The Use of Orthacryl Two-Dimensional Polyacrylamide Gel Electrophoresis to Identify and Compare the Subunit Polypeptides of Bovine Heart and Yeast Cytochrome c Oxidases*

(Received for publication, May 18, 1978, and in revised form, July 7, 1978)
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SUMMARY

A two-dimensional electrophoretic method which takes advantage of the "migration anomalies" experienced by some polypeptides on gels of different porosities has been successfully used to resolve the seven subunit polypeptides of yeast cytochrome c oxidase and the nine polypeptides associated with bovine cytochrome c oxidase. The two-dimensional maps provided by this method reveal clear differences between those two cytochrome c oxidases.

Recent studies have revealed that the protein component of cytochrome c oxidase from a variety of eukaryotes is an oligomer consisting of a number of different polypeptide subunits (1-15). Although the cytochrome c oxidases from Saccharomyces cerevisiae and Neurospora crassa have been convincingly demonstrated to contain seven different polypeptide subunits by the criteria of size, charge, antigenicity, and amino acid composition (2, 4, 16), the subunit composition of the enzyme from other eukaryotic species is less certain. For example, the subunit composition of bovine heart cytochrome c oxidase is still subject to some debate and has been reported by various authors to be anywhere from 2 to 10 different polypeptides (6-15). Part of the difficulty in identifying the subunits of this and other eukaryotic cytochrome c oxidases has been the almost exclusive reliance on SDS-polyacrylamide gel electrophoresis at one acrylamide concentration. While this technique is especially convenient for separating and analyzing the polypeptides of membranes and membrane-bound enzymes, such as cytochrome c oxidase, it suffers from the limited resolution inherent in a one-dimensional separation procedure and is inadequate for proteins which, like cytochrome c oxidase, are composed of some subunit polypeptides which exhibit "anomalous migration" (2, 14) and which have similar apparent molecular weights. In order to avoid these difficulties we have previously employed isoelectric focusing in polyacrylamide gels containing Triton X-100 and urea in parallel with SDS-polyacrylamide gel electrophoresis for an analysis of the subunit polypeptides of cytochrome c oxidase from Saccharomyces cerevisiae and the assessment of isolation procedures for the large scale purification of subunits for chemical and immunological studies (2, 16). Although successful, this approach is inconvenient and wasteful of material. It is therefore of limited usefulness for studies, such as those pertaining to the structure of mammalian tissue culture cell cytochrome c oxidases or to the assembly of cytochrome c oxidase in wild type or respiratory-deficient yeast cells, in which the amount of enzyme available may be limiting. Attempts to facilitate the analytical resolution of the subunits of yeast and bovine heart cytochrome c oxidase by combining Triton X-100-urea gel isoelectric focusing with sodium dodecyl sulfate-polyacrylamide gel electrophoresis into a two-dimensional system analogous to that described by O`Farrell (17) and modified by Ames and Nikaido (18) have been of limited success thus far due to the partial loss of some of the smaller subunits between first and second dimensions and the isoelectric precipitation of the more hydrophobic subunits (I, II, and III) of each enzyme in the first dimension gel.2

In this paper we describe an alternative two-dimensional method which is based on the migration anomalies experienced by some polypeptides during electrophoresis in gels of varying porosity and which allows for the complete resolution and recovery of all polypeptides of yeast and bovine heart cytochrome c oxidase.

EXPERIMENTAL PROCEDURES

Preparation of Cytochrome c Oxidase

Yeast cytochrome c oxidase (9.4 nmol of heme a per mg of protein) was isolated by previously published procedures as modified by George-Nascimento et al.3 Bovine cytochrome c oxidase (19.1 nmol of heme a per mg of protein) was prepared according to Kuboyama et al. (11).

Two-dimensional Gel Electrophoresis

Two-dimensional SDS-polyacrylamide gel electrophoresis was performed in a discontinuous buffer system (2) using 12.4% T - 3.13% C for the first dimension and 12.4, 16.4, or 20.4% T - 3.13% C for the second dimension.4 The first dimension was composed of the following buffer components: upper reservoir buffer, pH 8.94, 0.052 M Tris, 0.052 M HCl, 0.30 M Na3PO4, 0.059 M NaF, 0.052 M EDTA, 0.5% SDS; lower reservoir buffer, pH 8.94, 0.052 M Tris, 0.052 M HCl, 0.30 M Na3PO4, 0.059 M NaF, 0.052 M EDTA, 0.5% SDS; lower gel buffer, pH 8.8, 0.048 M Tris, 0.052 M EDTA, 0.052 M glycine. The second dimension contained the same buffer components except for upper gel buffer which was omitted since no stacking gel was used.

First Dimension—Electrophoresis in the first dimension was carried out in cylindrical gels (0.3 x 10 cm) cast in gel tubes which had an inner diameter of 0.3 cm and an outer diameter of 0.6 cm. Details concerning the preparation of resolving and stacking gels have been

* This research was supported by Grant GM21800 from the National Institutes of Health and Grant 75779 from the American Heart Association. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
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1 The abbreviation used is: SDS, sodium dodecyl sulfate.
2 R. O. Poyton and D. George, unpublished observations.
3 C. George-Nascimento, E. McKemnie, and R. O. Poyton, manuscript in preparation.
4 The nomenclature of Hjerten (19) is used to describe gel composition.
5 %T = grams of acrylamide plus grams of bisacrylamide per 100 ml of solution.
6 %C = 100 x g of bisacrylamide/T.
FIG. 1. Two-dimensional electrophoresis of yeast and bovine heart cytochrome c oxidases. First dimension gels were 12.4% T; second dimension gels were 12.4% T (top), 16.4% T (middle), or 20.4% T (bottom). A, yeast cytochrome c oxidase; B, composite derived from bovine heart (C) and yeast enzymes (A) dissociated under identical conditions and electrophoresed in parallel first and second dimension gels. Both first and second dimension gels were made from the same gel pouring and second dimension gels for the two enzymes at each of the three second dimension gel concentrations were run at the same time in the same electrophoresis unit (20). Gels were aligned by using both the top of the first dimension gel (arrow) and the top of the second dimension gel. Those spots identified with a Y refer to subunits of the yeast enzyme; those with a B refer to polypeptides of the bovine enzyme. C, bovine heart cytochrome c oxidase. The dotted lines and slash marks on the bottom gel indicate boundaries of components which were diffuse on the photograph of the gel.

Materials

Acrylamide and bisacrylamide were obtained from BDH (Gallard-Schlesinger). SDS (Sigma Chemical Co.) was recrystallized from 95% ethanol as described elsewhere (2).
RESULTS AND DISCUSSION

Two-dimensional Analysis of Yeast Cytochrome c Oxidase—Previous studies have demonstrated that subunits I (YI) and V (YV) of yeast cytochrome c oxidase exhibit "anomalous migration" on SDS-polyacrylamide gels (2). At high gel concentrations (11% T, 3% C, and above) necessary to resolve the smallest subunit, YIVII, from the dye front, the "anomalous migration" of subunit YI is not problematic since it does not co-migrate or reverse its position in the gel with any of the other subunits. However, as can be seen by comparing the marker tracts on the 12.4, 16.4, and 20.4% T second dimension gels (Fig. 1A), the "anomalous migration" of subunit YV is confusing since at 12.4% T, it co-migrates with subunit YIII, at 16.4% T, it migrates between subunits YIV and YVII, and at 20.4% T, it co-migrates with subunit YVII. On gels of higher concentration than 20.4% T or on 20.4% T gels which have been run for more than 150 min after the dye front has migrated off the gel bottom, subunit YV reverses position (i.e. low Rf) with subunit YIVII. These observations demonstrate the difficulty in identifying subunit YV by one-dimensional SDS-polyacrylamide gel electrophoresis and suggest the utilization of a two-dimensional gel system which takes advantage of "anomalous migration." As seen in Fig. 1A, the unequivocal identification of subunit YV can be achieved by merely varying the %T between first and second dimensions. Optimal resolution of all seven subunits is achieved by the combination of 12.4% T with 20.4% T in the first and second dimensions, respectively.

Two-dimensional Analysis of Bovine Heart Cytochrome c Oxidase—Recent studies have indicated that the apparent number of polypeptides associated with bovine cytochrome c oxidase and their order of migration on SDS-polyacrylamide gels is dependent on the gel system (7, 22). While it has been suggested that the variability may be the result of the different buffer systems employed, an examination of Fig. 1C reveals that the acrylamide concentration and hence gel porosity is more likely the cause. Indeed it is clear from an examination of the marker tracts in Fig. 1C that the number of polypeptides is dependent on the gel concentration and is 7, 6, and 9 on 12.4, 16.4, and 20.4% T gels, respectively. Ac can be seen from the two-dimensional behavior of the polypeptides, the decrease in apparent number of polypeptides on going from 12.4% T to 16.4% T gels results from the "anomalous migration" of subunit BIII and its co-migration with subunit BIV on 16.4% T gels. The increase in apparent number of polypeptides on going from 12.4% T to 20.4% T gels results from the reversal of migration order to subunits BIII and BIV from the improved resolution in that region of the gel where polypeptides BIII through BVI migrate.

Quantitative Recovery from Two-dimensional Gels—In order to determine if this technique permits the recovery of all of the protein components from the second dimension gels and to assess its usefulness for problems requiring the complete recovery of sample, we have determined the recovery of [3H]leucine-labeled yeast cytochrome c oxidase. Ac can be seen from Table I, the relative amounts recovered from first (12.4% T) and second dimension gels (20.4% T) are essentially identical and quantitative. This is in agreement with the observation that the first dimension gels which had contained either the yeast or bovine heart enzyme prior to electrophoresis into the second dimension never showed any Coomassie blue staining bands after electrophoresis into the second dimension gel. Thus, it appears that the protein components of both enzymes can be quantitatively transferred from first to second dimension gels and quantitatively recovered from second dimension gels. This technique should therefore be quite useful for studies, such as those directed at the stoichiometry or assembly of polypeptide subunits, which require the quantitative recovery of starting sample.

Comparison of Yeast and Bovine Cytochrome c Oxidases—In trying to determine the degree of similarity between yeast and bovine heart cytochrome c oxidases, it is interesting that the maximal number of polypeptides associated with the beef enzyme either by orthacryl two-dimensional gel electrophoresis or isoelectric focusing is nine, two more than the number of subunits in the yeast enzyme. Although it has been proposed that the two "extra" polypeptides associated with the bovine heart enzyme are contaminants and that the beef enzyme, like the yeast enzyme, is composed of seven subunits (6, 7, 22), a final decision regarding the subunit composition of the bovine heart enzyme must await more definitive chemical and functional analyses.

Aside from differences in polypeptide number it is clear from a comparison of the two-dimensional polypeptide maps (Fig. 1B) of both enzymes dissociated in SDS and electrophoresed under identical conditions that the behavior of the constituent polypeptides of both enzymes is quite different. Indeed, no subunit from the yeast enzyme co-migrates with any polypeptide constituent of the bovine heart enzyme under all three electrophoretic conditions. These results, together with recent observations which indicate clear cut chemical and immunological differences between the two enzymes, suggest that the subunits of each enzyme are not identical and indicate that some degree of caution must be exercised in using results derived from studies with both enzymes in proposing a generalized structural model for eukaryotic cytochrome c oxidases (cf. Refs. 23 and 24).

CONCLUSIONS

The method described here greatly facilitates the analysis of the subunit polypeptides of multisubunit complexes which, like cytochrome c oxidase, possess some polypeptides which experience migration anomalies in gels of different porosities. It has allowed for the unequivocal identification of the seven subunits of yeast cytochrome c oxidase and the nine polypeptides associated with bovine cytochrome c oxidase and should be most useful for future studies on the structure and assembly of cytochrome c oxidase from the variety of eukaryotes.

REFERENCES

The use of orthacryl two-dimensional polyacrylamide gel electrophoresis to identify and compare the subunit polypeptides of bovine heart and yeast cytochrome c oxidases.

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