Fast atom bombardment mass spectrometry and chemical analysis in determinations of acyl-blocked protein structures

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Peptide generation and fast atom bombardment mass spectrometry in combination with conventional chemical analysis was used to identify the blocking group and establish the N-terminal structure of six different proteins at the nanomole level. In this manner, the first terminal structures of three non-mammalian alcohol dehydrogenases were determined, demonstrating the presence of N-terminal acetylation in these piscine, amphibian, and avian enzymes. Similarly, two different yeast glucose-6-phosphate dehydrogenases and a minor variant of a human alcohol dehydrogenase were found to be acetylated. The exact end location of C-terminal structures was also established. Together, the analyses permit the definition of terminal regions and blocking groups, thus facilitating the delineation of remaining structures.

Mass spectrometry; Fast atom bombardment; Blocked peptide; N-terminal acetylation; C-terminal determination

1. INTRODUCTION

N-terminal structures of blocked peptides are derived from posttranslational modifications and require direct analysis of the blocking group. Acetylation is frequently found [1], but a number of other acyl group modifications has also been encountered, among them myristoylation [2]. Many dehydrogenases are acetylated (cf. [1,3]), but non-mammalian forms have received little attention and the nature of their blocking groups is not well established. Similarly, blocking groups in minor forms of mammalian proteins are of interest. We now report several dehydrogenase forms whose N-termini required special analysis both for the blocking group and the amino acid sequence. Fast atom bombardment mass spectrometry [4] in combination with chemically analyzed compositions and structures, permitted determination of the blocking group and the N-terminal sequence.

2. MATERIALS AND METHODS

2.1. *Protein isolation and purification*

The major alcohol dehydrogenase from the livers of cod, frog (*Rana perezi*), and chicken were purified [5-7], as was the minor component χ_2 of human class III alcohol dehydrogenase [8], which is equivalent to a glutathione-dependent formaldehyde dehydrogenase [9]. New forms of glucose-6-phosphate dehydrogenase, commercially available (Sigma) for two yeasts, *Saccharomyces cerevisiae* and *Pichia jadinii*, were also investigated [10]. All proteins were ^{14}C-carboxymethylated, cleaved with proteolytic enzymes, and the peptide mixtures then submitted to reverse phase HPLC for purification of the blocked, N-terminally derived fragments [5-7]. In this manner, 9 different peptides, ranging in size from 5 to 17 residues, were obtained in amounts of 1-10 nmol.

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2.2. *Fast atom bombardment mass spectrometry (FABMS)*

FABMS was performed with a VG 7070E double-focusing mass spectrometer equipped with a FAB ion source (used at 6 kV), an Ion Tech atom gun (producing 7 keV xenon atoms) and a VG 11-250 data system. The samples, 0.2-1 nmol, dissolved in 10% acetic acid were applied to the FAB target already covered with thioglycerol/2% trifluoroacetic acid. Spectra were recorded in positive ion mode using peak format or multi-channel acquisition (MCA). Recorded spectra were calibrated and the masses measured against glycerol (below 1200 Da) or caesium iodide (above 1200 Da).

Mass spectra of peptides with a molecular weight below 1200 Da were recorded at a resolution of 1000 or better giving the monoisotopic mass; when compounds with a molecular weight in excess of 1200 Da were studied, low resolution (non-resolved isotopic clusters) was used, giving the average mass [11].

3. RESULTS

Seven different N-terminally blocked peptides were analyzed from novel types of dehydrogenase. They were obtained by digestions of the carboxymethylated proteins with Lys-specific protease, trypsin, and staphylococcal Glu-specific protease, subsequent purification by reverse phase HPLC [5-7,10], and identification by lack of free N-termini upon attempts at direct sequencer analysis. The total compositions from

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Table I

Total compositions of peptides analyzed

Residue	Cod ADH		Frog ADH	Chicken ADH			*Saccharomyces cerevisiae* G6PDH	*Pichia jadinii* G6PDH	Minor variant of human ADH χ_2
	N-terminal peptide	C-terminal peptide	N-terminal peptide	N-terminal peptide	N-terminal peptide	C-terminal peptide			
Cys(Cm)		0.7 (1)			1.3 (1)				
Asx						1.1 (1)		2.0 (2)	1.1 (1)
Thr	0.9 (1)	0.9 (1)	0.9 (1)	0.9 (1)	1.1 (1)	1.1 (1)			
Ser		1.1 (1)		1.1 (1)	1.2 (1)	2.0 (2)	0.9 (1)	1.7 (2)	
Glx		1.1 (1)			1.1 (1)		1.1 (1)		1.1 (1)
Pro							1.2 (1)		
Gly	1.1 (1)	0.5 (0)	1.1 (1)	1.1 (1)	1.1 (1)	2.2 (2)	1.1 (1)	1.2 (1)	
Ala	1.0 (1)		2.0 (2)		2.0 (2)				1.1 (1)
Val	1.0 (1)	0.9 (1)		1.0 (1)	2.7 (3)	1.7 (2)	0.8 (1)		1.0 (1)
Ile		0.7 (1)			0.8 (1)	0.9 (1)			0.7 (1)
Leu		1.8 (2)			1.1 (1)	3.7 (4)			
Tyr								1.0 (1)	
Phe						0.9 (1)		1.0 (1)	
Trp					+ (1)				
Lys	0.9 (1)		1.1 (1)	0.9 (1)	2.8 (3)	1.1 (1)	1.0 (1)		1.0 (1)
Arg		0.9 (1)				1.7 (2)		1.1 (1)	
Sum	5	9	5	5	16	17	6	8	6

Values obtained are those derived from acid hydrolysis (6 M HCl/0.5% phenol, 110°C, 24 h) as expressed in molar ratios, with the number of constituent residues within parentheses. ADH, alcohol dehydrogenase, G6PDH, glucose-6-phosphate dehydrogenase.

acid hydrolysis (Table I) and the HPLC purification properties identified the peptides as 5–16-residue fragments. The likely C-terminal residues were deduced from the specificities of the proteases used to generate the peptides. Partial acid hydrolysis, secondary enzymatic digestions, and sequence analysis after deacylation using limited hydrolysis [5–7] established the structures except for the nature of the blocking group, and in some cases the nature of the blocked residue at position 1. In all cases, fast atom bombardment mass spec-

Table II

Structures of peptides analyzed

Enzyme	Amino acid sequence	Quasimolecular ion $[M+1]^+$	
		Determined (Da)	Calculated
Cod ADH			
Lys-cleaved N-terminal peptide	Ac-Ala-Thr-Val-Gly-Lys	517.4	517.3
Lys-cleaved C-terminal peptide	Cys(Cm)-Ile-Arg-Thr-Val-Leu-Ser-Leu-Glu	1091.6	1091.6
Frog ADH	Ac-Ala-Thr-Ala-Gly-Lys	489.2	489.3
Chicken ADH			
Lys-cleaved N-terminal peptide	Ac-Ser-Thr-Val-Gly-Lys	533.3	533.3
Glu-cleaved N-terminal peptide	Ac-Ser-Thr-Val-Gly-Lys-Val-Ile-Lys--Cys(Cm)-Lys-Ala-Ala-Val-Leu-Trp-Glu	1832.3	1833.2
Glu-cleaved C-terminal peptide	Gly-Phe-Asp-Leu-Leu-Arg-Thr-Gly-Lys--Ser-Ile-Arg-Ser-Val-Leu-Val-Leu	1875.6	1875.3
Human χ_2 ADH	Ac-Ala-Asn-Glu-Val-Ile-Lys	715.3	715.4
Pichia G6PDH	Ac-Ser-Tyr-Asp-Ser-Phe-Gly-Asp-Arg	988.4	988.4
Saccharomyces G6PDH	Ac-Ser-Glu-Gly-Val-Pro-Lys	658.3	658.3

Ac, acetyl; ADH and G6PDH, alcohol and glucose-6-phosphate dehydrogenases, respectively.

trometry was successful and established acetyl to be the blocking group, while the whole peptide sequence was confirmed in agreement with the total composition. The results, identifying the novel structures analyzed, are summarized in Table II, which also shows perfect agreement between mass spectrometric values actually obtained and calculated masses expected.

The method is also applicable to C-terminally derived peptides. One of these is the heptadecapeptide from chicken alcohol dehydrogenase (Table II), where conventional sequencer analysis had given inconclusive evidence for the C-terminal leucine, because the leucine residue at position −3, and the alternating Val-Leu pattern disturbed interpretations from phenylthiohydantoin analyses. Total composition (Table I) and fast atom bombardment mass spectrometry clearly established the existence of the C-terminal leucine, again resulting in excellent agreement between values obtained and those calculated (Table II). Similarly, the C-terminus of the cod enzyme was also verified (Table II).

4. DISCUSSION

Combined use of conventional analysis by chemical means and fast atom bombardment mass spectrometry greatly facilitated the identification of 9 different terminal peptides, 7 N-terminally blocked and two derived from C-terminal regions. In all cases, the results identify the nature of the blocking group and the exact length of the peptide, establishing the position of initiation and termination, and confirming the entire structure, in agreement with total compositions and partial sequence analysis.

The results show that non-mammalian forms of enzymes are frequently acetylated. The identification of the blocking groups and N-terminal peptides facilitated analysis of all fragments from these digests, eventually leading to complete structures of the proteins. In this manner, the successive duplications giving rise to the multiple forms of mammalian alcohol dehydrogenases could be traced in ancestral detail [6]. Similarly, the analysis of the glucose-6-phosphate dehydrogenases from yeast also allowed distinction of a new type [10] of this enzyme while analysis of χ_2 liver alcohol dehydrogenase showed this minor form not to differ from the major class III form in acetylation status. The combined use of conventional methods and fast atom bombardment mass spectrometry is efficient for analysis of complex peptide mixtures, giving rapid answers and high sensitivity.

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