NH_2-TERMINAL SEQUENCE OF YEAST INORGANIC PYROPHOSPHATASE

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SUMMARY. A NH_2-terminal sequence of 50 amino acids of inorganic pyrophosphatase has been established by Edman degradation.

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Considerable attention is given at present to a large group enzymes. Inorganic pyrophosphatase which hydrolyzed inorganic pyrophosphate and some esters of polyphosphoric acids, including ATP, is one of the better known representatives of the group. Important features of the protein catalyst such as the role of some amino acid residues in the manifestation of activity (1-4), formation of covalent enzyme-substrate intermediates (5,6) and the tertiary structure (7-10) have been established for pyrophosphatase from baker's yeast. Recently, the first results of an analysis of the primary structure of this enzyme have been reported (10).

In the present paper we describe a study of the NH_2-terminal sequence of inorganic pyrophosphatase by the automated Edman degradation.

MATERIALS AND METHODS

Inorganic pyrophosphatase was prepared from baker's yeast by column chromatography as described previously (11). All purification steps were performed in the presence of magnesium chloride (10^{-3}M). The enzyme thus obtained gave a single band

in disc gel electrophoresis at pH 9.2 (12). The activity of the purified enzyme was 2015 IU/mg (corresponding to 65 Kunitz' units/mg).

The sequence of the amino-terminal amino acids of inorganic pyrophosphatase was determined with a Beckman Protein-Peptide Sequencer Model 890, the reaction cup of which was modified according to the scheme of Model 890C. The Quadrol program (program D-XI) supplied by the manufacturer was employed. 1-Chlorobutane used for the extraction of thiazolinones contained 10^{-4}M dithiotreitol in order to improve the yield of serine and threonine. The thiazolinones liberated after each degradation cycle were converted to the corresponding thiohydantoins by treatment with 1 N HCl at 80° for 10 min. The phenylthiohydantoin amino acids were analyzed as such and as the trimethylsilyl derivatives by gas chromatography in a Beckman GC-45 unit (13). In some cases identifications were confirmed by thin layer chromatography. Some phenylthiohydantoins were hydrolyzed under reduced pressure at 105° in 6 N HCl containing 0.05% 2-mercaptoethanol for 24 hours to free amino acids which were analyzed using a Hitachi amino acid analyzer Model KLA-3b (14).

RESULTS

A sample (7.1 mg, 231 nmoles based upon the monomeric molecular weight of 30500) of native pyrophosphatase was applied in 0.8 ml of 50% acetic acid to the spinning cup of the Protein-Peptide Sequencer. The first were two blank cycles when phenylisothiocyanate was omitted from the program, then followed 50 cycles of the Edman degradation which were performed automatically.

The following partial sequence of residues at the NH_2-terminal of the molecule was elucidated:

H_2N-Thr-Tyr-Thr-Thr-( )-Gln-Ile-Gly-Ala-Lys
1 10
Asn-Thr-Leu-Glu-Tyr-Lys-Val-Tyr-Ile-Glu
15 20
Lys-Asp-Gly-Lys-Pro-Val-Ser-Ala-Phe-( )
25 30
Asp-Leu-Pro-Leu-Tyr-Ala-Asp-Lys-Glu-( )
35 40
( )-Leu-Phe-( )-Met-Val-Val-Glu-Ile-Glx
45 50

The phenylthiohydantoins were analyzed by gas liquid chromatography. In the case of residues 5,30,40,41,44 no clear-cut identification of phenylthiohydantoins was possible. The most likely residue in position 27 is that of serine, but this is not certain.

Designation Glx indicates that the residue is either a free acid or an amide. The yield of Thr-1 was 20% of the amount expected basing on the quantity of the protein monomer analyzed. Nine representative gas chromatogram are presented in Fig. 1.

DISCUSSION

This investigation deals with the NH_2-terminal sequence of inorganic pyrophosphatase prepared according to the method of Braga et al, which excludes the autolysis step (11). The use of the sequencer to study inorganic pyrophosphatase has resulted in sequence 1-50 being elucidated (Fig. 1). Sequence 1-20 confirmed the data obtained by Heinrikson et al (10).

Threonine was a single NH_2-terminal amino acid identified after the first degradation step. The automated Edman degradation carried through 50 cycles has provided a single NH_2-terminal sequence. From the data presented here, we have concluded

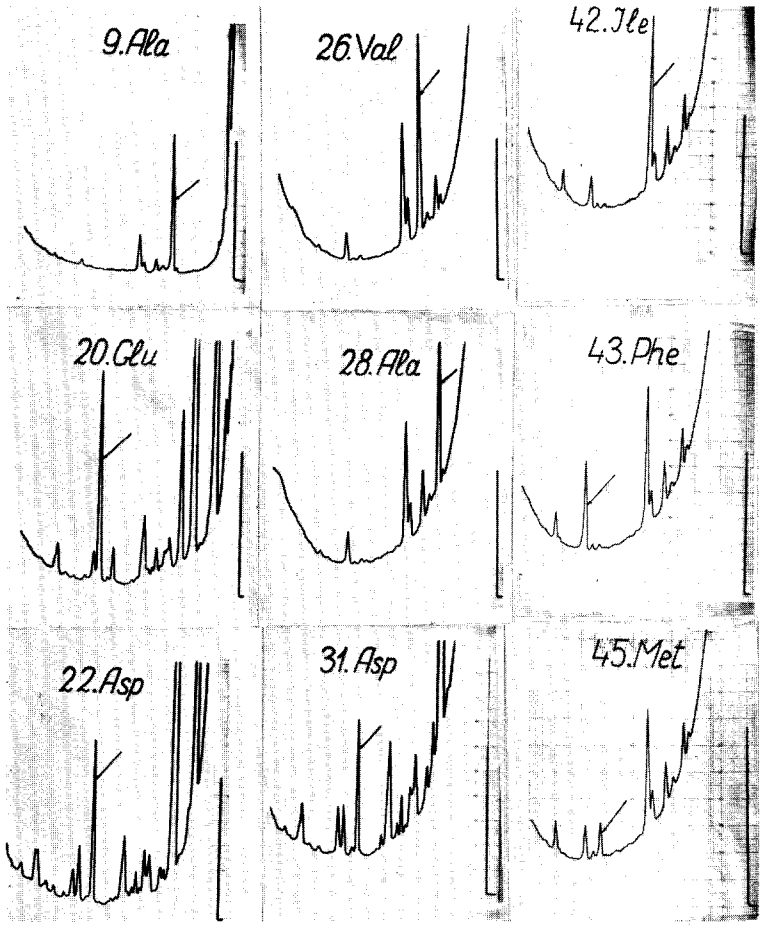

Fig. 1. Gas chromatography patterns of several phenylthiohydantoins produced during the automated Edman degradation of native yeast pyrophosphatase. Samples contained from 8% (cycles 9,20,22) to 12% (cycles 26,28, 31, 42, 43, 45) of the total phenylthiohydantoin removed in a given cycle. All the data were obtained at an attenuation of 800 with the exception of 9.Ala which was run at an attenuation of 1600. The phenylthiohydantoins of amino acids in positions 20,22,31 were determined as the trimethylsilyl derivatives.

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that native yeast inorganic pyrophosphatase is composed of two identical subunits, which is consistent with the results of other investigators (8-10).This finding is to replace our previous report where a tetrameric structure was proposed for this enzyme (15). The enzyme used in the latter work was obtained by the method including a long-time autolysis step. It seems

likely now that specific cleavage of a peptide bond near the middle of the protein molecule must have occured in these conditions, which affected the determinations of the NH_2-terminus and of the molecular weight of the enzyme.

It should be noted that the sequence contains one of the two methionine residues of the subunit. The investigation of cyanogen bromide cleavage products has shown that the fragments have molecular weight of 5000 (NH_2-terminal peptide), 12000 and 16000 (COOH-terminal peptide).

Certain gaps in the amino acid sequence may have arisen from the presence of carbohydrates. The latter were found in the enzyme and their nature and the mode of their bonding with the peptide backbone are presently under study in this laboratory.

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