Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 4883–4887, August 1982
Biochemistry

Protease La from *Escherichia coli* hydrolyzes ATP and proteins in a linked fashion

(*lon* gene/protein degradation/vanadate/energy requirement)

LLOYD WAXMAN AND ALFRED L. GOLDBERG

Department of Physiology and Biophysics, Harvard Medical School, 25 Shattuck Street, Boston, Massachusetts 02115

Communicated by Eugene P. Kennedy, May 6, 1982

ABSTRACT **The energy requirement for protein breakdown in *Escherichia coli* results from an ATP requirement for the function of protease La, the product of the *lon* gene. This novel serine protease contains an ATPase activity that is essential for proteolysis. ATP and protein hydrolysis show the same K_m for ATP (30–40 μM) and are affected similarly by various inhibitors, activators, and ATP analogs. Vanadate inhibited ATP cleavage and caused a proportionate reduction in casein hydrolysis, and inhibitors of serine proteases reduced ATP cleavage. Thus, ATP and protein hydrolysis appear to be linked stoichiometrically. Furthermore, ATP hydrolysis is stimulated two- to threefold by polypeptides that are substrates for the protease (casein, glucagon) but not by nonhydrolyzed polypeptides (insulin, RNase). Unlike hemoglobin or native albumin, globin and denatured albumin stimulated ATP hydrolysis and were substrates for proteolysis. It is suggested that the stimulation of ATP hydrolysis by potential substrates triggers activation of the proteolytic function.**

Escherichia coli, like mammalian cells, possesses a proteolytic system for the selective degradation of abnormal polypeptides, as may arise by mutation, biosynthetic error, or gene fusion (1–7). An important feature of the degradation of abnormal as well as normal proteins in bacterial and eukaryotic cells is a requirement for metabolic energy (2, 6, 8–10). Experiments with metabolic inhibitors in *E. coli* indicate that ATP is necessary for an initial endoproteolytic step in this process (6). Cell-free extracts from *E. coli* (11, 12) and reticulocytes (10, 13) have been developed in this laboratory in which the degradation of abnormal proteins is stimulated several-fold by ATP. Subsequently, *E. coli* was found to contain eight different endoproteases (10, 14, 15), of which only one, named protease La, is dependent on ATP and Mg^{2+} for activity.

This ATP requirement of protease La accounts for the energy requirement for the degradative process *in vivo* (16, 17), but the biochemical basis of this ATP effect is unclear. Nonhydrolyzable ATP analogs do not support proteolysis by this enzyme (11, 16, 18), and this process is prevented by inhibitors of ATPases, such as vanadate (16). To account for the ATP dependence of proteolysis in reticulocytes, Hershko, Rose, and co-workers (19, 20) suggested that this process involves multiple components, in which ATP is required not by a protease but for linkage of substrates to the polypeptide ubiquitin to enhance their susceptibility to proteases. However, ubiquitin had no effect on the activity of protease La (16).

Protease La has recently been shown to be the product of the *lon* gene (17). Mutants of *E. coli* that have a reduced capacity to hydrolyze abnormal proteins (called *deg*) were isolated by Bukhari and Zipser (5) and subsequently shown to map in the *lon* (*capR*) locus (7, 21). *In vivo*, the *lon* mutation decreases the endoproteolytic cleavages that require ATP (6). The identification of a single ATP-dependent protease in *E. coli* (10, 14, 22) suggested that protease La might either be the product of the *lon* gene or be regulated by it. Recently, Zehnbauer *et al.* (23) purified the *lon* gene product as a DNA-binding protein with a subunit molecular weight of 94,000. This purified protein was subsequently shown (17, 18) to be an ATP-requiring protease (M_r, 450,000) and to be identical to protease La by a variety of criteria (17).

The availability of homogeneous preparations of protease La made it possible to study the relationship between hydrolysis of ATP and proteolysis. Our results suggest an unusual type of proteolytic mechanism that may be characteristic of enzymes responsible for protein degradation within other cells.

METHODS

The purification of protease La is described in Fig. 1.

Protease Assay. The assay mixture (total vol, 200 μl) included 50 μl of a column fraction or 2 μg of purified enzyme, 0.5 mM ATP, 7.5 mM $MgCl_2$, 50 mM Tris·HCl (pH 7.8), and 20 μg of [^{3}H]casein (20,000 cpm) prepared by reductive methylation (24). After 1 hr at 30°C, the reactions were terminated by addition of 25 μl of 10% bovine serum albumin and 575 μl of 10% trichloroacetic acid, and acid-soluble radioactivity was determined (17).

ATPase Assay. The assay mixture (total vol, 25 μl) included 5 μl of a column fraction or 2 μg of purified enzyme, 50 mM Tris·HCl (pH 7.8), 5 mM $MgCl_2$, 0.5 mM [2,8-^{3}H]ATP (New England Nuclear, 100 cpm/pmol), and α-casein at 0.1 mg/ml. The mixture was incubated for 15 min at 30°C, and 2-μl aliquots were spotted on polyethyleneimine-cellulose plates (Baker) and chromatographed in 0.5 M KH_2PO_4 (25) to separate ATP from the ADP generated. Nonradioactive ATP and ADP (5 nmol) were cochromatographed with each sample to facilitate localization of the ^{3}H-labeled nucleotides under UV light. The appropriate spots were cut out and added to 4 ml of scintillation cocktail. An alternative ATPase assay was used in Fig. 4. The data reported are based on triplicate assays, and similar results were obtained with four different preparations of the protease.

RESULTS

The ATP-Dependent Protease Contains ATPase Activity. Protease La from *E. coli* was purified to homogeneity by sequential chromatography on phosphocellulose, DEAE-cellulose, Sephacryl S-300 (17), and heparin-agarose (Fig. 1). On gel filtration, a single peak of casein-degrading activity was observed but only in the presence of ATP and Mg^{2+}. This peak of proteolytic activity coincided with the peak of ATPase activity

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: p[NH]ppA, adenosine 5′-[β,γ-imido]triphosphate; p[CH_2]ppA, adenosine 5′-[β,γ-methylene]triphosphate.

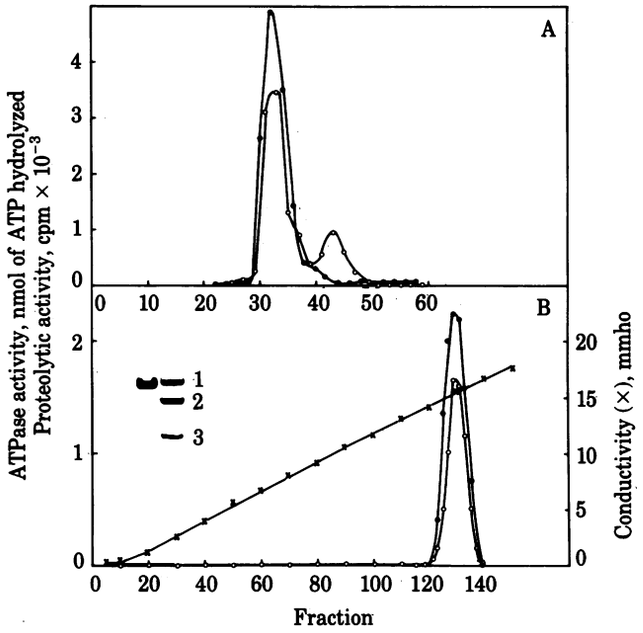

FIG. 1. (*A*) Gel filtration of protease La on Sephacryl S-300. Protease La was purified from 100 g of frozen *E. coli* by chromatography on phosphocellulose (P-11) and DEAE-cellulose (Whatman) as described (17). This material was concentrated by ultrafiltration in an Amicon cell with a PM-10 membrane and applied to a column of Sephacryl S-300 (Pharmacia) (1.5 × 60 cm) equilibrated with 5 mM Tris·HCl, pH 7.4/10 mM NaCl/0.1 mM EDTA/1 mM 2-mercaptoethanol/10% glycerol (buffer A); 1.5-ml fractions were collected and assayed for ATPase (○) and proteolytic activity (●) against [^{3}H]casein. (*B*) The pooled enzyme from the S-300 column was applied directly to an 8-ml column of heparin-agarose (Bethesda Research Laboratories) equilibrated with buffer A. Proteins were eluted with a linear gradient formed from 125 ml of buffer A and 125 ml of buffer A/0.75 M NaCl; 1.5-ml fractions were collected and aliquots were assayed for ATPase and protease activities. (*Inset*) After chromatography on heparin-agarose, 15 μg of enzyme was subjected to electrophoresis on a NaDodSO$_4$/10% polyacrylamide gel as described (26). Marker proteins: 1, phosphorylase b, M_r, 94,000; 2, bovine serum albumin, M_r, 67,000; 3, ovalbumin, M_r, 43,000.

(Fig. 1). When this material was applied to a heparin-agarose column, both activities bound very tightly but were eluted together in a NaCl gradient at a concentration of 0.4 M (Fig. 1). Moreover, when the active fractions were examined by NaDodSO$_4$/polyacrylamide gel electrophoresis, a single band with a molecular weight of 94,000 was found (Fig. 1 *Inset*). Thus, proteolytic and ATP-hydrolyzing activities reside within the same protein.

Using less pure preparations, we have previously established that protease La has neither protein kinase nor protein-adenylylating activity (16). In the ATPase assay used here, [^{3}H]ATP was hydrolyzed only to [^{3}H]ADP. Since no [^{3}H]AMP was detected, protease La has no phosphohydrolase activity. As shown in Table 1, the various nucleotides that support proteolysis were also hydrolyzed by protease La. ATP and dATP, which support proteolysis, are both good substrates for the ATPase. ADP, adenosine 5′-[β,γ-imido]triphosphate (p[NH]ppA), and adenosine 5′-[β,γ-methylene]triphosphate (p[CH$_2$]ppA), which do not support proteolysis, are not hydrolyzed, and adenosine 2′,3′-dialdehyde triphosphate was a poor substrate for both reactions. When added with ATP, ADP and p[NH]ppA effectively inhibited both activities, but p[CH$_2$]ppA did not. [The greater inhibition by p[NH]ppA is probably because it is a closer structural analog of ATP than p[CH$_2$]ppA (28).] Thus, protease La can hydrolyze a variety of adenosine nucleotides at rates that correlate roughly with their ability to support proteolysis. In contrast, UTP, CTP, and GTP were good substrates for the ATPase but supported proteolysis much less well than ATP for reasons that are unclear.

Table 1. Relative rates of hydrolysis of different nucleotides and their effects on casein degradation by protease La

Nucleotide	Relative rate: Nucleotide hydrolysis	Relative rate: Proteolysis
ATP	100	100
ATP in the absence of Mg^{2+}	0	2
ADP	0	2
dATP	100	92
p[CH$_2$]ppA	0	6
p[NH]ppA	0	2
CTP	82	31
GTP	113	14
UTP	77	22
Adenosine 2′,3′-dialdehyde triphosphate	23	30
ATP/p[CH$_2$]ppA	95	98
ATP/ADP	75	61
ATP/p[NH]ppA	52	40

Nucleotides were used at 0.5 mM. ATPase assays were carried out by using the colorimetric method described in Fig. 4 in the presence of α-casein at 0.1 mg/ml. Protease assays were carried out as described in Fig. 1 using 2 μg of purified protease La. The 2′,3′-dialdehyde derivative of ATP was synthesized as described by Easterbrook-Smith *et al.* (27).

The ATPase and proteolytic activities showed similar dependences on the concentration of ATP (Fig. 2). Analysis of these data (Fig. 2) by double reciprocal plots gave similar K_m values for the two reactions, 27 and 45 μM, respectively. Although the

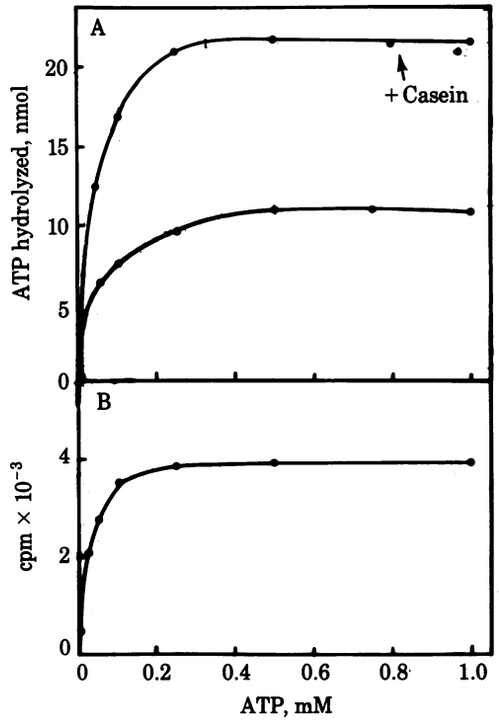

FIG. 2. ATP-dependences of protease (*A*) and ATPase (*B*) activities of purified protease La. Both activities were assayed in the presence or absence of α-casein at 0.1 mg/ml with various concentrations of ATP. In the protease assay, 2 μg of enzyme was used and, in the ATPase assay, 0.5 μg was used.

absolute values varied as much as 50% with different preparations of the enzyme and [^{3}H]casein, the K_m values for the two reactions were always similar (within a factor of two). In addition, Mg^{2+} was absolutely required for both the ATPase and the protease (Table 1), and 5–7 mM $MgCl_2$ gave maximal activity for both reactions in the presence of 0.5 mM ATP (data not shown).

Ubiquitin has been suggested to be a key component in ATP-stimulated proteolysis in reticulocytes (19, 20). However, over a range of concentrations (0.001–0.1 mg/ml), ubiquitin had no effect on the degradation of [^{3}H]casein or [^{3}H]ATP by protease La (Table 2). Thus, no additional factors other than ATP and Mg^{2+} are required for ATP-stimulated proteolysis.

Effects of Different Reagents on ATPase and Proteolytic Activities. Various experiments were undertaken to determine to what extent hydrolysis of ATP and proteolysis are linked. Diisopropyl fluorophosphate, which binds covalently to the active site of serine proteases (29), inhibits protease La (17). It also inhibits the ATPase, as does dansyl fluoride (Table 2), another reagent that appears to inactivate specifically serine proteases (30). The finding that these protease inhibitors reduced ATPase activity (both basal and protein-stimulated) suggests a tight coupling between these processes, although it is possible that these agents may also react at the ATPase site. On the other hand, vanadate, which inhibits a variety of ATP-utilizing enzymes (31), blocked both the ATPase and proteolytic functions of La, and the degree of inhibition of these two processes was quite similar with different vanadate concentrations (Fig. 3). In fact, the percentage of inhibition of ATP cleavage was directly proportional to the decrease in proteolysis.

At high concentrations, the sulfhydryl blocking agent iodoacetamide also inhibits both activities to a similar degree. Furthermore, both activities are severely inhibited by high concentrations of NaCl, and both are stimulated to the same degree by physiological concentrations of spermidine (ref. 32 and Table 2). Thus, the two hydrolytic functions of protease La are closely linked and could not be altered independently.

The Effect of Polypeptide Substrates on ATPase Activity. In defining the optimal conditions for measuring ATPase activity, we noted that ATP hydrolysis was significantly greater in the presence of casein than in its absence. As shown in Fig. 2, addition of α-casein at 0.1 mg/ml doubled the rate of ATP hydrolysis (α-casein itself had no detectable ATPase activity). At higher concentrations of casein, the ATPase activity was stimulated as much as three fold (Fig. 4) while the K_m for ATP was not significantly altered. It is noteworthy that the amount of casein required for half-maximal stimulation of the ATPase was approximately 40 μg/ml, which is similar to the K_m of the protease for casein (50 μg/ml; Fig. 4). The concentrations of casein needed for half-maximal stimulation of the two processes were

Table 2. Effects of different reagents on proteolysis and ATP hydrolysis by protease La

Compound	[^{3}H]Casein hydrolysis	ATPase
None	100	100
Ubiquitin (0.01 mg/ml)	100	100
Spermidine (5 mM)	150	140
Iodoacetamide		
2.5 mM	60	67
25 mM	2	0
Diisopropyl fluorophosphate		
(10 mM)	0	0
Dansyl fluoride		
0.5 mM	77	73
5 mM	24	25
Vanadate (10 μM)	50	53
NaCl (0.5 M)	8	40

Iodoacetamide, diisopropyl fluorophosphate, and dansyl fluoride were incubated with enzyme for 1 hr at 25°C before the assays were carried out. Controls incubated without these reagents showed a 10% loss in activity due to the presence of dimethyl sulfoxide (5%, vol/vol) used to solubilize diisopropyl fluorophosphate and dansyl fluoride. Previous incubation of the enzyme with purified bovine thymus ubiquitin (provided by G. Goldstein, Ortho Pharmaceuticals) for 30 min in the presence of ATP/Mg^{2+} also had no effect on either activity.

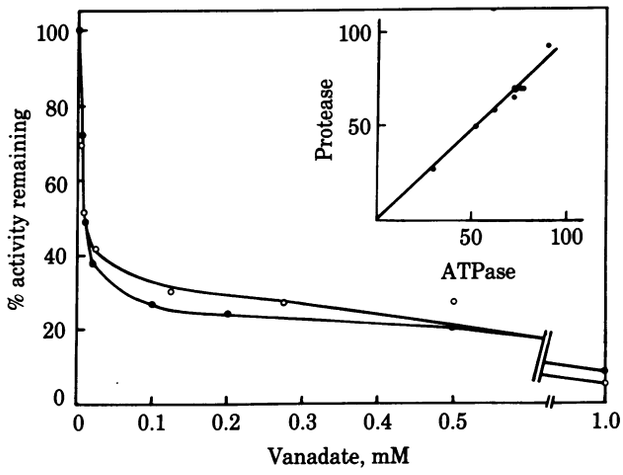

FIG. 3. Inhibition of the ATPase (●) and protease (○) functions of protease La by sodium vanadate. Basal ATPase activity in the absence of casein, which is also inhibited by vanadate, was subtracted from the values obtained in the presence of casein. (*Inset*) Data were replotted to examine percentage of inhibition of casein-stimulated ATPase as a function of percentage of inhibition of protease by vanadate.

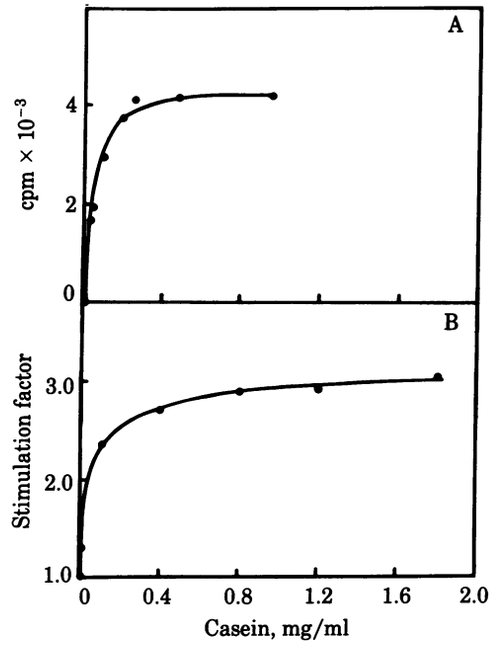

FIG. 4. Dependence of ATPase (*A*) and protease (*B*) activities of protease La on casein concentration. In this experiment, a colorimetric assay for ATPase activity was used, but identical results were obtained in similar experiments in which the standard assay was used. In this instance, 4 μg of purified protease was incubated in a volume of 200 μl under conditions identical to those used in the protease assay. After 30 min at 30°C, 100 μl of 3% $NaDodSO_4$ was added to inactivate the enzyme, and the phosphate released was determined as described by Ames (33).

Table 3. Effects of various polypeptides on ATPase activity of protease La

Polypeptide added	% stimulation	Substrate
α-Casein	100	Yes
Glucagon	90–100	Yes
Glucagon (trypsin treated)	0	—
Proinsulin	31	—
Insulin	5	No
Insulin		
A chain	0	—
B chain	0	—
Bovine serum albumin		
Native	0	No
Denatured	83	Yes
Globin	34	Yes
Hemoglobin	0	No
RNase	0	No
Lysozyme	0	No

ATPase assays were carried out as described in Fig. 1. Polypeptides were included at 0.1 mg/ml. Polypeptides examined as substrates for the protease were radiolabeled and assayed as described in *Methods*. To precipitate undegraded ^{125}I-labeled glucagon, the final concentrations of albumin and trichloroacetic acid were increased to 2% and 10%, respectively. Denatured albumin was prepared by reduction of the disulfide bonds in the presence of 6 M guanidine hydrochloride followed by alkylation with iodoacetamide (34) and succinylation to block lysine amino groups and thus improve solubility (35).

similar in other preparations, although the precise values varied.

Other polypeptides were examined to determine whether their ability to stimulate the ATPase correlated with their susceptibility to degradation. The degree of stimulation of the ATPase function varied widely (Table 3 and Fig. 5). Stimulation by glucagon was comparable with that of α-casein, while globin was significantly less effective and hemoglobin and insulin had no stimulatory effect. After incubation with trypsin, glucagon no longer stimulated the ATPase, which suggests that this treatment destroyed whatever structures in this peptide are recognized by the enzyme. Native bovine serum albumin and RNase were also ineffective at stimulating the ATPase activity but, after denaturation and covalent modification, bovine serum albumin (Table 3) was a good stimulator of the ATPase.

Several of these polypeptides were then labeled and tested as substrates for the protease. ^{125}I-Labeled glucagon and [^{14}C]globin were cleaved by protease La to acid-soluble products, while ^{125}I-labeled bovine serum albumin, RNase, insulin, and lysozyme were not. However, denatured ^{125}I-labeled bovine serum albumin was readily digested by the protease (Table 3). RNase, which failed to stimulate the ATPase significantly, also was not cleaved by protease La. Thus, although the ATPase activity is operative in the absence of exogenous factors, it is specifically enhanced by those polypeptides that are substrates for the enzyme. This unusual property provides further support for the conclusion that the two hydrolytic functions of protease La are closely coupled.

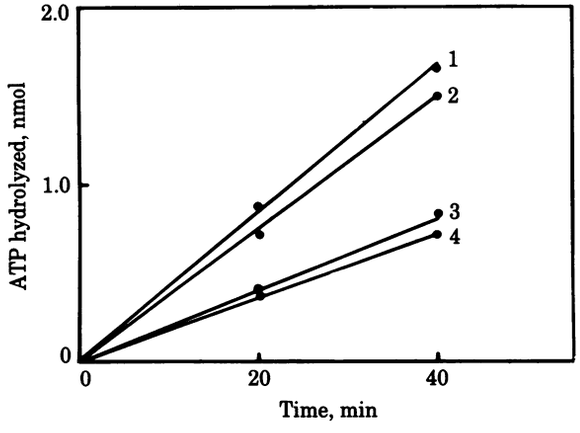

FIG. 5. Time course of ATP hydrolysis by protease La in the presence of various polypeptides at 0.1 mg/ml. Curves: 1, α-casein; 2, glucagon; 3, no added polypeptide; 4, bovine serum albumin.

DISCUSSION

Protease La may be representative of a new class of intracellular proteolytic enzymes in which ATP plays an important mechanistic role. Unlike other known endoproteases, protease La requires the hydrolysis of ATP for peptide bond cleavage. Rat liver mitochondria also possess an ATP-dependent system for degrading abnormal proteins (36), and an enzyme has been isolated from this organelle that has many of the properties of protease La (37). The recA protein from *E. coli* requires ATP as well as polynucleotides for the specific cleavage of the λ (4, 38) and lexA repressors (39). In this reaction, unlike that studied here, ATP need not be hydrolyzed, although the enzyme does contain an ATPase activity (40, 41). Recently, a nonlysosomal protease has been purified from rabbit red cells that is stimulated twofold by ATP but does not hydrolyze this nucleotide (42). This enzyme appears to play an essential role in the degradation of abnormal proteins (42), and a similar protease has been found in the cytoplasm of several mammalian tissues (10, 43). It is noteworthy that all these enzymes have unusually high molecular weights (450,000–550,000) and thus differ from well-characterized extracellular or lysosomal proteases (20,000–40,000). We suggest that the large size of these enzymes and their utilization of ATP underlie the exquisite selectivity and regulation of intracellular proteolysis.

The cleavage of ATP and the splitting of peptide bonds are indeed closely linked, perhaps coupled, functions, both of which reside in the same M_r 94,000 subunit (Fig. 1). A variety of agents that inhibit ATPases or serine proteases reduce both of these activities of protease La to a similar extent (Table 2 and Fig. 3). It has recently been found that inactive subunits produced by a *lon* mutant (*capR9*) also inhibit both enzymatic activities in parallel (44). On the other hand, certain molecules present intracellularly, including spermidine (Table 2) and DNA (45), stimulate both proteolytic and ATPase activities. Further evidence for the tight coupling of these two processes were the findings that (*i*) the ATPase activity is stimulated by polypeptides that are substrates (Table 3) and (*ii*) the decrease in ATP hydrolysis was directly proportional to the decrease in proteolysis (Fig. 3).

These observations suggest a stoichiometric relationship between the cleavage of peptide and high-energy phosphate bonds. However, the precise stoichiometry is difficult to resolve with the present assay, since it is not clear how many cleavages of [^{3}H]casein are necessary to generate acid-soluble radioactivity and whether further fragmentation of the peptides occurs after they have been rendered acid soluble. In addition, the apparent coupling between peptide bond cleavage and nucleotide hydrolysis seems to differ with different nucleoside triphosphates (Table 1) and when proteolysis is stimulated by DNA (45).

The fundamental mechanistic issue is how the energy from the hydrolysis of ATP serves in the enzymatic cleavage of proteins, because phosphorylation, adenylylation, and ubiquitin linkage to substrates have been ruled out (16). Labilization of the peptide bonds may require the transfer of phosphate from

ATP in a novel hydrolytic mechanism. Evidence for such a possibility comes from studies of the mammalian enzyme that catalyzes the ATP-dependent hydrolysis of the peptide bond in 5-oxoproline (46, 47). It is also possible that the hydrolysis of ATP may be needed to unwind regions of the polypeptide substrate to make them more accessible to hydrolysis.

Alternatively, ATP breakdown may cause a conformational change in the protease. For example, as a consequence of the substrate-induced ATP cleavage, the protease may assume a conformation in which the polypeptide is now accessible to the active site. In fact, we have recently found that some oligopeptides, unlike large polypeptides (Table 3), can be cleaved by protease La without activation of ATP hydrolysis. The finding that only substrate polypeptides activate ATP cleavage (Table 3) suggests that this step may be critical in the recognition of proteins to be degraded. Such a "triggering" mechanism could ensure that, in the absence of appropriate substrates (e.g., denatured proteins), the protease will not digest cellular proteins nonselectively.

We thank the members of this laboratory for their valuable suggestions, Mr. Timothy Meixsell for his assistance in certain studies, and Ms. Maureen Rush for her help in preparing this manuscript. These studies have been supported by grants from the National Institute of Neurological and Communicative Diseases and Stroke, the Juvenile Diabetes Foundation, the Kroc Foundation, and the Eli Lilly Company.

1. Goldberg, A. L. & Dice, J. F. (1974) *Annu. Rev. Biochem.* **43**, 835–869.
2. Goldberg, A. L. & St. John, A. C. (1976) *Annu. Rev. Biochem.* **45**, 747–803.
3. Holzer, H. & Heinrich, P. C. (1980) *Annu. Rev. Biochem.* **49**, 63–91.
4. Mount, D. W. (1980) *Annu. Rev. Genet.* **14**, 279–319.
5. Bukhari, A. I. & Zipser, D. (1973) *Nature (London) New Biol.* **243**, 238–241.
6. Kowit, J. D. & Goldberg, A. L. (1977) *J. Biol. Chem.* **252**, 8350–8357.
7. Gottesman, S. & Zipser, D. (1978) *J. Bacteriol.* **133**, 844–851.
8. Olden, K. & Goldberg, A. L. (1978) *Biochim. Biophys. Acta* **542**, 385–398.
9. Goldberg, A. L. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 2640–2644.
10. Goldberg, A. L., Strnad, N. & Swamy, K. H. S. (1980) *CIBA Found. Symp.* **75**, 227–251.
11. Murakami, K., Voellmy, R. W. & Goldberg, A. L. (1979) *J. Biol. Chem.* **254**, 8194–8200.
12. Voellmy, R. W. & Goldberg, A. L. (1981) *Nature (London)* **290**, 419–421.
13. Etlinger, J. D. & Goldberg, A. L. (1979) *Proc. Natl. Acad. Sci. USA* **74**, 54–58.
14. Swamy, K. H. S. & Goldberg, A. L. (1981) *Nature (London)* **292**, 652–654.
15. Goldberg, A. L., Swamy, K. H. S., Chung, C. H. & Larimore, F. S. (1981) *Methods Enzymol.* **80**, 680–702.
16. Larimore, F. S., Waxman, L. & Goldberg, A. L. (1982) *J. Biol. Chem.* **257**, 4187–4195.
17. Chung, C. H. & Goldberg, A. L. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 4931–4935.
18. Charette, M. F., Henderson, G. W. & Markovitz, A. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 4728–4732.
19. Ciechanover, A., Heller, H., Elias, S., Haas, A. L. & Hershko, A. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 1365–1368.
20. Hershko, A., Ciechanover, A., Heller, H., Haas, A. L. & Rose, I. A. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 1783–1786.
21. Markovitz, A. (1977) in *Surface Carbohydrates of the Prokaryotic Cell*, ed. Sutherland, I. (Academic, New York), 415–462.
22. Goldberg, A. L., Voellmy, R. & Swamy, K. H. S. (1979) in *Biological Functions of Proteinases*, eds. Holzer, H. & Tschesche, H. (Springer, Berlin), pp. 35–48.
23. Zehnbauer, B. A., Foley, E. C., Henderson, G. W. & Markovitz, A. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 2043–2047.
24. Rice, R. H. & Means, G. E. (1971) *J. Biol. Chem.* **246**, 831–832.
25. Belhadi, O., Santenac, A. & Fromageot, P. (1980) *J. Biol. Chem.* **255**, 11701–11709.
26. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
27. Easterbrook-Smith, S. B., Wallace, J. L. & Keech, D. B. (1976) *Eur. J. Biochem.* **62**, 125–130.
28. Yount, R. G. (1975) *Adv. Enzymol.* **43**, 1–56.
29. Hartley, B. S. (1960) *Annu. Rev. Biochem.* **29**, 45–72.
30. Vaz, W. L. C. & Schoellmann, G. (1976) *Biochim. Biophys. Acta* **439**, 194–205.
31. Macara, I. G. (1980) *Trends Biochem. Sci.* **5**, 92–94.
32. Tabor, C. W. & Tabor, H. (1976) *Annu. Rev. Biochem.* **45**, 285–306.
33. Ames, B. (1966) *Methods Enzymol.* **8**, 115–118.
34. Hirs, C. H. W. (1967) *Methods Enzymol.* **11**, 197–199.
35. Klapper, M. & Klotz, I. M. (1972) *Methods Enzymol.* **25**, 531–536.
36. Desautels, M. & Goldberg, A. L. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 1869–1873.
37. Desautels, M. & Goldberg, A. L. (1982) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **41**, 866.
38. Craig, N. L. & Roberts, J. W. (1980) *Nature (London)* **283**, 26–30.
39. Little, J., Edmiston, S., Pacelli, L. & Mount, D. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 3225–3229.
40. Roberts, J., Roberts, C., Craig, N. & Phizicky, E. (1978) *Cold Spring Harbor Symp. Quant. Biol.* **43**, 917–920.
41. Ogawa, T., Wabiko, H., Tsurimoto, T., Horri, T., Masukata, H. & Ogawa, H. (1978) *Cold Spring Harbor Symp. Quant. Biol.* **43**, 909–915.
42. Boches, F. S., Klemes, Y. & Goldberg, A. L. (1980) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **39**, 1682.
43. DeMartino, G. N. & Goldberg, A. L. (1979) *J. Biol. Chem.* **254**, 3712–3715.
44. Chung, C. H. & Goldberg, A. L. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 795–799.
45. Waxman, L., Chung, C. H. & Goldberg, A. L. (1982) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **41**, 865.
46. Van Der Werf, P. & Meister, A. (1975) *Adv. Enzymol.* **43**, 519–556.
47. Griffith, O. W. & Meister, A. (1981) *J. Biol. Chem.* **256**, 9981–9985.