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CATALYTIC ACTIVITY AND ARRANGEMENT OF SUBUNIT POLYPEPTIDES IN RAT LIVER CYTOCHROME *c* OXIDASE AS STUDIED BY PROTEOLYSIS

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Summary

Cytochrome *c* oxidase from rat liver was incubated with various proteinases of different specificities and the enzymic activity was measured after various incubation times. A loss of catalytic activity was found after digestion with proteinase K, aminopeptidase M and a mitochondrial proteinase from rat liver. In each case the decrease in enzymic activity was compared with the changes in intensities of the polypeptide pattern obtained after sodium dodecyl sulfate polyacrylamide gel electrophoresis. The susceptibilities of the subunit polypeptides of the soluble cytochrome *c* oxidase to proteinases were very different. Whereas subunit I was most susceptible, subunits V—VII were rather resistant to degradation. From the relative inaccessibility of subunits V—VII to proteinases it is likely that these polypeptides are buried in the interior of the enzyme complex.

Introduction

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—25]. Whereas the enzyme from *Saccharomyces cerevisiae* and *Neurospora crassa* have been demonstrated to consist of seven different polypeptide subunits [11,16], the subunit composition of the

Abbreviations: SDS, sodium dodecyl sulfate; TLCK, 1-chloro-3-tosylamido-7-amino-L-2-heptanone; TPCK, L-1-tosylamido-2-phenylethyl chloromethyl ketone; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

enzyme from other eukaryotic species is less certain. The number of polypeptide subunits varies between 2 and 10. Our recent studies on the isolation and subunit composition of cytochrome *c* oxidase from rat liver mitochondria revealed seven subunits [23]. To understand the mechanism of their assembly during the biogenesis of mitochondria, it is important to know the organisation of the multiple subunits of cytochrome *c* oxidase.

Comparative studies on the topography of cytochrome *c* oxidase within the inner mitochondrial membrane and the solubilized enzyme have been carried out. The probes included iodination with lactoperoxidase, coupling with *p*-diazoniumbenzenesulfonate, linking of bovine serum albumin and the binding of specific antibodies [26—28].

It is the purpose of the present paper to report studies on the topography of the soluble cytochrome *c* oxidase from rat liver mitochondria using proteinases as probes.

Materials and Methods

Chemicals used were of analytical reagent grade. Trypsin (TPCK treated) and α-chymotrypsin (TLCK treated) from beef pancreas, proteinase K from *Tritirachium album* Limber, and bromelain from pineapple were obtained from Merck, Darmstadt, F.R.G. Aminopeptidase M from pig kidney, elastase from pig pancreas, collagenase from *Clostridium histolyticum*, and papain from *Papaya carica* were purchased from Boehringer Mannheim GmbH, Mannheim, F.R.G. Ficin from fig-tree latex, cytochrom *c* from horse heart and phenylmethylsulfonyl fluoride were from Sigma, St. Louis, U.S.A. Pronase P was a product of Serva, Heidelberg, F.R.G. Proteinase B and carboxypeptidase Y from yeast were kindly supplied by Prof. H. Holzer. The mitochondrial proteinase from rat liver was prepared according to Jusic et al. [29].

Preparation of cytochrome c oxidase from rat liver mitochondria

Submitochondrial particles. Mitochondria were prepared from livers of SIV 50 rats (250—350 g) as described by Loewenstein et al. [30] with the following exception. A 0.07 M sucrose, 0.22 M mannitol and 2 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes) buffer, pH 7.4, was used [31] instead of 0.25 M sucrose in 1 mM Tris-HCl buffer, pH 7.5. Mitochondria were resuspended in the preparation buffer and converted to submitochondrial particles by sonication (Braun Labsonic 1510, micro-tip, three times 10 s) without exceeding 10°C. The particles were pelleted at 18 000 × *g* for 20 min in a Sorvall SS-34 rotor.

Submitochondrial particles suspended in 0.1 M sodium phosphate buffer, pH 7.5, were frozen at −20°C and thawed. The treatment was repeated twice and the yellow-red supernatant was removed by centrifugation at 18 000 × *g*. The precipitate was suspended in 0.2 M sodium phosphate buffer, pH 7.5, at a concentration of 20 mg protein/ml.

Deoxycholate-treated submitochondrial particles. To the frozen and thawed submitochondrial particles solid KCl (final concentration 0.97 M) was added and the pH was adjusted to 7.5 with a 5% NH_3 solution. Then 0.7 mg sodium cholate/mg of protein was added from a 10% (w/v), pH 8.5, stock solution.

After stirring for 20 min the mixture was centrifuged for 30 min at 95 000 × *g*. A yellow-red supernatant (discarded), containing most of cytochromes *b* and *c*, and about 6% of cytochrome *c* oxidase originally present in the submitochondrial particles, and a dark-brown precipitate was obtained. The precipitate was suspended in 0.1 M Tris-HCl buffer, pH 7.5 (protein concentration 20 mg/ml), and solid KCl was added to a final concentration of 1.0 M. Then 0.1 mg deoxycholate/mg of protein was given to the mixture and it was centrifuged for 25 min at 95 000 × *g*. This produced a faint red supernatant (discarded) and a brown precipitate. The precipitate was suspended in 0.1 M Tris-HCl buffer, pH 7.5 (protein concentration 13.5 mg/ml).

Soluble cytochrome c oxidase. To the deoxycholate-treated submitochondrial particles, solid KCl was added to a final concentration of 1.0 M; then 0.5 mg deoxycholate/mg of protein was given to the mixture and it was centrifuged for 25 min at 100 000 × *g*. This produced a brownish-green supernatant and a brownish-grey sediment (discarded). To this supernatant an equal volume of a 2.5% (w/v) sodium cholate solution, pH 8.5, was given, and then saturated $(NH_4)_2SO_4$ (neutralized) was added to make the solution 25% saturated. This mixture was centrifuged for 20 min at 35 000 × *g*. This produced a brown precipitate with a slight green tinge (discarded) and a brownish-green supernatant. The $(NH_4)_2SO_4$ concentration of the supernatant was raised to 38% saturation. After immediate centrifugation for 20 min at 35 000 × *g* brownish dark-green sediment with an oily appearance and a faint yellow supernatant was obtained. The sediment was dissolved in 0.1 M sodium phosphate buffer, pH 7.5, containing 1.5% sodium cholate and dialyzed against the same buffer for 15 h with three changes of the buffer solution. The dialysate was centrifuged at 15 000 × *g* for 20 min. The supernatant had a protein concentration of 11.9 mg/ml and was designated as 'deoxycholate-soluble cytochrome *c* oxidase'.

Proteinase treatment of the soluble cytochrome c oxidase

Cytochrome *c* oxidase solubilized with sodium deoxycholate (heme *a* content of 10.2 nmol/mg of protein) was incubated with the various proteinases in 50 mM sodium phosphate buffer, pH 7.5, at 0° C or 25° C for different times indicated in the legends to tables and figures. Aliquots were removed for the assay of cytochrome *c* oxidase activity or for preparations of samples for SDS-polyacrylamide gel electrophoresis.

Assay of cytochrome c oxidase activity

After incubation with proteinases, 10-μl aliquots of the reaction mixture were added to 100—300 μl of 50 mM sodium phosphate buffer, pH 7.5. Immediately after this dilution the cytochrome *c* oxidase activity was measured at 546 nm with a spectrophotometer thermostated at 25° C by following the oxidation of ferrocytochrome *c*. The reaction was initiated by the addition of 50 μl ferrocytochrome *c* (10 mg/ml in 10 mM potassium phosphate buffer, pH 7.1) to the solution of enzyme (20—50 μl) and 900 μl of 0.5% (v/v) Tween 80 in 10 mM sodium phosphate buffer, pH 7.5. The initial reaction rate (over a period from 0 to 30—60 s) was measured according to the method of Smith and Conrad [32]. The remaining activity of proteinase-treated cytochrome *c* oxidase was expressed as percentage of the activity of untreated cytochrome *c* oxidase.

SDS-polyacrylamide gel electrophoresis

Aliquots of 10 μl of the incubation mixtures consisting of soluble or membrane-bound cytochrome *c* oxidase and the serine proteinases were added to 3 μl of a 410 mM solution of phenylmethylsulfonyl fluoride in Me_2SO, and left at room temperature for 20 min. In the case of aminopeptidase M 5 μl of 14.5 mM EDTA was used to stop the reaction. The samples were then dissociated by adding 10—20 μl of dissociating buffer containing 12.5% (w/v) SDS, 6.5% (v/v) 2-mercaptoethanol, 40% (v/v) glycerol in 0.1 M sodium phosphate buffer, pH 7.0, and incubated at 37°C, for 90 min. Usually about 100 μg of protein was applied to the gel. In most cases electrophoresis was performed with 3-mm vertical slab gels (8 cm × 8 cm) containing 0.5% SDS, 10% glycerol and 15% total acrylamide with an acrylamide/bisacrylamide ratio of 32 : 1 in Tris-HCl, pH 8.8. The electrode buffer was similar to that described by Laemmli [33]. After about 2 h run at 40 mA per slab gel, gels were stained with 0.2% Coomassie blue containing 15% trichloroacetic acid in 50% methanol solution for 1 day and destained with methanol/water/acetic acid (5 : 5 : 1, v/v) solution. In order to check the occurrence of any anomalous migration behaviour of the various polypeptides of this enzyme, and furthermore to identify each subunit correctly, the electrophoresis was carried out with 10%, 12.5% and 20% polyacrylamide gels as well as with 10—20% step gels [34] with two kinds of acrylamide/bisacrylamide ratios (10 : 1 and 32 : 1) in each. In addition, polyacrylamide gel electrophoresis in SDS-8 M urea as described by Downer et al. [13] was also carried out. The gels were scanned at 570 nm with a Gilford spectrophotometer with a linear transport attachment. The scanning speed was 2.0 cm/min and the optical slit-width 0.25 mm. The amount of total protein added to the gels was in the range where the stained protein bands were linear with at least up to 30 μg of protein per band. For duplicate samples electrophoresed, the area under the peaks on the densitometric traces, determined by planimetry, agreed within 10%. When proteinase-treated and untreated samples were compared, changes in peak area of over 15% were therefore considered significant.

Protein concentration was determined by the method of Lowry et al. [35] and heme *a* content was estimated by the pyridine hemochromogen difference spectrum according to the method of Williams [36].

Results

The effect of proteinases of different specificities on the catalytic activity of cytochrome *c* oxidase from rat liver mitochondria was studied. Incubations were carried out at 0°C and the activity of cytochrome *c* oxidase was determined at different time intervals. As shown in Table I there are two groups of proteinases differing in respect to their action on cytochrome *c* oxidase activity. Essentially no marked changes in cytochrome *c* oxidase activity were observed with trypsin, α-chymotrypsin, elastase, collagenase, ficin, and papain even after 25 h of incubation. However, the action of bromelaine, yeast proteinase B, yeast carboxypeptidase Y, pronase P, proteinase K, aminopeptidase M and the mitochondrial proteinase on cytochrome *c* oxidase resulted in a significant loss of enzymic activity, particularly between 10 and

TABLE I

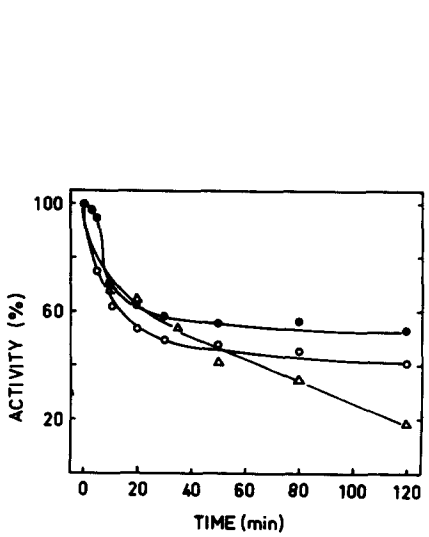
ACTIVITY OF CYTOCHROME *c* OXIDASE AFTER INCUBATION WITH VARIOUS PROTEINASES

0.24 mg of deoxycholate-solubilized cytochrome *c* oxidase of 10.2 nmol heme *a*/mg of protein were incubated in a total volume of 70 μl of 50 mM sodium phosphate buffer, pH 7.5, at 0°C with the various proteinases indicated in the first column. After various times of incubation, 10-μl aliquots were removed for cytochrome *c* oxidase activity measurement as described in Materials and Methods. Activity of proteinase-treated cytochrome *c* oxidase is expressed as percentage of untreated cytochrome *c* oxidase. The ratios in the last column are given as mg of cytochrome *c* oxidase/mg of proteinase.

Proteinases	Incubation time (h)						Cytochrome *c* oxidase/ proteinase ratio
	1	2	4	7	10	25	
Trypsin	99	90	86	92	96	92	2.4
α-Chymotrypsin	90	80	88	91	88	90	1.8
Elastase	96	92	90	91	100	91	1.9
Collagenase	97	91	90	97	92	96	2.4
Ficin	98	93	97	97	99	96	4.2
Papain	92	91	93	95	91	90	1.9
Bromelain	92	91	74	75	70	59	2.4
Proteinase B	83	73	80	78	74	38	1.3
Carboxypeptidase Y	89	86	87	90	89	70	6.7
Pronase P	89	90	77	79	70	22	4.1
Proteinase K	100	81	80	82	78	20	11
Aminopeptidase M	95	94	88	89	85	58	20
Mitochondrial proteinase	89	78	81	80	78	57	600

25 h of incubation. For all the digestions with proteinases the cytochrome *c* oxidase to proteinase ratios were comparable, except for aminopeptidase M and the mitochondrial proteinase. These two enzymes inactivated the cytochrome *c* oxidase in very low concentrations. In the following experiments cytochrome *c* oxidase was treated with proteinase K, aminopeptidase M and mitochondrial proteinase. The temperature of incubation was raised to 25°C (Fig. 1). The action of the aminopeptidase M on cytochrome *c* oxidase was characterized by a dramatic initial decrease of cytochrome *c* oxidase activity and a plateau at about 60% of the initial activity indicating an 'amputated' cytochrome *c* oxidase resistant to further degradation. This interpretation is further supported by the finding that up to 20-fold higher aminopeptidase M concentrations did not result in a plateau of lower activity (data not shown). In the case of the mitochondrial proteinase a similar biphasic activity change was observed, although there was no pronounced plateau of cytochrome *c* oxidase activity. Unlike aminopeptidase M and the mitochondrial proteinase, digestion with proteinase K resulted in a continuous decrease of cytochrome *c* oxidase.

In a recent paper [23] we described the purification of cytochrome *c* oxidase from rat liver mitochondria using hydrophobic chromatography. The enzyme characterized by a specific heme *a* content of 15.4 nmol/mg of protein was resolved into seven polypeptides after polyacrylamide gel electrophoresis (Fig. 2a). Hence it was of interest to correlate the activity changes of cytochrome *c* oxidase after proteolytic digestion with the subunit pattern of the enzyme. Therefore, cytochrome *c* oxidase incubated for different times with proteinase K, aminopeptidase M and the mitochondrial proteinase was analyzed



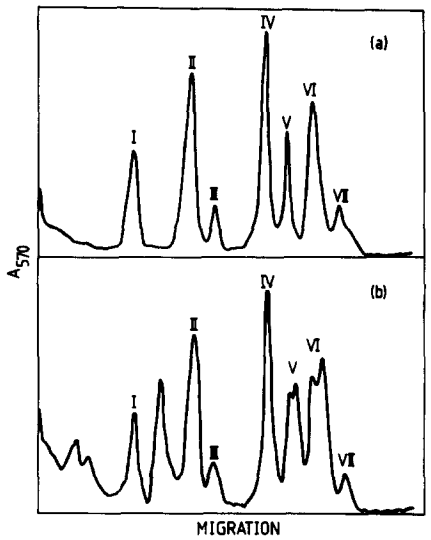


Fig. 1. Digestion of rat liver cytochrome *c* oxidase with proteinase K, aminopeptidase M and mitochondrial proteinase. Between 0.05 and 0.24 mg of deoxycholate-solubilized cytochrome *c* oxidase of 10.2 nmol heme *a*/mg of protein were incubated in a total volume of 100 μl of 50 mM sodium phosphate buffer, pH 7.5, at 25°C with proteinase K (△), aminopeptidase M (●), and mitochondrial proteinase (○). The reaction was stopped by 1 : 10 dilution with 50 mM sodium phosphate buffer, pH 7.5. Aliquots of the incubation mixture were assayed for cytochrome *c* oxidase activity. The oxidation reaction was initiated by adding 50 μl of ferrocytochrome *c* (10 mg/ml 10 mM potassium phosphate buffer, pH 7.05) to the mixture of 900 μl of 0.5% Tween 80 in 10 mM sodium phosphate buffer, pH 7.5, and 20—40 μl of the diluted reaction mixture. In the incubation mixtures the proteinase cytochrome *c* oxidase ratios were as follows: proteinase K/cytochrome *c* oxidase = 1/14; aminopeptidase M/cytochrome *c* oxidase = 1/20, and mitochondrial proteinase/cytochrome *c* oxidase = 1/599.

Fig. 2. Densitometric traces of SDS-polyacrylamide gels of soluble cytochrome *c* oxidase from rat liver mitochondria. The samples were dissociated in 0.1 M sodium phosphate buffer, pH 7.0, containing 12.5% (w/v) SDS, 6.5% (v/v) 2-mercaptoethanol and 40% (v/v) glycerol by incubation at 37°C for 90 min. 100 μg of the dissociated samples were applied on the gels. Electrophoresis was run on a 15% total polyacrylamide gel with an acrylamide/bisacrylamide ratio of 32 : 1 in the presence of 0.5% SDS and 10% glycerol as described in Materials and Methods. The gels were scanned at 570 nm, with full optical scale 0—1.0, slit-width 0.25 mm. (a) Soluble cytochrome *c* oxidase purified from rat liver mitochondria using hydrophobic chromatography (15.4 nmol heme *a*/mg protein). (b) Solubilized cytochrome *c* oxidase, designated as 'deoxycholate-soluble cytochrome *c* oxidase' (10.2 nmol heme *a*/ mg protein). The subunits of the enzyme are labelled I—VII.

by SDS-polyacrylamide gel electrophoresis. A representative gel scan of a cytochrome *c* oxidase preparation which has been digested with mitochondrial proteinase is shown in Fig. 3. Among various electrophoretic conditions, the one using 15% polyacrylamide and an acrylamide/bisacrylamide ratio of 32 : 1 without urea was found to give the best separation of the various polypeptides after digestion of the soluble enzyme with the various proteinases. Moreover, the time needed for an electrophoresis run was less than in the case of the urea-containing system. The decrease in intensity of the seven stained polypeptide bands of cytochrome *c* oxidase after proteinase K, aminopeptidase M and mitochondrial proteinase treatment is presented in Figs. 4—6. The contaminants with molecular weights higher than subunit I as well as the protein with an electrophoretic mobility between subunit I and subunit II were rapidly (within 5 min) degraded. Then the degradation of the cytochrome *c* oxidase

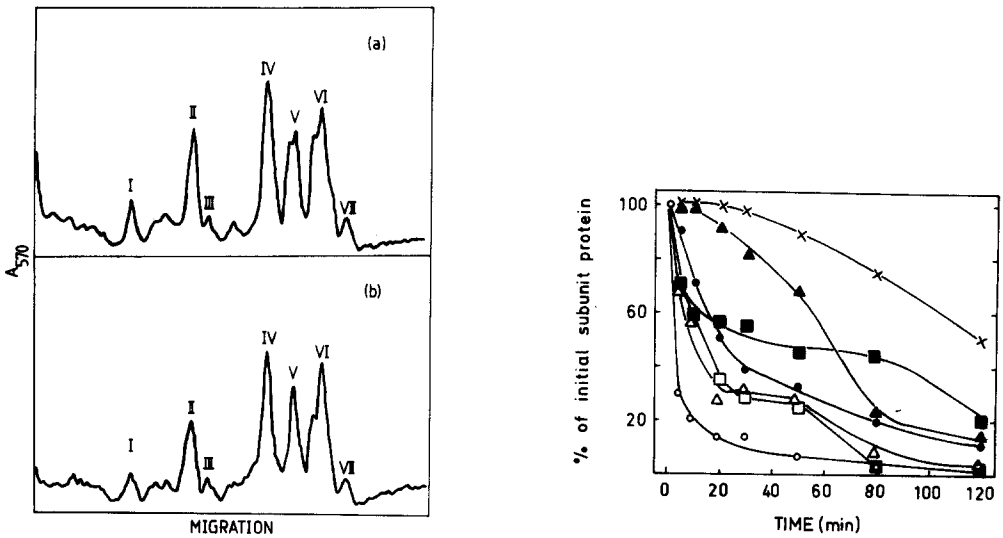


Fig. 3. Densitometric traces of SDS-polyacrylamide gels of soluble cytochrome *c* oxidase from rat liver mitochondria after incubation with mitochondrial proteinase. 0.42 mg of solubilized cytochrome *c* oxidase (10.2 nmol heme *a*/mg protein) was incubated with 0.7 μg of mitochondrial proteinase at 25°C in 50 mM sodium phosphate buffer, pH 7.5. After (a) 5 min and (b) 80 min incubation, the digestion was stopped by the addition of phenylmethylsulfonyl fluoride in Me_2SO. Conditions of dissociation, electrophoresis and gel scanning were the same as those described in the legend to Fig. 2.

Fig. 4. SDS-polyacrylamide gel electrophoresis of proteinase K-treated cytochrome *c* oxidase. 0.83 mg of solubilized cytochrome *c* oxidase (10.2 nmol heme *a*/mg protein) was incubated with 60 μg of proteinase K in a total volume of 80 μl at 25°C in 50 mM sodium phosphate buffer, pH 7.5. Digestion with proteinase K was stopped by addition of phenylmethylsulfonyl fluoride in Me_2SO to a final concentration of 95 mM. The sample was dissociated by SDS at 37°C for 90 min. 101 μg of protein was subjected to SDS-polyacrylamide gel electrophoresis as described in Materials and Methods. ○, I; △, II; □, III; ●, IV; ▲, V; ■, VI; X, VII.

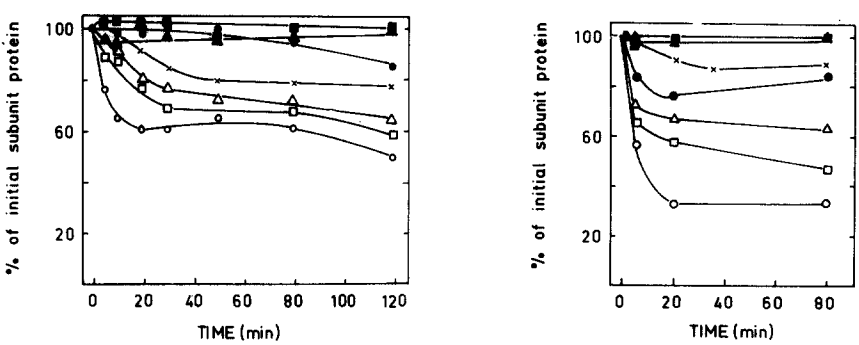


Fig. 5. SDS-polyacrylamide gel electrophoresis of aminopeptidase M-treated cytochrome *c* oxidase. 0.95 mg of solubilized cytochrome *c* oxidase (10.2 nmol heme *a*/mg protein) was incubated with 50 μg of aminopeptidase M in a total volume of 90 μl at 25°C in 50 mM sodium phosphate buffer, pH 7.5. Digestion with aminopeptidase M was stopped by the addition of EDTA to a final concentration of 5 mM. The sample was dissociated by SDS at 37°C for 90 min. 104 μg of protein was subjected to SDS-polyacrylamide gel electrophoresis as described in Materials and Methods. ○, I; △, II; □, III; ●, IV; ▲, V; ■, VI; X, VII.

Fig. 6. SDS-polyacrylamide gel electrophoresis of cytochrome *c* oxidase treated with mitochondrial proteinase. 0.42 mg of solubilized cytochrome *c* oxidase (10.2 nmol heme *a*/mg protein) was incubated with 0.7 μg of mitochondrial proteinase (specific activity 8.5 units/mg of protein [29]) in a total volume of 40 μl at 25°C in 50 mM sodium phosphate buffer, pH 7.5. Digestion with the proteinase was stopped by the addition of phenylmethylsulfonyl fluoride in Me_2SO to a final concentration of 95 mM. The sample was dissociated by SDS at 37°C for 90 min. 104 μg of protein was subjected to SDS-polyacrylamide gel electrophoresis as described in Materials and Methods. ○, I; △, II; □, III; ●, IV; ▲, V; ■, VI; X, VII.

TABLE II

SUSCEPTIBILITY OF PURIFIED SOLUBLE CYTOCHROME *c* OXIDASE SUBUNITS TO PROTEINASES

Proteinase	Order of susceptibility
Proteinase K	I > II,III > IV > VI > V > VII
Aminopeptidase M	I > II,III > VII > IV > V , VI
Mitochondrial proteinase	I > III > II > IV > VII > V , VI

subunits gradually occurred. As shown in Figs. 4—6 subunit I was most susceptible to proteolysis. Whereas subunit II was rather resistant to proteinase K digestion, subunits V and VI were most stable during incubation with aminopeptidase M or the mitochondrial proteinase. The order of susceptibility of the cytochrome *c* oxidase subunits to proteolytic degradation is summarized in Table II.

Discussion

As shown in the present study, there are two groups of proteinases which differ in respect to the inactivation and proteolytic degradation of soluble cytochrome *c* oxidase (Table I). Soluble cytochrome *c* oxidase was resistant to the action of trypsin and α-chymotrypsin. It was even possible to remove most of the protein contaminants from a partially purified cytochrome *c* oxidase preparation after incubation with these two proteinases. It was shown by SDS-polyacrylamide gel electrophoretic analysis after incubation of cytochrome *c* oxidase with elastase or collagenase that these enzymes had no effect at all on the protein pattern including protein contaminants.

Among the various proteinases used for the incubation of cytochrome *c* oxidase the proteinases of bacterial origin, such as pronase P, subtilisin (not shown in Table I) and proteinase K exhibited the highest capability to degrade the soluble enzyme substrate. The proteinase isolated from rat liver mitochondria also gave rise to a marked decrease in enzymic activity of cytochrome *c* oxidase.

The inactivation kinetics of cytochrome *c* oxidase incubated with aminopeptidase M, and exopeptidase, differed from those found for proteinase K and the mitochondrial proteinase (Fig. 1). A very slow initial decrease followed by a steep decrease and the formation of a plateau was observed. The lag phase in the cytochrome *c* oxidase activity curve of Fig. 1 may be due to the gradual loss of amino acids from the NH_2-terminal ends of the different cytochrome *c* oxidase subunits. When the exopeptidase degradation has reached a critical point, a conformational change may occur resulting in a rapid and partial loss of enzymic activity paralleled by inaccessibility to further proteolytic attack by the aminopeptidase M.

From the susceptibilities of the various subunit polypeptides of cytochrome *c* oxidase to proteolytic degradation (Figs. 3—6) it became clear that subunit I is most easily degraded by all proteinases. This finding is somewhat contradic-

tory to the results recently reported by Chan and Tracy [37]. These authors found that antisera against subunit I did not react with the soluble enzyme from beef heart mitochondria.

It is likely that the initial attack by proteinases might result in a drastic conformational change of the enzyme, which in turn leads to a more susceptible state of subunit I than that found in the 'native' soluble enzyme. Moreover, it cannot be excluded that the arrangement of the subunits of the soluble rat liver cytochrome *c* oxidase differs from that of the soluble enzyme from other tissues or organs. In the submitochondrial particles, however, subunit I is not accessible to proteolysis (Nagasawa-Fujimori, H., unpublished results). From this finding it may be concluded that subunit I is buried in the interior of the mitochondrial membrane. Using different surface probes Eytan and Schatz [26] have also found that subunit I is located in the interior of the mitochondrial membrane. After digestion of submitochondrial particles with proteinase K it was found that in addition to subunit I, subunit V was also resistant to degradation. Thus, subunit V might also be located in the interior of the mitochondrial membrane.

When the soluble cytochrome *c* oxidase was incubated with proteinase K a marked degradation of subunit V after an initial lag phase was observed. However, incubation of soluble cytochrome *c* oxidase with aminopeptidase M or the mitochondrial proteinase did not result in the removal of subunit V, contradicting to the finding with proteinase K. A plausible interpretation would be that subunit V is localized in the interior of the enzyme and is accessible only after extensive destruction of the surrounding subunits. In the case of proteinase K this process is relatively rapid due to the low specificity and the low molecular weight of the enzyme.

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References

1 Chuang, T.F. and Crane, F.L. (1971) Biochem. Biophys. Res. Commun. 42, 1076—1081
2 Keims, J.J., Yong, C.S. and Gilmour, M.W. (1971) Biochem. Biophys. Res. Commun. 45, 835—841
3 Kuboyama, M., Yong, F.A. and King, T.E. (1972) J. Biol. Chem. 247, 6375—6383
4 Weiss, H., Lorenz, B. and Kleinow, W. (1972) FEBS Lett. 30, 49—51
5 Capaldi, R.A. and Hayashi, M. (1972) FEBS Lett. 26, 261—263
6 Komai, H. and Capaldi, R.A. (1973) FEBS Lett. 30, 273—276
7 Rubin, M.S. and Tzagoloff, A. (1973) J. Biol. Chem. 248, 4269—4274
8 Mason, T.L., Poyton, R.O., Wharton, D.C. and Schatz, G. (1973) J. Biol. Chem. 248, 1346—1354
9 Sebald, W., Machleidt, W. and Otto, J. (1973) Eur. J. Biochem. 38, 311—324
10 Yamamoto, T. and Orii, Y. (1974) J. Biochem. 75, 1081—1089
11 Poyton, R.O. and Schatz, G. (1975) J. Biol. Chem. 250, 752—761
12 Briggs, N., Kamp, P.-F., Robinson, N.C. and Capaldi, R.A. (1975) Biochemistry 14, 5123—5128
13 Downer, N.W., Robinson, N.C. and Capaldi, R.A. (1976) Biochemistry 15, 2930—2936
14 Steffens, G. and Buse, G. (1976) Hoppe-Seyler's Z. Physiol. Chem. 357, 1125—1137
15 Phan, S.H. and Mahler, H.R. (1976) J. Biol. Chem. 251, 257—269
16 Werner, S. (1977) Eur. J. Biochem. 79, 103—110
17 Yu, C.-A. and Yu, L. (1977) Biochim. Biophys. Acta 495, 248—259
18 McGeer, A., Lavers, B. and Williams, G.R. (1977) Can. J. Biochem. 55, 988—994

19 Hundt, E. and Kadenbach, B. (1977) Hoppe-Seyler's Z. Physiol. Chem. 358, 1309—1314
20 Rosen, S. (1978) Biochim. Biophys. Acta 523, 314—320
21 Ades, I.Z. and Cascano, J. (1978) J. Bioenerg. Biomembranes 9, 237—253
22 Höchli, L. and Hackenbrock, C.R. (1978) Biochemistry 17, 3712—3719
23 Nagasawa, T., Nagasawa-Fujimori, H. and Heinrich, P.C. (1979) Eur. J. Biochem. 94, 31—39
24 Rascati, R.J. and Parsons, P. (1979) J. Biol. Chem. 254, 1586—1593
25 Tracy, R.P. and Chan, S.H.P. (1979) Biochim. Biophys. Acta 576, 109—117
26 Eytan, G.D. and Schatz, G. (1975) J. Biol. Chem. 250, 767—774
27 Eytan, G.D., Carrol, R.C., Schatz, G. and Racker, E. (1975) J. Biol. Chem. 250, 8598—8603
28 Bell, R.L., Sweetland, J., Ludwig, B. and Capaldi, R.A. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 741—745
29 Jusic, M., Seifert, S., Weiss, E., Haas, R. and Heinrich, P.C. (1976) Arch. Biochem. Biophys. 177, 355—363
30 Loewenstein, J., Scholte, H.R. and Wit-Peeters, E.M. (1970) Biochim. Biophys. Acta 223, 432—436
31 Greenawalt, J.W. (1974) Methods Enzymol. 31A, 310—323
32 Smith, L. and Conrad, H. (1956) Arch. Biochem. Biophys. 63, 403—413
33 Laemmli, U.K. (1970) Nature 227, 680—685
34 Chan, S.H.P. and Schatz, G. (1979) Methods Enzymol. 56, 223—228
35 Lowry, O.M., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
36 Williams, J.N., Jr. (1964) Arch. Biochem. Biophys. 107, 537—543
37 Chan, S.H.P. and Tracy, R.P. (1978) Eur. J. Biochem. 89, 595—605