Sequence homologies and structural similarities between the polypeptides of yeast and beef heart cytochrome *c* oxidase

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The homologous polypeptides in yeast and beef heart cytochrome *c* oxidase have been identified by sequence comparisons and structural similarities. The properties of individual polypeptides have been used to specify which components are extrinsic, and which intrinsic and bilayer spanning, in the cytochrome *c* oxidase complex.

1. INTRODUCTION

Cytochrome *c* oxidase is the terminal enzyme of the mitochondrial electron transfer chain, catalyzing the 4 electron reduction of molecular oxygen and coupling this reaction to the generation of a proton gradient across the mitochondrial inner membrane [1,2]. There are 4 prosthetic groups in the cytochrome *c* oxidase complex, 2 hemes and 2 copper atoms [3]. In prokaryotes, these are associated with 2 or 3 different polypeptides [4]. Cytochrome *c* oxidase in eukaryotes is considerably more complicated, there being as many as 12 or 13 different polypeptides in the purified enzyme complex [1,5,6].

Three of the polypeptides of the eukaryotic cytochrome *c* oxidase are the equivalents of the prokaryotic subunits. These are coded for on mitochondrial (mt) DNA and made in the mitochondrial matrix [7]. The remaining components are coded for on the nuclear genome and are made on cytoplasmic ribosomes. They are transported to the mitochondrion as precursor polypeptides and assembled with the mitochondrially-made components into a functional enzyme complex at the mitochondrial inner membrane [8].

Biochemical and genetic studies of yeast cytochrome *c* oxidase have established that enzyme from this source contains 9 different polypeptides, all of which have been sequenced [9–11]. Beef heart cytochrome *c* oxidase contains from 9 to 13 different polypeptides depending on the mode of isolation [1,5,6]. Twelve of the polypeptides that can copurify in beef heart cytochrome *c* oxidase preparations have been sequenced [6,12]. This makes it possible to examine homologies between polypeptides of the yeast and beef heart enzyme and thereby decide which polypeptides are common to diverse eukaryotes and which are unique to higher organisms. Here we describe such a comparison. The homologous polypeptides of the yeast and beef heart are identified and their properties compared.

2. EXPERIMENTAL

2.1. *Analysis of sequence homologies*

The sequences of the polypeptides of beef heart cytochrome *c* oxidase were aligned with those of the components of the yeast enzyme for comparison of sequence homologies using the procedure of Needleman and Wunsch [13]. A gap weight of 5.00 and length weight of 0.30 were used. The best alignments of Mt_I, Mt_{II} and Mt_{III} were obtained by including one gap in each. Optimal alignment of the remaining polypeptides, C_{IV}–C_{IX}, required no gaps to be included. The significance, *s*, of sequence homologies was calculated using the

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amino acid change procedure of Brennan et al. [14]. The pertinent formula is:

$$s = \frac{(0.939 - AAC)L^{1/2}}{0.236}$$

where AAC is the fraction of amino acids changed in a sequence of length L.

2.2. *Analysis for membrane spanning sequences*

The sequences of the polypeptides of beef heart and yeast cytochrome *c* oxidase were examined for likely transmembrane stretches using the procedure of Engelman and Steitz [15] which sums the free energies of transfer from a hydrophilic to hydrophobic medium of 20 amino acid stretches of the sequence. Values used for these free energies were taken from Von Heinje [16] and are A, 1.00; C, 1.51; D, −7,41; E, −5.90; F, 3.39; G, 0.00; H. −3.42; I, 2.51; K, −4.20; L, 2.41; M, 2.70; N, −2.91, P. −3.32; Q, −2.41; R, −11.30; S, −1.51; T, −0.91; V, 2.01; W, 2.01; Y, −1.12.

The sums of the free energy or index of hydrophobicity was plotted for each 20 residue sequence with each value being assigned to the residue number in the center of the stretch.

3. RESULTS AND DISCUSSION

3.1. *Polypeptide sequence homologies*

The polypeptide compositions of beef heart and yeast cytochrome *c* oxidase are given in table 1. Also compiled are molecular mass, N-terminal sequence data and the terminologies used in different laboratories to identify components, including a new terminology based on sequence comparisons that we have adopted in this laboratory. Homologies between the various polypeptides of the beef heart and yeast enzyme were calculated using the algorithm of Needleman and Wunsch [13]. The homologies between the mitochondrially synthesized polypeptides is much greater (38–56%) than between cytoplasmically made polypeptides

TABLE I. Comparison of Polypeptide Compositions of Yeast and Beef Heart Cytochrome c Oxidase

	Beef Enzyme		Nomenclatures			Yeast Enzyme		
Polypeptide	N terminus	Mr	Capaldi [1]	Kadenbach [5]	Buse [6]	N terminus	Mr	Homology
Mt I	MF1	56993	I	I	I	MVC	56000	56
Mt II	MAY	26049	II	II	II	DVP	26678	38
Mt III	MTH	29918	III	III	III	MTH	30340	43
C IV	AHG	17153	IV	IV	IV	AQT	14858	14
C V	SHG	12434	V	Va	V	SDA	12627	28
C VI	ASG	10670	a	Vb	VIa	QQK	14570	24
	ASA	9418	b	VIa	VIb			
	AED	10068	c	VIb	VII			
	STA	8480	VI	VIc	VIc			
C VII	FEN	6244	VIIs	VIIa	VIIIc	ANK	6603	13
C VIII	SHY	5541	VIIs	VIIc	VIIIa	VHF	5364	30
C IX	ITA	4962	VIIs	VIII	VIIIb	AIA	6303	17

The sodium dodecyl sulfate polyacrylamide gel on the left is of beef heart run according to Merle and Kadenbach [5].

(13–30%). The weakest homologies are for C_{IV}, C_{VII} and C_{IX} with values of 14, 13 and 17% identical amino acids, respectively. Fig.1 shows the sequence comparison for C_{IV}, listing the beef enzyme as well as sequence data for 2 isoenzyme forms of the yeast subunit. Polypeptides Cox 5a and Cox 5b of Cumsky et al. [17] represent two forms of C_{IV} (terminology in this paper) with 62% amino acid homology in the NH_2-terminal part of their sequences. As shown in fig.1, those amino acids common to yeast and beef are present in both forms of yeast C_{IV}.

Fig.2A shows the best alignment of polypeptides FEN (N-terminal) sequence) of beef and ANK of yeast. These 2 polypeptides have an overall sequence homology of only 13% identical amino acids according to Needleman and Wunsch [13] but their equivalence is obvious from the overall similarity of their N-terminal parts (residues 3–13 of the beef heart polypeptide). The significance of the sequence homology in this part was calculated according to Brennan et al. [14], As s value of 6.7 was obtained. This represents the number of standard deviations away from the mean, which in turn is the probability that the amino acid correspondence could arise by chance. The best alignment of the yeast polypeptide AIA was with polypeptide 1TA of the beef enzyme. These two sequences have only 8 identical residues, but 4 are in an 11 amino acid stretch at the C-terminal end of the hydrophobic (putative) transmembrane domain (see section 3.2). This homology represents 4.2 standard deviations above random chance.

Three polypeptides in beef heart cytochrome *c* oxidase appear to have no counterpart in the yeast enzyme. These begin with N-terminal ASA, AED and STA. The beef heart enzyme may contain at least one additional polypeptide (VIIb in the terminology of Merle and Kadenbach [5]) but see Buse and Meinecke [18]. No sequence data are available for this polypeptide.

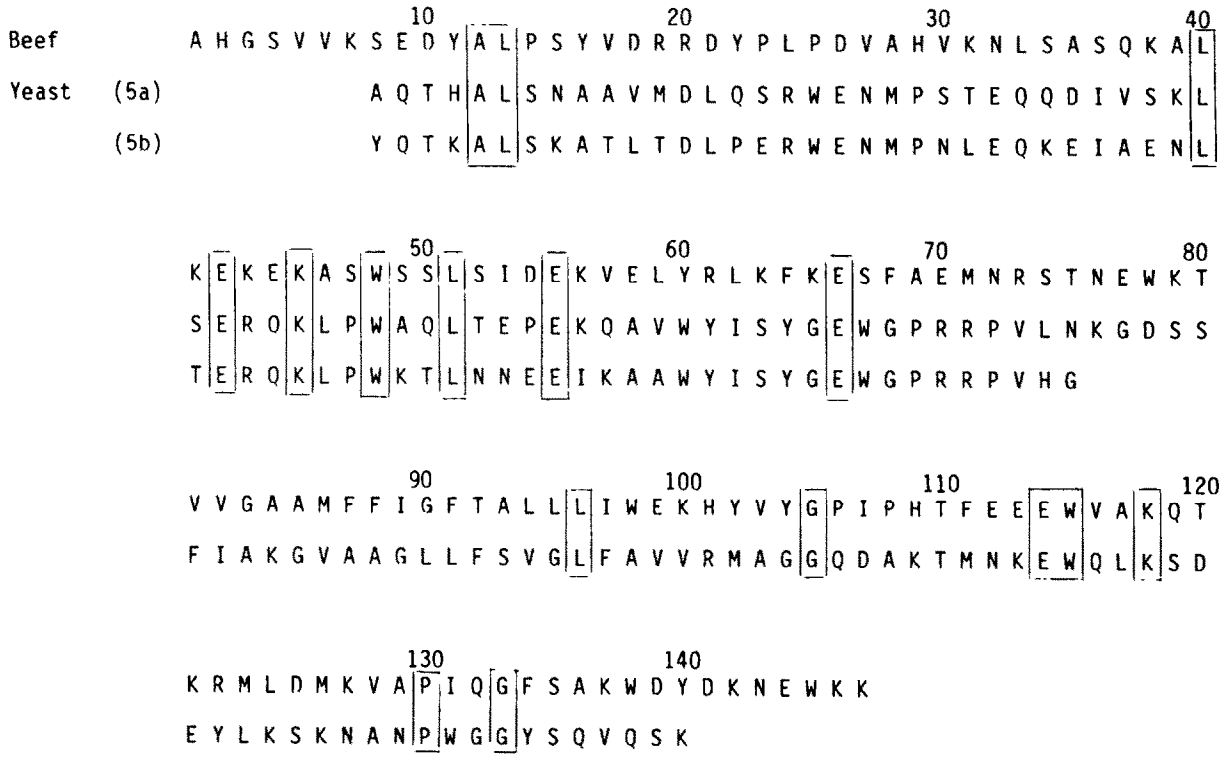

Fig.1. Sequence alignments of polypeptide C_{IV} from beef heart cytochrome *c* oxidase and the 2 forms of the equivalent polypeptide in yeast (Va and Vb according to Cumsky et al. [17]).

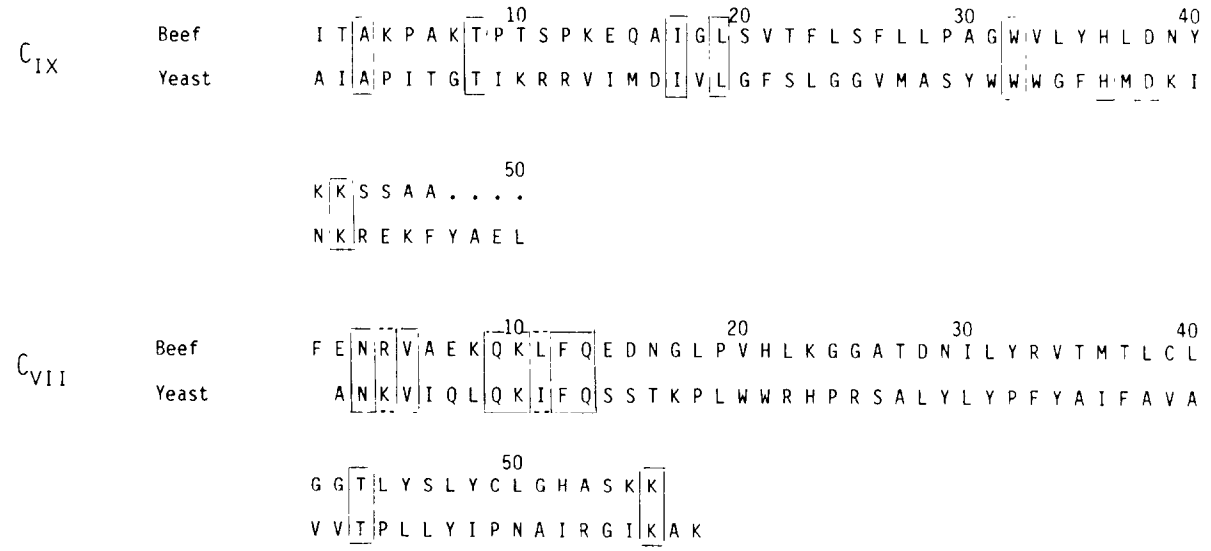

Fig.2. Sequene alignments of C_{IX} and C_{VII} of beef heart and yeast cytochrome *c* oxidase.

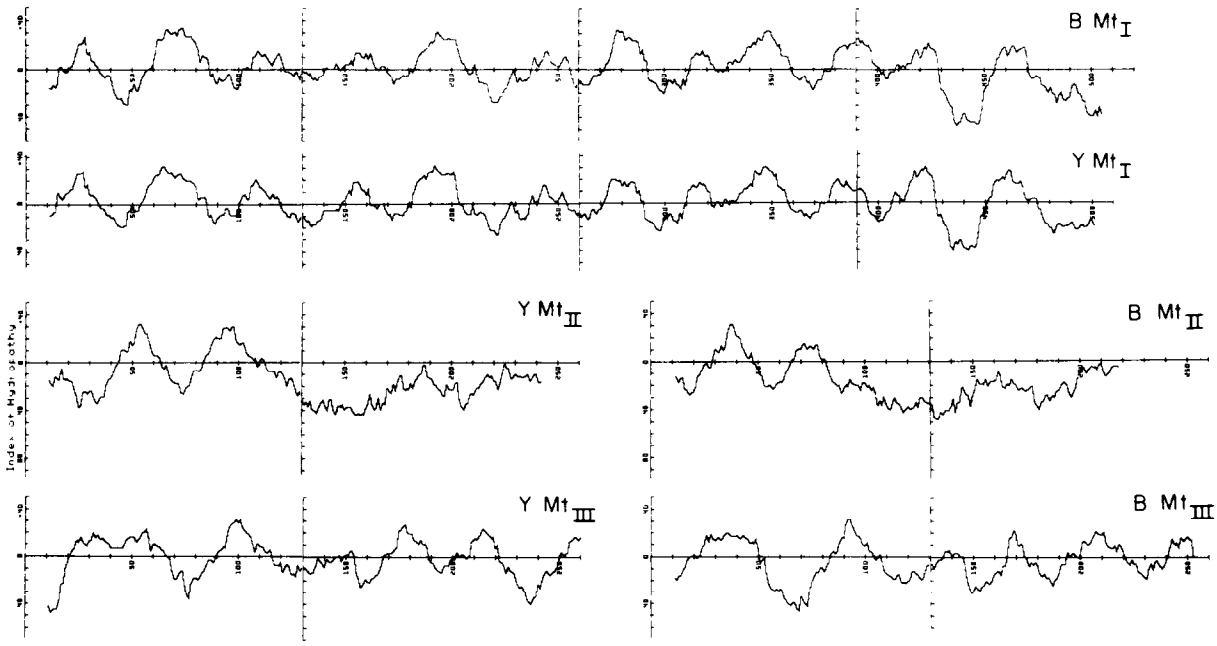

Fig.3. Hydropathy plots of the 3 mitochondrially coded polypeptides of yeast (Y) and beef heart (B) cytochrome *c* oxidase.

3.2. *Comparison of hydropathy profiles*

Protein derived from a common evolutionary precursor can diverge to the point of having very little sequence homology, and yet retain the functionally important three-dimensional structure. Two well-documented examples are cytochrome *c* and lysozyme [19]. In the case of cytochrome *c* oxidase, there are clear structural similarities between homologous subunits of the yeast and beef heart enzymes. Fig.3 shows hydropathy plots for the

mitochondrially coded subunits. The approach used was to sum the free energy for insertion (from water to a hydrophobic medium) of 20 amino acid long stretches of the sequence and to repeat this along the chain, i.e. 1–20, 2–21, etc. This value is plotted as a function of the residue number in the center of each stretch. The analysis has structural significance. The bilayer intercalated part of cytochrome *c* oxidase is mostly, if not wholly, made up of α-helices running perpendicular to the plane of the membrane, as judged from low angle X-ray studies [20,21]. A hydrophobic sequence of 20 residues arranged as an α-helix would span the hydrophobic interior (30 Å) of the bilayer. Fig.3 shows that the hydropathy of Mt_I from yeast and beef are very similar with 12 putative transmembrane sequences. Polypeptides Mt_{II} and Mt_{III} also have remarkably similar profiles in yeast and beef with 2 and 7 transmembrane stretches, respectively.

The hydropathy plots of the cytoplasmically made polypeptides C_{IV}–C_{IX} are shown in fig.4. The homologous polypeptides in yeast and beef show very similar profiles. For completeness, the plots of the three polypeptides ASA, AED and STA are shown in fig.5. In yeast cytochrome *c* oxidase there are 4 putative transmembrane sequences among the cytoplasmically made polypeptides, i.e. one

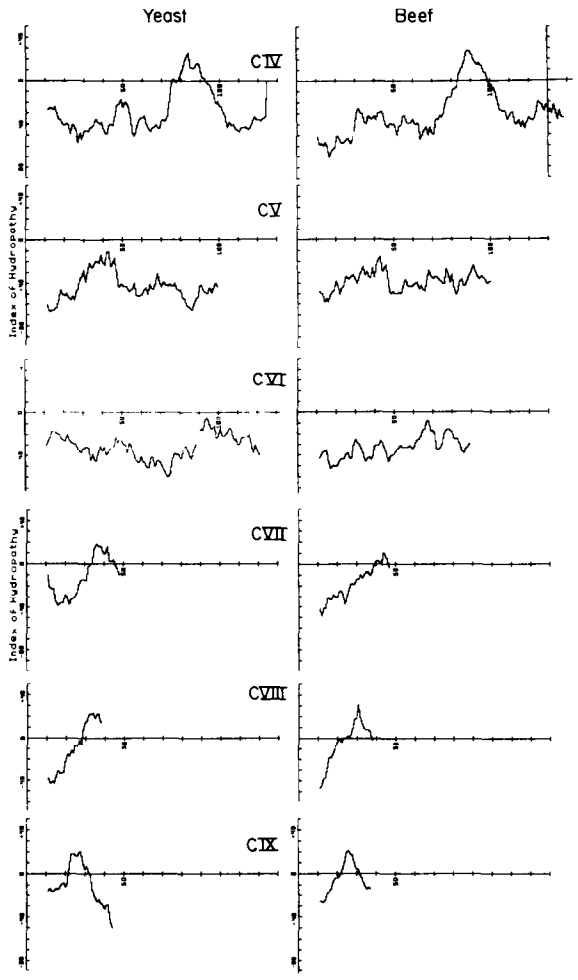

Fig.4. Hydropathy plots of the homologous polypeptides among the cytoplasmically synthesized components of yeast and beef heart cytochrome *c* oxidase.

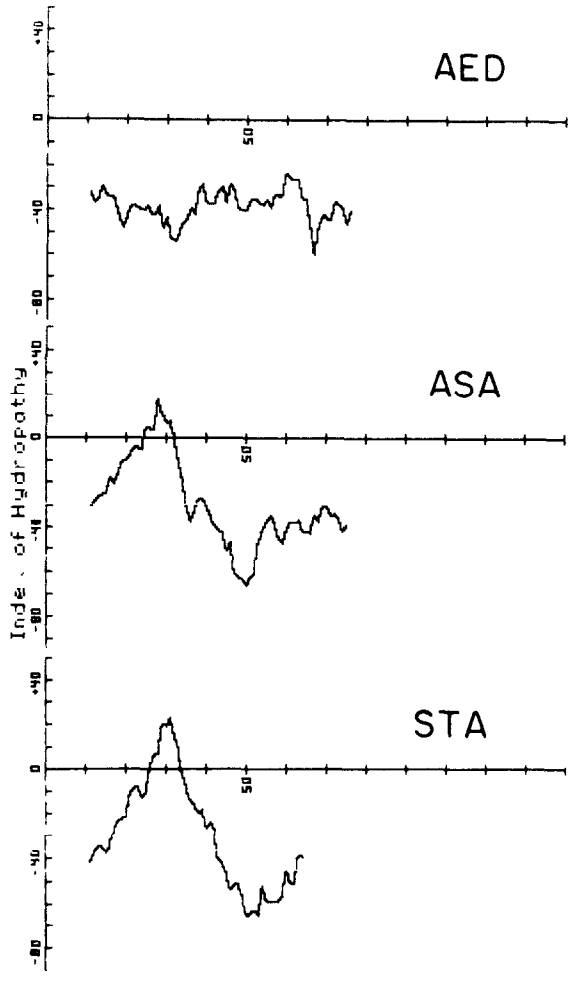

Fig.5. Hydropathy plots of 3 polypeptides of beef heart cytochrome *c* oxidase for which there is no equivalent subunit in the yeast enzyme. Polypeptides are identified by their 3 N-terminal amino acids.

each in C_{IV}, C_{VII}, C_{VIII} and C_{IX}, for a total of 25 in all the complex. In beef heart, C_{IV}, C_{VII}, C_{VIII} and C_{IX} contain one hydrophobic sequence and there is one putative transmembrane sequence each in ASA and STA, for a total of 27.

3.3. *Comparisons of physical properties and arrangement*

The similarity in properties of the homologous polypeptides in the yeast and beef heart cytochrome *c* oxidase extends beyond sequence comparisons and includes their physical properties and importantly, their arrangement in the complex. Three polypeptides of beef heart cytochrome *c* oxidase, C_V, C_{VI} and AED, are water-soluble after release from the enzyme complex by 50% pyridine [22]. C_V and C_{VI} are also released from the yeast enzyme complex as water-soluble polypeptides by treatment with 8 M Gdn HCl [23].

The implication of these solubility studies is that C_V, C_{VI} and AED are extrinsic to the complex. This is consistent with the hydropathy profiles. C_V, C_{VI} (and AED in beef heart) have hydrophilic amino acids distributed along their entire lengths (fig.4 and 5). These polypeptides are labeled when yeast or beef cytochrome *c* oxidase is reacted with water-soluble protein modifying reagents [24–26] but they are not labeled by bilayer intercalated probes including adamantane diazirine (beef heart) [27], iodonaphylazide (yeast) [28] or arylazidophospholipids (both beef heart and yeast) [29,30]. Polypeptides Mt_I, Mt_{II}, Mt_{III}, C_{IV}, C_{VII-IX} along with ASA and STA in beef heart along are all labeled by the lipophilic reagents, confirming that these components contribute the membrane spanning M_1 and M_2 domains. In the case of Mt_{II} and C_{IV}, the labeling from within the bilayer has been localized to the hydrophobic sequences seen in hydropathy plots [31,32].

4. SUMMARY

A comparison of the sequences of the polypeptide components of yeast and beef heart cytochrome *c* oxidase identifies 9 subunits as common to the enzyme of lower and higher eukaryotes. Based on structural comparisons these polypeptides fall into 2 classes, the 3 mitochondrially made polypeptides Mt_{I-III}, along with C_{IV} and C_{VI-IX}, are all intrinsic to the membrane continuum and span the lipid bilayer one or more times. C_V and C_{VI} appear to be extrinsic to the bilayer. Beef heart cytochrome *c* oxidase has 3 additional polypeptides not present in yeast, one of which, AED, appears to be extrinsic, the other 2 being intrinsic to the bilayer-intercalated domains. The function of the cytoplasmically made subunits in general, and the polypeptides found only in higher eukaryotes in particular, remains to be determined. The idea that these have a regulatory role is an attractive one which requires substantiation by careful structure-function relationship experiments.

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