N-Terminal Amino Acid Sequence of Taka-amylase A from *Aspergillus oryzae*

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Taka-amylase A (TAA) [EC 3.2.1.1 α-1,4-glucan 4-glucanohydrolase, *Aspergillus oryzae*) is a single polypeptide chain of molecular weight of 51,000 consisting of about 470 amino acid residues. The N- and C-terminal residues were alanine and serine, respectively. The amino acid sequences of the C-terminal 66 residues and the N-terminal few residues were characterized by both TLC and GLC. The repetitive yields were 94% and 97% for Ala-1 to Ala-4 and Phe-13 to Phe-19, respectively, based on the GLC data. However, the UV-absorbing spots, which did not correspond to any PTH-amino acids, appeared on the TLC plates and their amounts increased as the cycle proceeded. Therefore, we could not identify PTH-amino acids beyond the 25th cycle. These artifacts did not disturb GLC analysis of PTH-amino acid because they did not give peaks. These non-PTH materials might be derived from the low solubility of the sample in the coupling buffer and incomplete washing of the sequencing protein film because the protein film took times to be dissolved in Quadrol buffer in the coupling stage and was in gel stage in the washing stage.

In the next experiment, RCM-TAA was modified with Braunitzer's reagent III (3-isothiocyanato)naphthalene-1,5-disulfonic acid disodium salt) to improve the solubility in the coupling buffer prior to the automated degradation. RCM-TAA (36 mg) was dissolved in 3 ml of I M Quadrol buffer and solid Braunitzer's reagent (10-fold molar excess over total amino groups) was added. The mixture was incubated at 50°C for 60 min under a nitrogen atmosphere. The colloidal protein solution went into a clear solution within 10 min after addition of the reagent. Ten µl of triethylamine was added and the mixture was incubated for 60 min at 50°C. The mixture was desalted on a Sephadex G-25 column (2 x 100 cm) with 0.2% ammonium bicarbonate. The protein fraction was pooled and lyophilized. The modified RCM-TAA (15.6 mg) was dissolved in 0.7 ml of n-heptafluorobutylic acid (HFBA) which did not correspond to any PTH materials. PTH-Ile in TLC and their assignments were made by GLC (Shimadzu Gas Chromatogram GC-6A). A dehydro form of PTH-Thr was always detected together with PTH-Thr. PTH-Leu could not be distinguished from PTH-Ile in TLC and their assignments were made by GLC data.

In the first attempt, the lyophilized RCM-TAA (24 mg) was directly dissolved in the reaction cup of the sequencer by adding 0.7 ml of n-heptafluorobutylic acid (HFBA) under spinning the reaction cup and the solution was dried using the sample application program (No. 02772, described in Beckman sequencer Manual). The resulting uniform protein film was subjected to the degradation with the fast protein-Quadrol program. The first 25 residues were characterized by both TLC and GLC. The repetitive yields were 94% and 97%, for Ala-1 to Ala-4 and Phe-13 to Phe-19, respectively, based on the GLC data. However, the UV-absorbing spots, which did not correspond to any PTH-amino acids, appeared on the TLC plates and their amounts increased as the cycle proceeded. Therefore, we could not identify PTH-amino acids beyond the 25th cycle. These artifacts did not disturb GLC analysis of PTH-amino acid because they did not give peaks. These non-PTH materials might be derived from the low solubility of the sample in the coupling buffer and incomplete washing of the sequencing protein film because the protein film took times to be dissolved in Quadrol buffer in the coupling stage and was in gel stage in the washing stage.

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The modification of RCM-TAA with Braunitzer’s reagent III improved remarkably the solubility of the protein in the coupling buffer and reduced the amounts of the background materials at each cycle. The repetitive yield was also improved slightly. Therefore, all PTH-amino acids except for the first residue up to the 29th cycle could be identified by both TLC and GLC. The results of the first 25 cycles were identical with those of the first experiment. The sequence of N-terminal 29 residues in RCM-TAA deduced was shown in Fig. 1.

The present result could align four trypic peptides, whose sequences were previously determined (unpublished.

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results), and was in agreement with the sequence of one of the fragments derived from CNBr-treated RCM-TAA (unpublished results) but was in disagreement with the previously published N-terminal sequence of TAA, Ala-Gly-Asp,) and Ala-Gly-Asp-Glu-Ser-Ala-Leu-Thr,(4) which were deduced by classical method for several 2,4-dinitrophenyl (Dnp)-peptides in the partial acid hydrolyzates of Dnp-TAA. Since the present result was obtained by direct sequencing of whole RCM-TAA, the sequence shown in Fig. 1 is believed to be correct.

α-Amylase is widely distributed in the plant and animal kingdoms and plays an important role for the use of polysaccharides in vivo. Three α-amylases were isolated from different strains of Bacillus subtilis and their N-terminal sequences were reported,17–19) as shown in Fig. 1. The N-terminal residue of hog pancreatic-α-amylase was reported to be blocked with an acetyl group,20,21) but recent report described that its isoenzymes have the same N-terminal pyrrolidonecarboxyl-tyrosine sequence.21)

By comparing our result with those of bacterial and hog pancreatic α-amylases, no sequence similarity in their N-terminal regions was found. This is a striking finding in a viewpoint of structure and function relationship, because these enzymes exhibit the same specificity, catalysis of the hydrolysis of α-1, 4-glucoside linkage in starch, glycogen and related glucans and have similar enzymatic properties, calcium requirement and optimum pH (5–6).23,24) Therefore we expect that α-amylases from different origins have the sequence homologies in the enzymatically functional regions such as active site, calcium binding group and substrate binding site which express the same enzymatic functions and properties.

Further studies to complete the whole amino acid sequence of the enzyme are in progress in our laboratory.

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REFERENCES

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N-Terminal Sequence of Taka-amylose A