# CHANGES IN ORDER OF MIGRATION OF POLYPEPTIDES IN COMPLEX III AND CYTOCHROME c OXIDASE UNDER DIFFERENT CONDITIONS OF SDS POLYACRYLAMIDE GEL ELECTROPHORESIS

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SUMMARY: The order of migration of polypeptides in both cytochrome c oxidase and ubiquinone cytochrome c reductase has been found to differ depending on the gel conditions used. Thus the nomenclatures or numbering systems being used for the subunits of these membrane complexes by workers using Weber-Osborn gels is not the same as that being used in laboratories which use the Swank-Munkres or Fairbanks gel procedure.

SDS† polyacrylamide gel electrophoresis as yet provides the only convenient way of separating the polypeptides of a multicomponent complex for identification. The many approaches to this technique differ in the way the sample is dissociated, in the way the gel is polymerized, and in the buffer system used for electrophoresis. Among the various methods, the procedures of Weber and Osborn (1), Swank and Munkres (2), and Fairbanks et al. (3) have been used most commonly to study proteins in the mitochondrial inner membrane (4-8).

Most polypeptides migrate on gels as a function of their molecular weight, and the order of migration of a mixture of components is then the same no matter which of the gel techniques is used (1,9). However, several membrane proteins have been found to migrate anomalously, either because they bind more SDS than normal or because they are not unfolded by the denaturing detergent (10-12). These are properties which may be affected by the gel conditions, and it cannot be relied upon that the order of migration of polypeptides in membrane bound complexes is the same in all gel procedures. As we show here, there are clear differences in the order of migration of

† Abbreviations used in this paper are following: SDS, sodium dodecyl sulfate; Gdn HCl, guanidine hydrochloride.

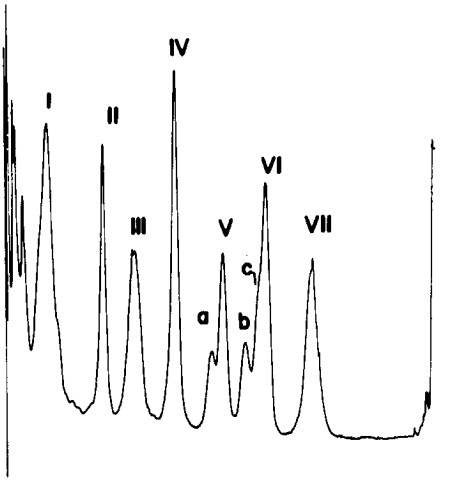


Figure 1: The polypeptide profile of beef heart cytochrome c oxidase run in the Swank-Munkres gel system on a 12.5% polyacrylamide gel (acrylamide-bisacrylamide 10:1). The enzyme was dissociated by incubating at 37°C for 2 hr in 3% SDS and 10 mM β-mercaptoethanol and 100 µg of this sample was applied to the gel. The subunits of cytochrome c oxidase are labeled I - VII. Several impurities were resolved by the gel. These are three low molecular weight components labeled a, b, and c, and small amounts of several large molecular weight impurities which are concentrated near the top of the gel.

subunits in both cytochrome c oxidase and ubiquinone cytochrome c reductase (complex III) under different gel conditions and this may be true for many other membrane systems.

MATERIALS AND METHODS: Beef heart mitochondria were isolated as described by Crane et al. (13). Complex III was prepared as described by Rieske (14). Samples of cytochrome c oxidase were prepared by various methods including procedures described by Yonetani (16), Sun et al. (17) and Kuboyama et al. (18). One sample of cytochrome c oxidase, prepared by the method of Kuboyama et al. (18), was the kind gift of Dr. Tsoo E. King of the State University of New York in Albany.

Protein concentrations were determined by a modification of the method of Lowry et al. (19) as described elsewhere (7), using bovine serum albumin as a standard.

Several different approaches to SDS polyacrylamide gel electrophoresis were used. Samples of cytochrome c oxidase and complex III were electrophoresed on 10% gels (acrylamide monomer) following exactly the procedures described by Weber and Osborn (1) and Fairbanks et al. (3). Both complexes were also run on Swank-Munkres type gels as described previously (7). Gels were fixed and stained as described by Downer et al. (7). The fixing step is particularly important for the staining of subunit III in cytochrome c oxidase.

TABLE I

Estimates of the Molecular Weight of the Subunits of Cytochrome c Oxidase by Different Gel Procedures

| Subunits | Weber-Osborn | Swank-Munkres | Fairbanks | MW on 6 M Gdn HCl Columns (taken from ref. 23) |
|---|---|---|---|---|
| I | 38000 | 35300 | 33000 | |
| II | 19000 | 25200 | 21300 | |
| III | 25000 | 21000 | 19000 | |
| IV | 13800 | 16200 | 14000 | 17000 |
| V | 6000 | 12100 | 12500 | 12500 |
| VI | 8600 | 6700 | 8500 | 9700 |
| VII | 4300 | 3400 | 4900 | 5300 |

Two dimensional gel electrophoresis was performed by a modification of the method of Wang and Richards (20) as detailed in the figure legends. Conditions used to dissociate the complex for electrophoresis are also described in the figure legends.

RESULTS AND DISCUSSION: The polypeptide profile of cytochrome c oxidase run on the Swank-Munkres gel system is shown in Fig. 1. Seven major bands were resolved, each representing a different subunit (7). These are numbered I through VII in the reverse of the order in which they migrated through the gel, i.e., subunit I is the largest and subunit VII the smallest polypeptide. Subunit III stains poorly with Coomassie blue. However this component is present in stoichiometric amounts with other subunits as judged by [$^{35}$S] DABS labeling of SDS dissociated enzyme (Downer N.W. and Capaldi R.A. unpublished results). Molecular weights estimated for each subunit are listed in Table I. Small amounts of three low molecular weight impurities were also resolved (labeled a, b and c). These impurities have been found in enzyme prepared by

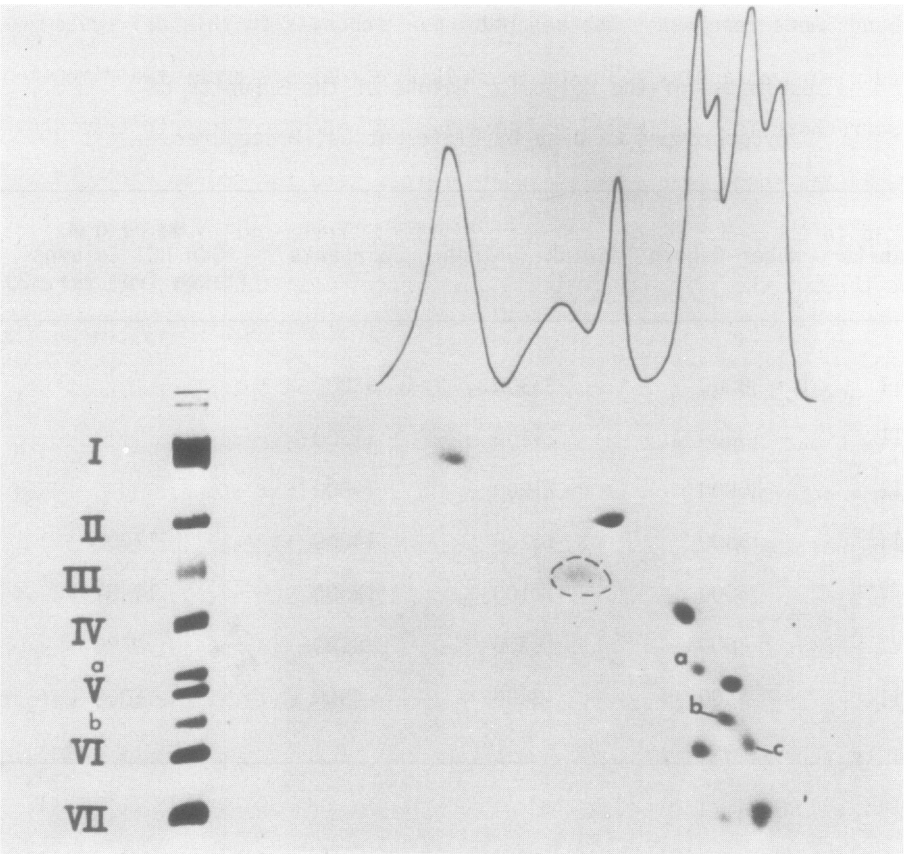


Figure 2: Two dimensional SDS polyacrylamide gel electrophoresis showing the relationship of bands observed in Weber-Osborn gels with those resolved in the Swank-Munkres gel system. Cytochrome c oxidase was dissolved in 3% SDS and 10 mM β-mercaptoethanol at 37°C for 2 hr. 150 μg of sample applied to a 10% polyacrylamide gel (acrylamide-bisacrylamide 40:1) and was electrophoresed in the Weber-Osborn buffer system. The separation of bands obtained is shown by the densitometric trace in the upper part of the figure. For the second dimension an unstained gel similar to that shown was fused to a slab made with 12.5% polyacrylamide (acrylamide-bisacrylamide 10:1) and polypeptides were electrophoresed through the slab in the Swank-Munkres buffer. The migrations of components from the tube gel were compared with a sample of purified cytochrome c oxidase at the left hand side of the slab.

any of the standard isolation procedures. Evidence that they are really impurities and not integral components of the enzymic complex has been presented elsewhere (7).

The polypeptide profile of cytochrome c oxidase run on the Weber-Osborn gel system is shown by the gel trace in the upper portion of Fig. 2. Again

seven bands were resolved. The separation of subunits in this gel system was compared with that achieved in the Swank-Munkres gel system by two dimension gel electrophoresis. An unstained but otherwise identical gel to that shown in Fig. 2, was fused to a slab gel with agarose, and the polypeptides first separated in the Weber-Osborn gel were electrophoresed out of the cylindrical gel and through the slab gel in the Swank-Munkres system (lower portion of Fig. 2). It can be seen that subunit III (using the numbering system obtained from the Swank-Munkres gels) runs behind subunit II on the Weber-Osborn gel as a very broad band. The gel shown in Fig. 2 and its companion applied to the slab, contained 150 μg of protein in order to detect subunit III. This component was not seen with the amount of enzyme usually applied to gels, i.e., 100 μg or less. Another change in migration is that subunit V ran ahead of VI on the Weber-Osborn gel. Subunit VI migrated with approximately the same molecular weight on both gel systems. Subunit V ran with a molecular weight of 6000 on Weber-Osborn gels and 12,100 on Swank-Munkres gels. The molecular weight of subunit V obtained by gel filtration in Gdn HCl was 12,500 (21). Clearly then, it is polypeptide V which migrates differently on the two gel systems.

There are important differences in the resolving power of Swank-Munkres and Weber-Osborn gels particularly for small molecular weight polypeptides and this is clearly demonstrated by the two dimensional gel in Fig. 2. On Swank-Munkres gels, subunit IV, V and VII and impurities a and b are each resolved into separate bands on the gel and only subunit VI and impurity c run as overlapping bands. On Weber-Osborn gels subunits IV through VII and the three impurities (a,b and c) run very close together as four overlapping bands and there is more than one component contributing to each of the peaks seen in the gel scan.

Some laboratories have used the Fairbanks gel procedure to separate the polypeptides of cytochrome c oxidase (5,22) and therefore the resolution of components in the gel system was compared with that obtained on the Swank-

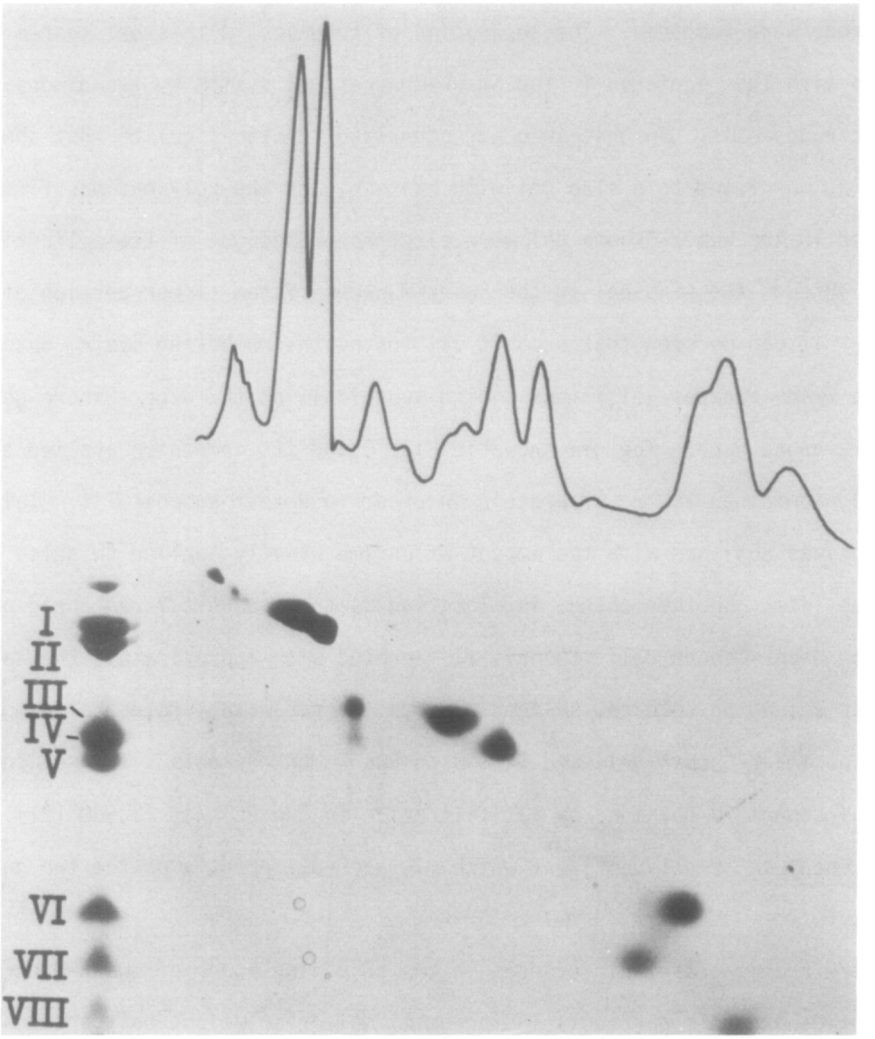


Figure 3: Two dimensional SDS polyacrylamide gel electrophoresis showing the relationship of the bands in complex III as separated on Weber-Osborn gels and Swank-Munkres gels. Complex III was dissociated in 3% SDS and 10 mM β-mercaptoethanol by incubating at room temperature for 2 hr and 100 μg of this sample was applied to a tube gel and electrophoresed in the Weber-Osborn buffer system. The separation of bands obtained is shown by the densitometric trace in the upper part of the figure. Polypeptide separated in this gel were electrophoresed into a slab gel and identified by the same procedures used for cytochrome c oxidase as described in the legend of Fig. 2.

Munkres system. The order of migration of subunits was found to be the same in the two gel systems and the molecular weights for individual subunits were similar (Table I).

TABLE II

Estimates of the Molecular Weight of the Polypeptides of Complex III by Different Gel Procedures

| Component | Weber-Osborn | Swank-Munkres |
|---|---|---|
| I | 46600 | 45500 |
| II | 41500 | 44500 |
| III | 38000† | 28600 |
| IV | 30000 | 26700 |
| V | 23300 | 24600 |
| VI | 12700 | 15000 |
| VII | 14300 | 9000 |
| VIII | 5600 | 4800 |

† The molecular weight of polypeptide III depends on the conditions used to dissociate Complex III as described in the text.

A comparison of the migration of the polypeptides of complex III on Weber-Osborn gels and Swank-Munkres gels was also made. This complex has been resolved into eight different polypeptides on Swank-Munkres gels (8). Eight bands were also seen on Weber-Osborn gels as shown by the gel trace in Fig. 3 but the order of migration of components was not the same. On Weber-Osborn gels, polypeptide VII (a component associated with cytochrome b) (8) ran behind polypeptide VI, (a component which copurifies with cytochrome $c_1$) rather than ahead of it as in the Swank-Munkres gel system (Fig. 3).

From Table II it can be seen that polypeptide III, (cytochrome b) (8) also migrated very differently on the two gel systems. It ran with a moleuclar weight of 28,600 on Swank-Munkres gels. On Weber-Osborn gels the migration

of polypeptide III depended on the conditions used to dissociate the sample for electrophoresis. It ran with a molecular weight of 41,500 and very close to polypeptides I and II when the enzymic complex was dissociated at room temperature for 2 hr; at 38,000 when the enzymic complex was dissociated at 37° C for 2 hr [as recommended by Weber and Osborn (1)] and at 32,000 in complex III dissociated by heating in SDS at 100° C for 3 min. These results suggest that cytochrome b is not denatured in SDS but is unfolded by heating or when 8 M urea is included along with SDS in the dissociation buffer (as in the Swank-Munkres gel system). Additional evidence that cytochrome b resists denaturation in SDS comes from the studies of Yu et al. (23) who have been able to purify the protein with non-covalently bound heme attatched and showing an absorbance maximum of 562 nm which is close to the absorbance maximum in the native protein (23).

In summary then, our data show that the migration of the polypeptides in both cytochrome c oxidase and complex III is different under different conditions of gel electrophoresis and this in part explains the different subunit structures that have been reported for both these complexes. Most significantly, the order of migration of subunits in both electron transport complexes is different under different gel conditions. This is an important finding because in the absence of a known function for many of the components, the subunits of both cytochrome c oxidase and complex III are almost always referred to by number based on their migration on gels. Our results indicate that the numbering system being used by workers employing Weber-Osborn gels is not the same as that being used in laboratories which use the Swank-Munkres or Fairbanks gel procedures. The analyses presented here will however facilitate comparison between results obtained by the different gel procedures. Also, the two dimensional gel systems described provide a way to optimize the separation of subunits for identification and should be useful for labeling and other modification studies of cytochrome c oxidase and complex III.

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