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Comparative Study of the Peptide Composition of Complex III (Quinol-Cytochrome *c* Reductase)

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Abstract

A comparative study has been made on the subunits of Complex III from beef heart, rat liver, *Neurospora*, and baker's yeast mitochondria. All of the subunits of the beef heart enzyme were similar to the counterpart subunit in rat liver Complex III, both with respect to their apparent molecular weights on SDS-polyacrylamide gels and their proteolytic digestion maps obtained in the presence of *S. subtilus* V8 protease. In contrast, the subunits of *Neurospora* and yeast Complex III varied considerably from the mammalian enzyme, as well as between themselves, the only exception being cytochrome *b* (subunit III). Less variation was observed in the electron transport peptides (IV–V) of higher and lower eukaryotes than in those subunits (I, II, VI–VIII) for which no functions are known. However, the data imply that subunits I, II, and VI–VIII are bona fide members of the complex, and that their functions within the complex, although unknown, are also somewhat conserved. Finally, the low-molecular-weight subunits of rat liver cytochrome oxidase and Complex III were compared. They appear to contain no subunits in common, implying different roles for these peptides in the two complexes.

Key Words: Mitochondria; Complex III; quinol-cytochrome *c* reductase; peptides; comparative analysis.

Introduction

Complex III (quinol-cytochrome *c* reductase) has now been isolated from several sources (Weiss and Kolb, 1979; Weiss *et al.*, 1978; Katan *et al.*, 1976; Siedow *et al.*, 1978; Hatefi *et al.*, 1962; Rieske *et al.*, 1964; Engel *et al.*, 1980; Gellerfors *et al.*, 1981). Although the composition of the redox centers of these preparations is similar, a wide variation in the numbers, and especially the sizes, of the subunits has been reported (Gellerfors and Nelson, 1975; Marres

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and Slater, 1977; Bell and Capaldi, 1976; Nelson and Gellerfors, 1978). Five of the eight subunits of Complex III have no known functions. Because of this, it has not been possible to determine if these peptides are necessary members of the complex or merely contaminants. In attempts to partially answer this question, we have undertaken a comparative analysis of the various Complex III preparations. These studies disclose a number of similarities between the

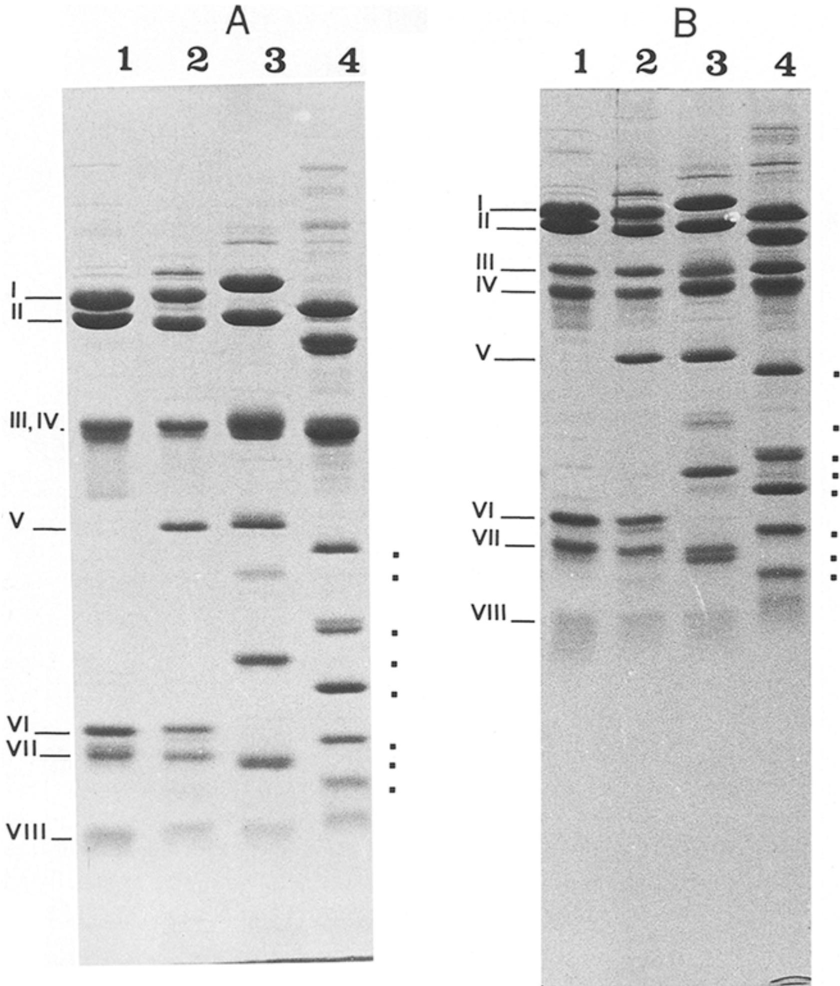


Fig. 1. Comparison of the peptides of Complex III from several sources. Complex III from beef heart (lane 1), rat liver (lane 2), *Neurospora crassa* (lane 3), and baker's yeast (lane 4) were subjected to SDS-electrophoretic analysis on either 12.5% (A) or 16% (B) polyacrylamide gels.

individual subunits of both higher and lower eukaryotic cells, which support the belief that they are bona fide subunits of the enzyme, even though no function can be assigned to them.

Materials and Methods

Complex III was isolated from beef heart (Riccio *et al.*, 1977) and rat liver (Gellerfors *et al.*, 1981) mitochondria as described. Complex III from *Neurospora crassa* was a generous gift from Prof. Hans Weiss, and that from *Saccharomyces cerevisiae* was generously supplied by Prof. Graham Palmer, Houston, Texas.

The complexes were resolved on SDS-polyacrylamide slab gels in the buffer system of Laemmli (1970). The bands to be analyzed were cut from the gels, and proteolytic digestion maps were made by the method of Cleveland *et al.* (1977) using *Staphylcoccus aureus* protease V8 or chymotrypsin. Apparent molecular weights of the peptides were determined using the following protein standards: bovine serum albumin (68,000), ovalbumin (46,000), carbonic anhydrase (29,000), trypsin inhibitor (21,000), cytochrome *c* (12,400), aprotinin (6,800), and the β chain of insulin (3,600).

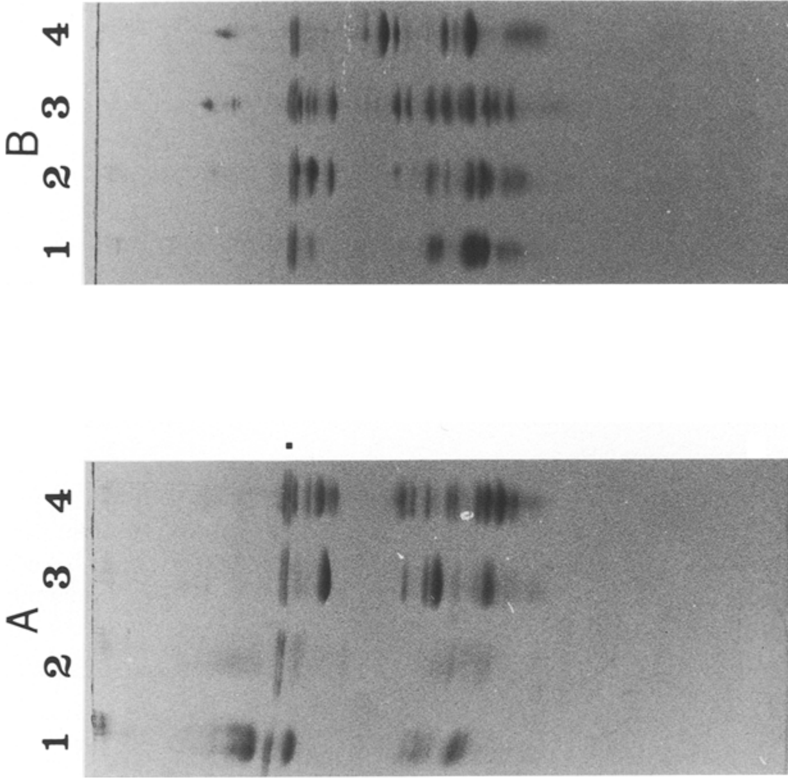
Results

Figure 1 shows the electrophoretic resolution of Complex III from beef heart (lane 1), rat liver (lane 2), *Neurospora crassa* (lane 3), and baker's yeast (lane 4) on both 12.5% (Fig. 1 A) and 16% (Fig. 1 B) polyacrylamide

Table 1. Molecular Weights of Complex III Subunits

Peptide	Molecular weights $\times 10^{-3}$			
	Beef heart	Rat liver	*Neurospora*	Yeast
I	49.5	50.7	51.6	47.5
II	46.8	46.2	46.8	42.7
III	33.5 (38.0)[a]	33.5 (38.0)	33.5 (38.0)	32.7 (38.0)
IV	33.5	33.5	33.5	32.7
V	25.0[b]	24.5	24.5	22.6
VI	12.0	12.0	15.8	17.6
VII	10.2	10.2	9.6	14.5
VIII	5.0	5.7	5.2	11.5
IX				7.9
X				5.8

[a] Apparent molecular weights determined on 16% polyacrylamide gels. All other molecular weights were identical on 12 and 16% gels.
[b] From Gellerfors *et al.* (1981).


B
1
2
3
4
A
1
2
3
4

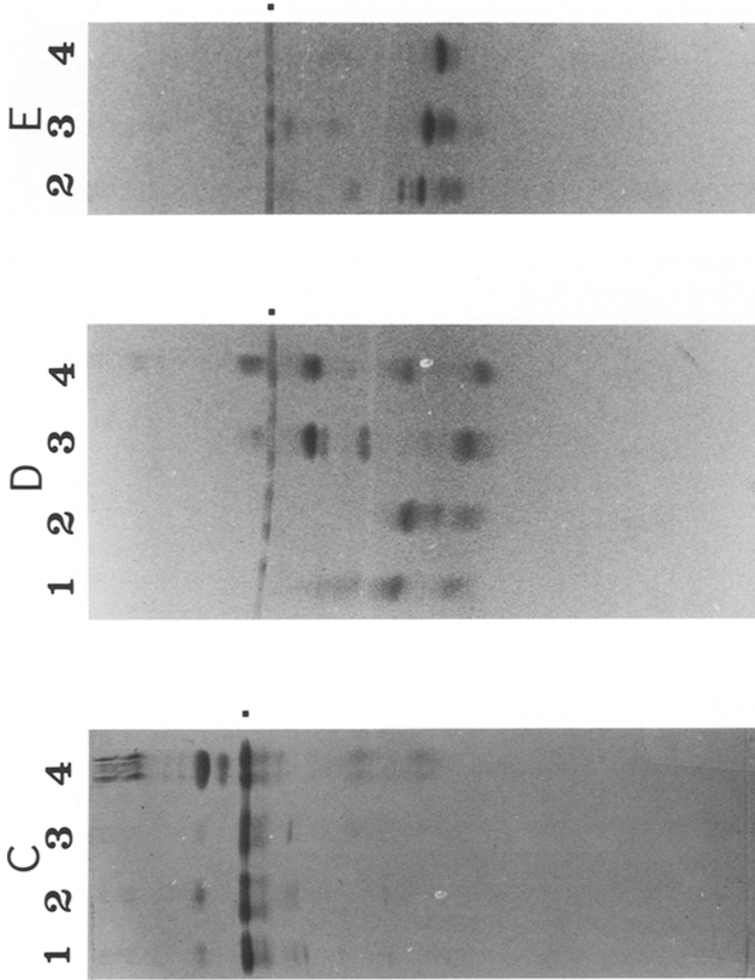


Fig. 2. Peptide map analysis of subunits I–V of Complex III isolated from several sources. Individual subunits were sliced from polyacrylamide gels after separation as shown in Fig. 1, and then subjected to peptide map analysis by the method of Cleveland using 1 μg *S. aureus* V8 protease. (A) subunit I, (B) subunit II, (C) subunit III, (D) subunit IV, and (E) subunit V from beef heart (lane 1), rat liver (lane 2), *Neurospora* (lane 3), and baker's yeast (lane 4). The dot indicates the location of protease V8 on each gel.

gels containing SDS. The eight major bands present in the mammalian enzymes are numbered. The dots indicate the lower-molecular-weight peptides of yeast and *Neurospora* which are either absent from, or whose mobilities differ from, those of rat and beef heart. It can be seen that the apparent molecular weights of the rat liver and beef heart subunits are very similar. Although the beef heart enzyme (lane 1) used in the study lacks the iron sulfur peptide (subunit V) (Riccio *et al.*, 1977), earlier experiments from this laboratory indicate that this subunit has the same molecular weight in both enzymes (Gellerfors *et al.*, 1981).

A rather substantial difference in the apparent molecular weights is observed between the subunits of Complex III from mammalian sources and from lower eukaryotes (Fig. 1). These differences are most pronounced in the lower-molecular-weight regions (subunits VI–VIII). However, even subunits I and II vary to some extent. The electron transfer peptides (III, IV, V), on the other hand, were, with the exception of yeast subunit V, similar in size in all four species, suggesting that these peptides might be more highly conserved. In line with this reasoning, cytochrome *b* (subunit III), the only mitochondrial translation product in Complex III, exhibits identical apparent molecular weights and identical anomalous electrophoretic behavior in all four preparations of Complex III. In each case, cytochrome *b* comigrates with cytochrome c_1 on 12.5% gels but is well resolved, exhibiting higher apparent molecular weights than cytochrome c_1, on 16% gels. This is also in keeping with the high degree of conservation of cytochrome *b* as determined from sequence analysis of the genes from several sources (Anderson *et al.*, 1981; Bibb *et al.*, 1981; Nobrega and Tzogoloff, 1980). The apparent molecular weights of the various subunits are summarized in Table I.

A more extensive comparison was made by peptide map analysis of the individual subunits. Figure 2 shows the peptide maps obtained from subunits I–V with V8 protease from *Staphylococcus aureus*. Subunits I–IV from rat liver and beef heart gave similar, if not identical, peptide maps. Subunit V from beef heart was not analyzed. In contrast, the peptide maps obtained from yeast and *Neurospora* differed considerably from each other as well as from their mammalian counterparts. Subunit II from *Neurospora* was the only lower eukaryotic peptide which exhibited apparent homology with its mammalian counterpart. Cytochrome *b* (subunit III), however, was resistant to V8 under these conditions in all four preparations, again suggesting a conservation in the primary and/or tertiary structures.

Similar peptide analysis of the lower-molecular-weight subunits was more difficult due to the lack of resolution of the digestion products in the separation system. However, it could be concluded that subunits VI and VII from rat liver and beef heart were similar, as determined from V8 digestion patterns (Fig. 3). Similarities in subunits VI and VII could also be established from the rat liver and beef heart peptides upon digestion with chymotrypsin,

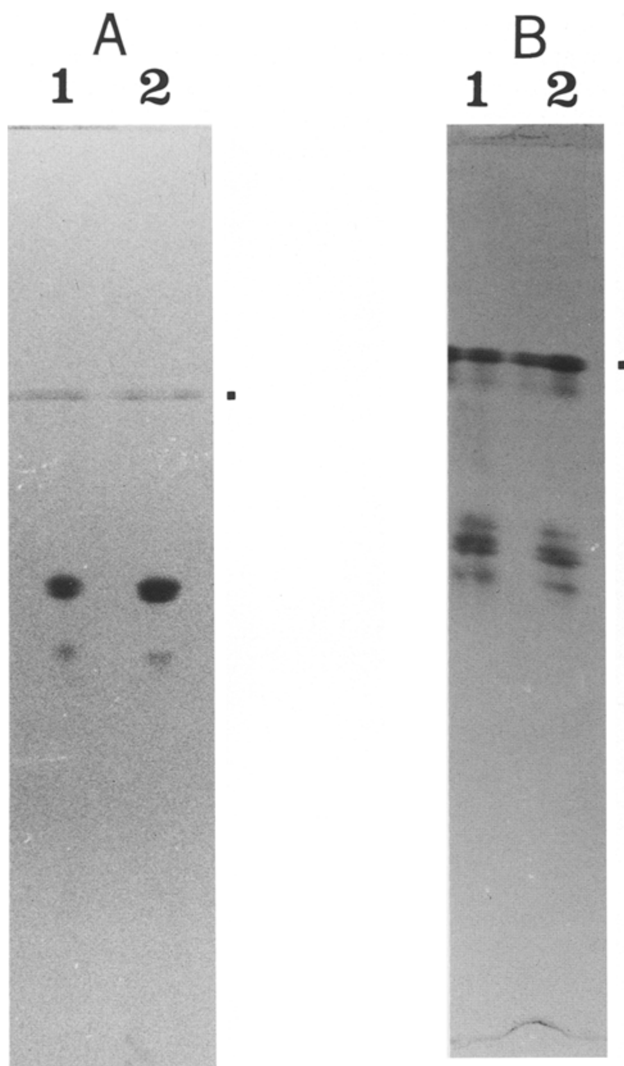


Fig. 3. Peptide map analysis of subunits VI and VII from mammalian Complex III. Peptide map analysis was done as in Fig. 2. (A) Subunit VI, and (B) subunit VII from rat (lane 1) or beef heart (lane 2). The dots indicate the location of V8 protease.

since these subunits from both sources were not attacked. Subunit VIII could not be analyzed with this method.

It was also of interest to determine if cytochrome oxidase and Complex III from the same source contained certain subunits in common. This is particularly important for the lower-molecular-weight peptides for which

functions are not known, and for which a large variation in both the numbers and sizes have appeared in the literature. Figure 4 shows that the rat liver cytochrome oxidase subunit V and rat liver Complex III subunit VI have similar apparent molecular weights, and are the only peptides which are consistently similar in the two preparations. However, V8 digestion products

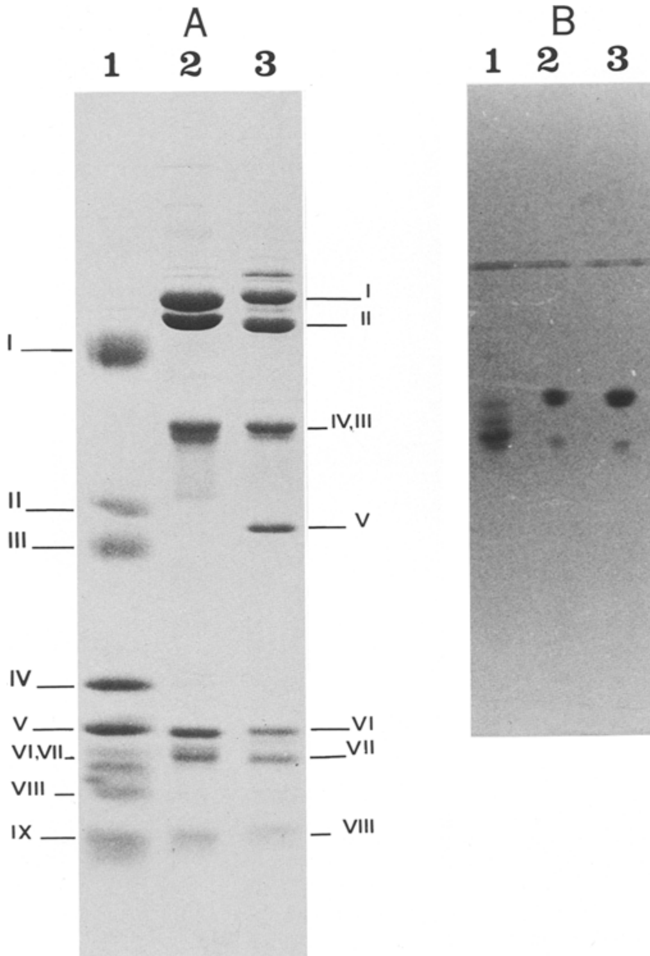


Fig. 4. Comparison of the peptides of cytochrome oxidase and Complex III. Cytochrome oxidase was isolated from rat liver, and Complex III was isolated from rat liver and beef heart as described in Materials and Methods. (A) Resolution of the complexes on 12.5% polyacrylamide gels. Rat liver cytochrome oxidase (lane 1), beef heart complex III (lane 2), and rat liver Complex III (lane 3). (B) peptide map analysis of rat cytochrome oxidase subunit V (lane 1), rat liver Complex III subunit VI (lane 2), and beef heart Complex III subunit VI (lane 3). Peptide analysis was done as in Fig. 2. The dot indicates the location of V8 protease.

of the cytochrome oxidase subunit is very different from that of both the beef and rat Complex III subunit (Fig. 4). We conclude that the two enzymes share no common subunits.

Discussion

A number of interesting observations have emerged from the present comparative analysis of Complex III. One is that the counterpart subunits in rat liver and beef heart Complex III are very similar, both with respect to their apparent molecular weights and the peptide maps obtained with *S. aureus* V8 protease and with chymotrypsin. This was observed for all subunits analyzed (I–VII). Thus, among the mammals, we can probably expect little tissue or species variation in these subunits. This result is to be expected for cytochrome *b* (subunit III), which is encoded in mitochondrial DNA and which has been shown by nucleotide sequence analysis of the genes to be highly conserved in man, mouse, and yeast (Anderson *et al.*, 1981; Bibb *et al.*, 1981; Nobrega and Tzogoloff, 1980). Additional electron-transfer proteins such as cytochrome c_1 and the iron sulfur protein might also be expected to exhibit a high degree of structural conservation. The remaining peptides of Complex III (I, II, and VI–VIII) have no known functions, and we, therefore, have no basis for predicting the need for genetic conservation. The similarities between the same subunits in rat liver and beef heart Complex III suggest, however, a reasonably conserved primary structure in these peptides. This might be expected if the peptides served the same functions in the complex from different organisms. In any event, the similarities observed support the belief that these peptides are bona fide subunits of Complex III and not contaminants.

Both tissue- and species-specific variations have been reported in the molecular weights of the small subunits of mammalian cytochrome oxidase (Kadenbach *et al.*, 1981, 1982; Kadenbach and Merle, 1981). As reported above, this does not appear to be the case for the small subunits of mammalian Complex III, at least with those experimental conditions used. In line with the results from cytochrome oxidase, however, we observed considerable variety between the low-molecular-weight subunits of Complex III of higher and lower eukaryotes. Thus, it is possible that, as with cytochrome oxidase (Kadenbach *et al.*, 1981, 1982; Kadenbach and Merle, 1981), more genetic variation has been tolerated with the smaller subunits of Complex III than with the electron-transferring peptides.

Cytochrome *b* from the four species analyzed was relatively less sensitive to V8 protease digestion than were the other subunits, in spite of the fact that V8 protease is specific for glutamic acid residues, and that five to six glutamic acid residues have been deduced from nucleotide sequence analysis of human

(Anderson *et al.,* 1981), mouse (Bibb *et al.,* 1981), and yeast (Nobrega and Tzogoloff, 1980) cytochrome *b* genes. This resistance to digestion suggests that the tertiary structure of cytochrome *b* might also be preserved in all four species, even in the presence of SDS. Such structural similarities could account for the identical anomalous behavior of these peptides on SDS-gel (Fig. 1).

The variation observed in the molecular properties of the small subunits of Complex III (Gellerfors *et al.,* 1975; Marres *et al.,* 1977; Bell and Capaldi, 1976) and cytochrome oxidase (Kadenbach *et al.,* 1982), and the unknown functions of these peptides, promted us to investigate the possibility that these two respiratory chain complexes might contain cytoplasmically translated subunits in common. Analysis of the rat liver enzymes demonstrates, however, that this is not the case. If, as has been suggested (Kadenbach *et al.,* 1981; Kadenbach and Merle, 1981), the low-molecular-weight subunits function in some regulatory capacity, it would appear that either the mechanism of regulation differs in Complex III and cytochrome oxidase or that it requires different peptides.

Acknowledgments

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