TISSUE-SPECIFIC GENES CODE FOR POLYPEPTIDE VIa OF BOVINE LIVER AND HEART CYTOCHROME *c* OXIDASE

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1. Introduction

Cytochrome *c* oxidase from higher eucaryotes is composed of 12–13 different protein components (review [1–3]). This was concluded from:

(i) The occurrence of 12 protein bands after SDS gel electrophoresis in enzyme preparations from many species and tissues [4,5];
(ii) The isolation of 12 different polypeptides from bovine heart cytochrome *c* oxidase by gel chromatography, and the proof of 12 different N-terminal amino acid sequences [6];
(iii) The occurrence of all components in the complex in stoichiometric amounts [4,7];
(iv) The immunoprecipitation of 12 polypeptides from a mitochondrial Triton X-100 lysate with a specific antiserum against subunit IV [8].

Recent work suggests that the functional properties of the enzyme are connected with the 3 large mitochondrially synthesized subunits. The 2 heme *a* groups are assumed to be localized in subunits I and II, the 2 copper atoms in subunit II, which also binds cytochrome *c*, and the proton-pumping activity within subunit III (review [2,3]). The function of the other, cytoplasmically synthesized polypeptides remained unknown.

Comparison of the gel electrophoretic polypeptide pattern of cytochrome *c* oxidases from rat liver and heart revealed differences in the apparent M_r-values of polypeptides VIa [9]. (The nomenclature of [4] is used, if not otherwise stated.) Similar differences were found for polypeptide VIII of liver and heart cytochrome *c* oxidases from chicken, pig and bovine and for polypeptide VIIa from pig and bovine [5].

In a kinetic study of cytochrome *c* oxidases from bovine liver and heart, differences in the V_{max} and K_m-values for the 2 enzymes were observed [5,10]. It was concluded that vertebrates contain tissue-specific isoenzymes of cytochrome *c* oxidase.

Here, the N-terminal amino acid sequences of cytochrome *c* oxidase polypeptides VIa from bovine liver and bovine heart are compared. The data indicate that 2 different genes code for polypeptide VIa of the enzymes from liver and heart.

2. Materials and methods

Cytochrome *c* oxidases were isolated from freshly prepared or from frozen bovine liver and heart mitochondria by use of Triton X-114 and Triton X-100 as in [4]. SDS–polyacrylamide slab-gel electrophoresis was performed as detailed in [4] with 18% acrylamide and 6 M urea in the separation gel. For isolating proteins from gels, the brush in the stacking gel was omitted. Polypeptide VIa was isolated essentially as in [8] from Coomassie blue-stained gels by extraction with 1% SDS and ion-pair precipitation with triethylamine acetate from acetone. The yield of protein reactive in the Edman degradation was low, either due to the very poor solubility of proteins extracted from gels or to carbamylation of N-terminal amino acids by isocyanate formed from urea. Polypeptide VIa from the bovine heart enzyme, designated VIb [11], was isolated on a larger scale for determination of its complete sequence by Biogel P-10 chromatography in 10% acetic acid [11].

The N-terminal amino acid sequences of gel-extracted proteins were determined with an automatic Beckman sequencer as in [12], that of proteins isolated by gel chromatography as detailed in [11].

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3. Results and discussion

The polypeptide composition of isolated cytochrome *c* oxidase from bovine liver and heart is compared in fig.1 by SDS gel electrophoresis. The apparent M_r-values of most subunits of the 2 enzymes are identical. However, polypeptides VIa, VIIa and VIII of the liver and heart enzyme, corresponding to polypeptides VIb, VIIIc and VIIIb, according to the nomenclature in [11], appear at different positions on the gel. Polypeptides VIa and VIII have a smaller, polypeptide VIIa has a higher apparent M_r in the liver enzyme compared to that of the heart. The same differences in apparent M_r-values were also obtained if the enzymes were isolated from mitochondria in the presence of a mixture of protease inhibitors (phenylmethane sulfonylchloride, L-1-chloro-3-[4-tosylamino]-7-amino-2-heptanone and L-1-chloro-3-*p*-tosylamido-4-phenyl-2-butanone).

To decide whether the different apparent M_r-values are due to post-translational modifications or to different primary structures of the corresponding polypeptides, the N-terminal amino acid sequences of the 2 polypeptides VIa were determined. Table 1 compares the sequences for the first 11 amino acids. Whereas some positions have identical amino acids (positions 2,4,6,9), others show conservative (positions 3,7,11) or radical exchanges (position 5,8). The stimilarity of the 2 sequences suggests a common phylogenetic precursor of the 2 polypeptides. The possibility that polypeptide VIa from liver may represent a proteolytic fragment of the corresponding polypeptide from heart can be excluded, because the sequence of the liver polypeptide does not occur in the further sequence of polypeptide VIa from heart. The sequence of the liver polypeptide VIa has also not been found in any other polypeptide of bovine heart cytochrome *c* oxidase (unpublished).

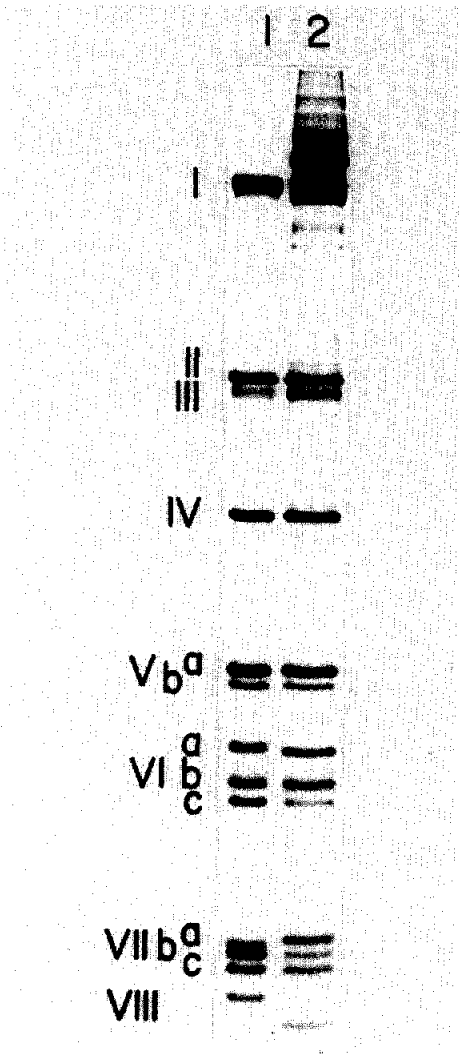

Fig.1. Comparison of the polypeptide pattern of isolated cytochrome *c* oxidase from bovine liver and heart. SDS gel electrophoresis was performed as in [4]. About 10 μg enzyme protein were applied to each lane: (1) bovine heart; (2) bovine liver cytochrome *c* oxidases.

Table 1
N-terminal amino acid sequence of polypeptide VIa of cytochrome *c* oxidase from bovine liver and heart

Heart:	H_2N–Ala–Ser–Ala–Ala–Lys–Gly–Asp–His–Gly–Gly–Thr
Liver:	H_2N–Ser–Ser–Gly–Ala–Thr–Gly–Glu–Glu–Gly–Ser–Ala

The amino acid sequence of the heart polypeptide has been determined with polypeptide VIa isolated by gel chromatography [11] and was corroborated by sequence analysis of polypeptide VIa isolated from Coomassie blue-stained gels. The sequence of the liver polypeptide has been performed twice with samples extracted from SDS gels. The yield of amino acids during Edman-degradation was 10–20% (section 2)

A preliminary analysis of the N-terminal amino acid sequences of several other cytoplasmically synthesized polypeptides from bovine liver and heart cytochrome *c* oxidases, revealed mainly identical amino acids, but also some exchanges (unpublished). From these data we conclude that polypeptides VIa, and several other cytoplasmically-made polypeptides, from bovine liver and heart cytochrome *c* oxidases are coded by different genes. The structure of these polypeptides is currently being determined.

Since different apparent M_r-values were also found for polypeptide VIa from rat liver and rat heart cytochrome *c* oxidase, for polypeptide VIIa from pig and for polypeptide VIII from chicken and pig [4], we suggest polypeptides VIa, VIIa and VIII to represent tissue-specific components of cytochrome *c* oxidases from vertebrates. The functional meaning of cytochrome *c* oxidase isoenzymes may be understood by a tissue-specific regulation of respiratory function including electron- and/or proton-translocation in complex IV of the respiratory chain of the mitochondrial membrane (see [2]). This assumption is supported by different kinetic properties found for cytochrome *c* oxidases of bovine liver and heart [5,10].

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