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CORE PROTEIN I AS AN ARTIFACT IN COMPLEX III

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Summary

SDS electrophoresis gels of complex III from yeast mitochondria were run after incubation of the enzyme at several different temperatures. It was found that the intensity of the more slowly moving core protein band was substantially affected by the incubation temperature. Four low molecular weight polypeptides were eluted from gels electrophoresed after preincubation of the enzyme at 15° C for 12 hours. These polypeptides were then incubated for 5 minutes at 100° followed by SDS gel electrophoresis. A polypeptide with the same molecular weight as the anomalous core protein was resolved.

SDS gel patterns of complex III isolated from beef heart reveal the existence of eight major polypeptide components including cytochrome b, cytochrome c_1, proteins which copurify with isolated cytochromes b and c_1, a non-heme iron protein, an antimycin binding protein, and two components assigned as core proteins (1). The migration patterns of the two core proteins were found to be anomalous, differing on gels prepared with different concentrations of acrylamide monomer (2). Furthermore the origins of core proteins I and II have not been elucidated.

A similar pattern of behavior is found with highly purified complex III from bakers yeast. However we have observed that the intensity of the largest molecular weight band (core protein I) can be altered by incubations and we present evidence here that this species is an aggregate of lower molecular weight peptides present in the complex.

Methods

Cytochrome b-c_1 complex was prepared from commercial bakers yeast according to the procedure of Siedow et al (3). 0.2 ml of the preparation containing 3.2 mg protein and 9 nmoles protoheme/mg protein was diluted to

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three milliliters in the following solution: 0.1 M potassium phosphate, pH = 7.4, 0.5% cholate, 1.0 mM EDTA. The complex was then pelleted at 100,000 x g for 100 minutes in a Beckman model-L ultracentrifuge using a type 65 rotor at 40,000 rpm. This served to remove unbound detergents used during the purification procedure. The pellet was resuspended in water containing 1% dodecyl-sulphate, 10% glycerol, 50 mM Na_2CO_3, 50 mM dithiothreitol, and 10% 2-mercaptoethanol* and allowed to incubate for 12 hours at 25°. 7.5% Sodium dodecyl-sulphate gels were run according to the procedure of Weber and Osborn (4). Molecular weight determinations were performed using the following standards: bovine serum albumin (68,000), creatine kinase (40,000), chymotrypsinogen (25,700), myoglobin (17,200), and cytochrome c (11,700). Staining and destaining was performed according to the procedure outlined by Swank and Munkres (5).

Pellets were prepared following the procedure outlined above and incubated for 12 hours at either 10°, 15°, or 20°. One additional pellet was prepared and incubated at 25° for 12 hours followed by 100° for 5 minutes. 7.5% Gels were run according to the procedure outlined.

An additional pellet was resuspended in protein dissolving solution and incubated for 12 hours at 15°. Four gels were then overloaded, two with 50 and two with 100 microliter samples of this preparation and electrophoresed as described by Weber and Osborn (4). Upon completion of the run the polypeptides migrating faster than the visible cytochrome c_1 band were processed in the following manner. The appropriate portion of the gel was cut into several pieces using a clean disposable razor. These slices were immersed in liquid nitrogen, frozen, and ground to a fine powder, transferred to a test tube and allowed to incubate for 12 hours at 15° in protein dissolving solution. An aliquot was removed after the 12 hour incubation and further incubated for 5 minutes at 100°. (It is important that the cold temperature be maintained at all times. The protein dissolving solution is stored at 10° until use, added cold and allowed to warm up to 15° during the incubation. Neither pellets nor processed gels are removed from ice during the resuspension procedure.)

After incubation gels were electrophoresed as described (4). These gels were stained, destained, then scanned on a Gilford gel scanning machine at 560 nm with a slit width of 0.01 mm, chart speed of 1 minute per inch and transport speed of 1 cm/minute.

Results

Highly purified complex III from bakers yeast exhibits 7 well defined bands when incubated at 100° prior to electrophoresis on 7.5% SDS gels (Fig. 1). From the measured mobility and interpolation on a semi-log plot prepared from standard proteins, the apparent molecular weights were 45,000, 39,000 (core proteins I and II), 31,000 (cytochrome c_1), and 25,000 (iron sulfur protein). Two polypeptides which copurify with cytochrome c_1 and cytochrome b had apparent weights of 21,000 and 18,000 respectively. By comparison

* This solution was adapted from that used by Marres and Slater (2).

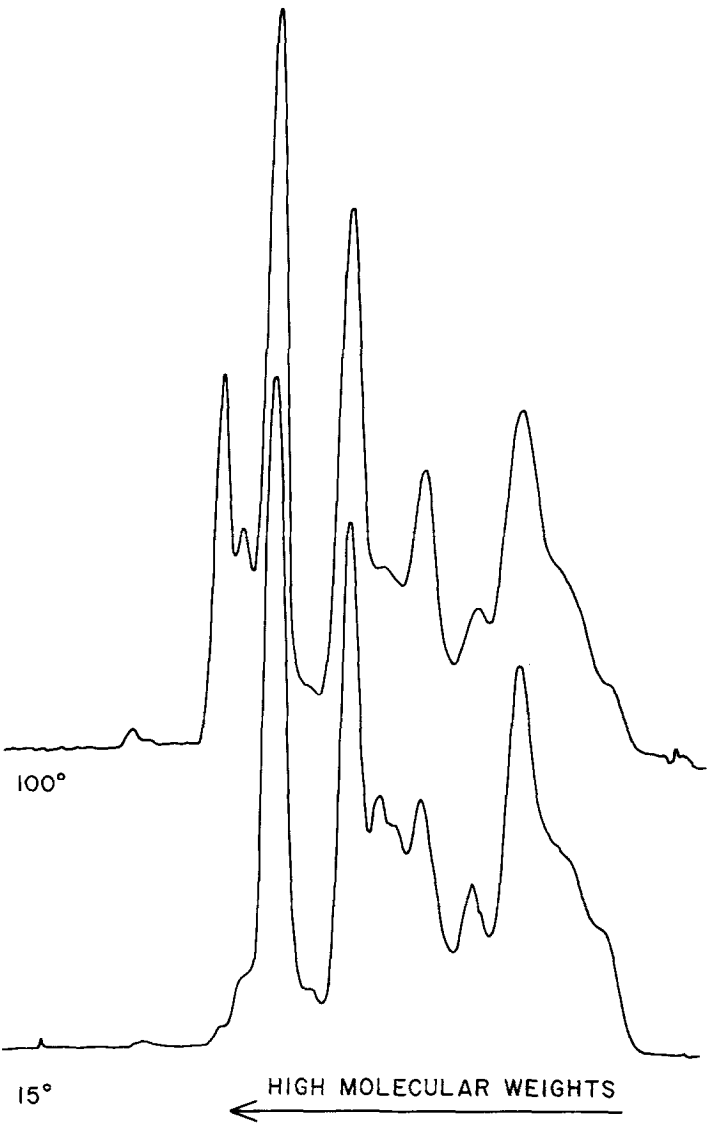

Fig. 1. Scans of gels electrophoresed after incubation at two different temperatures. Upper trace shows position of bands on gel electrophoresed after incubation at 100°. Lower trace shows position of bands on gel electrophoresed after incubation at 15°.

with gel patterns obtained with purified cytochrome b it was found that the major b polypeptide migrated with core protein II on Weber-Osborn 7.5% gels and between cytochrome c_1 and core protein II on Weber-Osborn 10% gels. Marres and Slater (2) have previously reported that the mobility of the major b polypeptide depends strongly on the porosity of the gel. A faint band was

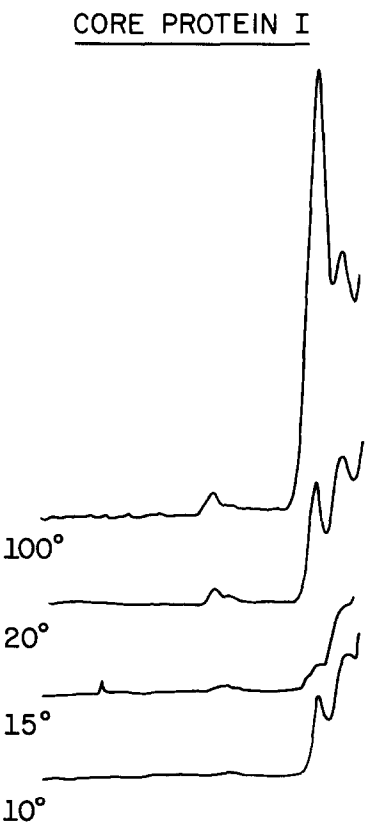

Fig. 2. Intensity of core protein I band following incubation at four different temperatures as shown.

observed between the two core proteins as well as a faint band at 70,000; this may be a result of contamination by succinate dehydrogenase (6). The antimycin binding protein had a weight of 13,500. Gels run after incubation at 15° were found to be virtually free of the polypeptide with a molecular weight of 45,000 while incubation at both 10° and 20° produced intermediate amounts of the species as judged by the density of staining (Fig. 2).

The source of the variable amount of material migrating with an R_f appropriate for 45,000 was established as follows: Complex III was incubated at 15° and the mixture subjected to electrophoresis as usual. The section of gel containing the polypeptides smaller than cytochrome c_1 was isolated and these polypeptides extracted (see experimental section). The extract

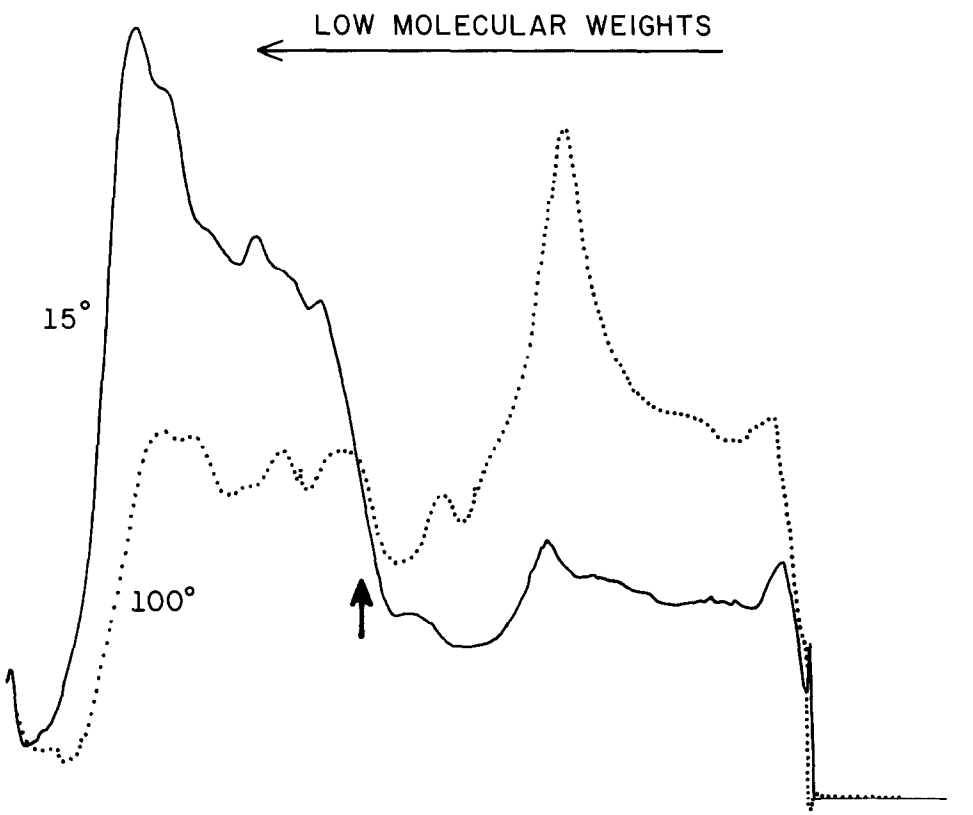

Fig. 3. Scans of gels run after incubation at either 15° or 100° of protein previously recovered from the low molecular weight region.

was divided into two parts, one of which was reincubated at 15° and the other incubated at 100° in the same denaturing solvent. The two samples were electrophoresed once more and the mobility pattern of the polypeptides in the two samples established by staining.

It was found that incubation of the extract at high temperature produced a darkly staining band with a mobility corresponding to the upper core protein (Fig. 3). A second less strongly staining band with an R_f midway between those of the two "core" proteins was also produced by this treatment. Both bands were very faint in the aliquot which had been maintained at 15° during the second incubation. From a comparison of the densitometric traces run on both the high and low temperature gels it was established that the

Table 1: Comparison of the changes in integrated areas in high and low R_f regions after heating at 100°.

Incubation Temperature	% Low Mol. Wt.	% High Mol. Wt.
15°	67%	33%
100°	37%	63%

Percentages in above table calculated after weighing portions of densitometric tracings of gels after two different incubations of recovered protein: data of Fig. 3. The space between the high and low molecular weight regions is indicated by the arrow in Fig. 3.

increase in protein stain observed in the region of low R_f quantitatively accounted for the loss in staining capacity in the region of high R_f (Table 1).

Discussion

After denaturation at high temperatures the SDS gel patterns observed with complex III isolated from bakers yeast are very similar to those observed with the beef heart complex with respect to both number and approximate molecular weight of the individual components. The gel patterns that are observed are commonly interpreted in terms of polypeptides associated with cytochromes b and c_1, the iron-sulfur protein, and two larger molecular weight subunits assigned as core proteins (7,8).

We have found however that the observation of the presence of the largest subunit depends upon the temperature at which the initial denaturing incubations are performed and it is possible to select incubation conditions which do not yield any polypeptides with molecular weights larger than those associated with core protein II. Furthermore when the low molecular weight polypeptides are isolated after low temperature denaturation and electrophoresis and subsequently incubated at 100° substantial amounts of core protein I are produced and the quantity of stain associated with the newly formed large molecular weight material correlates well with the amount of stain which disappears from the low molecular weight region.

These data make it very likely that at least in yeast, core protein I is an artifact produced from one or more of the low molecular weight components

of complex III by incubation at elevated temperatures. The pattern of loss of stain from the low molecular weight region is consistent with the participation of several low molecular weight polypeptides in the formation of "core protein I."

There have been several studies using membrane impermeant and cross linking reagents which purport to characterize the relative disposition of the subunits within the intact complex, and the relationship of the subunits of the inner mitochondrial membrane (8,9). In these studies core protein I has featured predominantly in the interpretation of the observed data. The results reported here require that the results of such experiments be reexamined and alternative interpretations considered.

Acknowledgements

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