than that of trypsin bearing Gly in place of Asp225. In API, the side chain of lysine of the substrate can fit in the pocket and bind to Asp225 by electrostatic interaction. Since the side chain of arginine is longer than that of lysine, arginine has difficulty fitting into the shallow binding pocket of API. This is consistent with our suggestion that Asp225 is the substrate specificity determinant of API.

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Pieter D. van Wassenaar. Substrate Specificity of Savinase Toward β-Casein. (Unilever Research Laboratory, Olivier van Noorlaan 120, 3133 AT Vlaardingen, The Netherlands)

Improvement of proteolytic enzymes regarding stability and specificity is studied by production and evaluation of recombinant proteases for detergent applications. The specificity of the wild-type enzyme, Savinase, was characterized using β-casein as model substrate. Substrate degradation and product formation was followed in time, generated peptides were isolated and analyzed by N-terminal sequence analysis. The outcome of the analysis revealed 19 cleavage sites. In general, a positional preference regarding the P4 to P1′-sites could be observed. Comparison of these sites with known cleavage sites of proteolytic enzymes toward β-casein showed that nine of them are also observed for chymotrypsin (Keil, 1987). No significant (predicted) structural (Chou and Fasman, 1974) criteria seem to be of major importance for proteolytic attack by Savinase toward β-casein. As is known for aspecific proteases, peptide sequences containing phosphorylated residues or proline residues were observed difficult to be hydrolyzed. The adopted method for isolation and sequencing generated peptides is very well suited to characterize mutant proteases regarding specificity or stability and the effect of environmental conditions during washing experiments.

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Shaoliang Li, Dorjbal Dorjsuren, Susumu Tsunasawa, and Fumio Sakiyama. Cleavage of Peptide Bonds at $CySO_3H$ and CM-Cys with an Endoprotease (Blase) from *Bacillus licheniformis*. (Institute for Protein Research, Osaka University, Suita, Osaka, Japan)

Endoprotease "Blase" is an extracellular serine-protease isolated from *Bacillus licheniformis* and reported to specifically cleave peptide bonds at the C-terminal side of glutamic acid (Kakudoh *et al.*, 1991). However, during the investigation on the cleavage pattern of peptide substrate with highly purified Blase, we found that this enzyme can cleave peptide bonds at the C-terminal sides of aspartic acid, cysteic acid, and S-carboxymethylated cysteine in addition to glutamic acid. In this communication, we present the results of an investigation on the substrate specificity of Blase and evaluate its preference of glutamic acid to other acidic amino acids.

The substrate specificity of Blase was examined by digestion of several model proteins and peptides at a substrate-to-enzyme ratio of 100:1 (mol/mol). The digestion was performed at 37°C for an appropriate time with or without 4 M urea.

The digestion products were separated on reversed-phase HPLC using a trifluoroacetic acid/acetonitrile gradient and a cleavage site was determined by both N-terminal