Note

Fluorogenic Substrate of Aspergillus Aspartic Proteinase

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The extracellular aspartic proteinase from Aspergillus saitoi (EC 3.4.23.6) can activate trypsinogen and chymotrypsinogen A at acidic pHs. Primary cleavage sites at the phenylalanyl24-phenylalanyl25 and Ieucyl15-tyrosyl16 bonds in the oxidized insulin B-chain were found with the aspartic proteinase. Unfortunately, the proteinase does not hydrolyze any of the commercially available small synthetic peptides, amides, or esters. In this study, a sensitive, fluorometric assay for the aspartic proteinase from Aspergillus is described.

r-Butoxycarbonyl-leucyl-seryl-threonyl-arginyl-4-methylcoumaryl-7-amide (Boc-LSTR-MCA), Boc-IEGR-MCA and, Boc-QRR-MCA were purchased from the Peptide Institute, Ina, Mino-shi, Osaka, Japan. Leucine aminopeptidase was from Sigma, U.S.A.

The aspartic proteinase from Aspergillus saitoi was purified from the commercial product, “Molsin” (Seishin Pharmaceutical Co., Ltd., Tokyo) as in an earlier paper. The optimum pH of the aspartic proteinase for the hydrolysis of Boc-LSTR-MCA was estimated as pH 3.5. The HPLC chromatogram of a 24-hr hydrolysate showed that the retention times of R-MCA, Boc-LSTR, and Boc-LSTR-MCA in the enzymatic hydrolysate were respectively 25, 28, and 31 min.

According to the Lineweaver-Burk plots, the Km and kcat values for the hydrolysis of Boc-LSTR-MCA at pH 3.5 were 0.11 ± 0.03 mM and 1.32 ± 0.01 x 10^-3 sec^-1, respectively.

Enzymatic hydrolysis has been found for two substrates, Boc-LSTR-MCA and Boc-IEFR-MCA, at pH 3.5. Both of the substrates have similar structural properties: (1) the length of the peptides in N-acylpeptide-MCA is 4 amino acid residues, (2) the P1′ position of the two substrates is occupied by arginine residue, and (3) the P3 position in both substrates is occupied by hydrophobic amino acids such as leucine and isoleucine. About 8% of the activity was shown by Boc-QRR-MCA. For all of these substrates, R-MCA was released.

The Km and kcat values for the trypsinogen activation at pH 3.0 were reported as 0.023 mM and 0.14 sec^-1 by Abita et al. The catalytic efficiency, kcat/Km, of the aspartic proteinase for the activation of trypsinogen is about 500-fold higher than that for the hydrolysis of Boc-LSTR-MCA (Table I). Although the catalytic efficiency of the enzyme for hydrolysis of the fluorogenic substrate, Boc-LSTR-MCA, is low, the fluorometric assaying can be considered a rapid, sensitive and useful one for the aspartic proteinase from Aspergillus.

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