Scope of the ATP–ubiquitin system for intracellular protein degradation

J. Saus, J. Timoneda, J. Hernández-Yago and S. Grisolía

Instituto de Investigaciones Citológicas de la Caja de Ahorros de Valencia, Amadeo de Saboya, 4, Valencia-10, Spain

Received 8 April 1982

Proteolysis *Ubiquitin* *Reticulocytes* *Proteases* *Mitochondria* *Cytosol*

1. INTRODUCTION

Intracellular proteolysis and mechanisms of regulating such processes are not clear. An ATP-dependent proteolytic system has been described in rabbit reticulocytes. This system is composed of a heat-stable polypeptide of 8500–9000 M_r, designated ATP-dependent proteolysis factor I (APF-I), identified as ubiquitin [1] and detected in rat liver [2]. The substrates tested with U–ATP-protease (ubiquitin–ATP-dependent protease system) have been [^{3}H]globin, ^{125}I-lactalbumin, ^{125}I-lactoglobin and ^{125}I-BSA. It is not clear if only denatured proteins are substrates from this system [3]. Therefore, we have tested whether the U–ATP-protease from rabbit reticulocytes can degrade enzymes of mitochondrial and of cytosolic location. There was no ubiquitin–ATP-dependent proteolysis with fresh ornithine transcarbamoylase (OTC), carbamoyl phosphate synthetase (CPS), glutamate dehydrogenase (GDH), pyruvate kinase (PK), lactate dehydrogenase (LDH), glucose-6-phosphate dehydrogenase (G6PDH), as assessed by activity measurements or with ^{125}I-iodinated GDH, CPS and G6PDH. Some small ATP-dependent proteolysis was observed with ^{125}I-PK. The ATP–ubiquitin system appears to be of limited scope.

2. MATERIALS AND METHODS

2.1. *Enzymes*

CPS from rat liver was purified and assayed for activity as in [4] and GDH from rat liver as in [5]. OTC (a gift from A. Navarro) was purified from rat liver and assayed as in [6]. GDH from beef liver, PK from rabbit muscle and G6PDH from yeast were purchased from Boehringer.

2.2. *Protein iodination*

Proteins were iodinated for 5 min as in [7] with Na^{125}I (13–17 mCi/μg; 100 mCi/ml, 250 μCi/mg protein). Free $^{125}I^-$ was removed by filtration using Sephadex G-25 fine equilibrated with 0.05 M sodium phosphate buffer (pH 7.5) pre-saturated with BSA. Protein fractions from the column, containing $<5\%$ of trichloroacetic acid-soluble $^{125}I^-$ were pooled (spec. act. 10^4–10^5 cpm/μg protein).

2.3. *Isolation and assay of components of the ubiquitin–ATP–system*

Partially purified fraction I and fraction II were obtained from rabbit reticulocytes [8]. The U–ATP-protease was assayed according to [9]. Unless indicated otherwise, incubation mixtures contained in 100 μl 100 mM Tris–HCl (pH 7.6); 0.2 μmol dithiothreitol, 1 μmol phosphocreatine, 10 μg phosphocreatine kinase, 0,5 μmol $MgCl_2$, 25 μg partially purified APF-I, 1.3 mg fraction II, 50 nmol ATP, and 2 μg enzyme or BSA. All incubations were for 2 h at 37°C. Loss of enzyme activity, or liberation of trichloroacetic acid-soluble radioactivity for ^{125}I-proteins was measured. Results are expressed as the percentage of trichloroacetic acid-soluble radioactivity after 2 h incubation, corrected for the percentage at zero time.

3. RESULTS AND DISCUSSION

Here, the U–ATP-protease under the conditions in [9] liberated ~ 13% of the trichloroacetic acid-soluble radioactivity from ^{125}I-BSA, in good agreement with [9]. However, when we used native enzymes, there was no or at best slight loss of activity from the enzymes tested (table 1,2). Neither

Published by Elsevier Biomedical Press
00145793/82/0000–0000/$2.75 © 1982 Federation of European Biochemical Societies

Table 1

Effect of the U–ATP-protease on several cytosolic enzymes (% proteolysis or activity after 2 h at 37°C)

Protein	– ATP	+ ATP
^{125}I-BSA (Control)	1.5	12.5
Pyruvate kinase	108	103
Lactate dehydrogenase	103	112
Glucose-6-phosphate dehydrogenase	105	105

Incubations were done as in section 2 using 10 μg enzymes listed or 2 μg ^{125}I-BSA

Table 2

Effect of the U–ATP-protease on several mitochondrial enzymes (% proteolysis or activity after 2 h at 37°C)

Protein	– ATP	+ ATP
^{125}I-BSA	1.5	12.5
Glutamate dehydrogenase (beef liver)	100 (108)	99 (91)
Carbamoyl phosphate synthetase	115 (80)	143 (83)
Ornithine transcarbamoylase	95 (123)	111 (110)

Incubations were done as in section 2 using 100 μg of the proteins listed, except for OTC where 2.5 μg were added. The figures between parenthesis were obtained using 3 μg of the enzymes listed

APF-I nor fraction II alone had any effect on the activities of the enzymes tested.

Given that only denatured and/or modified proteins can be substrates for the U–ATP-protease, ^{125}I-labelled proteins were tested. With ^{125}I-GDH there were no significant differences in the effects produced with either the complete system or the components separately, which could be ascribed to the U–ATP-protease (fig.1). However, fraction II had some proteolytic activity 'per se'. Also, when incubating ^{125}I-BSA or other labelled proteins for 2 h (without the U–ATP-protease) there was some liberation (~ 2%) of trichloroacetic acid-soluble radioactivity.

When non-specific degradation due to fraction II was subtracted (see fig.2), no significant proteolysis was seen with the different enzymes tested. It should be noted that greater activities with ^{125}I-BSA as substrate have been obtained with

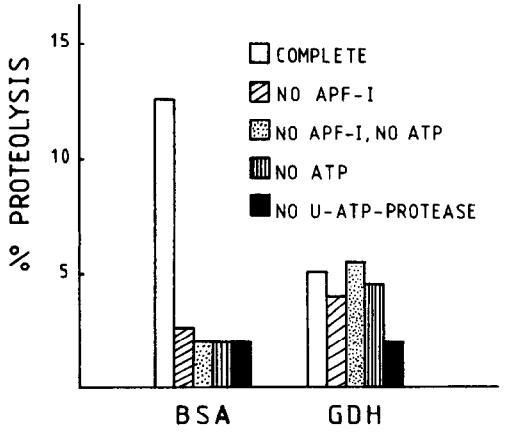

Fig.1. Effect of different components of the U–ATP-dependent proteolytic system on ^{125}I-BSA and ^{125}I-GDH from rat liver. Assayed as in section 2 except where indicated.

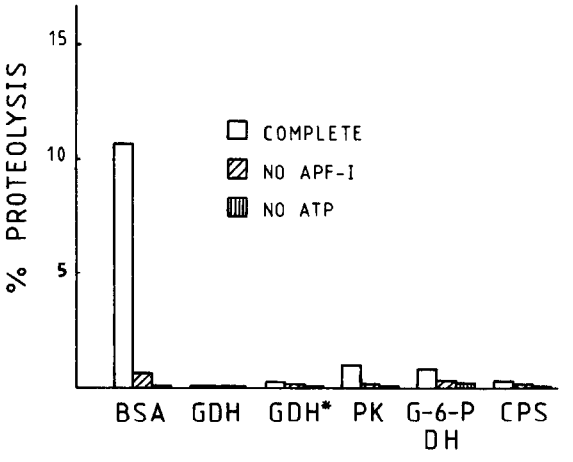

Fig.2. Proteolysis of BSA and of some iodinated enzymes. Assayed as in section 2 using the indicated iodinated proteins. Where indicated ATP or APF-I were excluded. Liberation of radioactivity produced by F-II alone has been subtracted from the indicated values. GDH* means glutamate dehydrogenase from beef liver.

some preparations of fraction II (23%), in agreement with [10]; however, the results with native and iodinated enzymes were also negative (not shown).

In [11] we presented evidence for the existence of both lysosomal and non-lysosomal mechanisms for mitochondrial protein degradation. The U–ATP-protease may be involved in mitochondrial protein degradation during the reticulocyte maturation process [12].

We have tested both native and partially modified (by iodination) mitochondrial enzymes as substrates for the U–ATP-protease and found no significant inactivation and/or degradation of native or iodinated mitochondrial enzymes tested with U–ATP-protease under the same conditions that cause extensive proteolysis of BSA. We also tested a number of cytosolic enzymes and again no evidence was found for inactivation with the U–ATP-protease. Thus, under these conditions, the enzymes tested are not substrates for the U–ATP-protease. The activity of U–ATP-protease is very low; i.e., ~ 0.2–0.4 μg BSA are hydrolyzed in 2 h with ~ 1.3–2 mg fraction II protein. Thus, the U–ATP-protease may be a highly specialized system and of a limited scope.

ACKNOWLEDGEMENTS

This research has been supported in part by the 'Comisión Asesora de Investigación Científica y Técnica' and the 'Fondo de Investigaciones Sanitarias' of Spain. J.S. is an 'S. Ochoa' Fellow of the Ayuntamiento de Valencia.

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