Phys. Sci. 240, 117.
Prince, R. H., and Woolley, P. R. (1973), *Bioorg. Chem. 2,* 337.
Riepe, M. E., and Wang, J. H. (1968), *J. Biol. Chem. 243,* 2779.
Robinson, R. A., Paabo, M., and Bates, R. G. (1969), *J. Res. Natl. Bur. Stand. 734,* 299.
Schowen, R. L. (1975), *Faraday Symp. Chem. Soc. 10,* 167.
Sharma, M. M., and Dankwerts, P. V. (1963), *Trans. Faraday Soc. 59,* 386.
Silverman, D. N., and Tu, C. K. (1975), *J. Am. Chem. Soc. 97,* 2263.
Silverman, D. N., and Tu, C. K. (1976), *J. Am. Chem. Soc. 98,* 978.
Stein, P. J., Merrill, S. P., and Henkens, R. W. (1977), *J. Am. Chem. Soc. 99,* 3194.
Steiner, H., Jonsson, B.-H., and Lindskog, S. (1975), *Eur. J. Biochem. 59,* 253.
Steiner, H., Jonsson, B.-H., and Lindskog, S. (1976), *FEBS Lett. 62,* 16.
Sudmeier, J. L., and Bell, S. J. (1977), *J. Am. Chem. Soc. 99,* 4499.
Thorslund, A., and Lindskog, S. (1967), *Eur. J. Biochem. 3,* 117.
Tu, C. K., and Silverman, D. N. (1975), *J. Am. Chem. Soc. 97,* 5935.
Vaara, I. (1974), Inaugural Dissertation, UUIC-B22-2, Uppsala University.
Verpoorte, J. A., Mehta, S., and Edsall, J. T. (1967), *J. Biol. Chem. 242,* 4221.
Wang, J. H. (1968), *Science 161,* 378.
Wang, J. H. (1970), *Proc. Natl. Acad. Sci. U.S.A. 66,* 874.
Wissburn, K. F., French, D. M., and Patterson, A. (1954), *J. Phys. Chem. 58,* 693.
Woolley, P. (1975), *Nature (London) 258,* 677.
Yeagle, P. L., Lochmüller, C. H., and Henkens, R. W. (1975), *Proc. Natl. Acad. Sci. U.S.A. 72,* 454.

Polypeptides in the Succinate–Coenzyme Q Reductase Segment of the Respiratory Chain†

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ABSTRACT: Complex II (succinate–coenzyme Q reductase) was resolved into ten different polypeptides by polyacrylamide gel electrophoresis. Four polypeptides, C_{II-1}, C_{II-2}, C_{II-3}, and C_{II-4} with molecular weights of 70 000, 24 000, 13 500, and 7000, were present in large amounts in all preparations examined. C_{II-1} and C_{II-2} are the flavoprotein and iron–sulfur protein, respectively, of succinate dehydrogenase; C_{II-3} and C_{II-4} have not been functionally identified. Six polypeptides were present in much smaller amounts as judged by staining intensity, and each of these comigrated with components in complex III. The amino acid compositions of several of the minor components in complex II were identical with that of an equivalently migrating polypeptide in complex III. We conclude that succinate–coenzyme Q reductase contains four different polypeptides and is contaminated with variable amounts of complex III when isolated as complex II.

Succinate–coenzyme Q reductase or complex II provides one of the two major entry points for electrons into the cytochrome containing portion of the electron transfer chain (for review, see Hatefi, 1976). The question of how many components function in transferring electrons from succinate via coenzyme Q to cytochrome *b* and the structural relationship between these components and the bc_1 segment of the respiratory chain remain obscure. Complex II but not purified succinate dehydrogenase will recombine with purified complex III to reconstitute succinate cytochrome *c* reductase activity (Davis and Hatefi, 1971a,b). This indicates that there are components in complex II other than succinate dehydrogenase which are required for efficient electron transfer from succinate to oxygen. However, these components have not been identified nor their role established. As a first step in this direction we have examined the polypeptide composition of complex II and here identify two polypeptides as likely candidates for the functional and/or structural link between succinate dehydrogenase and the cytochrome portion of the respiratory chain.

Experimental Procedure

Complex II was prepared from beef heart mitochondria as described by Ziegler and Rieske (1967). Protein concentrations were determined by the method of Lowry et al. (1951). Heme *b* and c_1 concentrations were determined as described by Williams (1964). Flavin analysis was performed as described by Singer et al. (1971).

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis was performed as described by Weber and Osborn (1969) and by Swank and Munkres (1971). Two-dimensional gel electrophoresis was performed as described by Smith and Capaldi (1977), using the Weber–Osborn buffer system in the first dimension and the Swank–Munkres system in the second dimension.

For preparative gel electrophoresis, a slab gel (0.5 cm × 15 cm × 12 cm) of 10% acrylamide and 0.67% bisacrylamide was used. Complex II (8 mg), dissociated in 2% sodium dodecyl sulfate, 1% β-mercaptoethanol, 4 M urea by boiling for 1 min, was applied to the top of the gel in a volume of 500 μL and the sample was electrophoresed through the gel in the Swank–

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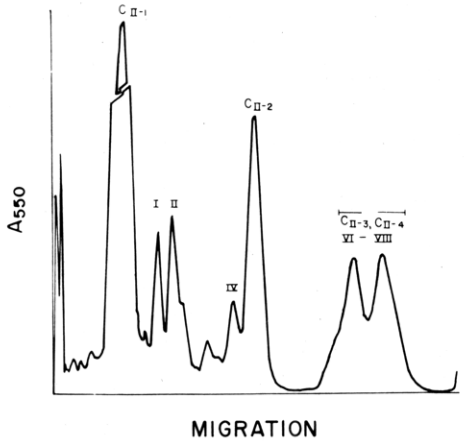

FIGURE 1: Densitometric trace of a sodium dodecyl sulfate–polyacrylamide gel of beef heart complex II. The sample (60 μg), dissociated in 1% sodium dodecyl sulfate and 1% β-mercaptoethanol by incubating at 37 °C for 2 h, was applied to a 10% gel and electrophoresed under the Weber–Osborn (1969) buffer conditions.

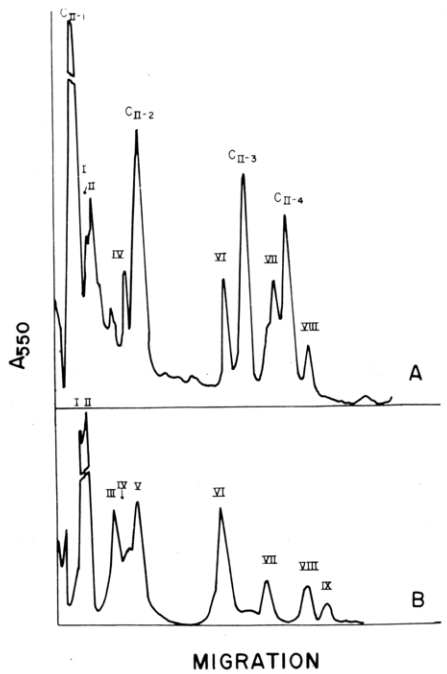

FIGURE 2: Densitometric traces of sodium dodecyl sulfate–polyacrylamide gels of beef heart complex II (A) and complex III (B) run on 9% gels in the Swank–Munkres (1971) buffer conditions.

Munkres buffer system. Slices (5-mm thick) taken vertically through the middle and at each end of the slab were stained with Coomassie blue to localize protein. Polypeptides were sliced out, the gel was macerated, and protein was eluted from the acrylamide by shaking in 1% sodium dodecyl sulfate, 5 mM sodium bicarbonate for 48 h. These solutions were filtered and dialyzed against distilled water to remove salts, and then protein was precipitated by dialyzing against 95% ethanol and collected by centrifugation. The purity of each purified polypeptide was checked by sodium dodecyl sulfate gel electrophoresis on tube gels prior to amino acid analysis.

Samples for amino acid analysis were hydrolyzed for 24 h with 6 N HCl in a sealed tube under vacuum in a 100 °C oven. A drop of phenol was included to protect tyrosine from being oxidized. HCl was removed by rotary evaporation at 30 °C and samples were analyzed in a Technicon automatic amino acid analyzer according to the method of Spackman et al. (1958).

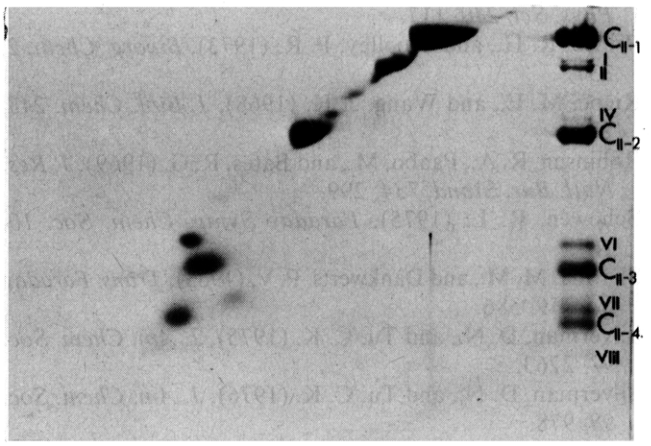

FIGURE 3: A two-dimensional sodium dodecyl sulfate–polyacrylamide gel electrophorogram showing the resolution obtained when samples of complex II were run in the Weber–Osborn gel system (10% gel) in the horizontal dimension and in the Swank–Munkres gel system (9% gel) in the vertical dimension. The procedure for making these gels is described in Smith and Capaldi (1977).

TABLE I: Estimated Molecular Weights of Polypeptides in Complex II.

Component	Gel system: Weber–Osborn	Gel system: Swank–Munkres	Equivalently migrating polypeptides in complex III[a]
C_{II-1}	73 000	70 000	
I	46 600	45 500	Core protein I
II	41 500	44 500	Core protein II
IV	31 000	27 500	{Cytochrome *b* / Cytochrome c_1}[b]
C_{II-2}	24 000	24 000	Cytochrome c_1 associated
VI	12 700	15 000	
C_{II-3}	13 700	13 500	
VII	14 300	9 000	Cytochrome *b* associated
C_{II-4}	12 500	7 000	
VIII	5 600	4 800	

[a] From Bell and Capaldi (1976). [b] These components migrate very close together on both the gel systems used.

Results

Polypeptide Composition of Complex II. Preparations of complex II used in this study contained from 4 to 5 nmol of covalently bound flavin and 3 to 4 nmol of cytochrome *b* per mg of protein. Cytochrome c_1 was present in all preparations in amounts ranging from 0.3 to 1.5 nmol/mg of protein, the largest amounts being found in samples of lower flavin content.

The polypeptide profile of a sample of complex II of high flavin content is shown in Figure 1. The gel used contained 10% acrylamide and electrophoresis was carried out under the Weber–Osborn buffer conditions (Weber and Osborn, 1969). Four highly stained bands were resolved with molecular weights of 73 000, 24 000, 14 000, and 12 000. In addition, three less intensely stained bands were visible with molecular weights of 46 600, 41 500, and 31 000. This profile is similar to that published by Davis and Hatefi (1971) and by Capaldi (1974) for purified complex II.

The polypeptide profile of complex II run on a 9% gel containing bisacrylamide to acrylamide in the ratio 1:10 (instead of 1:33, as in Weber–Osborn gels) and run in the Swank–Munkres buffer system is shown in Figure 2 and is compared with the polypeptide profile of complex III obtained under identical conditions. Complex II was separated into 10 dif-

TABLE II: Comparison of the Amino Acid Composition of Polypeptides in Complexes II and III.

Amino acid	$C_{II}C_{i}$ II		$C_{II}C_{III}$[a] IV		$C_{II}C_{III}$[a] VI		C_{II-3}
Lys	5.4	4.8	4.3	5.5	5.7	5.8	4.6
His	3.0	3.0	2.9	2.6	1.4	0.5	2.8
Arg	4.2	4.9	5.0	5.7	6.0	6.0	5.0
Asp	7.7	8.5	9.2	8.9	10.5	9.4	5.7
Thr	4.9	5.3	5.4	3.9	3.8	3.8	5.7
Ser	8.1	7.7	7.7	6.6	6.8	6.8	9.0
Glu	9.9	9.4	8.3	10.8	17.3	17.5	11.7
Pro	4.3	5.2	9.2	9.5	4.5	6.0	5.3
Gly	8.7	7.9	9.0	8.3	7.5	6.5	9.6
Ala	11.3	11.6	8.5	8.7	8.3	8.3	9.3
Val	7.4	7.6	4.1	6.5	4.8	6.4	5.3
Met	1.8	2.0	3.7	2.8	2.4	3.3	2.3
Ile	5.2	4.2	2.9	3.0	3.3	3.6	3.0
Leu	11.0	11.1	10.1	10.8	10.7	11.1	14.1
Tyr	3.4	3.2	5.0	2.7	4.3	2.4	2.0
Phe	3.7	3.6	4.7	3.7	2.7	2.6	4.6

[a] From Bell and Capaldi (1976).

ferent bands. There were four darkly stained bands labeled C_{II-1}, C_{II-2}, C_{II-3}, and C_{II-4} which did not have an equivalent in complex III and six less densely stained bands each of which comigrated with a band seen in complex III. The lightly stained components are labeled by the number given to the equivalent band in complex III. Components C_{II-1}, C_{II-2}, C_{II-3}, and C_{II-4} were present in the largest amounts in preparations containing the highest flavin content. The lightly staining components were diminished in the purest preparations of complex II (by flavin content) and were in greatest amounts in impure preparations (low flavin) which contained relatively high amounts of c_1 heme.

Two of the four major bands, C_{II-1} and C_{II-2}, which are respectively the flavoprotein and non-heme iron containing subunits of succinate dehydrogenase, were released from complex II by perchlorate treatment (results not shown: see also Davis and Hatefi, 1971; Coles et al., 1972). Components C_{II-3} and C_{II-4} have not been previously identified.

The two-dimensional gel shown in Figure 3, in which complex II was run in a Weber–Osborn gel in the horizontal dimension followed by a Swank–Munkres gel in the second dimension, represents the optimal resolution of components so far achieved. Fourteen different spots were resolved including $C_{II\ 1-4}$, bands labeled I, II, IV, VI, VII, and VIII and minor contaminants (as judged by staining intensity) in the molecular weight range 45 000 to 33 000. The two-dimensional gel shows clearly that the two broad and low molecular weight bands seen on the Weber–Osborn gels each contain several components among which are polypeptides that run with very different molecular weights in different gel systems and can actually change places in different gels. Thus, C_{II-3} and VII both run ahead of the polypeptide labeled VI on Swank–Munkres gels but run behind this component (with a larger apparent molecular weight) on Weber–Osborn gels. The molecular weights of the various components estimated from the different gel systems are listed in Table I. A tentative identification of several components as polypeptides of complex III can be made based on their migration under different gel conditions (Table I). These identifications are supported by amino acid compositional data as detailed in the next section.

Purification and Amino Acid Composition of Components of Complex II. Several polypeptides that ran as single bands on Swank–Munkres gels were purified by preparative gel electrophoresis. The amino acid compositions of II, IV, and VI are listed in Table II and these are compared with the amino acid compositions of the equivalently migrating components in complex III. The amino acid compositions of II and VI are essentially identical with those of core protein II and the cytochrome c_1 associated polypeptide of complex III, respectively, while the amino acid composition of IV is quite similar to that of cytochrome c_1 from complex III.

In the course of our studies of complex III we have prepared an antibody against a cytochrome c_1-enriched fraction of this complex that contains only the cytochrome c_1 hemoprotein (polypeptide IV) and VI (Bell and Capaldi, unpublished studies). This antibody was found to cross-react with complex II (but not cytochrome *c* oxidase, or oligomycin-sensitive ATPase) and this is taken as additional evidence that the cytochrome c_1 hemoprotein, the c_1-associated polypeptide, or both, are present in complex II.

The amino acid composition of C_{II-3} is also listed in Table I. No component of similar composition was found in complex III or cytochrome *c* oxidase.

Discussion

The polypeptide composition of complex II or succinate–coenzyme Q reductase has been examined previously by Davis and Hatefi (1971) and by Capaldi (1974) and the complex shown to contain at least seven different polypeptides. However, these studies did not use optimally resolving gel conditions. On Swank–Munkres gels, ten different polypeptides can be resolved in purified complex II. Four (C_{II-1}, C_{II-2}, C_{II-3}, and C_{II-4}) are clearly present in much larger amount than others. They are present in the same relative amounts in all preparations and are found in the highest amounts in preparations of high flavin content. Other polypeptides are present in all preparations of complex II but are diminished in amount in the purest preparations. Most of the minor components in complex II preparations appear to be components of complex III (see also Hatefi, 1976). The bands labeled I and II comigrate with core proteins I and II in complex III and the amino acid composition of band II is essentially identical with that of core protein II purified from complex III. Band IV migrates very close to the position at which the cytochrome c_1 hemoprotein migrates in gels of complex III. Spectral measurements show that this hemoprotein is present in low amounts in complex II and the amino acid composition of IV is similar to that of cytochrome c_1. The differences found between the amino acid

composition of band IV and that of the purified hemoprotein may result from contamination of IV with polypeptide III of complex III, a cytochrome *b* apoprotein which runs very close to IV in the gels used here. Band VI seen in complex II has an amino acid composition essentially identical with the cytochrome c_1 associated polypeptide of complex III and antibody against IV and VI of complex III cross-reacts with complex II. There are bands which comigrate with VII and VIII of complex III. Band VII changes places with band VI when Weber-Osborn and Swank-Munkres gels are compared, in an analogous manner to VII and VI of complex III (Capaldi et al., 1977).

Our results, therefore, indicate that complex II as isolated contains four major components which are contaminated with small and variable amounts of complex III. These four components may comprise the functional unit of succinate-coenzyme Q reductase. Polypeptides C_{II-1} and C_{II-2} with molecular weights 70 000 and 25 000 are the subunits of succinate dehydrogenase; the role of C_{II-3} and C_{II-4} with molecular weights of 13 500 and 7000 remains to be established. The fact that complex II but not purified succinate dehydrogenase is capable of interacting with complex III to reconstitute succinate-cytochrome c reductase activity (Davis and Hatefi, 1971) suggests that one or both of these small molecular weight components are necessary for linkage of succinate dehydrogenase to the bc_1 segment of the respiratory chain. Preliminary cross-linking experiments with complex II indicate that the 13 500 molecular weight component is the primary binding site of succinate dehydrogenase with the membrane (Merli, unpublished results). It is possible that either this component or the 7000-dalton component is the cytochrome *b* seen in complex II (Davis et al., 1973) but such an identification must await purification of these polypeptides in native form.

With resolution of the polypeptides in complex II, the composition of the whole succinic oxidase system has now been carefully examined in our laboratory and some interesting generalizations about the components involved in this major sequence of the respiratory chain can be made. Succinic oxidase includes at least 20 different polypeptides (4 in succinate-coenzyme Q reductase, 9 in reduced coenzyme Q-cytochrome c reductase[1] (Bell and Capaldi, 1976) and 7 in cytochrome *c* oxidase (Downer et al., 1976)). Of these, half have molecular weights below 20 000 and five have molecular weights below 10 000. This preponderance of small molecular weight polypeptides as yet appears to be unique to the mitochondrial membrane.

Four of the twenty polypeptides are synthesized on mitochondrial ribosomes while the remainder are made in the cytoplasm (Schatz and Mason, 1974). Two of the components, cytochrome *b* of complex II and subunit I of cytochrome *c* oxidase, are particularly hydrophobic with polarities well below 40% (Bell and Capaldi, 1976; Briggs et al., 1975), and these are mitochondrial in origin. The challenge now is to determine the arrangements of these 20 components in the mitochondrial inner membrane; hopefully, this will lead to an understanding of the mechanism of electron transfer in the succinic oxidase segment of the respiratory chain.

[1] Recent studies have identified a polypeptide (IX) of molecular weight 4400 as a component of complex III in addition to those discussed in Bell and Capaldi (1976).

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References

Bell, R. L., and Capaldi, R. A. (1976), *Biochemistry 15,* 996.

Briggs, M. M., Kamp, P. F., Robinson, N. C., and Capaldi, R. A. (1975), *Biochemistry 14,* 5123.

Capaldi, R. A. (1974), *Arch. Biochem. Biophys. 163,* 99.

Capaldi, R. A., Bell, R. L., and Branchek, T. (1977), *Biochem. Biophys. Res. Commun. 74,* 425.

Coles, C. J., Tisdale, H. D., Kenney, W. C., and Singer, T. P. (1972), *Physiol. Chem. Phys. 4,* 301.

Davis, K. A., and Hatefi, Y. (1971a), *Biochem. Biophys. Res. Commun. 44,* 1338.

Davis, K. A., and Hatefi, Y. (1971b), *Biochemistry 10,* 2509.

Downer, N. W., Robinson, N. C., and Capaldi, R. A. (1976), *Biochemistry 15,* 2930.

Hatefi, Y. (1976), in The Enzymes of Biological Membranes, Vol. 4, Martinosi, A., Ed., New York, N.Y., Plenum Press, pp 1-42.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951), *J. Biol. Chem. 193,* 6328.

Schatz, G., and Mason, T. L. (1974), *Annu. Rev. Biochem. 43,* 51.

Singer, T. P., Salach, J., Hemmench, P., and Ehrenberg, A. (1971), *Methods Enzymol. 18B,* 416.

Smith, R. J., and Capaldi, R. A. (1977), *Biochemistry 16,* 2629.

Spackman, D. H., Stein, W. H., and Moore, S. (1958), *Anal. Chem. 30,* 1196.

Swank, R. T., and Munkres, K. D. (1971), *Anal. Biochem. 39,* 462.

Weber, K., and Osborn, M. (1969), *J. Biol. Chem. 244,* 4406.

Williams, J. R. (1964), *Arch. Biochem. Biophys. 107,* 537.

Ziegler, D., and Rieske, J. (1967), *Methods Enzymol. 10,* 231.