Tissue-Specific Differences between Heart and Liver Cytochrome *c* Oxidase†

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ABSTRACT: Bovine liver cytochrome *c* oxidase has been isolated and the subunit structure of this preparation compared with that of the bovine heart enzyme. Of the 10 nuclear-coded subunits, 3 were different in the 2 tissue forms, having different migrations in sodium dodecyl sulfate–polyacrylamide gel electrophoresis, different antigenicities to antibodies made against the heart subunits, and different N-terminal amino acid sequences. Subunit ASA of heart begins with the N-terminal sequence of SSG in liver and is different in 17 of the first 33 residues including a deletion of 2 residues in the liver isoform of this subunit. Subunit C_{VII} of liver differs from its heart counterpart in 6 of the first 37 residues while subunit C_{IX} from liver differs from the heart isoform in 15 of the first 25 residues. No differences between tissue types were observed in partial sequencing of the remaining nuclear-coded subunits. Recently, the major portion of the sequence of subunit C_{IX} from rat liver has been obtained by cloning and sequencing of the cDNA for this polypeptide [Suske, G., Mengel, T., Cordingley, M., & Kadenbach, B. (1987) *Eur. J. Biochem. 168*, 233–237]. There is a greater sequence homology of the rat and bovine liver forms of C_{IX} than there is between the bovine heart and liver isoforms.

Cytochrome *c* oxidase, the terminal part of the respiratory chain, catalyzes a reduction of molecular oxygen to water, coupled to the translocation of protons across the mitochondrial inner membrane [reviewed in Capaldi et al. (1983) and Wikstrom et al. (1984)]. The proton gradient so generated is used to drive ion transport or is used for ATP synthesis. Cytochrome *c* oxidase is an integral membrane complex composed of 3 mitochondrially coded polypeptides and 10 nuclear-coded subunits [reviewed in Capaldi et al. (1987) and Kadenbach et al. (1987)]. Recent evidence suggests that the three mitochondrially coded subunits are the catalytic core of the enzyme, containing the prosthetic groups and involved in the proton pumping function (Ludwig & Schatz, 1980; Raitio et al., 1987; Holm et al., 1987). The role of the nuclear-coded and cytoplasmically synthesized subunits is just now beginning to be explored in any detail. The available evidence suggests that some of these subunits are involved in regulation of catalytic activity [reviewed in Kadenbach (1986) and Kadenbach et al. (1987)].

One indication that cytochrome *c* oxidase is under physiological regulation is the presence of tissue-specific forms of the enzyme (Kadenbach et al., 1987). The existence of isoenzymes of cytochrome *c* oxidase is indicated by studies of patients with mitochondrial diseases, where the enzyme can be defective in one tissue but not in another, for example, when muscle is affected but not heart or liver (DiMauro et al., 1987; Bresolin et al., 1985). More evidence for tissue-specific forms of cytochrome *c* oxidase has come from the pioneering work of Kadenbach and colleagues [reviewed in Kadenbach et al. (1987)]. These workers first showed that some of the subunits of the enzyme have different migration on sodium dodecyl sulfate ($NaDodSO_4$)–polyacrylamide gels (Kadenbach et al., 1983). They then demonstrated differences in sulfhydryl content for some of the subunits in different tissues (Stroh & Kadenbach, 1986). Also, Kuhn-Nentwig and Kadenbach (1985) have claimed to find differences in immunoreactivity of all of the nuclear-coded subunits in different tissues.

The antibody binding experiments of Kuhn-Nentwig and Kadenbach (1985) used antibodies made to denatured subunits of the rat liver enzyme. These were reacted with isolated mitochondria from adult and fetal rat tissues using the enzyme-linked immunosorbent assay (ELISA) method. The different reactivities observed could be due to isoforms of the various subunits, as proposed, or could be due to different amounts of nonspecific binding to the multitude of proteins in the different mitochondrial preparations. The studies of Nakagawa and colleagues (Nakagawa et al., 1985; Nakagawa & Miranda, 1986) indicate another potential difficulty in identifying tissue-specific isoforms of an enzyme by immunological approaches. These workers claimed to have obtained evidence for different heart and skeletal forms of human cytochrome *c* oxidase subunit C_{IV} by immunohistochemistry with a monoclonal antibody to the heart form (Nakagawa et al., 1985). However, on further analysis, the difference in immunoreactivity was found to result from a difference in shielding of the antibody binding site. The subunit C_{IV} antibody reacted equally well with heart or muscle when samples were denatured prior to immunoassay (Nakagawa & Miranda, 1986).

From the above, it remains to be established how many of the nuclear-coded subunits of cytochrome *c* oxidase are present in isoforms and the extent to which these differ in different tissues. The straightforward way to address these questions is by isolating the enzyme from various tissues, purifying the subunits, and then sequencing each one. The sequences of all of the subunits of beef heart cytochrome *c* oxidase have been determined already [e.g., see Anderson et al. (1981), Erdwig and Buse (1985), and Tanaka et al. (1979)]. Here we describe isolation and characterization of beef liver cytochrome *c* oxidase. Partial sequence data show that 3 of the 10 nuclear-coded subunits are present in tissue-specific isoforms.

Experimental Procedures

Large-Scale Preparation of Beef Liver Mitochondria. Adult bovine liver, obtained at the slaughterhouse from a freshly killed animal, was kept on ice and processed promptly. The outer membrane was removed and the liver tissue dissected

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into small slices and allowed to drain. Tissue in 400-g amounts was suspended to 1 L in 0.25 M sucrose, 10 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), and 5 mM ethylenediaminetetraacetic acid (EDTA) buffer, pH 7.4 (STE buffer), and homogenized in a Waring blender at high spped for 10–15 1-s bursts. The homogenate was adjusted to 3 L in STE buffer and poured through a strainer to remove undisrupted tissue. The suspension was centrifuged at 600*g* for 10 min in a Sorvall centrifuge; the supernatant was decanted through a double layer of cheesecloth and then centrifuged at 15000*g* for 10 min. The pelleted mitochondria were washed 3 times in STE buffer or until the supernatant was clear of hemoglobin. The mitochondria were resuspended to approximately 50 mg/mL in the same buffer and stored at −20 °C.

Purification of Cytochrome c Oxidase. Cytochrome *c* oxidase was isolated from beef liver mitochondria according to Capaldi and Hayashi (1972) but with an additional washing step of the green pellet after deoxycholate treatment of the mitochondrial membrane fraction. For this step, the green membrane was suspended in 1% potassium cholate and 10 mM sodium phosphate (pH 7.4) and then centrifuged at 100000*g* for 15 min at 4 °C. A red supernatant was decanted and the pellet resuspended in 1% potassium cholate for ammonium sulfate fractionation, as described previously (Capaldi & Hayashi, 1972).

Beef heart cytochrome oxidase was purified by the published procedure (Capaldi & Hayashi, 1972).

Separation of Polypeptides by Gel Filtration on Bio-Gel P60. Beef liver cytochrome *c* oxidase (20–25 mg in 0.6 mL) was dissolved and dissociated in 0.4 mL of 20% $NaDodSO_4$ containing 2% (v/v) β-mercaptoethanol. The dissociated enzyme was allowed to stand for 30 min at room temperature, loaded on the top of a Bio-Gel P60 (<400) column (1.5 × 116 cm), previously equilibrated with 2% $NaDodSO_4$, and eluted at 1 mL/h. The eluent was monitored at 280 nm.

Purification of Subunits Using Reversed-Phase High-Performance Liquid Chromatography (HPLC). Separation of subunit mixtures obtained from the Bio-Gel P60 column was performed essentially as described by Takamiya et al. (1987). HPLC was performed on a Beckman gradient liquid chromatograph using a Brownlee Laboratories column (type RP-300 Aquapore Octyl, 300-Å pore size, 7 μm spherical 100 × 4.6 mm) and guard column (type RP-8, 15 × 3.2 mm) at a flow rate of 0.5 mL/mm. The solvents used for chromatography ("A") 0.05% (v/v) triethylamine and 0.05% (v/v) trifluoroacetic acid in 100% acetonitrile or ("B") 0.05% (v/v) triethylamine and 0.05% (v/v) trifluoroacetic acid in 5% (v/v) acetonitrile.

Protein Sequencing. Sequence analysis was performed using an Applied Biosystems Model 470A protein sequenator and an Applied Biosystems Model 120A phenylthiohydantoin (PTH) analyzer. CNBr cleavage of subunits was conducted as described by Sacher et al. (1979).

$NaDodSO_4$–Polyacrylamide Gel Electrophoresis. Polyacrylamide gel electrophoresis in $NaDodSO_4$ was carried out according to Kadenbach et al. (1982) using 25 cm long gels, 0.12 cm thickness, with 19.2% acrylamide and 0.5% *N,N'*-methylenebis(acrylamide).

Immunodetection of Subunits on Gels. Beef heart and beef liver cytochrome *c* oxidases, run on $NaDodSO_4$–polyacrylamide gels as described, were transferred electrophoretically to nitrocellulose paper by a modification of the method of Towbin et al. (1979) using a Hoefer TE22 transblot apparatus at 400 mA for 3–5 h and a buffer of 0.19 M glycine, 0.02 M Tris, 0.1% $NaDodSO_4$, and 20% methanol. The nitrocellulose paper was incubated with subunit-specific antibodies raised in rabbits or with monoclonal antibodies raised in mice, and the immunoreactive polypeptides were diluted by reaction of horseradish peroxidase conjugated goat anti-rabbit antibodies (Bio-Rad) (Hawkes et al, 1982).

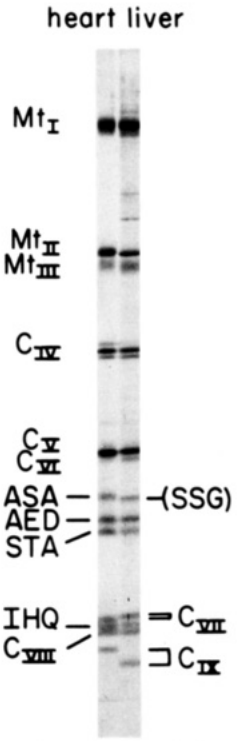

FIGURE 1: $NaDodSO_4$–polyacrylamide gel electrophoresis of beef heart and beef liver cytochrome *c* oxidase. The subunit nomenclature is according to Capaldi et al. (1986). This corresponds to the subunit numbering system of Kadenbach et al. (1983) as follows: Mt_{I-III}, C_{IV} = I–IV; C_V, C_{VI} = VaVb; ASA = VIa; AED = VIb; STA = VIc; C_{VII}, IHQ, C_{VIII} = VIIa, −b, and −c, respectively; C_{IX} = VIII.

Other Assays. Protein concentrations were determined according to Lowry et al. (1951). Cytochrome *c* oxidase activity was measured as described by Vik and Capaldi (1977).

RESULTS

Purification and Characterization of Beef Liver Cytochrome c Oxidase. Cytochrome oxidase was isolated from beef liver mitochondria using a modification of the method of Hayashi and Capaldi (1972). Submitochondrial particles were treated with deoxycholate and KCl (0.3 mg/mg and 1 M, respectively), and the green membrane was collected by centrifugation, as described previously. This pellet was washed once in 1% cholate and 10 mM phosphate (pH 7.4) prior to cholate and ammonium sulfate precipitation steps. The low-salt washing step improved the removal of *b*-type cytochromes, and cytochrome *c* oxidase was precipitated between 35% and 43% ammonium sulfate in different preparations. The liver enzyme was spectrally identical with beef heart cytochrome *c* oxidase and had a heme *a* content in the range 8.3–9.5 nmol/mg of protein and a specific activity in the range of 220–290 s^{-1}, when assayed according to Vik and Capaldi (1977).

Subunit Structure of the Liver Enzyme. The polypeptide compositions of beef liver and beef heart cytochrome *c* oxidase were compared by $NaDodSO_4$–polyacrylamide gel electrophoresis, using a procedure modified from the original method of Kadenbach et al. (1982). Both the liver and the heart enzyme were resolved into 13 different subunits as shown in Figure 1. The different subunits are labeled in this figure according to Capaldi et al. (1986). Most of the subunits have

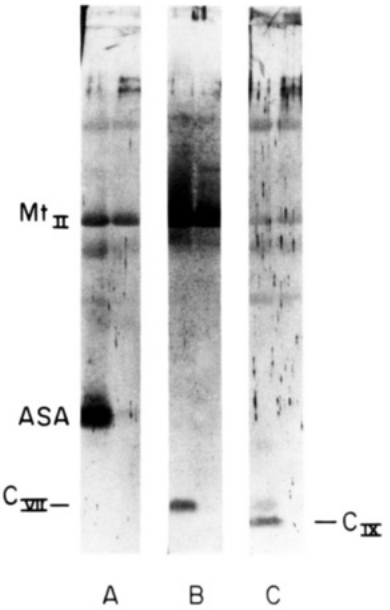

FIGURE 2: Antigenic differences between subunits of the beef heart and beef liver cytochrome *c* oxidase. Equal amounts (30 μg) of enzyme from heart (left lanes) and liver (right lanes) were separated into subunits by $NaDodSO_4$–polyacrylamide gel electrophoresis and the subunits transferred to nitrocellulose for reaction with antibodies against beef heart ASA (gel A), C_{VII} (gel B), and C_{IX} (gel C). The blots in gels A and B included antibody against subunit Mt_{II} as a control. The polyclonal antibody against subunit C_{IX} was less specific and reacted to a small extent with Mt_{II}, C_{IV}, and C_{VII}.

the same migration in both preparations. Three polypeptides, ASA, C_{VII}, and C_{IX}, migrated differently in liver from heart, as first reported by Kadenbach et al. (1982).

Antibody Cross-Reactivity of the Liver and Heart Enzyme. Antibodies have been obtained against each of the subunits of beef heart cytochrome *c* oxidase for use in our ongoing studies of mitochondrial myopathies [e.g., see Takamiya et al. (1986)]. These were examined for reactivity against beef liver cytochrome *c* oxidase by using the Western blotting technique of Towbin et al. (1979). Polyclonal antibodies specific to C_{IV}, ASA, AED, STA, C_{VII}, C_{VIII}, C_{IX}, and IHQ, respectively, along with the two monoclonal antibodies against C_{IV} and one monoclonal antibody each against C_V, C_{VI}, and IHQ were used. Most of these antibodies reacted equally well with both the liver and heart enzyme. However, antibodies to three of the beef heart subunits, namely, against ASA, C_{VII}, and C_{IX}, reacted poorly or not at all to the corresponding beef liver subunits, as shown in Figure 2.

Separation and Purification of the Subunits of Beef Liver Cytochrome c Oxidase. All of the subunits of beef liver cytochrome *c* oxidase could be purified from a single aliquot of enzyme using the procedure of Takamiya et al. (1986) developed with the beef heart enzyme. The first step in this procedure involved denaturation of the enzyme in 2% $NaDodSO_4$ and elution on a Bio-Gel P60 column. Subunits Mt_I, Mt_{II}, Mt_{III}, and C_{IV} were obtained in pure form from this column. Subunits C_V and C_{VI} and the liver equivalent of ASA eluted in one peak. This contrasts with the heart enzyme where C_V and C_{VI} eluted together, with subunit ASA coeluting with STA and AED. This last broad peak from the column of the liver enzyme contained the four smallest subunits (C_{VII-IX} and IHQ) eluting as a mixture.

Subunit mixtures were resolved by reverse-phase high-performance liquid chromatography (HPLC) as described by Takamiya et al. (1987) (Figure 3). The purity of each subunit preparation was checked by $NaDodSO_4$–polyacrylamide gel electrophoresis.

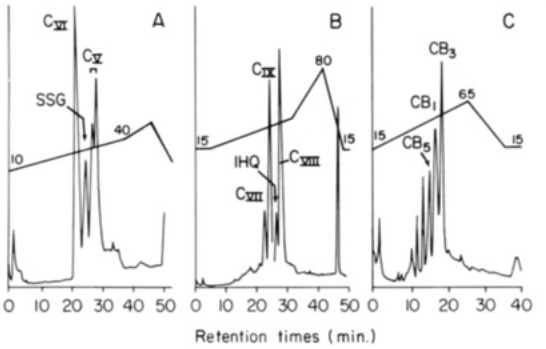

FIGURE 3: HPLC separation of bovine liver subunits and CNBr fragments of bovine liver subunit C_{IV}. (A) Fraction from the Bio-Gel P60 column containing C_V, C_{VI}, and SSG. (B) A fraction from the Bio-Gel P60 column containing the four smallest subunits C_{VII}–C_{IX} and IHQ. (C) Separation of the CNBr fragments of beef liver subunit C_{IV}. The cleavage profile obtained for beef heart subunit C_{IV} was identical (not shown). The straight-line plots over the elution profile show the solvent gradient used. The numbers indicate the percent of solvent B used in different steps.

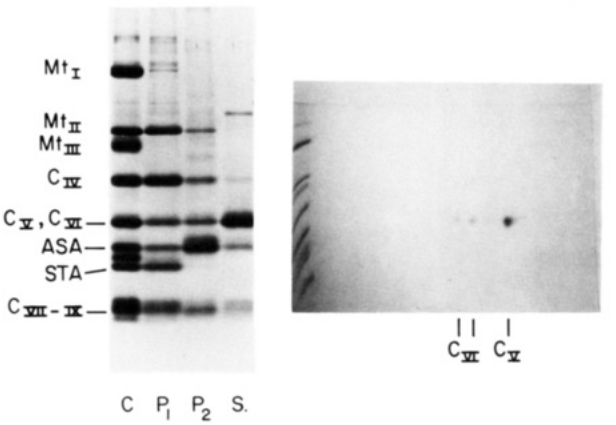

FIGURE 4: Separation of subunits C_V and C_{VI} of the liver enzyme by pyridine extraction and isoelectric focusing separation of these subunits. (Left panel) Fractionation of cytochrome *c* oxidase by pyridine treatment: C = control enzyme; P_1 = pellet of material not dissolved in 50% pyridine–50% water solution; P_2 = pellet of precipitated protein after dilution of the supernatant of step 1 to 5% pyridine with distilled water; S = supernatant of protein soluble in the 5% pyridine solution. (Right panel) Isoelectric focusing separation of C_V and C_{VI} of the liver enzyme. Subunit C_V runs with an isoelectric point of 5.2; C_{VI} always ran as two spots with isoelectric points of 5.9 and 6.2, possibly because of incomplete reduction of disulfide bonds. Isoelectric focusing of the beef heart subunits gave an identical pattern, and mixing of liver and heart C_V and C_{VI} failed to show differences between the two tissue forms of these subunits.

Subunits C_V and C_{VI} were also isolated by the pyridine–water extraction method (Figure 4, left panel) (Darley-Usmar et al., 1987). These subunits are water soluble as released by this procedure. The isoelectric points of the heart and liver forms of C_V and C_{VI} were compared by isoelectric focusing gel electrophoresis and found to be identical (Figure 4, right panel).

Amino Acid Sequencing of Liver Cytochrome c Oxidase Subunits. All of the nuclear-coded subunits of the liver enzyme were subjected to N-terminal sequencing. Table I lists the data obtained for subunits C_V, C_{VI}, STA, IHQ, and C_{VIII}. In each case, the amino acid sequence of the liver form was identical with that of the equivalent polypeptide isolated from the heart enzyme. These sequencing data represent a minimum of 27% of the full sequences of C_{VI} and STA, and as much as 55% of the sequence of C_{VIII}. The subunit equivalent of AED in liver had a blocked N terminus, as is the case with the heart form (Steffens et al., 1979). This subunit was cleaved

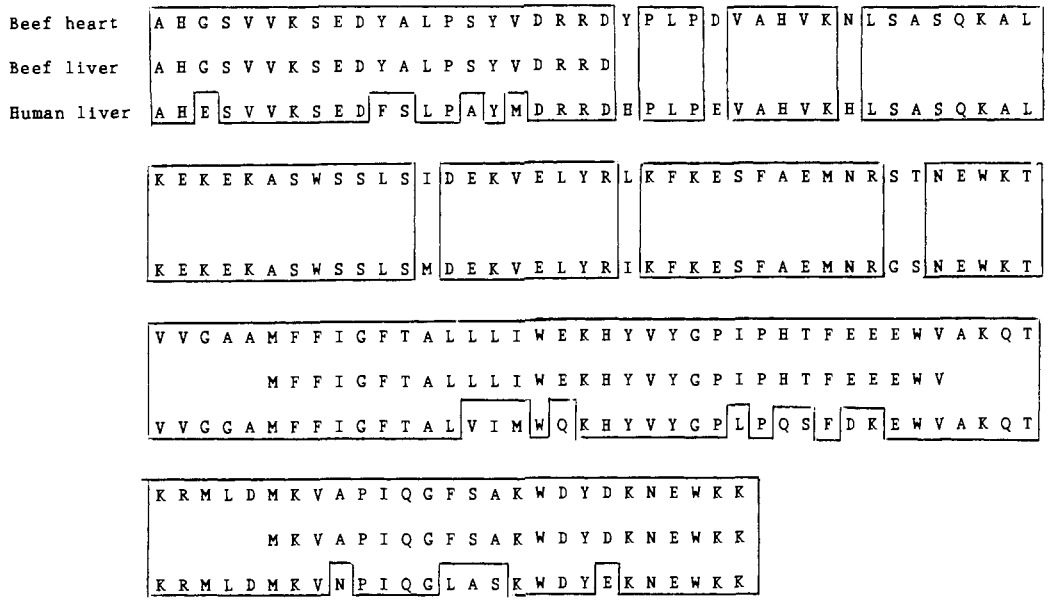

FIGURE 5: Sequences of beef heart and beef liver subunit C_{IV} along with the sequence of C_{IV} from human liver.

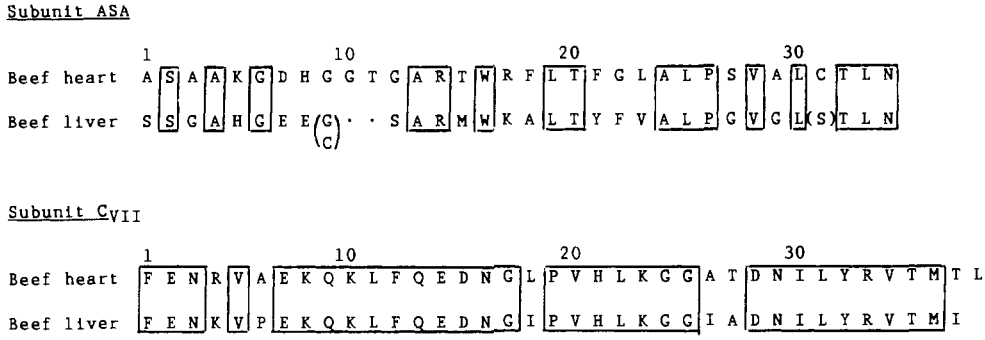

FIGURE 6: Sequence comparison of the N-terminal parts of the beef heart and beef liver forms of subunits ASA (SSG in liver) and C_{VII}.

by CNBr into two fragments which could be resolved by HPLC. Sequencing of the C-terminal fragment gave data for 25 residues near the center of the polypeptide (Table I). This sequence was identical with that of the heart form of this subunit.

Figure 5 shows sequence data on the liver form of subunit C_{IV}. N-Terminal sequencing of the intact polypeptide gave the first 22, 23, and 19 residues, respectively, in 3 determinations, each on a different preparation of the subunit. In every case, the N terminus of this subunit proved to be ragged with a second sequence present that began at residue 3, i.e., GSVV. This proteolytically modified subunit C_{IV} represented 10–20% of the polypeptide present. The liver form of subunit C_{IV} was cleaved by CNBr into four major fragments that were separated by HPLC. The CNBr fragmentation patterns obtained on HPLC and by $NaDodSO_4$–polyacrylamide gel electrophoresis were identical for the liver and heart forms of this subunit (Figure 3). CB_4 was sequenced for 31 residues and CB_5, a fragment of 22 residues, was sequenced completely. In all, 74 of the 147 residues in subunit C_{IV} were determined, with no differences being found between the heart and liver forms of this polypeptide.

Recently, the full sequence of subunit C_{IV} from human liver cytochrome *c* oxidase has been obtained by sequencing of the cDNA for this polypeptide (Zeviani et al., 1987). Human liver subunit C_{IV} differs from beef heart in 27 of 147 residues (Figure 5). Of these 27 amino acid substitutions, 19 are in regions for which we have obtained sequence data on the beef liver form of subunit C_{IV}, and in every case, the beef liver residue is the same as beef heart rather than the human liver form.

Table I: Sequences of Nuclear-Coded Subunits of the Liver Enzyme That Are the Same as Heart Cytochrome *c* Oxidase

subunit	sequence	no. of amino acids[a]	% determined[b]
C_V	SHGSHETDEE^{10}FDARWVTYF-N^{20}KPDIDAWELR^{30}KGMN	109	31
C_{VI}	ASGGGVPTDE^{10}EQATGLEREV-^{20}MLAARK	98	27
STA	STALAKPQMR^{10}GLLARRLRF-H^{20}	73	27
IHQ	IHQKRAPDFH^{10}DKYGNAVLA-S^{20}GA	56	39
C_{VIII}	SHYEEGPGKN^{10}IPFSVENKWR-^{20}LLAMMT	47	55
AED	T^{24}AKGGDV^{30}SVCEWYRRVY^{40}KSLCPI	85	27

[a] Number of amino acids in the sequence of the heart subunit. [b] Percentage of the liver sequence determined.

Three subunits proved to be different in liver and heart on the basis of sequencing data. Figure 6 shows the N-terminal sequences of subunits ASA and C_{VII} from liver and compares these with the sequences of the heart isoforms of these subunits. Figure 7 compares the sequences of C_{IX} from liver and heart. Also included is the sequence of C_{IX} from rat liver (Suske et al., 1987) and from the yeast, *Saccharomyces cerevisiae* (Wright et al., 1986). Regions of sequence homology between beef heart and beef liver are enclosed in boxes. Regions of

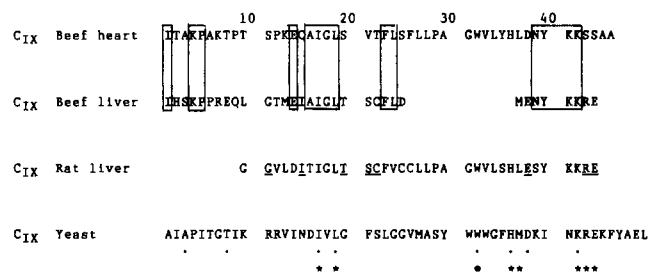

FIGURE 7: Sequences of beef heart, beef liver, rat liver, and yeast subunit C_{IX}. Homologues between the beef heart and liver sequences are boxed. Homologues between rat liver and beef liver where there is no homology with the heart subunit are underlined. Sequence homologies between yeast C_{IX} and the heart subunit are shown by dots; homologues between the yeast and liver subunits marked by asterisks.

sequence that are the same in rat liver and beef liver, but different in beef heart, are underlined while regions of homology between yeast and the mammalian subunit are indicated by asterisks.

DISCUSSION

The results of this study of beef liver cytochrome *c* oxidase confirm the original findings of Kadenbach and colleagues [e.g., see Kadenbach et al. (1982, 1987)] that there are tissue-specific isoenzyme forms of this terminal respiratory chain complex. Unresolved is how many of the subunits of this enzyme occur as isoforms. Originally, Kadenbach et al. (1982) identified three subunits as having different migrations in $NaDodSO_4$–polyacrylamide gel electrophoresis under highly resolving conditions. More recently, Kuhn-Nentwig and Kadenbach (1985) have proposed that all 10 of the nuclear-coded subunits of cytochrome *c* oxidase have tissue-specific forms. This suggestion is based on antibody binding experiments, which, as discussed in the introduction, are unreliable.

We have used a Western blot technique to examine the antigenic differences between subunits of heart and liver cytochrome *c* oxidase. Antibodies made against purified subunits of beef heart cytochrome *c* oxidases were reacted with beef heart and beef liver cytochrome *c* oxidase that had been denatured in $NaDodSO_4$ and then electrophoresed to resolve the subunits. Antibodies to most subunits reacted equally well with heart and liver enzyme. The exceptions were polyclonal antibodies made to ASA, C_{VII}, and C_{IX} respectively, each of which reacted well with the beef heart enzyme but poorly or not at all with the liver form of the enzyme.

Conclusive evidence for tissue-specific forms of ASA, C_{VII}, and C_{IX} was obtained by sequencing of these polypeptides. The liver form of subunit C_{VII} was sequenced from the N terminus for 37 residues, which represents 53% of the entire sequence. There were six residues different in this part of the polypeptide. These are all conservative changes that did not introduce new ionic residues, alter the net charge, or change the hydrophobicity of the subunit.

Sequence data for the liver form of ASA were obtained by N-terminal sequencing for 33 residues, constituting more than one-third of the full sequence. The liver form differed from heart in its N-terminus SSG instead of ASA, in deletion of 2 amino acids (residues 10 and 11 of the heart sequence), and in a total of 17 of the 33 positions covered by the liver sequence data. These changes increased the net negative charge in the N-terminal part of the liver form compared with the heart form by the loss of one Lys (K_5 in heart is replaced by H) and in addition of one carboxyl ($H_8 \rightarrow E$).

Three-fourths of the sequence of the liver form of C_{IX} was obtained (33 of 44 amino acids) by N-terminal sequencing (first 25 residues) and by sequencing of a C-terminal CNBr fragment. The liver form of C_{IX} was different from the heart form in 15 of the N-terminal 25 amino acids, and in 4 of 6 C-terminal residues.

Recently, Suske et al. (1987) have sequenced a cDNA clone coding for all but the N-terminal nine residues of rat liver C_{IX}. A comparison of the full sequence of beef heart C_{IX} with the partial sequences of this subunit from beef liver and rat liver shows some interesting features. Beef liver and rat liver C_{IX} have the same C terminus, i.e., KKRE, that is significantly different from the heart form, i.e., KKSSAA. The two liver C_{IX} subunits are also the same at residues 25–28, i.e., TSC. In heart, this sequence is SVT. For the overlapping regions of sequence (residues 9–25 and 37–44), the homology between rat liver and beef liver (59%) is higher than that between beef liver and beef heart (46%).

The sequence of rat liver C_{IX} covers that part of the beef liver subunit missing in our analysis. In this region (residues 20–36), which includes part of the hydrophobic, putative transmembrane domain, the homology between liver (rat) and heart (beef) is 82%; i.e., 9 of the 11 residues are the same.

It is interesting to compare the sequence of the mammalian C_{IX} with the equivalent polypeptide of yeast cytochrome *c* oxidase. The homology between these is localized to the C terminus. There are 5 conserved residues in the sequence between 32 and 44 (numbering system for beef) for yeast and beef liver, but only 3 residues are the same in this stretch when yeast and beef heart are compared.

The liver and heart forms of 7 of the 10 nuclear-coded subunits were indistinguishable in our studies. Subunit C_{IV} was studied most extensively because of previous evidence to suggest that this subunit occurs in tissue-specific isoforms. Thus, C_{IV} is one of the most different subunits antigenically in different tissues according to Kuhn-Nentwig and Kadenbach (1985) and had been claimed to be tissue specific in humans (Nakagawa et al., 1980) (see the introduction). Finally, there are two forms of subunit C_{IV} in yeast that are coded by different genes (Cumsky et al., 1987).

In our studies, beef heart and beef liver subunit C_{IV} reacted identically with antibodies and showed identical CNBr fragmentation patterns, and the amino acid sequence of the polypeptide from the 2 sources was the same in the 74 of the 147 residues that could be compared by using our data for the liver subunit. This similarity in sequence includes 19 of the 27 amino acid changes found between beef heart and human liver C_{IV}. The other eight residue differences were not in segments of polypeptide sequenced in our study. There are no homologies between the beef and human liver sequences that are not found in heart, as is the case for subunit C_{IX} as discussed above.

Our findings, that the heart and liver forms of C_{IV} appear to be identical, are consistent with recent molecular biological studies. Bachman et al. (1987) have found only one expressed gene for subunit C_{IV} in the bovine genome, along with a pseudogene. Zeviani et al. (1987) have shown a single identically sized RNA transcript for subunit C_{IV} in human muscle and liver and Hela cells.

Partial sequences of the liver forms of C_V, C_{VI}, AED, STA, and C_{VIII} were the same as for the equivalent heart subunits. The liver and heart forms of subunit C_V and C_{VI} had identical isoelectric points. Thus, our results suggest three classes of subunits in mammalian cytochrome *c* oxidase. The first class includes the three mitochondrially coded subunits and represents the functional core of the complex with the prosthetic groups and redox-linked proton pump. The second class in-

cludes nuclear-coded subunits that are identical in heart and liver tissues, with the third class being the tissue-specific components such as ASA (SSG), C_{VII}, and C_{IX}.

There is accumulating evidence that the nuclear-coded subunits are involved in the regulation of cytochrome *c* oxidase activity. It has been found that the electron-transfer activity of the enzyme is sensitive to anions in general, and nucleotides in particular. Huther and Kadenbach (1986) and Bisson et al. (1987) have each shown that ATP binding significantly reduces the high-affinity phase of cytochrome *c* oxidase activity. Bisson et al. (1987) have shown that ATP causes a conformational change in the enzyme that alters the cytochrome *c* binding site. It is probably significant that subunits identified as involved in ATP binding, and components localized to the cytochrome *c* binding site, include the subunits identified in our study as being tissue-specific subunits.

Registry No. Cytochrome *c* oxidase, 9001-16-5.

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