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# Identification and Partial Purification of a Heart Mitochondrial Membrane Proteinase[1]

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## Abstract

Membrane-bound proteinase activity was demonstrated by a solid-phase assay system in both beef heart and rat liver mitochondria. The activity was sensitive to SH reagents and assorted proteinase inhibitors. Although stimulated by nonionic detergents, it became labile when solubilized by detergents. The proteinase activity from heart mitochondria copurified with the ADP:ATP translocator protein. Gel electrophoresis of this preparation revealed the translocator polypeptide as well as a number of minor components. In solubilized mitochondria the ADP:ATP translocator polypeptide slowly disappeared upon standing at 0°C as revealed by polyacrylamide gel electrophoresis under denaturing conditions. The loss of this polypeptide was prevented by addition of proteinase inhibitors as well as the translocator affinity ligand, carboxyatractylate. These observations confirm the presence of an integral membrane proteinase in mitochondria and suggest a structural and enzymatic interaction between the proteinase and the ADP:ATP translocator.


**Key Words:** Membrane proteinase; ADP:ATP translocator.

## Introduction

Interest in the turnover and processing of membrane proteins has stimulated attempts to identify proteinases involved in these functions. Mitochondria appear to possess a processing proteinase since preprotein precursors to cytoplasmically synthesized mitochondrial adenosine triphosphatase (Nelson and Schatz, 1979), carbamyl phosphate synthetase (Raymond and Shore,

[1]Abbreviations: PMSF, phenylmethanesulfonyl fluoride; TPCK, L-1-tosylamido-2-phenylethylchloromethyl ketone; TLCK, 1-chloro-3-tosylamido-7-amino-L-2-heptanone; NEM, *N*-ethylmaleimide; PCMBS, *p*-chloromercuriphenylsulfonic acid; SDS, sodium dodecyl sulfate; MOPS, morpholinopropane sulfonate; [$I_{50}$], concentration of inhibitor required to give 50% inhibition.

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1979), and cytochrome *c* oxidase (Mihara and Blobel, 1980; Lewin *et al.*, 1980) have been identified. Several recent reports have described proteinase activity associated with mitochondrial membranes (Katunuma *et al.*, 1975; Jusic *et al.*, 1976; Aoki, 1978). The enzymes responsible for these activities have been purified after their release from mitochondrial membranes by high salt washes. Their release from membranes with salt and their solubility in the absence of detergents indicate these proteinases to be peripherally and not integrally associated with the membrane. In addition, these enzymes appear to be cell specific in that they originate from mast cells (Woodbury *et al.*, 1978; Haas *et al.*, 1979) or bone marrow cells (Aoki, 1978). I have described a proteinase that is integrally associated with the rat liver inner mitochondrial membrane in that it could not be extracted with salt but only by detergent (Hare, 1978). This enzyme was detected by measuring the release of radioactivity from [$^{125}$I]insulin-coated Sepharose beads as a function of time. In this report I describe the partial purification of an integral proteinase from heart mitochondria that is identical in its properties to the liver enzyme. This mitochondrial proteinase copurifies with the ADP:ATP translocator and appears responsible for proteolytic loss of the translocator protein in solubilized mitochondria.

## Materials and Methods

### *Materials*

The following reagents were obtained from Sigma Chemical Co.: carboxyatractylate, bovine insulin, bovine insulin B chain, twice-recrystallized trypsin, soybean trypsin inhibitor, PMSF, TPCK, TLCK, *p*-aminobenzamidine, and PCMBS. NEM was purchased from K&K Laboratories, $\epsilon$-aminocaproic acid from Calbiochem, and hydroxylapatite (Biogel HT) from Biorad Laboratories. Na$^{125}$I was acquired from New England Nuclear. All other chemicals were reagent grade or better.

### *Methods*

Beef heart mitochondria (Low and Vallin, 1963) and rat liver mitochondria (Bustamante *et al.*, 1977) were prepared and utilized immediately or stored at $-20$°C. Rat liver mitochondria mitoplasts (inner membrane plus matrix) were prepared as described (Greenawalt, 1974). Rat liver hepatocytes were prepared by collagenase perfusion (Moldevs *et al.*, 1978) and from these a crude mitochondria fraction was prepared (Gellefors and Nelson, 1979).

Proteinase assays were performed as described previously (Hare, 1978) with [$^{125}$I]insulin or [$^{125}$I]insulin B chain as a substrate covalently bound to

Sepharose 4B (Cuatrecasas, 1969). All assays were conducted at 23°C for 15 min in 0.1 M Tris-HCl, 0.001 mM EDTA, 0.25% Triton X-100 (pH 8.2). Activity is expressed in the following manner: unit of activity = [cpm released/min in the presence of enzyme − cpm released/min in the absence of enzyme] $\times 10^{-3}$. Specific activity is expressed as units/mg protein. Isocitrate dehydrogenase was assayed as described (Plaut, 1969).

Membrane-bound proteinase was partially purified from beef heart mitochondria by following the scheme of Riccio *et al.* (1975, 1977) for purification of the ADP:ATP translocase. Thawed beef heart mitochondria were washed with 0.25 M sucrose, 0.1 M Tris-HCl (pH 7.4) and resuspended to 20 mg protein/ml. A 5-ml portion of the washed mitochondria was diluted to 20 ml with 2 M LiCl, 0.05% (w/v) Triton X-100, 0.02 M MOPS (pH 6.8) and centrifuged at 85,000 $\times$ *g* for 30 min. The pellet was resuspended in 9 ml of 2.5% Triton X-100, 0.04 M NaCl, 0.02 M MOPS (pH 6.8), incubated at 0°C for 30 min, and centrifuged at 85,000 $\times$ *g* for 60 min. The extract was applied to a column (2.5 $\times$ 3.5 cm) of hydroxylapatite equilibrated in 0.5% Triton X-100, 0.01 M NaCl, 0.02 M MOPS (pH 6.8). The protein eluting in the pass-through volume (3.9 mg in 7–8 ml) was concentrated with an Amicon PM10 membrane to 2.5 ml under nitrogen pressure. A 0.5-ml portion of the concentrated eluate was then applied to a Sepharose 6B column (1 $\times$ 20 cm) equilibrated with 0.5% Triton X-100, 0.01 M NaCl, 0.02 M MOPS (pH 7.4); 0.5 ml fractions from the column were collected and assayed for proteinase activity and protein concentration. Pooled fractions from the major protein peak are referred to herein as the ADP:ATP translocator preparation.

Protein was measured by the Lowry method (Lowry *et al.*, 1951). Polypeptide components were visualized by staining (Fairbanks *et al.*, 1971) after their separation by SDS-urea polyacrylamide gel electrophoresis (Downer *et al.*, 1976; Hare *et al.*, 1980). $M_r$ values were calculated as described (Hare *et al.*, 1980).

## RESULTS

### *Proteinase Assay*

The solid-phase assay system used in this investigation was previously described and measures radioactivity released proteolytically from [$^{125}$I]insulin beads (Hare, 1978). The radioactivity released by mitochondria or known proteinases was linear with both time and enzyme concentration (Hare, 1978). Mitochondria from several sources exhibit similar specific proteinase activity (Table I). The release of radioactivity from the [$^{125}$I]insulin-coated beads could be caused by deiodination or oxidative or reductive

**Table I.** Proteinase Activity of Mitochondria in Solid-Phase Assay System

| Preparation | Specific activity* |
|---|---|
| Beef heart mitochondria | 6.3 |
| Rat liver mitochondria | 4.1 |
| Hepatocyte mitochondria | 2.0 |
| Trypsin | 8,000 |

*[cpm released/min/mg protein] $\times 10^{-3}$.

cleavage of insulin intrachain cystine residues rather than endoproteolysis. Both of the former activities have been described from rat liver cytosol (Brinke *et al.*, 1980; Chandler and Jarandani, 1972). A deiodination mechanism was excluded by demonstrating that radioactivity released from the substrate beads in the presence of trypsin or rat liver mitochondria eluted from a Sephadex G-25 column, previously equilibrated with 0.15 M NaCl, 1 M acetic acid, 6 M urea, as a single broad peak one void volume ahead of $Na^{125}I$ eluting in the totally included volume (data not shown). Disulfide oxidation or reduction can be excluded by the ability of [$^{125}$I]insulin B chain to serve as a more effective substrate for the mitochondrial enzyme than [$^{125}$I]insulin (Table II). In addition, the enzymatically released radioactive products from solid-phase bound insulin chromatograph as smaller products than isolated A chain on a Sephadex G-25 column in 0.15 M NaCl, 1 M acetic acid, 6 M urea.

### *Properties of the Mitochondrial Proteinase*

In the solid-phase assay system, detergent is an essential component of the reaction mix to achieve enzyme-mediated release of radioactivity from the insulin-coated beads. Triton X-100 was the most effective of the detergents tested (Lubrol WX, Tween 80, cholate, deoxycholate) when used at a concentration of 0.2% or greater. SDS at 0.25% concentration was completely inhibitory to activity. On the other hand, nonionic detergent had no effect on trypsin activity in the same assay system. Whether detergent was necessary for substrate accessibility or enzyme activation is not known. When mitochondrial membranes were incubated with free [$^{125}$I]insulin, no detectable trichlo-

**Table II.** Mitochondria-Catalyzed Release of Radioactivity from Insulin-Coated Sepharose Beads

| Substrate | [$^{125}$I]Insulin | [$^{125}$I]Insulin B chain |
|---|---|---|
| cpm added to assay system | $4.0 \times 10^5$ | $3.1 \times 10^5$ |
| cpm released by 0.1 mg mitochondrial protein in 15 min | $1.9 \times 10^3$ | $2.4 \times 10^3$ |
| Percent of added cpm released in 15 min | 0.48 | 0.78 |

**Table III.** [$I_{50}$] for Various Proteinase Inhibitors

| Inhibitor | Rat liver mitochondria | Beef heart mitochondria | Partially purified proteinase |
|---|---|---|---|
| PMSF | $\gg$1 mM | — | — |
| $\epsilon$-Caproic acid | $\gg$1 mM | — | — |
| Soybean trypsin inhibitor | $\gg$ 1 mM | — | — |
| TPCK | 5 $\mu$M | 2 $\mu$M | 0.5 $\mu$M |
| TLCK | 20 $\mu$M | 20 $\mu$M | 10 $\mu$M |
| *p*-Aminobenzamidine | 0.15 mM | 1 mM | 0.5 mM |
| NEM | 0.5 $\mu$M | 0.8 $\mu$M | 0.5 $\mu$M |

roacetic acid-precipitable counts were released in the absence of detergent, while the presence of detergent interfered with acid precipitation.

Mitochondrial proteinase activity exhibits a pH optimum of 8.1–8.3 and is sensitive to all SH group reagents tested (PCMB, PCMBS, 5,5′-dithiobis-[2-nitrobenzoic acid], NEM) as well as the proteinase inhibitors TPCK, TLCK, and *p*-aminobenzamidine. As shown in Table III, the [$I_{50}$] for a variety of proteinase inhibitors is similar for both rat liver and beef heart mitochondria activity. Rat liver hepatocyte mitochondria also showed similar [$I_{50}$] values for the inhibitor tested (data not shown). Possible interference of SH inhibitors with substrate rather than proteinase can be excluded by the finding that SH reagent-pretreated mitochondria show greatly decreased activity. Intact or freeze–thawed rat liver mitoplasts were treated with 2 mM SH reagent at 0°C and washed several times before assay. As shown in Table IV, membrane-impermeable PCMBS partially inhibited membrane proteinase activity in both intact and freeze–thawed mitoplasts. PCMBS apparently did not enter the mitochondria during pretreatment since it did not inhibit a matrix enzyme, isocitrate dehydrogenase, when added to intact mitoplasts (Table IV) but did inhibit completely the same enzyme in broken mitochondria (results not shown). Mitoplasts or mitochondria pretreated with

**Table IV.** Effect of PCMBS Treatment of Rat Liver Mitoplasts on Proteinase Activity

| Preparation | Additions to mitoplasts before washing and assay[a] | Percent control specific activity | |
|---|---|---|---|
| | | Proteinase[b] | Isocitrate dehydrogenase[c] |
| Intact mitoplasts | — | 100 | 100 |
| Intact mitoplasts | 2 mM PCMBS | 59 | 114 |
| Freeze–thawed mitoplasts | — | 106 | — |
| Freeze–thawed mitoplasts | 2 mM PCMBS | 55 | — |

[a] Mitoplasts were suspended to 10 mg protein/ml in 0.25 M sucrose, 0.01 M Tris-HCl (pH 7.4). After 30-min incubation at 0°C with PCMBS, the mitoplasts were washed three times in 10 times their original volume.

[b] Control specific activity was 3.3 cpm released/min/mg protein $\times$ $10^{-3}$.

[c] Control specific activity was 0.2 $\mu$moles/min/mg protein.

2 mM NEM with or without freeze–thawing exhibited only 20% of control activity. Since NEM is able to pass through membranes while PCMBS cannot, these experiments suggest that an essential SH of the proteinase is exposed to the exterior of the mitochondrial inner membrane and is at least partially accessible to water-soluble SH reagents.

A variety of chromogenic proteinase substrates was tested to determine if a hydrolytic activity purified into the rat liver inner mitochondrial membrane. Except for the trypsin substrate α-*N*-benzoyl-DL-arginine-*p*-nitroanilide, no chromogenic substrate was efficiently hydrolyzed by inner membrane preparations. The latter substrate probably detects the same proteinase described by Jusic *et al.* (1976) since this activity could be solubilized by 0.5 M potassium phosphate (pH 8.5). There thus appears to be no suitable chromogenic substrate for the membrane proteinase.

### *Partial Purification of the Heart Proteinase*

I have previously shown that the solid-phase proteinase activity in a rat liver homogenate purifies into the rat liver mitochondrial inner membrane fraction (Hare, 1978). Since beef heart mitochondria appear to possess activity which is identical to that of rat liver mitochondria in all examined properties, and, in addition, are amenable to established fractionation approaches, I used the former as a source for proteinase purification. A variety of fractionation methods were tested for their ability to achieve selective enrichment of heart mitochondrial proteinase activity. During these studies it was found that the proteinase activity copurified with ADP:ATP translocase (Table V), purified by Riccio *et al.* (1975) by enrichment for specifically bound [$^{35}$S]carboxyatractyloside, an affinity inhibitor of the ADP:ATP exchange reaction. The partially purified proteinase showed similar inhibitor sensitivity to the membrane-bound starting material (Table III). From the point of extraction, proteinase activity becomes labile (50% inactivation in

**Table V.** Partial Purification of Mitochondrial Inner Membrane Proteinase

| | Proteinase activity | | | Adenyltranslocase[c] | |
|---|---|---|---|---|---|
| Fraction | $SA_a$ | Total | Purification | μmol CAT/g protein[b] | Purification |
| Mitochondria | 3.28 | 344 | (1.0) | 1.88 | (1.0) |
| 2 M LiCl, 0.05% Triton washed | 3.62 | 344 | (1.1) | 3.14 | (1.7) |
| 2.5% Triton extract | 6.15 | 320 | (1.9) | 3.90 | (2.1) |
| Hydroxylapatite "pass-through" | 35.4 | 138 | (10.8) | 16.9 | (9.0) |
| Sepharose 6B | — | — | | 18.6 | (9.9) |

[a] Specific activity as defined in Materials and Methods.
[b] Carboxyatractyloside specifically bound.
[c] Riccio *et al.* (1975).

12 h at 0°C) and is partially inactivated by pressure concentration and Sepharose gel filtration. Nevertheless, proteinase activity remaining in the eluted fractions of the Sepharose 6B column migrated with the major peak of protein (Fig. 1) which represents ADP:ATP translocator protein (Riccio *et al.*, 1975). Pretreatment of mitochondria with 10 $\mu$M carboxyatractyloside, which protects the translocase from inactivation as described (Ricco *et al.*, 1975), did not alter the recovery of proteinase activity from the hydroxylapatite column. Dissociation of the concentrated hydroxylapatite pass-through fractions in SDS and urea and their resolution by polyacrylamide gel electrophoresis (Fig. 2, trace A) demonstrated the presence of a major polypeptide with an apparent $M_r \sim 29{,}000$ and six minor polypeptides. The 29,000 $M_r$ polypeptide is the ADP:ATP translocator protein (Riccio *et al.*, 1975).

*Proteolysis of the ADP:ATP Translocator in Isolated Mitochondria*

Aquila *et al.* (1978) monitored proteolytic loss of ADP:ATP translocator protein in detergent-solubilized mitochondria at 0°C over a 21-h period by visualizing loss of the stained translocator protein in SDS polyacylamide gels. They found that the protein was stabilized against proteolytic degradation by preloading mitochondria with the affinity ligands bongkrekate or carboxyatractylate. I repeated these experiments to determine whether known inhibitors of the membrane proteinase could also protect the ADP:ATP translocator protein against degradation. As shown in Fig. 2, washed beef heart mitochondria, protected with carboxyatractylate (trace D), NEM (trace E), or TPCK (trace F), during a 53-h incubation at 0°C, retained the 29,000 $M_r$ translocase polypeptide which was lost in the absence of these reagents (trace C). Freshly thawed mitochondria (trace B) were included for reference. No proteolytic disappearance of the translocase polypeptide occurred in the absence of detergent.

## Discussion

This report presents data that support the existence of a unique proteinase with mammalian mitochondria. The solid-phase system used to assay this enzyme was designed to detect endoproteinase activity of membrane-bound enzymes that might require the presence of detergent for expression of enzyme activity. The use of insulin B chain as an alternative substrate to insulin and column chromatography of the radioactive products released from the solid-phase-attached substrate support the interpretation that release of radioactivity results from endoproteolysis rather than disulfide bond cleavage or deiodination. Moreover, known proteinases catalyzed the release of radioactivity from the same substrate. Since the activity was only

partially purified from mitochondria, the activity detected in the assay system could represent more than one proteinase.

Several proteinases have recently been purified from liver and bone marrow mitochondria. The properties of these enzymes as well as those of lysosomes and other cellular compartments contrast with those of the membrane-bound proteinase described in this report. Previously described mitochondrial proteinases appear to be cell specific. The enzyme described by Jusic *et al.* (1976) appears specific for mast cells (Woodbury *et al.*, 1978). The enzyme from bone marrow cells described by Aoki (1978) was found in granulocytes and erythroblasts but not lymphocytes. The proteinase described and purified by Katunuma *et al.* (1975) is specific for atypical mast cells (Haas *et al.*, 1979). The proteinase activity described here, on the other hand, is endogenous to both heart and liver hepatocyte mitochondria. Since characteristics of the activity are similar in both tissues, this activity seems to be more universally distributed and not unique to particular cells or tissues. The inner mitochondrial membrane location, slightly alkaline pH optimum, and membrane-bound disposition distinguish the mitochondrial proteinase from lysosomal cathepsins. The true membrane-bound nature of the mitochondrial proteinase is emphasized by earlier fractionation studies (Hare, 1978), the inability to solubilize the enzyme with high or low ionic strength buffers (Hare, 1978), the instability of the enzyme in detergent-extracted form, a detergent requirement for expression of its activity, and its copurification with another integral membrane protein, the ADP:ATP translocator. Except for the latter, these characteristics compare with those of the protein-processing peptidases of microsomal (Shields and Blobel, 1978) and bacterial (Lazdunski *et al.*, 1979) origin and contrast with those of previously described mitochondrial proteinases (Katunuma *et al.*, 1975; Jusic *et al.*, 1976; Aoki, 1978). Except for a thiol-containing carboxypeptidase described by Haas and Heinrich (1979), previously described mitochondrial endoproteinases (Katunuma *et al.*, 1975; Jusic *et al.*, 1976; Aoki, 1978) are sensitive to PMSF but not SH reagents. By contrast, the membrane-bound endoproteinase is sensitive to SH reagents, two trypsin inhibitors (*p*-aminobenzamidine and TLCK), and one chymotrypsin inhibitor, TPCK. If only one enzyme is detected by the assay system, this enzyme must not be a serine proteinase but a papainlike sulfhydryl proteinase. The effect of TPCK and TLCK on the enzyme is consistent with it having an active-site cysteine since these affinity reagents are known to alkylate cysteine as well as histidine residues at the active-site of proteinases (Whitaker and Perez-Villasenor, 1968). Of the two alkylating inhibitors, TPCK has an [$I_{50}$] 4–20 times lower than TLCK. The essential SH group is likely exposed to the external surface of the mitochondrial inner membrane, as suggested from its inhibition by impermeant reagents in mitoplasts (Table IV).

Copurification of the mitochondrial proteinase and the ADP:ATP translocator may be either fortuitous or have functional or structural significance. Their copurification explains the proteolytic removal of 5000 and 10,000 $M_r$ polypeptides from purified 29,000 $M_r$ ADP:ATP translocator polypeptide that occurs except when the latter is protected by carboxyatractylate (Klingenberg *et al.*, 1978). Even the most purified preparations of translocator probably contain traces of the membrane proteinase. The slight separation of the bulk of protein, comprising mostly translocator protein, and proteinase activity observed upon Sepharose chromatography of the combined, concentrated hydroxylapatite pass-through fractions (Fig. 1) suggests a nonidentity of the translocator and proteinase. The association of these two enzymes with different polypeptides is further suggested by the finding of a number of minor polypeptides in addition to translocator polypeptide in SDS-urea polyacrylamide gels of the translocator preparation (Fig. 2). Moreover, treatment of mitochondria with carboxyatractylate had no effect on proteinase activity. The susceptibility of the translocase to proteolysis by the SH-sensitive proteinase could represent a structural association of the two enzymes or an unusual proteolytic sensitivity of the translocase.

The function of the membrane-bound, sulfhydryl proteinase is unknown. Four possibilities exist: (1) processing of newly translocated polypeptides in transit from the cytoplasm to their proper disposition in the mitochondrial membrane, (2) turnover of mitochondrial proteins, (3) elimination of translational excess, or (4) regulation of enzyme activity. The characteristics of the proteinase appear compatible with the first function. Indeed, the partially

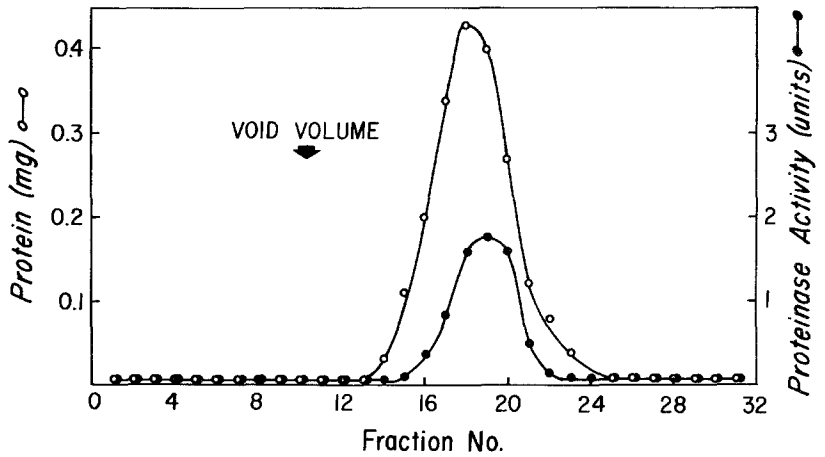


**Fig. 1.** Sepharose 6B chromatography of concentrated hydroxylapatite pass-through fractions. Chromatography was performed as described in the text, and fractions were assayed for protein concentration and proteinase activity.

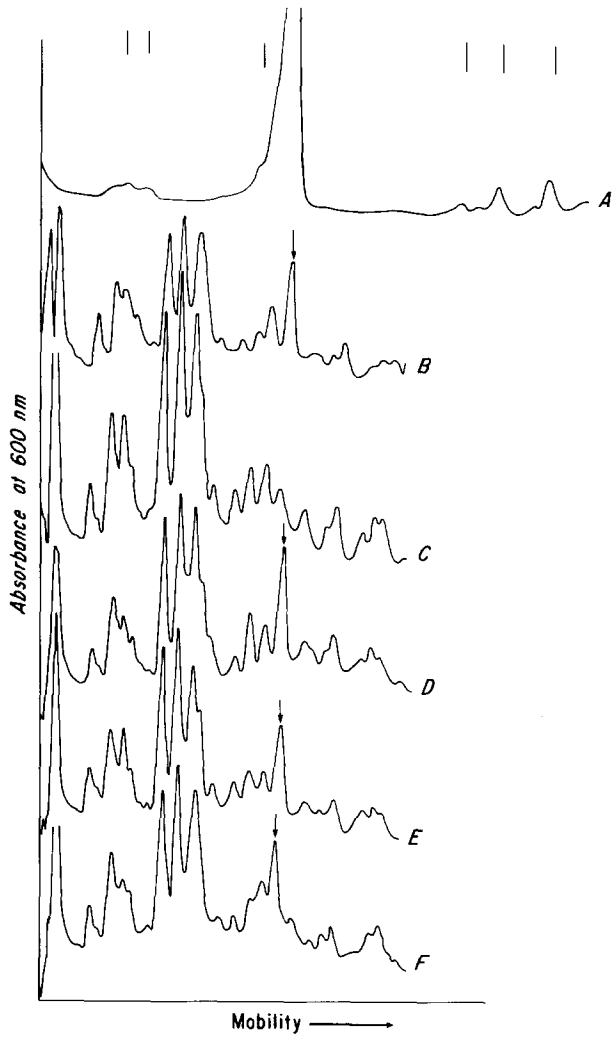


**Fig. 2.** SDS-urea polyacrylamide gel electrophoresis of the ADP:ATP translocator after hydroxylapatite chromatography and in incubated membranes. (A) 20 $\mu$g adenyltranslocase preparation; (B) 100 $\mu$g freshly thawed beef heart mitochondria; (C, D, E, F) 100 $\mu$g beef heart mitochondria solubilized in 0.1 M $(NH_4)_2SO_4$, 0.05 mM EDTA, 0.02% $NaN_3$, 5% Triton X-100, 0.005 M Tris-HCl (pH 8.2), and incubated for 53 h at 0°C with, respectively, no additions (C), 20 mM carboxyatractylate (D), 2 mM NEM (E), or 2 mM TPCK (F). After the incubation, samples B, C, and D were made 2 mM NEM. All samples were dissociated for 20 min at 50°C in 1% mercaptoethanol, 4% SDS, 8 M urea, and 0.01 M $K_2PO_4$ (pH 8.3). Lines next to trace A mark the position of minor polypeptide components present in adenyltranslocase preparation. Arrows mark position of ADP:ATP translocator polypeptide.

purified enzyme should be tested to see if it can correctly process proteins in cell-free systems. A role for the enzyme in processing, however, does not explain the unusual susceptibility of the translocator protein to proteinase action. The membrane localization of the proteinase would be consistent with a role for it in protein turnover since mitochondrial inner membrane polypeptides are degraded at disparate rates (Hare and Hodges, 1982). Perhaps the membrane proteinase is specifically concerned with the degradation of the translocator protein, a possibility which, if true, would explain an enzymatic and possible physical association between these two proteins. It may be characteristic of those cellular enzymes which play key roles in metabolism to each have their own proteinase to which they are particularly sensitive by conformation or structural association. In the case of the translocator protein, addition of detergent to membranes causes it to become more accessible or sensitive to the action of the proteinase in the absence of its affinity ligand. Membrane-associated or ligand-bound translocase is resistant to proteinase attack. A role for the proteinase in removal of excess translation products is suggested by two reports (Gear *et al.*, 1974; Wheeldon *et al.*, 1974) of mitochondrially translated proteins that are produced in excess of their cytoplasmic counterparts necessary for functional integration into the membrane and that are found to be rapidly proteolytically removed. These reports, however, were based upon experiments with isolated mitochondria, and this phenomenon may not occur *in vivo*. The ADP:ATP translocator in *Neurospora* is cytoplasmically synthesized (Hackenberg *et al.*, 1978), appears not to require mitochondrial gene products to function, and is probably not produced in excess of that which can function in the membrane. Speculation about a role for the membrane proteinase in regulation of translocase function would be premature in light of the paucity of information on this topic (Vignais and Lauquin, 1979; Akerboom *et al.*, 1977).

Although purification of the membrane proteinase to homogeneity would be difficult in light of its lability and association with the ubiquitous translocator protein, it may be possible to identify its position on SDS-polyacrylamide gels with radioactive alkylating inhibitors. Also the ease with which the enzyme can be partially purified from a readily available source of mitochondria lends its use to cell-free experiments.

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