TRYPSIN CLEAVAGE OF UBIQUINONE–CYTOCHROME *c* REDUCTASE (COMPLEX III)

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1. Introduction

Recent studies have shown that ubiquinone–cytochrome *c* reductase (complex III) is a multipeptide aggregate containing *b* heme, c_1 heme and a non-heme iron center in the molar ratio 2:1:1 [1–5]. We have been able to resolve the complex into nine different polypeptides with molecular weights ranging from 50 000–4000 [5,6]. At least three polypeptides contain redox groups including a cytochrome *b* apoprotein (mol. wt 31 500 and band III on gels); cytochrome c_1 (29 000, band IV) and the non-heme iron protein (25 000, band V) [1,4,5].

Complex III is almost certainly a structural unit as well as a functional segment of the mitochondrial inner membrane, and we are presently attempting to derive a picture of the arrangement of components in this aggregate. Several different types of probes can be used for such studies including proteolytic enzymes which provide important information about the surface exposure of the component polypeptides.

Studies by Baum et al. [1] have shown that complex III activity is susceptible to proteolysis. Further, these workers found that there was selective cleavage of components by both trypsin and chymotrypsin. Unfortunately their study was completed before highly resolving systems of SDS–polyacrylamide gel electrophoresis were available, and the susceptibility of all of the polypeptides in the complex to proteolytic cleavage was not monitored.

We have examined the effect of trypsin in complex III. Our results confirm and extend the findings of Baum et al. [1] and supplement the data from other approaches currently being used to determine the structure of the ubiquinone–cytochrome *c* reductase segment of the respiratory chain.

2. Materials and methods

Lyophilised trypsin and soybean trypsin inhibitor were both purchased from Worthinton Biochemical Company. Complex III was isolated from beef heart mitochondria by the method of Rieske [7]. Protein concentrations were determined by the method of Lowry et al. [8] using bovine serum albumin as a standard.

Complex III (5 mg/ml), suspended in 0.67 M sucrose, 0.05 M Tris–HCl, 1 mM histidine, pH 8.0, was reacted with trypsin (0.1 mg/ml) at room temperature either without further additions (oxidized form) or in the presence of sodium dithionite (reduced form). Aliquots were removed at various time intervals and the reaction was quenched by adding trypsin inhibitor (4 mg/mg protease) dissolved in the same sucrose–Tris buffer. Each aliquot was examined for duroquinol–cytochrome *c* reductase activity as described previously [5].

Samples for gel electrophoresis, prepared by heating at 100°C in 3% SDS, 3% β-mercaptoethanol, were run on 9% acrylamide gels (0.9% bisacrylamide) in the Swank-Munkres buffer system [9]. Gels were stained and destained as described by Downer et al. [10]. Densitometric traces of the gels were obtained at 550 nm with a Gilford linear scanner attached to a Beckman DU spectrometer using a 5 mm × 10 cm quartz cuvette.

3. Results

The effect of low levels of trypsin (0.02 mg/mg complex III) on the polypeptide profile of oxidized and reduced complex III was monitored by SDS–polyacrylamide gel electrophoresis (fig.1) and this was correlated with the loss of electron transfer activity for both redox states (fig.2). Relatively few polypeptides were cleaved under conditions in which there was extensive loss of activity. Polypeptide IX was found to disappear from the polypeptide profile most rapidly and much faster than activity was lost. Polypeptide V was also cleaved and the disappearance of this component from the gel scan appeared to coincide with the loss of electron transfer activity in both redox states. Other components were not rapidly cleaved by low levels of trypsin as shown in fig.3. However, most were attacked at a significant rate in the presence of 0.1 mg trypsin/mg complex III. Polypeptide I was the most resistant to cleavage and could be clearly identified in the gel profile of samples of complex III which had been incubated with high levels of trypsin at room temperature for 6 h (fig.4a). This component along with all the others was rapidly digested when the complex was denatured and dissociated with 3% SDS before proteolysis (fig.4b).

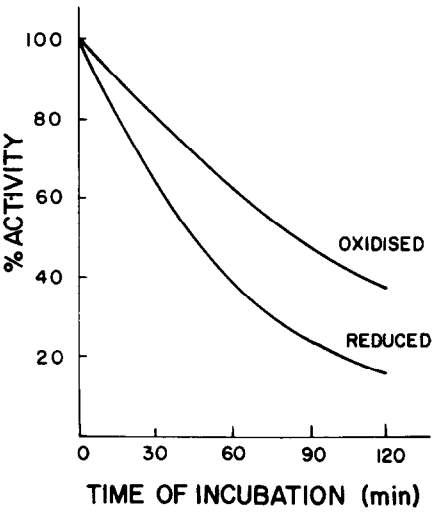

Fig.2. Loss of duroquinol–cytochrome *c* reductase activity for both oxidized and reduced complex III in the presence of trypsin (0.02 mg/mg complex III). The loss of activity when complex III in either redox state was incubated for 2 h at room temperature without proteases was 5–10%.

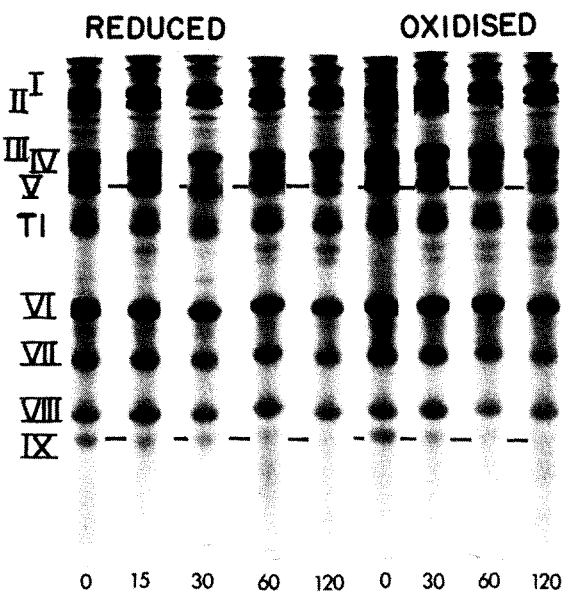

Fig.1. Polyacrylamide gels showing the cleavage of polypeptides in oxidized and reduced complex III by trypsin (0.02 mg/mg complex III) when incubated at room temperature for the lengths of time indicated. Components are labeled by Roman numerals. TI, trypsin inhibitor.

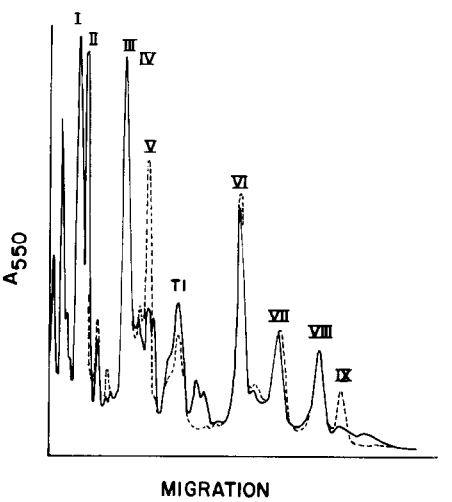

Fig.3. A densitometric trace of a 9% gel of oxidized complex III (solid line) after digestion with trypsin (0.02 mg/mg complex III) for 2 h at room temperature. The dotted line shows the densitometric trace of a control sample of complex III to which trypsin inhibitor had been added before the protease.

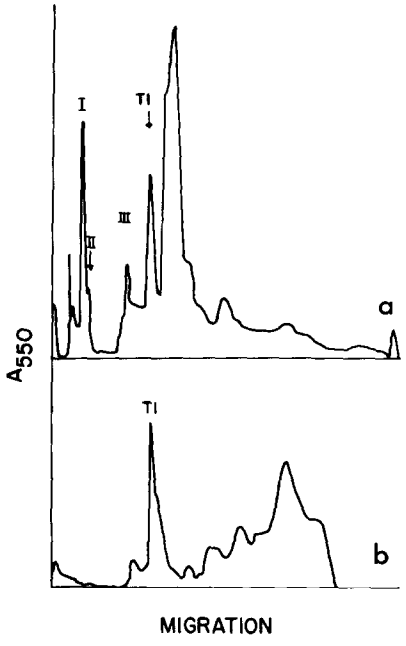

Fig.4. Densitometric traces of complex III after incubation with trypsin (0.1 mg/mg complex III) for 6 h at room temperature (upper trace) and after incubation with the same level of trypsin but for 1 h and after the complex had first been dissociated and denatured with 3% SDS (lower trace).

4. Discussion

The experiments described here were undertaken as part of our long term study of the topology of complex III. We have been conducting labeling studies using [^{35}S]DABS to determine the arrangement of the various polypeptides both in the isolated complex III and as the complex sits in the mitochondrial inner membrane (Bell, R. L. and Capaldi, R. A, in preparation). These studies have provided evidence that most of the polypeptides in complex III are exposed at the surface of the aggregate, in apparent contrast to cytochrome *c* oxidase where one or two of the subunits are not available for reaction with [^{35}S]DABS ([11] Ludwig, B., Downer, N. W. and Capaldi, R. A., in preparation).

It has proved very difficult to obtain unambiguous evidence that polypeptide IX is exposed in complex III from labeling experiments because this polypeptide is small (about 1% of the total protein complex) and incorporates very few counts under our reaction conditions. However, the trypsin digestion studies provide a clear indication that this polypeptide is exposed at the surface of complex III in both redox states.

Our results confirm the original finding of Baum et al. [1] that cleavage of the non-heme iron protein by trypsin occurs at a rate comparable to that at which electron transfer activity is lost. This suggests that the non-heme iron protein is involved directly in electron transfer (which could be tested by a careful EPR study of the effect of proteolytic digestion on the *g* 1.91 center) and/or that the polypeptide is important to the functional integrity of the complex as a whole, a possibility which is more difficult to test directly.

Finally, trypsin cleavage experiments suggest that the conformational change in going from oxidized to reduced complex III is not large. Only the rate of cleavage of V appears to be altered in the different redox states. We have not detected any large changes in the structure of oxidized or reduced complex III from DABS labeling experiments (Bell, R. L. and Capaldi, R. A. unpublished results). It may be then that oxidoreduction involves only small and localized structural rearrangements, one of which must occur in the region of the non-heme iron protein.

Acknowledgements

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References

[1] Baum, H., Silman, H. I., Rieske, J. S. and Lipton, S. H. (1967) J. Biol. Chem. 242, 4876–4887.
[2] Das Gupta, V. A. and Rieske, J. S. (1973) Biochem. Biophys. Res. Commun. 54, 1247–1254.
[3] Hare, J. F. and Crane, F. L. (1974) Sub. Cell. Biochem. 3, 1–25.
[4] Gellefors, P. and Nelson, B. S. (1975) Eur. J. Biochem. 52, 433–443.
[5] Bell, R. L. and Capaldi, R. A. (1976) Biochemistry 15, 996–1001.

[6] Capaldi, R. A., Bell, R. L. and Branchek, T. (1977) Biochem. Biophys. Res. Commun. 74, 425–433.
[7] Rieske, J. S. (1967) in: Methods Enzymol., Vol. X (Estabrook, R. W. and Pullman, M. E. eds) pp. 239–245, Academic Press, New York, London.
[8] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, (1951) J. Biol. Chem. 193, 265–275.
[9] Swank, R. T. and Munkres, K. D. (1975) Anal. Biochem. 39, 462–477.
[10] Downer, N. W., Robinson, N. C. and Capaldi, R. A. (1976) Biochemistry 15, 2930–2936.
[11] Eytan, G., Carrol, R. C., Schatz, G. and Racker, E. (1975) J. Biol. Chem. 250, 8598–8603.