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AMINO ACID SEQUENCE OF BOVINE HEART CYTOCHROME OXIDASE SUBUNIT IV (ALBANY)

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SUMMARY- The preliminary data on the amino acid sequence of subunit IV from bovine heart cytochrome oxidase (Albany) is presented. The subunit consists of 97 amino acids linked together in a single polypeptide chain. The sequence was established by the isolation, purification and sequencing of some of the tryptic, chymotryptic and thermolytic and Staphylococcus aureus protease peptides. This subunit is present in all cytochrome oxidase preparations. It corresponds to polypeptide VIa in cytochrome oxidase (Aachen) and subunit a in cytochrome oxidase (Eugene).

Our laboratories are currently determining the amino acid sequences of the subunits of bovine heart cytochrome oxidase for future structure-function studies as well as for clarifying the subunit composition of the oxidase (1-4). There is considerable confusion as to the number and kinds of subunits present. Buse et al. (6-12) are also determining the amino acid sequence of bovine heart cytochrome oxidase and it was decided in 1978 at a cytochrome oxidase symposium at Rokkosan, Japan, that the two groups would independently determine the amino acid sequences of the various subunits for purpose of obtaining accurate sequence data and for determination of the kinds of subunits present in each of the preparations of the oxidase (13).

In the present communication, we wish to present the preliminary sequence data of subunit IV(Albany). The complete amino acid sequence of this subunit has not been published to the best our knowledge.

EXPERIMENTAL PROCEDURES

Materials- Highly purified phospholipid-depleted bovine heart cytochrome c oxidase was isolated as reported previously (14). Large scale

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isolation of subunit IV (Albany) was carried out as described by Wei and King (15). Trypsin, chymotrypsin, carboxypeptidase A-DFP were purchased from Worthington Biochemical Co. Carboxypeptidase B was purchased from Millipore Corp. Thermolysin was purchased from Cal. Biochem. and Staphylococcus aureus protease was purchased from Pierce Chemical Co. 1,3-Propane sultone, tri-n-butyl phosphine and iodoacetic acid were purchased from Aldrich Chemical Co. Methanesulfonic acid was purchased from Eastman Kodak Co. Anhydrous trifluoroacetic acid and β-mercaptoethanol were purchased from Pierce Chemical Co. Hexafluoroacetone was purchased from PCR Research Chemical Co., Gainesville, Fla. Water used for HPLC was redistilled from deionized water. Urea, reagent grade from Mallinkrodt, was purified by three recrystallizations from ethanol, the first two of which contained activated charcoal. Solvents used for HPLC were purchased from Burdick and Jackson, Muskegon, Mich. All of the sequencing reagents were of sequanal quality and were purchased from Pierce Chemical Co.

Methods- Carboxymethylation of subunit IV was accomplished as reported by Crestfield et al. (16). The sulfopropylated derivative was prepared as described by Rüegg and Rüdinger (17), though often with larger excesses of reagents and for longer reaction times.

Amino acid composition of the protein and peptide samples were determined using the Beckman Model 121 MB automatic amino acid analyzer (18). When necessary the samples were hydrolyzed for 24, 48, and 87 hours using twice distilled constant boiling HCl (5.7 N) including 2% phenol.

Automated NH_2-terminal amino acid determination was performed with a Beckman 890C Protein Sequencer using a double cleavage program with 1.0 M Quadrol coupling buffer. Manual sequencing was done essentially by the procedure of Tarr (19). Pth-amino acids were identified by HPLC [Tarr (20)] or GC [Pisano and Bronzert (21)], thin layer chromatography [Tarr (19)], and amino acid analysis after hydrolysis to form amino acids [Van Orden and Carpenter (22)].

Carboxypeptidase digestions were performed as described by Ambler (23). Tryptic and chymotryptic digestions were performed for various periods of time ranging from 45 minutes to 52 hours on the S-sulfopropyl derivative in 0.2 N HFA-NH_4, pH 8.0 or 0.2 M NEM-acetate, pH 8.0 at 37°C using enzyme/substrate molar ratios of 1:100. Reactions were stopped by addition of glacial acetic acid to pH 2. TLCK-chymotrypsin was made according to the method of Carpenter (24) described for making TPCK-trypsin. Staphylococcus aureus V-8* protease digestion was carried out for 24 hours with 1:37.5 molar ratios at 37°C in 0.1 M potassium phosphate (pH 7.8), 0.1 M ammonium bicarbonate (pH 7.8), or 0.1 M ammonium acetate (pH 4.0). The digestion mixtures contained 0.002 M EDTA and some digests contained 2 M urea. Reactions were terminated by lowering the pH to 2 with glacial acetic acid. Thermolysin digests was performed at molar ratios of 1:150 for 2.5 hours in 0.1 M HFA-NH_4 (pH 8.1), 5 M urea and 0.01 M $CaCl_2$. The reaction was stopped by the addition of glacial acetic acid to pH 2.0

HPLC runs were performed with an Altex Model 330 Gradient Liquid Chromatograph equipped with a programmer and a Hitachi 100-10 spectrophotometer. Reverse phase chromatography was accomplished using Ultrasphere 5 μ RP-18 or RP-8 columns (Altex) or μ-bondapak phenyl column (Waters Associates). Digestion mixtures, adjusted to pH 2, were centrifuged, or made 70% in acetonitrile and centrifuged, then lyophilized or dried under N_2. The sample was dissolved in starting buffer or starting buffer plus 4 M urea or guanidine-HCl for injection onto the reverse phase support columns. Gradients for initial separation of enzyme digests were generally 3-5 minutes per percent to 40% followed by 1 minute per percent gradients to 100% organic. Aqueous buffers included 0.1% TFA, 0.02 M HFA-ammonium (pH 7.2), or potassium phosphate (pH 2.2). Ninety percent acetonitrile or 90% n-propanol were used as organic eluants and included the same salt concentrations as used with the starting buffers.

High pressure ion exchange reverse chromatography was done with Hitachi Gel 3013-N resin as described by Takahashi et al. (25). The column

was equilibrated with 4 M urea and gradients to 0.25 M methane sulfonic-ammonia (pH 2.8) including 50% acetonitrile, 25% isoproanol, and 4 M urea were used to elute the polypeptides. Samples were dissolved in 8 M urea-0.1 M ammonium bicarbonate and injected at 25°C. The column temperature was raised in a 15 minute period to 70°C after injection. The column was washed with 0.5 M ammonium perchlorate (pH 2.8), 60% acetonitrile, 4 M urea at 70°C after each run. Fractions were desalted using SEP-PACK C-18 sample preparation cartridges (Waters Associates) eluting the peptides with 40% n-propanol-0.1% TFA.

RESULTS

Amino acid composition and End Group of Subunit IV (Albany). The amino acid composition of subunit IV is summarized in Table I. There is good agreement between the determined amino acid composition and the value obtained from the sequence data. The lower histidine and lysine content are due to over-sulfopropylation of the subunit due to the vigorous conditions needed to sulfoproplate the cysteine residues.

Manual Edman degradation of the CysCm-subunit IV yielded Pth-alanine as the sole amino acid. Carboxypeptidase A digestion of the CysCm-subunit IV yielded the COOH-terminal amino acid histidine followed by the simultaneous release of leucine and alanine and finally glutamine which indicated the following COOH-terminal sequence, -Gln(Leu,Ala)His-COOH.

Sequence of the Residues 1-54. The sequence determination of residues 1-54 from the NH_2-terminal of subunit IV, with the exception of the identification of all the Pth-hydroxyamino acids was possible by automated Edman degradation. The sequencing of peptides derived from the subunit IV were needed to establish the identity of the hydroxyamino acids.

TABLE I. Amino acid composition of Bovine Heart Cytochrome Oxidase Subunit IV

Amino acid	Residues	From Sequence	Amino acid	Residues	From Sequence
Asparate	8.23	8	Isoleucine	4.65	5
Threonine	7.1	7	Leucine	7.00	7
Serine	5.2	5	Tyrosine	1.79	2
Glutamate	12.2	12	Phenylalanine	1.04	1
Proline	6.57	7	Lysine	4.37	6
Glycine	8.94	9	Histidine	2.41	4
Alanine	8.00	8	Tryptophan	1.37	2.
Cysteine	2.21	3	Arginine	4.04	4
Valine	5.84	6			
Methionine	0.95	1			
Total					97

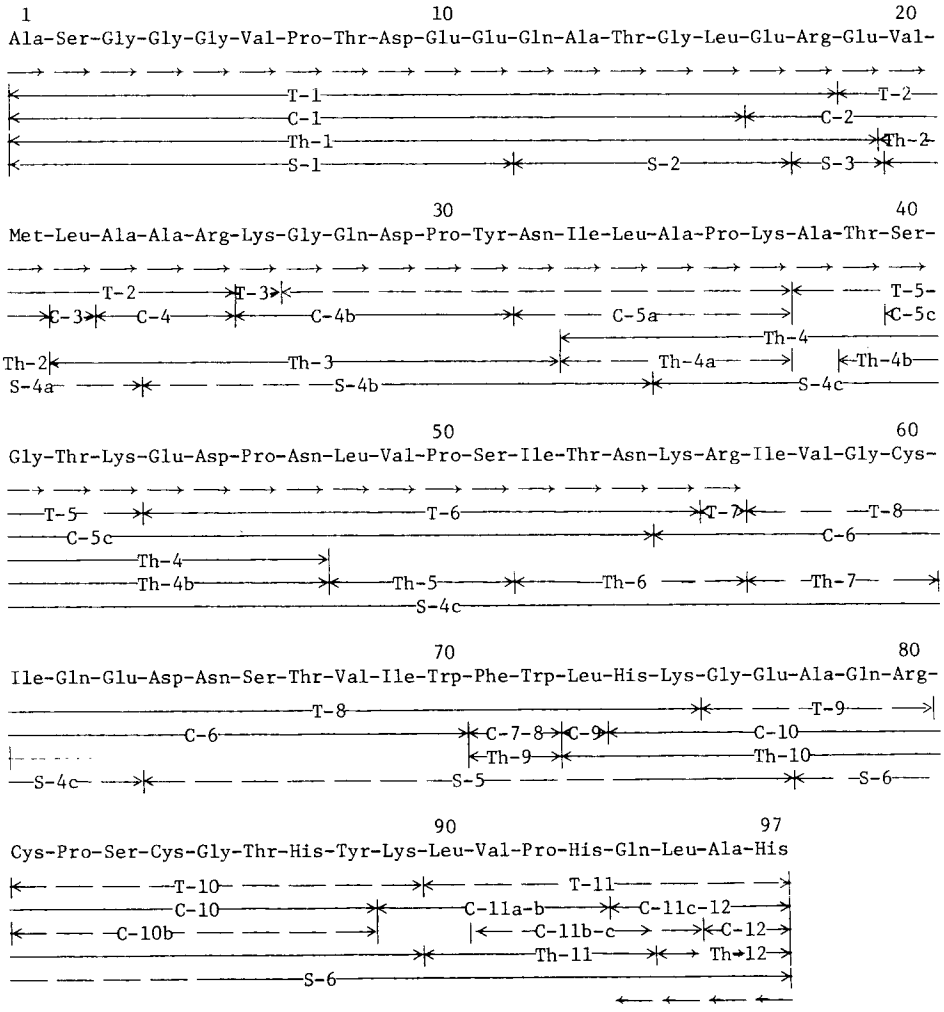

Figure 1

Sequence of Residues 55-98. The tryptic, chymotryptic, thermolytic and S. aureus derived peptides and the partial sequencing of the peptides sufficient to establish the complete sequence of subunit IV are summarized in Fig. 1. All of the peptides were purified by HPLC by methods to be described elsewhere.

DISCUSSION

Table II summarizes the various subunits reported to be present in the 3 preparations of bovine heart cytochrome oxidase as well as the molecular weights and the sequence status of the subunits. Also indicated are the correlations between the different subunit numbering systems used

TABLE II. The subunits of Bovine Heart Cytochrome Oxidase

Capaldi (26)		Steffens and Buse (12)		King et al. (13)	
I.	35,400[a]	I.	36,000[a] Formyl-MFIN[b]	I.	40,000[a]
II.	24,100[a]	II.	26,021[a] Formyl-MAYP	II.	21,000[a]
III.	21,000[a]	III.	22,000 (M) THQ		
IV.	16,000[a]	IV.	17,153[a] AHGS	III.	14,800[a]
a.	[a]	VIa.	14,000 ASGG	IV.	13,500[a]
V.	12,400	V.	12,436[a] SHGS	V.	12,346[a]
b.		VIb.	11,000 ASAA		
c.		VIc.	11,000[a] ATAL		
VI.	8,200	VII.	10,026 Acetyl-AEDI	VI.	10,026
VII.	4,400[a]	VIIIa.	5,541 SHYE	VII.	7,600
		VIIIb.	6,000 ITAK		
		VIIIc.	6,000[a] FEDR		

a = Apparent mol. wt.
b = NH_2-terminal sequence

by the different groups which is now firmly established using the primary structure information.

Cytochrome c oxidase subunit IV (Albany) [Yu and Yu, (14)] has the same N-terminal sequence as that of subunit VIa of Buse et al. (12), and is probably the same polypeptide referred to as element "a" by Capaldi (26). The question still remains as to whether this polypeptide has a function directly relating to that of cytochrome c oxidase. Yu and Yu (14) have reported that this subunit is naturally disulfide bonded to subunit VI [Buse et al's VII (121)]. Ludwig et al. (27) found that element "a" is reduced but not eliminated as are b and c in limited trypsin treated cytochrome oxidase which still retains activity. Buse et al's subunit VIa and Capaldi et al's (28) element "a" have an apparent molecular weight of about 14,000 when analyzed by SDS-urea polyacrylamide gel electrophoresis. The tentative sequence of subunit IV (Albany) given in this paper estimates the molecular weight to be somewhat less, approximately 10,529 in contrast to 13,500 found by SDS-polyacrylamide gel electrophoresis (cf. Wei and King (15); and references cited therein). At any rate, the actual molecular weights from sequence for some proteins are quite different from those estimated by gel electrophoresis. This deviation is not unique for subunit IV or some of the other subunits of the oxidase. Cytochrome c_1 and the small peptide associated with "2-band" c_1, among others, are also

different [Wakabayashi, S. et al (20,29)]. The data accumulated from this sequence study along with previously reported data will hopefully allow chemical modification studies of the intact enzyme to proceed to the primary structure level.

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