Structure Determination of N-Terminal-blocked Peptide from Hog Kidney Aldose 1-Epimerase by Tandem Mass Spectrometry

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The blocked-N-terminal structure of hog kidney aldose 1-epimerase (mutarotase, EC 5.1.3.3) was determined to be Ac-Val-Ser-Val-Thr-Arg-Ser-Val-Phe-Gly-Asp⋯ by a coupling of conventional methods (enzymatic digestion, amino acid analysis, and Edman sequencing) and tandem mass spectrometry. The side-chain fragmentations observed in the high-energy product ion spectra gave unambiguous sequence information about the arginine-containing N-terminal structure.

Aldose 1-epimerase (mutarotase, EC 5.1.3.3), which catalyzes interconversion of α- and β-anomers of aldoses,1) is widespread in microorganisms, higher plants, and mammalian tissues.2) Mutarotase is an important enzyme to be used in the glucose oxidase-peroxidase method for the measurement of glucose. We are analyzing the primary structure of a mutarotase from hog kidney. In the procedure of analysis, the presence of a blocking group on the N-terminal amino group was suggested,3) because the N-terminal amino acid was not detected by a gas-phase peptide sequencer (Applied Biosystems). A small amount of an N-terminal-blocked peptide, B-LY, was isolated from a peptide mixture prepared by a Lys-C proteinase digestion.3) This paper describes the structural analysis of the N-terminal-blocked peptide by liquid secondary ion (LSI) mass spectrometry4) and LSI tandem mass spectrometry (MS/MS).5–14)

As the amino acid analysis of B-LY suggested that the peptide consists of 20 amino acid residues and contains an arginine residue, the peptide was redigested with trypsin. The tryptic digest comprised two peptides, B-TR1 and TR2, as expected. One of the tryptic peptides, TR2, was found to be Ser-Val-Phe-Gly-Asp-Leu-Pro-Ser-Gly-Ala-Gly-Thr-Val-Gln-Lys on a gas-phase peptide sequencer, but the N-terminal of B-TR1 was not identified. The amino acid analysis of B-TR1 suggested that the peptide contained five amino acid residues, i.e., arginine, serine, threonine, and two valine residues. To determine the structure of the peptide and the blocking group, the peptide was analyzed by mass spectrometry.

A reversed-phase HPLC-purified sample of B-TR1 was concentrated by vacuum centrifugation. A portion of the sample was concentrated with an appropriate liquid matrix (glycerol, glycerol-1,2,3,3-d₄, or a mixture of dithiothreitol and dithiodythreitol) to minimize sample adsorption on a polypropylene microtube. An HPLC eluate containing 50–60 pmol of the peptide was concentrated with 2 µl of matrix, and a 1-µl sample of the residual solution was put on the sample holder of an EEBB-geometry LSI tandem mass spectrometer (VG 70-4SE), which was described elsewhere.12) All of the mass spectra were obtained in the positive ion mode at an accelerating voltage of 8 kV.

Both the LSI mass spectra of B-TR1 in glycerol and glycerol-1,2,3,3-d₄ clearly showed the protonated molecule of the peptide at m/z 603. A high resolution mass analysis of the peptide (resolution: 10,000; matrix ions were used as the reference masses) showed that the accurate mass of the protonated ion was 603.3473. The calculated mass for the protonated molecule of a pentapeptide containing arginine, serine, threonine, and two valines is 561.3360, and the difference between these two values, 42.0113, well agreed with the mass of C₉H₈O (calcd. value: 42.0106) corresponding to an acetyl substitution. Thus, the N-terminal-blocking group of the peptide was identified as an acetyl group.

The peptide sequence of B-TR1 was analyzed by LSI tandem mass spectrometry. The product ion spectrum from the protonated molecule of the peptide (m/z 603) was obtained as follows and is shown in Fig. 1. The precursor ion was separated by MS-1 of the tandem instrument at a resolution of 1,000, and the ion was collisionally activated in a gas cell located at the intermediate region of the instrument. Helium was used as the target gas and was introduced into the gas cell to give approximately 80% attenuation of the precursor ion beam. The laboratory frame collision energy was 4 keV, since the gas cell was floated at 4 kV. The product ion spectrum was obtained by a linked scanning of MS-2,13) the resolving power of which was adjusted to the unit resolution at the precursor-ion mass.

The C-terminal residue of B-TR1 is presumed to be arginine, since the terminal was liberated by the tryptic digestion, and hence there are 12 (4/2) possible sequences for the other four residues in the peptide. All of the possible sequences were tested from the viewpoint of consistency between the observed product ions and the expected fragmentations8,9) for each sequence. Among the 12 possible sequences, only the sequence Ac-Val-Ser-Val-Thr-Arg can explain most of the major product ions. As shown in Fig. 1, the complete series of y ions and some of the a and b ions expected for the proposed sequence were observed in the product ion spectrum. Moreover, the complete series of z + 1, w, and v ions for the sequence were observed as well. The latter observation was consistent with the presence of a basic residue, arginine, at the C-terminal. It is well known that such basic residues often yield abundant C-terminal ions.9) The observed product ion spectrum contains no complete series of C-terminal ions for any of the other 11 sequences. Consequently, the structure of B-TR1 was deduced to be Ac-Val-Ser-Val-Thr-Arg.

Abbreviations: LSI, liquid secondary ion; MS/MS, tandem mass spectrometry; MS-1, first half of the analyzer of a tandem mass spectrometer; MS-2, second half of the analyzer of a tandem mass spectrometer.
Gln-Lys by using the peptide sequencer. This sequence corresponded to the sequence of the C-terminal 11 residues of TR2. The N-terminal of the other Asp-N peptide, B-AN2, was blocked, and its structure was analyzed by mass spectrometry like B-TR1.

The LSI mass spectrum of B-AN2 showed a protonated molecule at m/z 993 (data not shown), and its accurate mass (993.5391) agreed with the expected one (993.5369: calc. for the protonated molecule of Ac-Val-Ser-Val-Thr-Arg-Ser-Val-Phe-Gly). The product ion spectrum from the protonated molecule agreed with the expected sequence, as shown in Fig. 2. It is quite reasonable that the z+1, w, and v ions were observed only in the series of the N-terminal side of Arg^5 and the a ions near the C-terminal, which involve the arginine residue, were observed at high relative intensity.

In conclusion, the N-terminal structure of hog kidney aldose
1-epimerase was determined to be Ac-Val-Ser-Val-Thr-Arg-Ser-Val-Phe-Gly-Asp-Leu-Pro-Ser-Gly-Ala-Gly-Thr-Val-Gln-Lys. A coupling of conventional methods (enzymatic digestion, amino acid analysis, and Edman sequencing) and tandem mass spectrometry was extremely useful for the structural analysis of the N-terminal-blocked structure. The side-chain fragmentations\(8-9\) observed in the high-energy product ion spectra gave unambiguous sequence information about the arginine-containing peptides. The amounts of the samples consumed in the whole mass spectrometric studies were approximately 100 pmol for each peptide. The total primary structure of the enzyme will be reported elsewhere.

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References