

# Enzyme catalysis: a new definition accounting for noncovalent substrate- and product-like states

Daniel L. Purich

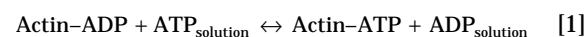
Biological catalysis frequently causes changes in noncovalent bonding. By building on Pauling's assertion that any long-lived, chemically distinct interaction is a chemical bond, this article redefines enzyme catalysis as the facilitated making and/or breaking of chemical bonds, not just of covalent bonds. It is also argued that nearly every ATPase or GTPase is misnamed as a hydrolase and actually belongs to a distinct class of enzymes, termed here 'energases'. By transducing covalent bond energy into mechanical work, energases mediate such fundamental processes as protein folding, self-assembly, G-protein interactions, DNA replication, chromatin remodeling and even active transport.

Nearly half a century ago, the Enzyme Commission began the daunting task of systematically classifying and naming enzyme-catalyzed reactions<sup>1–3</sup>. Six reaction types (oxidation–reduction, group transfer, hydrolysis, group addition–elimination, isomerization and ligation) were used to classify enzymes in terms of attendant changes in covalent bonding. During the preparation of a new treatise detailing the ~4500 known enzyme-catalyzed reactions, I questioned whether all biological catalysts can still be so neatly classified as oxidoreductases, transferases, hydrolases, lyases, isomerases or ligases. The simple and succinct definition of an enzyme as a biological catalyst implies that an enzyme accelerates a chemical reaction without altering its equilibrium. However, by limiting the term 'chemical reaction' to the making or breaking of covalent bonds, the current system excludes catalyzed reactions involving substrate-like and product-like states that differ only with respect to their noncovalent-bonding interactions, and also classifies certain synthase-like enzymes as hydrolases. These considerations suggest that the definition of enzyme catalysis must be reconsidered in a manner that accounts for reactions involving transformations of noncovalent bonds.

Reactions involving changes in noncovalent-bonding interactions

There is a diverse range of biochemical processes that involves reactant-like and product-like states representing rearrangements in noncovalent-bonding interactions (Table 1). Tabulated are reactions that: (1) strictly involve noncovalent interactions; (2) are mechanistically linked to the Gibbs free energy of ATP or GTP hydrolysis, or to another thermodynamic driving force such as a solute gradient; and (3), for example proteins such as GroEL and GroES, that catalyze the noncovalent folding of other proteins. For reactions belonging to type (3), the energy of ATP hydrolysis is used to expel unproductive folding intermediates after a period of time determined by the intrinsic rate of ATP hydrolysis<sup>4</sup>.

The case of ATP exchange with actin-bound ADP in the presence of profilin illustrates an important process that strictly entails changes in noncovalent bonding<sup>5</sup>. Profilin is an actin-regulatory protein that accelerates the conversion of one long-lived protein–ligand complex (actin–ADP) into another (actin–ATP) (Eqn 1):



The following are indisputable facts about the profilin-catalyzed exchange of actin-bound nucleotide: (1) profilin enhances the nucleotide exchange rate by a factor of 150; (2) at catalytic concentrations, profilin has no effect on the overall equilibrium of the exchange reaction; and (3) profilin weakens the interaction of actin with nucleotide and stabilizes a nucleotide-free transition state<sup>5,6</sup>.

If profilin were to catalyze the ATP-dependent transphosphorylation of actin-bound ADP, this actin-regulatory protein would certainly fit the classical definition of an enzyme. However, the reaction catalyzed by profilin only involves the breaking and making of noncovalent bonds. This prompts the question: should profilin be regarded as an enzyme? Consider the reaction coordinate diagrams for a classical enzymatic reaction and a factor-mediated ligand-exchange reaction (Fig. 1). Except for differences in their activation energies, both are stepwise transformations that increase reaction rate. The 10<sup>2.5</sup> rate enhancement observed in the presence of profilin is unquestionably paltry when compared with the 10<sup>17</sup>–10<sup>19</sup> value for orotidine-5'-phosphate decarboxylase<sup>7</sup>. The much higher rate enhancement factor for orotidine-5'-phosphate decarboxylase reflects the extremely low rate of the uncatalyzed reference reaction<sup>7</sup>. Rearrangements in noncovalent bonding are faster than uncatalyzed rates of reactions involving changes in covalency, and this is reflected in their respective activation energies. Whereas enzymologists focus on the magnitude of enhancement factors, individual reactions within metabolic pathways need only be fast enough to prevent bottlenecks.

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**Table 1. Catalyzed reactions involving substrate-like and product-like states differing in their noncovalent bonding and/or position**

Reaction type	Examples
Facilitated exchange of protein-bound ligands	Profilin-promoted exchange of actin-bound nucleotides Exchange factors acting on G proteins
Chaperonin-mediated reactions	Protein folding Assembly of multi-subunit protein complexes
Molecular motors	Myosin locomotion on actin filaments Kinesin and dynein locomotion on microtubules
Cytoskeleton self-assembly	ATP-dependent actin filament assembly GTP-dependent microtubule self-assembly
Polymerase processivity	ATP-dependent clamp-loading onto DNA polymerases Contact-transfer polymerization of actin during actin-based motility
Active transport	Sodium and/or potassium ATPase ATP synthase
Carrier-mediated transport	Sugar transport Amino acid transport

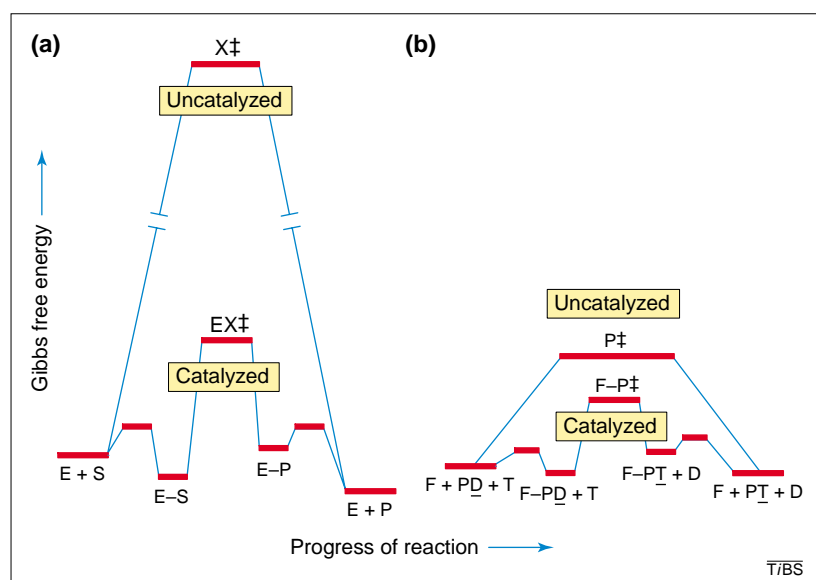
Motile cells have developed a motility complex that uses profilin to mobilize actin-ATP in the form of profilin-actin-ATP, thereby accelerating the actin polymerization rate by a factor of 200–500. Again, profilin acts catalytically without any effect on the equilibrium constant for actin filament assembly<sup>8</sup>. Actin-based cell crawling has a limiting rate of  $1 \mu\text{m s}^{-1}$ , corresponding to the addition of  $\sim 500$  actin monomers per second to the growing end of each actin filament<sup>9</sup>. Based on the intracellular concentration of actin monomers, the enhancement

of filament assembly mediated by profilin is sufficient to maintain the fast rates of actin-based cell motility, and any greater rate enhancement offers no selective advantage.

**A more encompassing definition of enzyme catalysis**  
As there are so many instances in which biological catalysis is not attended by changes in covalent bonding, I offer a broader definition: enzymes catalyze the making and/or breaking of chemical bonds by promoting substrate and/or product access to the transition state. (This statement deliberately avoids specifying how an enzyme promotes catalysis; for example, by stabilizing enzyme transition states, destabilizing the ground state, reorganizing active-site solvent molecules, enabling fluctuating motions within protein domains, vibrationally coupling atomic motions, and managing entropic and/or enthalpic contributions.)

Although this definition seems no more encompassing than existing definitions of enzyme catalysis, the crucial difference is the use of the phrase 'chemical bond' in place of 'covalent bond'. In his book *The Nature of The Chemical Bond*<sup>10</sup>, Linus Pauling offered the following guiding comment: 'We shall say that there is a chemical bond between two atoms or groups of atoms in case that the forces acting between them are such as to lead to the formation of an aggregate with sufficient stability to make it convenient for the chemist to consider it as an independent molecular species.' Because many protein conformational states and numerous protein-ligand complexes are sufficiently long-lived to exhibit chemically definable properties, their formation and/or transformation must be considered as chemical reactions. Thus, by admitting a more encompassing definition of enzyme catalysis, it becomes clear that profilin is a remarkably versatile enzyme, one that speeds up two entirely different reaction types attended by changes in noncovalent-bonding interactions.

There are other special cases of enzyme catalysis, including those agents that catalyze noncovalent self-assembly of macromolecular and supramolecular structures. In this context, catalysts of protein folding and refolding, cytoskeletal filament assembly and chromatin condensation should be regarded as enzymes. With modest tinkering, Pauling's prescient comment can be extended to include the persistent, chemically definable position of a solute relative to the faces of a membrane. In this way, the proposed definition of enzyme catalysis also treats membrane transporters as specialized enzymes. The Enzyme Commission now treats transporters as enzymes, recognizing that a change in the position of a metabolite with respect to a membrane defines substrate-like and product-like states. Boyer's binding change mechanism for ATP synthase<sup>11</sup> and the Mackinose-Jencks calcium pump



**Fig. 1.** Typical reaction coordinate diagrams for catalyzed and uncatalyzed reactions involving changes in covalent or noncovalent bonding. Except for differences in the magnitude of the activation energy ( $\Delta E_{act}$ ), reactions involving changes in covalent and/or noncovalent bonding follow the same basic reaction scheme. (a) Classical enzymatic process showing progress of the classical covalent bond-altering reaction in the absence and presence of enzyme catalysis. (b) Facilitated ligand exchange, showing progress of the ligand-exchange reaction. Abbreviations: D, nucleoside 5'-diphosphate; E, enzyme; F, exchange-promoting factor; P, nucleotide-binding protein; PD, protein-nucleoside 5'-diphosphate complex; P‡, protein with vacant nucleotide site; PT, protein-nucleoside 5'-triphosphate complex; S, substrate; T, nucleoside 5'-triphosphate; X, reaction intermediate.

**Table 2. Reactions and equilibrium constants for hydrolase-, synthetase- and energase-catalyzed reactions<sup>a</sup>**

Catalyst	Reaction	Equilibrium constant
Hydrolase	Releases $\Delta G_{\text{hydrolysis}}$ as heat $\text{ATP} + \text{H}_2\text{O} \leftrightarrow \text{ADP} + \text{P}_i$	$K_{\text{eq}} = [\text{ADP}][\text{P}_i]/[\text{ATP}]$
Synthetase	Couples $\Delta G_{\text{hydrolysis}}$ to $\Delta G_{\text{biosynthesis}}$ $\text{ATP} + \text{Glu} + \text{NH}_3 \leftrightarrow \text{Gln} + \text{ADP} + \text{P}_i$	$K_{\text{eq}} = [\text{Gln}]/[\text{Glu}][\text{NH}_3] \times [\text{ADP}][\text{P}_i]/[\text{ATP}]$
Energase	Couples $\Delta G_{\text{hydrolysis}}$ to $\Delta G_{\text{conformation-change}}$ or $\Delta G_{\text{work}}$ $\text{ATP} + \text{state 1} + \text{H}_2\text{O} \leftrightarrow \text{state 2} + \text{ADP} + \text{P}_i$	$K_{\text{eq}} = [\text{state 2}]/[\text{state 1}] \times [\text{ADP}][\text{P}_i]/[\text{ATP}]$

<sup>a</sup>Abbreviations:  $\Delta G$ , change in Gibbs free energy;  $\text{P}_i$ , inorganic phosphate.

model<sup>12,13</sup> certainly reinforce the idea that transporter mechanisms are inherently similar to other enzyme mechanisms.

**Energases: a distinct class of enzyme-catalyzed reactions**  
Discoveries of the past two decades have convincingly demonstrated the pervasiveness of mechanochemical proteins that transduce the Gibbs free energy of nucleotide hydrolysis into some form of useful work. The product of these reactions can be described as a form of translational movement, rotation or solute gradient. Under normal physiological conditions, nucleotide hydrolysis is stoichiometrically coupled to the production of an increment of useful work. 'Energase' is offered as a new term that: (1) treats these mechanochemical systems as a distinct enzyme class; (2) uses a root word (energy) known in all modern languages; and (3) reinforces the idea that the energy of changes in chemical bonding is transduced into mechanical energy. The term energase also fits nicely alongside oxidase, reductase, hydrolase, lyase, isomerase and ligase.

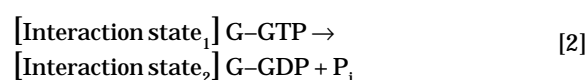
The present Enzyme Commission classification does not explicitly account for the noncovalent 'work steps', thereby treating energases as hydrolases. That energases constitute a separate class becomes obvious when we consider equilibrium constants for three different ATP-dependent reactions (Table 2), without specifying how metal ions are involved or how protons are released. The fundamental nature of the mechanical work step in energase reactions is illustrated by treating conformation-state<sub>1</sub> as a substrate-like species and conformation-state<sub>2</sub> as a product-like species. The relative abundance of these conformational states (i.e.  $[\text{state}_2]/[\text{state}_1]$ ) must be explicitly indicated when writing the chemical reaction and the equilibrium constant. Although the quotient  $[\text{ADP}][\text{P}_i]/[\text{ATP}]$  is common to all three reactions, energases actually share much more in common with synthases than with hydrolases. One simply cannot ignore the substrate-like and product-like species that differ only in the energetics of their noncovalent interactions.

Ironically, the hydrolytic activities of energases often become exaggerated when steps in an energase reaction are uncoupled by various treatments or agents. A good example is the tubulin GTPase. Strict

stoichiometric coupling of tubulin incorporation and GTP hydrolysis occurs under normal physiological conditions, but is lost in the presence of colchicine, which blocks microtubule self-assembly<sup>14,15</sup>. Tubulin only exhibits an enhanced capacity to hydrolyze GTP in the presence of colchicine. Similar statements apply to the abilities of other uncoupling agents to increase the hydrolase activities of ATP synthase and other ATP-dependent transporters.

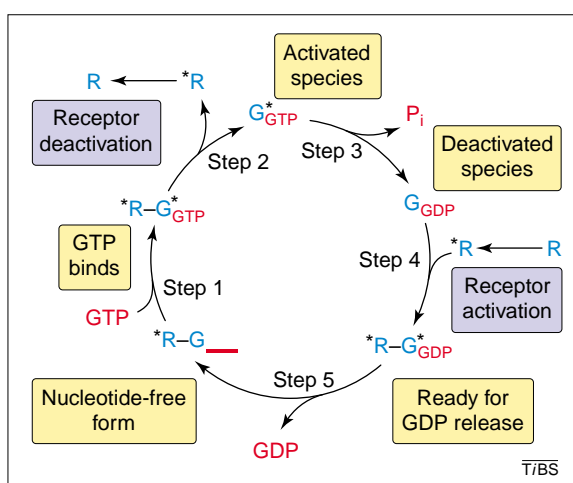
Another relevant example of energase action is the ATP-dependent clamp-loader that permits DNA polymerases to remain associated with the DNA template through many rounds of phosphodiester bond synthesis. Upon loading the lock-washer-shaped clamp, DNA polymerase cannot dissociate from its DNA template, and the polymerase processively replicates several thousand bases before dissociating<sup>16–18</sup>. As the holoenzyme moves along the replication fork, the polymerase continuously extends DNA on the leading strand; however, a second polymerase molecule acting on the lagging strand releases its sliding clamp upon synthesis of each Okazaki fragment. The polymerase must then return to the advancing replication fork where it reattaches to another clamp that the clamp-loader has deployed at an upstream RNA primer. The clamp-loader does not merely hydrolyze ATP, it is an energase that accomplishes an increment of mechanical work as it attaches the clamp around DNA.

The GTP-regulatory protein superfamily of GTPases<sup>19,20</sup> represents yet another example of energases that use the Gibbs free energy of GTP hydrolysis to modulate the affinity of noncovalent-binding interactions. Effective G-protein-mediated regulation of hormone receptors stems from GTPase-linked modulation of agonist and antagonist potency. G proteins are enzymes that exist in at least two states (Eqn 2):



in which the strength of noncovalent interactions depends on the energy difference between the interaction sites.

A characteristic feature of all catalyzed processes is that the unmodified catalyst is regenerated after



**Fig. 2.** Complete reaction cycle for the G protein GTPase reaction. GTP binds to a complex of activated receptor (\*R) and GTP-regulatory protein (G) (Step 1), resulting in receptor release (Step 2). The activated G protein  $G^{*}_{GTP}$  is then free to stimulate the activity of other downstream signal transduction reactions. When GTP is hydrolyzed (Step 3), the resulting GDP-containing G protein is deactivated; its reactivation must await the binding of activated receptor (Step 4), which is followed by GDP release (Step 5).

each reaction cycle. Although the processes presented above do not exhibit the high turnover numbers observed with many enzymes, each of these systems participates in what can be regarded as a long-lived catalytic cycle. This is illustrated in Fig. 2 for receptor-mediated nucleotide transformations associated with G-protein-coupled receptors. In this scheme, the activated receptor (designated \*R) binds to the G protein and reloads the nucleotide-binding site with GTP. In this respect, unless \*R is available, the catalytic cycle of the G protein is arrested at the  $G_{GDP}$  stage.

#### Energases that modify enzyme performance

The free energy of nucleoside 5'-triphosphate hydrolysis can also produce substrate-like and product-like conformational states that alter an enzyme's ability to catalyze a reaction. Two notable examples are ATP sulfurylase ( $ATP + \text{sulfate} \rightarrow \text{AMP-sulfate} + \text{pyrophosphate}$ ), which transduces the energy of GTP hydrolysis to modulate both substrate binding and catalytic performance<sup>21</sup>, and nicotinate phosphoribosyltransferase (nicotinate + phosphoribosylpyrophosphate  $\rightarrow$  nicotinate D-ribonucleotide + pyrophosphate), which hydrolyzes ATP for similar reasons<sup>22</sup>. Any change in substrate affinity alters the Michaelis constant, thereby altering one or more of the other parameters in the Haldane relationship ( $K_{eq} = V_f K_{mr} / V_r K_{ml}$ ). These and

related enzymes should be accorded two separate Enzyme Commission classifications, as is the current practice for other bifunctional enzymes.

#### Concluding remarks

Although the systematic classification of enzymes on the basis of organic chemistry was logical, modern biochemistry has shown that metabolism necessarily includes many other reactions that must be written in terms of changes in the strength of noncovalent interactions. The involvement of molecular motors in contractile processes, intracellular organelle trafficking and cell crawling constitutes just another branch of metabolism, as does the building up and tearing down of macromolecular and supramolecular structures. The interested reader should note the recent classification of ATP-driven nucleic acid helicases as coupling factors that transduce P–O–P bond-scission energy into the mechanical work of separating and rearranging complementary strands of double-helical nucleic acids<sup>23</sup>.

The underlying principles of biological catalysis apply equally well to the breaking and making of covalent bonds, coordinate covalent bonds and the much weaker noncovalent bonds. The induced-fit hypothesis<sup>24</sup> provided an early impetus for learning how binding of substrate reorients otherwise functionally inactive groups on an enzyme to form an active catalyst. Initial active-site reorganization and later steps in catalysis involve noncovalent-binding interactions in the form of conformational changes. Although most enzymes employ noncovalent interactions to facilitate covalent bond scission, the 'energases' use  $\Delta G_{\text{covalent-bond-scission}}$  (the change in the Gibbs free energy during covalent-bond-scission) to modify noncovalent interactions. Energases are endowed with the capacity to accomplish mechanical work by transducing covalent bond energy into substrate-like and product-like conformational states. In both cases, the greatest challenge for the enzyme mechanic is to establish unambiguously the role of noncovalent interactions in catalysis.

Finally, as teachers, we must seek better ways to promote student comprehension of biochemistry's organizing principles. The current classification system obscures salient differences between hydrolase and 'energase'; we should therefore embrace a more encompassing definition that treats structural metabolism as an integral component of cellular metabolism and reinforces the idea that enzymes are the universal mediators of metabolism. This new definition of enzyme catalysis erases many of the artificial barriers between biochemistry and cell biology, thereby expanding the dominion of enzymology.

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#### References

- Hoffmann-Ostenhof, O. (1953) Suggestions for a more rational classification and nomenclature of enzymes. *Adv. Enzymol.* 14, 219–260
- Dixon, M. and Webb, E.C. (1958) Enzyme reactions. In *Enzymes*, pp. 182–241, Academic Press
- Webb, E.C. (1992) *Enzyme Nomenclature – 1992: Recommendations of the Nomenclature*

Committee of the International Union of Biochemistry and Molecular Biology, Academic Press

- Wang, J.D. and Weissman, J.S. (1999) Thinking outside the box: new insights into the mechanism of GroEL-mediated protein folding. *Nat. Struct. Biol.* 6, 597–604
- Purich, D.L. and Allison, R.D. (2000) Nucleotide

exchange factors. In *Handbook of Biochemical Kinetics*, pp. 519–520, Academic Press

- Kinosian, H.J. *et al.* (2000) Interdependence of profilin, cation, and nucleotide binding to vertebrate non-muscle actin. *Biochemistry* 39, 13176–13188
- Radzicka, A. and Wolfenden, R. (1995) A proficient enzyme. *Science* 267, 90–93



- 8 Kang, F. *et al.* (1999) Profilin promotes barbed-end actin filament assembly without lowering the critical concentration. *J. Biol. Chem.* 274, 36963–36972
- 9 Stossel, T.P. (1993) On the crawling of animal cells. *Science* 260, 1086–1094
- 10 Pauling, L. (1945) Resonance and the chemical bond. In *The Nature of the Chemical Bond*, p. 3, Cornell University Press
- 11 Boyer, P.D. (1997) The ATP synthase – a splendid molecular machine. *Annu. Rev. Biochem.* 66, 717–749
- 12 Makinose, M. (1973) Possible functional states of the enzyme of the sarcoplasmic calcium pump. *FEBS Lett.* 37, 140–143
- 13 Jencks, W.P. (1989) How does a calcium pump pump calcium? *J. Biol. Chem.* 264, 18855–18858
- 14 MacNeal, R.K. and Purich, D.L. (1978) Stoichiometry and role of GTP hydrolysis in bovine microtubule assembly. *J. Biol. Chem.* 253, 4683–4687
- 15 Purich, D.L. and Angelastro, J.M. (1994) Microtubule dynamics: bioenergetics and control. *Adv. Enzymol.* 69, 121–154
- 16 Kelman, Z. and O'Donnell, M. (1995) DNA polymerase III holoenzyme: structure and function of a chromosomal replicating machine. *Annu. Rev. Biochem.* 64, 171–200
- 17 Kornberg, A. and Baker, T. (1992) Prokaryotic DNA polymerases other than *E. coli* Pol I. In *DNA Replication*, 2nd edn, p. 165, W.H. Freeman
- 18 Bertram, J.G. *et al.* (2000) Molecular mechanism and energetics of clamp assembly in *Escherichia coli*. The role of ATP hydrolysis when  $\gamma$  complex loads  $\beta$  onto DNA. *J. Biol. Chem.* 275, 28413–28420
- 19 Gilman, A.G. (1987) G proteins: transducers of receptor-generated signals. *Annu. Rev. Biochem.* 56, 615–649
- 20 Vale, R.D. (1996) Switches, latches, and amplifiers: common themes of G proteins and molecular motors. *J. Cell Biol.* 135, 291–302
- 21 Leyh, T.S. (1999) On the advantages of imperfect energetic linkage. *Methods Enzymol.* 308, 48–70
- 22 Grubmeyer, C.T. *et al.* (1999) Energy coupling through molecular discrimination: nicotinate phosphoribosyltransferase. *Methods Enzymol.* 308, 28–48
- 23 von Hippel, P.H. and Delagoutte, E. (2001) A general model for nucleic acid helicases and their 'coupling' within molecular machines. *Cell* 104, 177–190
- 24 Koshland, D.E., Jr (1960) The active site and enzyme action. *Adv. Enzymol.* 22, 45–98

# Insulin-degrading enzyme: embarking on amyloid destruction

Igor V. Kurochkin

Several human disorders are caused by or associated with the deposition of protein aggregates known as amyloid fibrils. Despite the lack of sequence homology among amyloidogenic proteins, all amyloid fibrils share a common morphology, are insoluble under physiological conditions and are resistant to proteolytic degradation. Because amyloidogenic proteins are being produced continuously, eukaryotic organisms must have developed a form of proteolytic machinery capable of controlling these aggregation-prone species before their fibrillization. This article suggests that an intracellular metalloprotease called insulin-degrading enzyme (IDE) is responsible for the elimination of proteins with amyloidogenic potential and proposes a mechanism for the selectivity of the enzyme. In this respect, IDE can also be referred to as ADE: amyloid-degrading enzyme.

Fibrillar protein deposits, known as amyloid plaques, accompany or cause several medical disorders, the most notable of which are Alzheimer's disease, type 2 (non-insulin-dependent) diabetes and the spongiform encephalopathies such as Creutzfeldt–Jakob disease<sup>1</sup>. Although the primary sequences of the protein constituents of amyloid fibrils differ substantially from one disease to another, all of the fibrils are remarkably similar morphologically: all amyloid fibrils contain a  $\beta$ -sheet structure in which the peptide strands run perpendicular to the fibril axis<sup>2</sup>. Once insoluble amyloid fibrils have formed, the process is effectively irreversible under physiological conditions.

Therefore, cells must have developed mechanisms to eliminate amyloidogenic species before their aggregation. One such mechanism involves molecular chaperones, which promote proper protein folding and prevent the aggregation of misfolded proteins. However, amyloid-forming proteins are inherently unfolded and cannot be refolded by the action of chaperones. Another mechanism requires the proteasome, which eliminates improperly folded proteins that are prone to aggregation<sup>3</sup>. However, the involvement of proteasomes in the selective elimination of the subset of misfolded proteins that form amyloid fibrils has yet to be demonstrated. Recent reports suggest that the removal of amyloid-forming proteins, particularly small proteins, is performed by an intracellular protease called insulin-degrading enzyme (IDE) (Table 1)<sup>4–8</sup>.

IDE: an evolutionary conserved metalloendoprotease with an unusual zinc-binding motif  
IDE, also known as insulysin or insulinase, is a thiol-metalloendopeptidase, active at neutral pH, that was first postulated to be responsible for insulin proteolysis *in vivo* more than 50 years ago<sup>9</sup>. IDE has been purified from several mammalian tissues, including red blood cells, skeletal muscle, liver and brain<sup>10,11</sup>. The purified enzyme consists of a single polypeptide chain with a mass of 110 kDa on denaturing polyacrylamide gels. Under nondenaturing conditions, IDE has an apparent molecular weight of 300 kDa, suggesting that it exists as a dimer or trimer. Purified IDE has a very high affinity for insulin ( $K_m = \sim 20$  nM)<sup>11</sup>. Isolation and sequencing of the degradation products generated from insulin by the action of IDE indicate that the enzyme cleaves the A chain of insulin at two sites and the B chain at seven sites<sup>12</sup>. Numerous observations suggest that IDE is the principal enzyme involved in insulin processing in cells; for example: (1) insulin breakdown products found *in vivo* can be generated by purified IDE; (2) microinjection of monoclonal antibodies directed against the enzyme inhibits cellular degradation of insulin; (3) inhibitors of purified IDE

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