THE ACETYLATED NH_2-TERMINUS OF CaATPase FROM RABBIT SKELETAL MUSCLE SARCOPLASMIC RETICULUM: A COMMON NH_2-TERMINAL ACETYLATED METHIONYL SEQUENCE

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SUMMARY : The amino-terminus of the CaATPase from rabbit skeletal muscle sarcoplasmic reticulum was obtained by treating the reduced and alkylated enzyme with pronase with subsequent isolation of the acetyl peptides by ion-exchange chromatography and electrophoresis. Two peptides, N-Acetyl-Met-Glu and N-Acetyl-Met-Glu-Ala-Ala were co-purified. For all amino-terminally acetylated methionyl peptides reported so far, penultimate residues are found to be either aspartic or glutamic acids.

INTRODUCTION : CaATPase from rabbit skeletal muscle is the enzyme responsible for active transport of Ca^{++} into sarcoplasmic reticulum. It consists of a single polypeptide with molecular weight close to 100,000 (1-3). Its amino acid composition has been determined (2-4). However, there are conflicting reports on the NH_2-terminus of the polypeptide; alanine has been identified in one study (4) and no free NH_2-terminal residue was obtained in another (5). This paper presents the NH_2-terminal amino acid sequence of the CaATPase. This information is required to determine the linear arrangement of the fragments of the molecule obtained by limited tryptic digest (4,5) as well as the entire sequence.

METHODS : Pronase was purchased from Calbiochem. Iodoacetamide and cyanogen bromide were products of Aldrich. Dansyl chloride[1] was from Pierce. AG 50W-X2 and AG 1-X2 resins were obtained from Bio-Rad. Urea from Baker was deionized with mixed bed resin before use. All other chemicals were reagent grade.

CaATPase (1), delipidated according to Folch et al (6), was solubilized in sodium dodecyl sulfate. After passing through AG 1-X2 resin (7), it was alkylated with iodoacetamide (8) and

[1] Abbreviation, Dansyl : 5-dimethylaminonaphthalene-1-sulfonyl

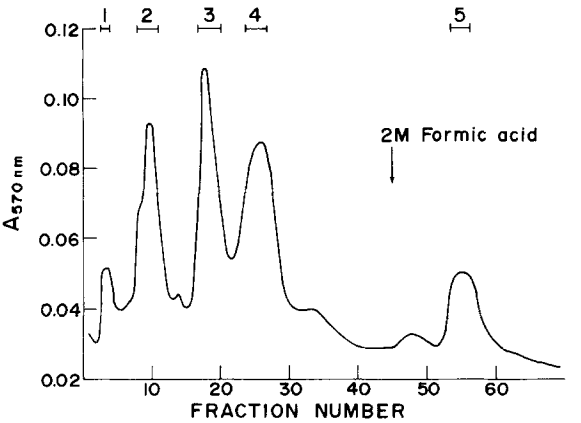

Figure 1 : Separation of AG 50W-X2 effluent on AG 1-X2. A_{570nm} of alkaline ninhydrin is plotted against fraction number. Fractions were pooled as indicated by the horizontal bars and denoted peak 1-5.

dialysed exhaustively against water at 4°C in the dark. The suspension was digested with 3% by weight pronase for 16 hours at room temperature. The pH was kept near 8 by additions of NaOH. The acidified (to pH 3) solution was clarified by centrifugation and passed through a column of AG 50W-X2 (H^+ form, 200-400 mesh, 2.0 x 4.5 cm). The effluent containing materials unretarded by the column was collected and lyophilized. The residue was fractionated by chromatography on an AG 1-X2 column (formate form, 200-400 mesh, 0.8 x 16 cm) equilibrated and developed with 0.1M ammonium formate, 0.1M formic acid, pH 4.5 at a flow rate of 15 ml per hour. Fractions of 2 ml were collected at room temperature. Peptides were monitored by alkaline ninhydrin, as shown in Figure 1.

Peptides were separated on thin-layer cellulose by either chromatography or electrophoresis (9) and stained with either ninhydrin-cadmium (10) or chlorine-o-tolidine (11). Cleavage at methionyl residues was performed, after reduction at 45°C in 25% β-mercaptoethanol (12), in 70% formic acid at room temperature for 18 hours with 100 fold molar excess of cyanogen bromide over methionine. Homoserine was analysed by the method of Ambler (13).

RESULTS : Both dansylation (14) and the cyanate method of Stark (15) failed to yield an NH_2-terminal residue for the CaATPase, suggesting a blocked or cyclized NH_2-terminus. Chromatography of the unretarded materials from the AG 50W-X2 column on AG 1-X2 resin yielded five distinct peaks which were further fractionated

TABLE 1

AMINO ACID COMPOSITIONS OF AG 50W-X2 EFFLUENT

AND OF INDIVIDUAL PEAKS OF AG 1-X2 *

	AG 50W-X2	AG 1-X2						
	effluent	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	3a	3b
Asp	0.63	1.68	0.32			2.88		
Thr	0.30	1.16	0.35			0.42		
Ser	0.47	1.37	0.78			1.15		
Glu	1.63	0.67	1.06	1.14	1.05(1)	2.39		1.04(1)
Pro	0.11	0.96						
Gly	0.28	1.06	0.32			0.87		
Ala	1.00	1.00	1.00	1.90	0.14(0)	1.00		1.95(2)
Cys**	0.58	1.53	0.64	0.98		0.28	1.00(1)	
Met	1.09	0.57		0.92	0.96(1)+			0.99(1)+

* Amino acids of less than 0.10 are omitted ; values are expressed as molar ratio ; numbers in parentheses are closest integers ; Thr and Ser are corrected for 5% and 10% destructions respectively.

** Present as carboxymethylcysteine ; corrected for 10% destruction.

\+ Same result if determined as methionine sulfone.

by thin-layer chromatography and electrophoresis at pH 3.5. Peaks 1, 2 and 5 were heterogeneous. Peak 3 was resolved into two spots denoted 3a (more anodic) and 3b by electrophoresis. Peak 4 appeared to be homogeneous. The amino acid compositions are shown in Table 1.

Structure of 3a : The electrophoretic mobility of the material in peak 3a was found to be different from that of the synthetic N-Acetyl, S-carboxyamidomethylcysteine. Thus, this material was likely to be derived from the cyclization of internal S-carboxyamidomethylated cysteines of CaATPase. Such a cyclization, analogous to that of closely related glutamate yielding pyrrolidonecarboxylic acid, has been observed with comparable rates (16).

Structure of 4 (Glx,Met) : After cleavage of peak 4 by cyanogen bromide, only free glutamic acid was obtained. Since this peptide also has two negative charges at pH 6.5, it was determined to be N-blocked-Met-Glu.

Structure of 3b (Glx,Ala_2,Met) : After cyanogen bromide cleavage and electrophoresis at pH 2.0, a tripeptide (Glx,Ala_2) with NH_2-terminal glutamic acid was obtained. This tripeptide must have the sequence Glx-Ala-Ala and originate from the COOH-terminus of peptide 3b due to the lack of homoserine. Since peptide 3b has two net negative charges at pH 6.5, it was determined to be N-blocked-Met-Glu-Ala-Ala.

Nature of the NH_2-terminal blocking group : The blocking groups on both peptides 4 and 3b were found to be acetyl groups by the method of Takagi and Doolittle (17).

DISCUSSION : In the present study, two peptides, N-acetyl-Met-Glu and N-acetyl-Met-Glu-Ala-Ala, were isolated from a single pronase digestion of CaATPase with molar yields of 22.5% and 11.5% respectively. The low yields were partly due to losses on ion-exchange chromatography and incomplete hydrolysis of CaATPase by pronase. However, a combined yield of 34% strongly indicates that N-acetyl-Met-Glu-Ala-Ala originates from the NH_2-terminus of CaATPase.

It is generally accepted that methionine donated by methionyl-$tRNA^f_{Met}$ is used in the initiation of the synthesis of eukaryotic

TABLE 2

SEQUENCES OF PROTEINS WITH NH_2-TERMINAL METHIONINE

Sources	NH_2-terminal sequences	Reference
Bovine lens α-crystalline	N-acetyl-Met-Asp-Ile-Ala	18
Turnip yellow mosaic virus coat	N-acetyl-Met-Glu-Ile-Asp	19
Rabbit muscle tropomyosin	N-acetyl-Met-Asp-Ala-Ile	20
Bovine cardiac troponin C	N-acetyl-Met-Asp-Asp-Ile	21
Human adenylate kinase	N-acetyl-Met-Glu-Glu-Lys	22
Equine renal metallothionein-1B	N-acetyl-Met-Asp-Pro-Asn	23
Rabbit muscle CaATPase	N-acetyl-Met-Glu-Ala-Ala	--
Bovine hemoglobin β	Met-Leu-Thr-Ala	24
Cape Cobra Neurotoxin β	Met-Ile-Cys-His	25
Murine H-2K	Met-Pro-His-Leu	26
Bovine bradykinin	Met-Lys-Arg-Pro	27
Camel hemoglobin β	Met-His-Leu-Thr	28
D-amino acid oxidase	Met-Arg-Val-Val	29
Chick brain α-tubulin	Met-Arg-Glx-Ser	30

proteins (31). The NH_2-termini of some proteins are acetylated after synthesis before or after the initiation methionines are removed. However, the residues which follow the N-acetylmethionines in proteins are always aspartic or glutamic acids, as shown in Table 2.[2] The negative charge may serve as a recognition site for the acetylating mechanisms or as an inhibitor of the deacetylation enzymes. Since all the acetylated methionyl peptides fall into a general pattern, it is likely that all these methionines are, as in the case of bovine α-crystalline (18), initiation methionines which are subsequently acetylated.

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