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The NH_2-terminal residues of rat liver proteasome (multicatalytic proteinase complex) subunits, C2, C3 and C8, are $N\alpha$-acetylated

Fuminori Tokunaga, Rie Aruga*, Sadaaki Iwanaga*, Keiji Tanaka°, Akira Ichihara°, Toshifumi Takao+ and Yasutsugu Shimonishi+

*Department of Molecular Biology, Graduate School of Medical Science, and *Department of Biology, Faculty of Science, Kyushu University 33, Fukuoka 812, Japan, °Institute for Enzyme Research, The University of Tokushima, Tokushima 770, Japan* and *+Institute for Protein Research, Osaka University, Suita, Osaka 565, Japan*

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Rat liver proteasome (multicatalytic proteinase complex) is a 20S-ring shaped particle having a molecular mass of 750 kDa, and is composed of at least 13 non-identical components ranging from 21 to 31 kDa in size. We found here that the NH_2-terminal residues of all the known 13 components, except for C5, are not reactive to phenylisothiocyanate. Among them, components C2, C3 and C8 are blocked in their NH_2-termini with $N\alpha$-acetyl-Met, $N\alpha$-acetyl-Ala, and $N\alpha$-acetyl-Ser, respectively. The NH_2-terminal portions of C2, C3, and C8 exhibit sequence similarity to one another, but that of the non-blocked component C5 differs from those of C2, C3, and C8.

$N\alpha$-acetylation; Proteasome; Multicatalytic proteinase

1. INTRODUCTION

Rat liver proteasome (multicatalytic proteinase complex) is composed of at least 13 nonidentical components, named C1 to C10-II, ranging in size from 21 to 31 kDa, its total molecular mass being 750 kDa [1–3]. Previously, we reported the whole cDNA sequences of components C2 and C3 and deduced their amino acid sequences. C2 is the largest component in proteasome and composed of 263 amino acids [4]. C3 consists of 234 residues and has a possible tyrosine phosphorylation site [5]. The covalent structure of both C2 and C3 are similar to each other, showing 31% sequence identity. The corresponding components obtained from *Drosophila* proteasome (PROS-35 gene product) also show high sequence identity with C2 (52%) and C3 (38%) [5,6]. Although proteasomes are known to occur in yeast, invertebrates and mammals [7], their NH_2-terminal residues at protein level have not yet been analyzed. Thus, this series of studies was conducted to clarify the NH_2-terminal sequences of components of rat liver proteasome.

2. MATERIALS AND METHODS

Components C2, C3, C5 and C8 of rat liver proteosome, purified by using reversed-phase HPLC [3], were reduced, *S*-pyridylethylated, and then digested with lysyl endopeptidase (Wako Pure Chemicals, Co., Ltd., Tokyo) as described previously [4,5]. The peptide fragments in the digests were separated by HPLC using a Chemcosorb 7 ODS-H column (Chemco, Tokyo) and the peptides were eluted with a linear gradient of acetonitrile in 0.1% trifluoroacetic acid. Amino acid analysis of peptides was performed by PICO TAG (Waters, Millipore Corp.), according to the instruction manual, after hydrolyzing each sample in vapor of 6 M HCl at 110°C for 20 h. Amino acid sequence analysis was performed with a gas-phase sequencer (Applied Biosystems 477A)-120A phenylthiohydantoin analyzer on-line system. For identification of the NH_2-terminal peptides derived from C2 and C3, all the peptide fragments were subjected to amino acid analysis and their compositions were compared with the whole amino acid sequences deduced from cDNA sequences of C2 and C3. Regarding the NH_2-terminal peptide derived from C8, a peptide fragment unreactive to Edman's reagent was taken and digested further with 0.066 units of acyl amino acid releasing enzyme (Takara Shuzo, Kyoto) in 10 mM phosphate buffer, pH 7.2, at 37°C, for 36 h, and the products were separated by HPLC under the conditions as described above. The FAB mass spectra were recorded in a JEOL double-focusing mass spectrometer (JMS-HX100) equipped with an FAB ion source and a data processor (DA-5000) [8].

Correspondence address: S. Iwanaga, Department of Biology, Faculty of Science, Kyushu University 33, Fukuoka-812, Japan

Abbreviations: FAB, fast atom bombardment; HPLC, high performance liquid chromatography

3. RESULTS AND DISCUSSION

The NH_1-terminal sequences of all the known components of rat liver proteasome, purified as described previously [3], were determined after reduction with dithiothreitol and subsequent *S*-pyridylethylation. However, no phenylthiohydantoin amino acids were detected up to 10 cycles of Edman degradation, indicating that the NH_2-termini of all the components except for C5 are blocked. The NH_2-terminal sequence of C5 was determined as shown in Fig. 1. On the other hand, cDNA sequence analysis showed that the NH_2-terminus of C2 is Met [4]. To identify the block-

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          1         10        20        30
C2:   Ac-MFRNQYDNDVTVWSPQGRIHQIEYAMEAVK-
C3:   Ac-AERHGYSFSLTTFSPSGKLVQIEYALAAVA-
C8:   Ac-SSIGIGYDLSASTFSPDGRVFQVEYAMK-

C5:   NH2-RFSPYAFNGGTVLAIAGEDF-
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Fig. 1. NH_2-terminal sequences of components C2, C3, C8, and C5. Identical residues among C2, C3 and C8 are boxed.

ing group, the amino acid composition of the lysyl endopeptidase peptide C2-G, derived from the NH_2-terminus of C2, was determined (Table I). The peptide C2-G was found to be composed of 30 amino acids with a calculated molecular mass of 3625.7 [4]. In FAB-mass analysis, its $[M+H]^+$ signal was osberved at 3667.9, as shown in Fig. 2A, suggesting that the NH_2-terminal Met was blocked with an acetyl group. In the case of C3, the isolated NH_2-terminal peptide C3K29 was expected to possess the sequence M-A-E-R-G-Y-S-F-S-L-T-T-F-S-P-S-G-K based on the cDNA sequence [5], but amino acid analysis indicated that C3K29 does not contain initiator Met residue (Table I). While its $[M+H]^+$ signal was observed at 1876.8 (Fig. 1B), the calculated molecular mass of the sequence Ala^2-Lys^{18} was 1834.9, suggesting that the NH_2-terminal Ala is blocked with an acetyl group. Regarding component C8, the cDNA sequence of which is not known, the lysyl endopeptidase peptide C8K46 derived from its NH_2-terminal portion was

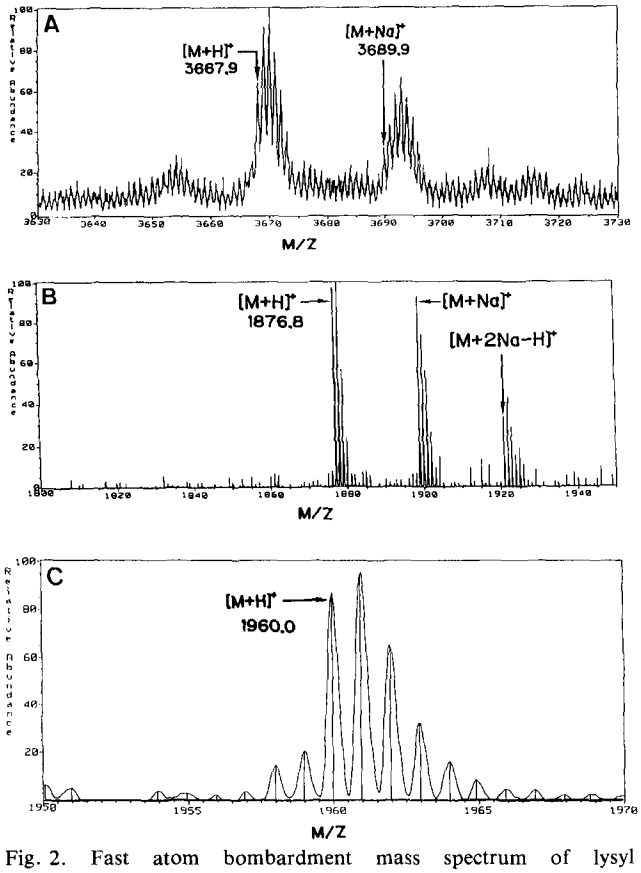

Fig. 2. Fast atom bombardment mass spectrum of lysyl endopeptidase peptides derived from the NH_2-terminal of C2, C2G (A), C3, C3K29 (B), and C8, C8K46T23 (C). M denotes molecular weight.

Table I

Amino acid compositions of NH_2-terminal peptides derived from C2, C3 and C8

Amino acid	C2-G	C3K29	C8K46T20	C8K46T23	C8K46T23[d] (AARE)
Asp	3.8 (4)[a]			1.7 (2)	1.9 (2)[b]
Glu	4.8 (5)	1.1 (1)[a]	2.0 (2)[b]		
Ser	1.0 (1)	3.5 (4)		4.9 (5)	3.8 (4)
Gly	1.1 (1)	2.3 (2)		3.5 (3)	3.3 (3)
His	0.6 (1)				
Arg	2.0 (2)	1.2 (1)		1.2 (1)	1.0 (1)
Thr	1.0 (1)	1.8 (2)		2.1 (2)	1.8 (2)
Ala	1.7 (2)	1.0 (1)	1.1 (1)	1.2 (1)	1.1 (1)
Pro	1.2 (1)	1.0 (1)		1.1 (1)	1.0 (1)
Tyr	1.7 (2)	1.0 (1)	1.1 (1)	1.1 (1)	1.0 (1)
Val	2.8 (3)		1.9 (2)		
Met	1.6 (2)	(1)	0.4 (1)		
Ile	1.7 (2)			1.2 (1)	1.0 (1)
Leu		0.8 (1)		1.1 (1)	1.0 (1)
Phe	1.0 (1)	1.7 (2)	0.9 (1)	1.2 (1)	1.0 (1)
Trp	0.8[c] (1)				
Lys	1.0 (1)	0.8 (1)	1.0 (1)		
Total	30	18	9	19	18

The values are residues per molecule; values in parentheses are taken from cDNA ([a]) or amino acid sequence data ([b]). [c] Determined after hydrolysis for 20 h with 3 M mercaptoethanesulfonic acid. [d] The peptide C8K46T23 was isolated by HPLC after treatment with acyl amino acid releasing enzyme (AARE)

analyzed. The peptide C8K46 was digested further with trypsin and the resulting two peptides, C8K46T20 and C8K46T23, were isolated by HPLC (data not shown). Their amino acid compositions are shown in Table I. The sequence of C8K46T20 was determined to be V-F-Q-V-E-Y-A-M-K, indicating that this peptide was derived from the COOH-terminal portion of C8K46. The peptide C8K46T23, on the other hand, was unreactive to Edman's reagent. However, after treatment of the peptide C8K46T23 with acyl amino acid releasing enzyme, it was possible to determine its sequence as follows: S-I-G-T-G-Y-D-L-S-A-S-T-F-S-P-D-G-R. Since C8K46T20 contains 5 Ser residues (Table I), these results suggested that the NH_2-terminus of this peptide was blocked with a modified Ser residue. In FAB-mass analysis, C8K46T23 showed the $[M+H]^+$ signal at 1960.0, a value which is the same with the theoretical value (1959.9) calculated for the peptide having an *Nα*-acetyl-Ser residue at the NH_2-terminus (Fig. 2C). It can, therefore, be concluded that the NH_2-terminal residues of mature forms of components C2, C3 and C8, which constitute rat liver proteasome, are all *Nα*-acetylated. Moreover, their NH_2-terminal sequences are highly similar to one another, as can be seen in Fig. 1. The *Nα*-acetylation of the NH_2-terminal Met,

Ala and Ser residues has been known to occur very frequently [9]. On the other hand, component C5 contains a free NH_2-terminal Arg residue and the sequences up to 20 residues are quite different from those of components C2, C3 and C8, suggesting that it belongs to another structural family of the proteasomal particles.

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