Characteristics of an ATP-dependent proteolytic system of rat liver mitochondria

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Rat liver mitochondria contain an ATP-dependent proteolytic system which is localized on the outside of the inner membrane. It is capable of utilizing both the ATP produced within the mitochondria as well as that supplied externally. The system is dependent on Ca^{2+}. Its physiological function is seen in the normal breakdown of mitochondria during their turnover. The system may be selective for the breakdown of the inner membranes.

Proteolysis *ATP-dependent proteolysis* *Ca^{2+}-dependent proteolysis* *Mitochondrial proteolysis*

1. INTRODUCTION

It has been assumed that mitochondrial breakdown proceeds via lysosomes [1–4]. We demonstrated that the breakdown of mitochondria of reticulocytes during maturation involves selective ATP-dependent proteolysis [5–8], triggered by the attack of a specific lipoxygenase [9]. In exploring the possibility that mitochondria of cells other than reticulocytes might also be the target of ATP-dependent proteolysis, a process widely distributed in nature [10–13], we found such a system in rat liver mitochondria [7,8]. It differs from that of reticulocytes in two respects:

(i) In being mitochondrial membrane bound and not cytosolic;

(ii) By its lack of requirement for a preceding attack by lipoxygenase [14].

Here, we describe the characteristics of the ATP-dependent proteolytic system of rat liver mitochondria with respect to its dependence on pH, temperature and time as well as supporting evidence for the utilization of both endogenously and externally supplied ATP. The system appears to be calcium dependent.

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2. MATERIALS AND METHODS

The experimental procedures were employed as in [14], the only modifications being that the animals were decapitated and that sucrose was used instead of mannitol.

ATP, ADP, carboxyatractyloside (CAT), succinate and antimycin A were used in final concentrations of 5.7×10^{-3} M, 1.5×10^{-3} M, 50×10^{-6} M, 9.9×10^{-3} M and 5×10^{-6} M, respectively. Usually the incubations were carried out for 2 h at 37°C and pH 7.6. Proteolysis was measured by the lysine method [6] and was expressed in terms of lysine liberation from mitochondrial proteins during the incubation period.

3. RESULTS AND DISCUSSION

Fig.1–3 shows the pH, time and temperature characteristics of the ATP-dependent proteolytic system of rat liver mitochondria. In comparison with the cytosolic system of reticulocytes [6] there are similarities with respect to the pH profile, with very low rates of proteolysis at pH ≤ 7 and the time dependence with a decline of rate particularly after 120 min. There is, however, a significant difference with respect to the temperature dependence. Whereas the curve of the liver system is flat in the range of 27–42°C, in the reticulocyte pro-

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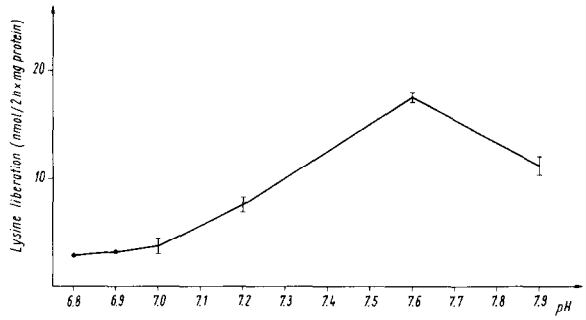

Fig.1. pH dependence of ATP-dependent proteolysis in rat liver mitochondria in the presence of ATP. The incubations were carried out for 2 h at 37°C in Tris–HCl buffers of appropriate pH (75 mM); mean ± SEM of 2–3 separate expt are given.

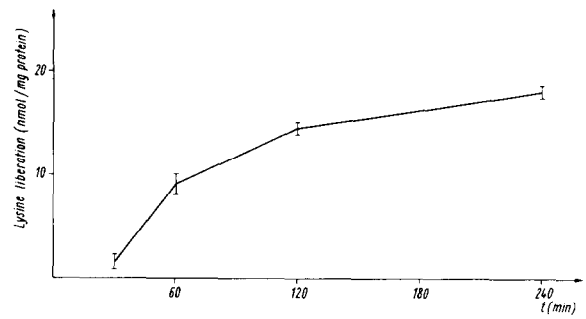

Fig.2. Time dependence of ATP-dependent proteolysis of rat liver mitochondria in the presence of ATP. The incubations were carried out at pH 7.6 and 37°C; means ± SEM of 3 separate expt are given.

teolysis is nearly such at 27°C and exhibits a sharp peak at 37°C.

In fig.4 are presented data which help to specify the localization of the proteolytic system. The data of the first 2 and the last 2 bars indicate:

(i) That ATP produced within the mitochondria is utilized for proteolysis;

(ii) That proteolysis may be augmented by increased ATP supply produced by addition of ADP and succinate.

Inhibition of the respiratory chain by antimycin A as expected decreased proteolysis greatly under these conditions. The fact that carboxyatractyloside (bar 3) inhibited the utilization of endogenously produced ATP for proteolysis indicates that the adenine nucleotide translocator is a necessary component of the system and strongly supports the assumption that the proteolytic system is located on the outer side of the inner mitochondrial membrane. It may be surmised that the proteolytic system is in close vicinity of the adenine nucleotide translocator through which there may be a recycling of the ATP utilized and presumably hydrolyzed during proteolysis. A comparison of the results under endogenous conditions with those with externally supplied ATP (bars 4–6) indicates:

(i) That the endogenous system if supplied with ADP and succinate may approach the maximal capacity with abundant ATP supply,

(ii) That neither antimycin A nor carboxyatractyloside interfere strongly with the system with external ATP supply.

The lack of requirement for the adenine nucleotide translocator supports the conclusion that the proteolytic system is directly accessible from the outside of the mitochondria.

The proteolytic system of rat liver mitochondria does not require preceding action of lipoxygenase [14]. Table 1 presents data which demonstrate that it is strongly dependent on Ca^{2+}. From the fact that the mitochondria under usual conditions are prepared with 1 mM EDTA one may conclude that the proteolytic system has a high calcium af-

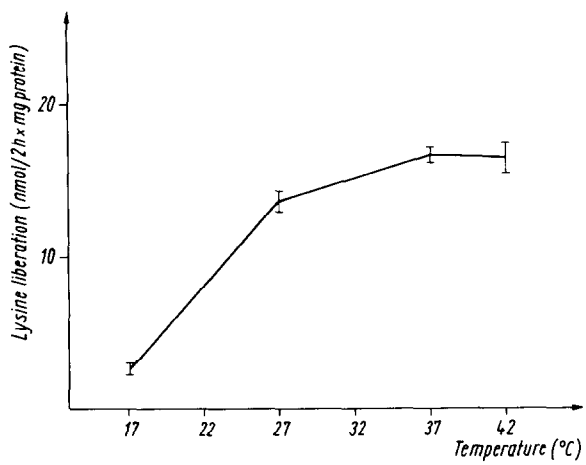

Fig.3. Temperature dependence of ATP-dependent proteolysis in rat liver mitochondria in the presence of ATP. The incubation was carried out for 2 h at pH 7.6; mean ± SEM of 2–3 separate expt are given.

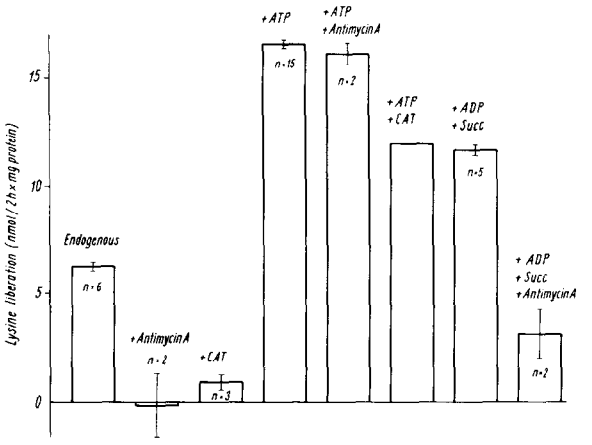

Fig.4. ATP-dependence and localisation of proteolysis in rat liver mitochondria. The incubations were carried out for 2 h at pH 7.6 and 37°C; (details in section 2).

finity, probably in the μM-range. Addition of the local anaesthetic tetracain in high concentrations had only a minor inhibitory effect on proteolysis (not shown), which argues against the involvement of phospholipases as triggers for proteolysis. It would appear therefore that it is proteolysis itself which is calcium-dependent. Proteases even with high affinity for calcium have been described [15,16]. It remains to be established whether the proteolytic system is a two-step cascade, the first being Ca^{2+}- and the second ATP-dependent.

As far as the physiological significance of the membrane-bound ATP-dependent proteolytic system discovered by us is concerned, one should bear in mind that we studied a mitochondrial population in a steady state. Consequently it contains only a small percentage of newly synthesized proteins. Therefore the function of ATP-dependent proteolysis is not to be sought in co- or post-translational processing [17,18], nor can it be reasonably assumed that 'structurally abnormal' proteins produced by isolated mitochondria with a high frequency of biosynthetic errors [19] are the substrates. On the contrary ATP-dependent proteolysis must be connected with a physiological process. It may be suggested that the system discovered by us is instrumental in the normal breakdown of mitochondria as part of their regulated turnover. The system bound to the internal membrane may be selective for the breakdown of this membrane, whereas the cytosolic system discovered in mouse organs including the liver [12] and in rat liver [13] may attack the outer membrane of mitochondria. The difference in the half-times of inner and outer mitochondrial membranes [20] are in consonance with such a suggestion.

Thus it has been established in two biological systems, (i) the maturational process of reticulocytes, and (ii) in normal liver, that mitochondria are the targets and substrates of ATP-dependent proteolysis. Therefore it is reasonable to assume that such ATP-dependent proteolytic systems are physiological mechanisms rather than means for the removal of abnormal proteins which may never arise during the life time of a normal cell.

Table 1

Effect of EGTA on ATP-dependent proteolysis of rat liver mitochondria in the presence of ATP

Expt. no.	Protein breakdown	
	− EGTA	+EGTA
1	18.5	− 3.4
2	16.0	− 0.5
3	16.1	3.1
$\bar{x}$ ± SEM	$\bar{x}$ = 16.9 ± 0.5	$\bar{x}$ = −0.3 ± 1.1

EDTA was replaced by 1 mM EGTA during isolation which was also present in the incubation medium. The incubation was carried out for 2 h at pH 7.6 and 37°C

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