GPa and 2550°C (Figs. 1 and 2) and that the dissolution of Si is enhanced with increasing pressure. Therefore, Si and O could be important light elements in the core. Concurrently, the SiO_2 content of the mantle would be substantially reduced from its primitive one by core formation in the deep magma ocean. In the experiments, however, entry of Mg into molten iron was not observed. Thus, the core formation should have strongly affected the chemistry of the mantle. The light elements that were incorporated, on the other hand, certainly play an important role in the dynamics and evolution of the core.

High-pressure mineral physics has extensively contributed to the solution of the question, "What and how is the Earth's interior?" The answer generates more specific and comprehensive new questions, "Why and when was the answered situation caused?" This is because the Earth has evolved for 4.5 billion years, and its present status reflects all the time-integrated results of various events and complicated processes.

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Proteases in *Escherichia coli*

Chin Ha Chung

Cells make mistakes. Sometimes the proteins of bacterial and animal cells are not synthesized correctly. This can happen by accident through biosynthetic error or mutation, or can be induced by the incorporation of amino acid analogs or by stresses such as high temperatures (*1*). Because such abnormal polypeptides with highly aberrant structures are nonfunctional and are frequently toxic to cells, it is to the cell's advantage to eliminate them. This essential cellular process is accomplished by the action of proteolytic enzymes. The mechanisms by which these enzymes digest abnormal proteins have been illuminated by the study of the proteases of the bacterium *Escherichia coli*.

Since the early 1980s, nine distinct endoproteases have been isolated from *E. coli* (see table) (*2*). Seven of these, proteases Do, Re, Mi, Fa, So, La, and Ti, are serine proteases that hydrolyze large proteins such as casein and globin. Two other enzymes, proteases Ci and Pi, are metalloproteases that degrade smaller polypeptides, such as insulin and short amino-terminal fragments of β-galactosidase. Proteases Mi and Pi are periplasmic enzymes, while all others are cytoplasmic and therefore may potentially degrade intracellular proteins.

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Protease Do is a serine protease with an unusually high molecular mass of about 500 kD (*3*). This enzyme in vitro catalyzes limited cleavage of the Ada protein, which takes part in the repair of methylated DNA, and of the IciA protein, which is an inhibitor of replication initiation of the *E. coli* chromosome (*4*). In addition, protease Do is identical to the *htrA* gene product, and mutations of the gene result in the loss of cell viability at high temperatures and loss of its ability to degrade alkaline phosphatase fusion proteins (*5*). Proteases Re and So degrade oxidized glutamine synthetase but not the native form of the protein (*6*). Protease So also degrades signal peptides after their release from precursor proteins in vitro (*7*). In addition, protease Re has recently been identified as Tsp (tail-specific protease), which in vitro catalyzes the degradation of a bacteriophage λ repressor variant (*8*).

An intriguing feature of protein breakdown in bacteria, as well as in animal cells, is its requirement for metabolic energy (*1*). For example, in *E. coli*, inhibitors of energy metabolism greatly reduce hydrolysis of most abnormal proteins and certain regulatory proteins. This energy requirement would not be expected on the basis of thermodynamics or from the properties of typical proteolytic enzymes. Studies on the mechanistic basis of this energy requirement have led to the isolation of two *E. coli* proteases, La and Ti, which are dependent on adenosine triphosphate (ATP) and Mg^{2+} for activity.

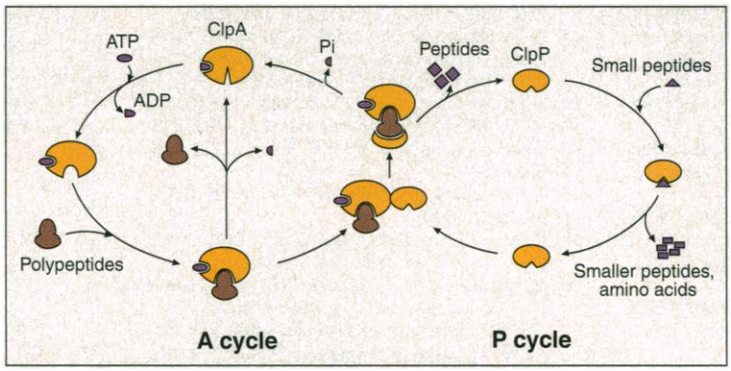
The protease La is the product of the *lon* gene (*9*). This enzyme is essential for the degradation of most abnormal proteins and certain normal short-lived polypeptides, in-

Soluble endoproteases from *E. coli*. Abbreviations: DFP, diisopropyl fluorophosphate; NEM, *N*-ethylmaleimide; and GS, glutamine synthetase.

Protease	Gene	Size (native) (kD)	Substrate	Inhibitor	ATP-dependency
Do	*htrA*	48 (500)	Casein, IciA, Ada	DFP	No
Re	*tsp*	82 (82)	Casein, oxidized GS	DFP	No
Mi		110 (110)	Casein	DFP	No
Fa		110 (110)	Casein	DFP	No
So		77 (140)	Casein, oxidized GS, signal peptides, Ada	DFP	No
La	*lon*	87 (450)	Casein, SulA, RcsA, λN	DFP, NEM, ADP	Yes
Ti	*clpA*	84 (140)		NEM	Yes
	clpP	21 (240)	Casein, ClpA	DFP	
Ci		54 (54)	Insulin	*o*-phenanthroline	No
Pi	*ptr*	110 (110)	Insulin	*o*-phenanthroline	No

cluding SulA, an inhibitor of cell division (*10*). La is a heat-shock protein and can be induced when large amounts of abnormal proteins are produced in *E. coli* (*11*). It is composed of four identical 87-kD subunits, each of which contains a site for ATP hydrolysis. One unusual property of this enzyme is that protein substrates stimulate its ATP-cleaving activity, and this process is linked stoichiometrically to proteolysis (*12*). Two molecules of ATP are consumed for each peptide bond cleaved in proteins. In addition to proteins, protease La cleaves several synthetic peptides with bulky hydrophobic residues in the P1 position (for example, glutaryl-Ala-Ala-Phe-methoxy-naphthylamide) (*13*). This peptide hydrolysis also requires ATP, but unlike protein degradation, only needs the binding of ATP not ATP cleavage. Thus, nonmetabolized analogs of ATP support the cleavage of the peptide but allow only limited cleavage of protein substrates. Another unusual property of this enzyme is that protein substrates stimulate the peptide-hydrolyzing activity. Thus, the enzyme seems to function through a multistep mechanism, in which protein substrates and ATP initially bind to allosteric sites, the occupancy of which activates the proteolytic site. This complex mechanism seems to help ensure against inappropriate or excessive proteolysis in the cytoplasm.

The second ATP-dependent enzyme is protease Ti, also called Clp (*3*, *14*). This enzyme also degrades abnormal proteins, such as those containing canavanine, an analog of arginine. In addition, Ti rapidly degrades proteins with abnormal amino acids at their amino termini (*15*). Unlike protease La, however, protease Ti consists of two different polypeptide subunits, both of which are required for proteolysis. When isolated, component A (ClpA) behaves as a dimeric complex of 84-kD subunits and contains the ATP-cleaving site (*14*). Component P (ClpP), which is a heat-shock protein, is a multimer of 21-kD subunits and contains the serine active site for proteolysis. Isolated ClpA also has protein-activated adenosine triphosphatase (ATPase) activity, which in the reconstituted enzyme is linked to protein breakdown. However, ClpP can hydrolyze a few synthetic peptides with bulky hydrophobic residues in the P1 position (for example, succinyl-Leu-Tyr-amidomethylcoumarin), even though ClpP by itself cannot degrade protein substrates (*16*). Accordingly, the reconstituted enzyme degrades the peptide similarly to ClpP alone and at rates that are similar in the presence and absence of ATP. In addition, this peptide, unlike protein substrates, does not stimulate ATP hydrolysis by ClpA and also does not affect the protein-activated ATPase activity (see figure).



A model for ATP-dependent proteolysis by protease Ti. In the A cycle, ATPase activity of ClpA is activated by proteins that are potential substrates for ClpP. Therefore, without ClpP, this cycle is futile. In the presence of ClpP, however, ATP hydrolysis may induce a conformational change in ClpP, which allows the protein substrates access to the active site and thereby permits rapid protein hydrolysis. After proteolysis, adenosine diphosphate (ADP) may remain bound or compete with ATP to block the inherent ATPase activity of ClpA. In the P cycle without ClpA, only small peptides are accessible to and degraded by the active site of ClpP.

In the absence of ATP hydrolysis by ClpA, the peptidase site in ClpP is presumably inaccessible to large protein substrates. Thus, ATP hydrolysis must unmask or enlarge the peptidase site so that it can also digest large proteins. This mechanism is supported by the finding that ClpP can form a tight complex with ClpA only in the presence of ATP (*17*). In the presence of nonhydrolyzable analogs of ATP, ClpP can form a complex with ClpA but is unable to degrade proteins. Therefore, Ti seems to function through a multistep mechanism, in which protein substrates initially interact with ClpA, thereby activating ATP cleavage and inducing a conformational change in ClpP, which allows rapid hydrolysis by the peptidase site.

Clearly, ATP hydrolysis is not required for the peptide bond cleavages by proteases La and Ti. Therefore, in the two *E. coli* ATP-dependent proteases, nucleotide hydrolysis is not required thermodynamically for peptide bond cleavage, but instead the energy-linked reactions seem to allow access to (Ti) or formation of (La), the proteolytic site.

The *lon clp* double mutants retain 20 to 30% of the wild-type ATP-dependent degradative capacity toward canavanine-containing polypeptides (*17*). One of the candidates for the residual ATP-dependent proteolytic activity is ClpB, which is a close homolog of ClpA (*18*). ClpB also has a protein-activated ATPase activity, but, when isolated, behaves as a tetrameric complex of 93-kD subunits, unlike dimeric ClpA (*19*). In addition, the *clpB* gene also encodes a 79-kD polypeptide from the same reading frame as 93-kD ClpB but from an internal translational initiation. Because both ClpB and ClpP are heat-inducible proteins, a model ATP-dependent protease, a ClpB-ClpP complex, has been proposed to exist and be crucial for protection against thermal stress in *E. coli* (*20*). However, purified ClpB cannot replace ClpA in supporting the proteolytic activity of ClpP. Therefore, *E. coli* may still contain another ClpP-like proteolytic component that associates with ClpB to form a new type of ATP-dependent protease.

Although much has been learned about the mechanism of ATP-dependent proteolysis by proteases La and Ti, many questions concerning the selectivity of the degradation process remain unanswered. It is unclear, for example, how the proteases can distinguish abnormal polypeptides and short-lived regulatory proteins from the bulk of normal cell proteins. An important goal for future research is to isolate the ATP-requiring protease activity for abnormal proteins that remains in the *lon clp* double mutants. In addition, as yet we know little about the specific physiological roles of the other soluble, ATP-independent proteases (Do, Re, Mi, Fa, So, Ci, and Pi). Since bacterial cells would likely recognize cloned and overproduced eukaryotic proteins as foreign and abnormal to themselves, further progress in understanding *E. coli* proteases and construction of mutants lacking their activities in proper combinations will not only be of physiological and biochemical interest but also prove to be of practical importance for biotechnology.

References and Notes

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Double Resonance Spectroscopy and Molecular Dynamics

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During the past two decades, numerous laser spectroscopic techniques have been introduced that aim at unraveling the structure and dynamics of excited molecular species. The technique of optical-optical double resonance multiphoton ionization (OODR-MPI) has been one of the latecomers of the family. It is, nevertheless, also one of the most promising.

Even for simple molecules, electronic spectra tend to be so complex that it is difficult to properly assign the transitions, in spite of the fact that data may be taken at a resolution sufficient to see molecular rotations. Double resonance spectroscopy, developed by Field and co-workers (*1*), proves to be an incisive tool in this respect. Here, we are dealing with two consecutive resonance transitions involving three molecular energy levels, 1, 2, and 3. In general, double resonance experiments are performed as follows: First, a tunable laser is locked to the frequency of a known molecular transition 1 → 2. Thanks to the narrow linewidth of state-of-the-art tunable lasers, this can easily provide finer-than-rotational spectroscopic resolution. Scanning the wavelength of a second tunable laser then gives rise to a very simple, easy-to-interpret spectrum of 2 → 3, as a result of the restricted selection rules of 1 → 2 → 3. By tuning the first laser to successive rovibronic transitions of 1 → 2 and repeating the scanning of the second laser, one can accumulate systematic and unambiguous spectroscopic data of 2 → 3.

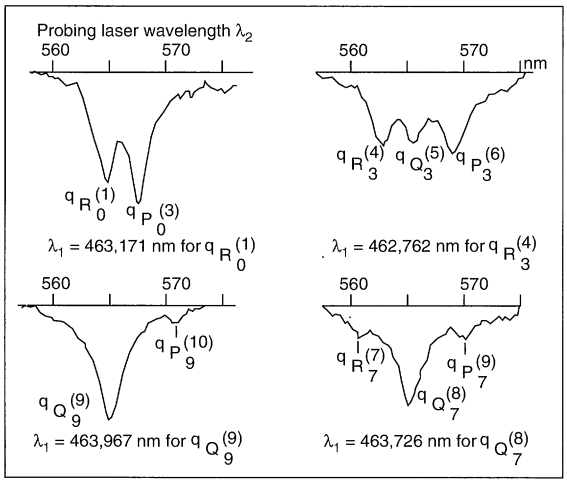

Fig. 1. Ion dip spectrum for NH_3 $\tilde{C}'\,{}^1A_1$ $v' = 1 \rightarrow {}^1A_2''$ $v_2'' = 1$ transitions obtained by locking the $\tilde{C}' \leftarrow \tilde{X}$ pump laser and scanning the probe laser (*4*).

Multiphoton excitation by tunable lasers in the optical region (*2*) allows access to high-lying electronic states of molecules, which would have required very expensive tunable vacuum ultraviolet light sources. Ionic detection competes favorably with photon counting; with a little care and a number of simple tricks, the former is capable of providing higher signal-to-noise ratios and higher sensitivity. These features combine to make OODR-MPI an extremely versatile spectroscopic technique, whose potential has not yet been fully exploited.

We have used a variant of OODR-MPI known as ion dip spectroscopy (IDS) to obtain rotational spectra of the very short-lived predissociating species NH_3 $\tilde{A}\ {}^1A_2''$. Ito and co-workers have performed detailed studies on large molecules by IDS (*3*). Our present endeavor deals with a short-lived species with a lifetime of around 10^{-13} s (*4*). Each rotational line is broadened to several tens of wave numbers because of the short lifetime, and in conventional spectroscopy there is always complete overlap of the rotational lines with the adjacent ones to form a diffuse spectrum. With IDS, we first performed three-photon excitation to pump ground state NH_3 to the $\tilde{C}'$ state, and from there, another photon brought it to the ionization continuum. Then, with the first laser locked to a specific rovibronic transition $\tilde{C}'(v', J') \leftarrow \tilde{X}$, we scanned a second laser to bring about the stimulated emission $\tilde{A}(v, J) \xleftarrow{h\nu} \tilde{C}'(v', J')$. IDS is in essence an ionic detection version of stimulated emission pumping (SEP) first developed by Kinsey, Field, and their colleagues (*5*). Whenever the second laser hit such a transition, the $\tilde{C}'(v', J')$ species would have an additional depletion channel besides the usual one going to ionization, which caused a dip in the ion signal (Fig. 1).

Although IDS lines of iodine were obtained earlier (*6*), the lines in our spectra for NH_3 were much more pronounced, owing to the fact that the $\tilde{A}$ state predissociates fast enough to make the reverse process $\tilde{A} \rightarrow \tilde{C}'$ very unlikely. We worked out the selection rule that for each $\tilde{C}' \leftarrow \tilde{X}$ rovibronic transition, the follow-up $\tilde{A} \leftarrow \tilde{C}'$ transitions could be at most three, and these were readily assignable. It was also simple to determine the line center positions. Accordingly, a "composite" rotational spectrum could be constructed (Fig. 2), and the rotational constants B and C and the band origins could be obtained by least-square fits. Noting that for many molecules, the first excited electronic state is a short-lived predissociating state, our method should work for many of them, as long as their lifetimes are longer than a few tens of femtoseconds.

Once we found the vibrotational levels of the $NH_3\tilde{A}$ state with IDS, we proceeded to explore the $\tilde{B}$ state by straight double resonance $\tilde{B} \leftarrow \tilde{A} \leftarrow \tilde{X}$ (*7*). Because $\tilde{B}$ is a degenerate electronic state, Jahn-Teller splitting can occur as a result of coupling between the electrons and nuclear vibrations. If the levels were purely vibrational, the symmetry should be E. Owing to the Jahn-Teller effect, however, each of these levels splits into E and A. Thanks to the OODR technique, we actually observed this splitting by distinguishing vertical bands of PQR structure, characteristic of E symmetry, from parallel bands of PR structure, characteristic of A symmetry. By these means, we successfully studied the Jahn-Teller effect rotationally, assigning each type of band

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