Which Enzyme Uses What Tricks?

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Enzymes bring about reaction rate acceleration through a number of tricks. Of the tricks used to bring down activation energy, some are entropic, and others are enthalpic in nature. Various tools are recruited and relied on by each enzyme in different proportions. All these are within the realm of simple physical and chemical explanations – the combined effect however is quite dramatic! While there is no common formula, each enzyme uses a combination of these tricks to achieve the objective (Fig. 7.1). Indeed, *each enzyme is a biological experiment*, just the same way evolutionary biologist Ernst Mayr described evolution of each species.

Lysozyme Hen egg white lysozyme is a well-dissected example of catalytic principles. A large number of contacts to the substrate (peptidoglycan) provide good binding. Through these specific interactions, both Glu35 and Asp52 are brought in proximity to the susceptible glycosidic bond and properly oriented for catalysis. Being present at the same active site, the two –COOH groups behave quite differently – Glu35 is a general acid–base, while Asp52 is a nucleophile. The pKa of Glu35-COOH is attenuated to 6.0 (from the expected pKa of 4.3). The notion of Asp52-COO^- stabilizing the developing carbenium ion on the C1-anomeric carbon was recently revised to indicate its role as a nucleophile in covalent catalysis (see section 6.5 in the previous chapter) (Kirby 2001; Vocadlo et al. 2001). Straddling the -$(NAM\text{-}NAG)_n$- polymer across the lysozyme active site cleft involves binding sub-sites for each sugar residue (Fig. 7.1). The binding of the fourth sugar residue is unfavorable and the weakest. It is thought that interactions at other sub-sites are exploited to accommodate the D sugar in a distorted, half-chair conformation – resembling the *TS*. Thus except for metal ion catalysis, all other tricks are brought to bear on its substrate by lysozyme.

Proteases Each catalytic solution in biology is unique to the reaction in question. But there can be different possible solutions to the same problem. Hydrolysis of a

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Fig. 7.1 Substrate binding at the lysozyme active site. The binding free energy for each sugar residue at each sub-site is shown. Lysozyme cleaves the substrate between sugar residues occupying sub-sites D and E

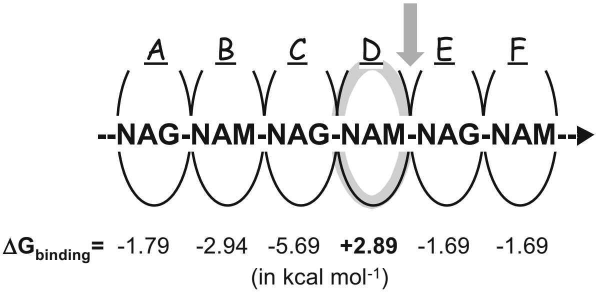
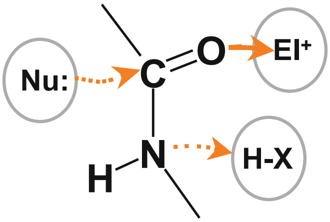


Fig. 7.2 A schematized protease active site. Typically it displays a nucleophile (Nu:), an electrophile (El^{-}), and a general acid–base group for proton transfers



peptide bond is a case in point. Proteins and peptides constitute significant biomolecular components in all life forms. Consequently, peptide bond hydrolysis is also a universal requirement.

The problem associated with peptide bond hydrolysis is threefold: (a) water being a poor nucleophile needs to be activated for attack, (b) the amine product resulting from peptide hydrolysis is a poor leaving group, and (c) amide (peptide) bonds are quite stable due to resonance (partial double bond character). In comparison, an ester is about 3000 times more reactive, and a *p*-nitrophenyl ester is 300,000 times more so! Nature has invented suitable tools to overcome these three hurdles. Rate accelerations of up to 10^{10} times the uncatalyzed rates have been achieved. Peptide bond hydrolysis is an addition–elimination reaction that goes through a tetrahedral reaction intermediate. In fact, formation of tetrahedral intermediate ensures that the peptide bond is weakened by eliminating resonance stabilization (no partial double bond!). The chemical apparatus at the protease active site provides suitable functional groups to interact with C, N, and O atoms of the peptide bond. These include (1) a nucleophile-like water (H-OH), Ser-OH, or Cys-SH to attack the carbonyl carbon, (2) a general base to accept the proton from the nucleophilic –OH, (3) an electrophilic group(s) to stabilize the oxyanion formed, and (iv) a general acid to protonate the amine – which is a poor leaving group. These features create the catalytic forces common to all proteases and are schematically shown in Fig. 7.2.

As expected, protease active site extensively interacts with the substrate and freezes one of its conformations. Some proteases have distinct sub-sites to accommodate and bind substrate amino acid residues around the scissile peptide bond. The binding specificity may feature binding pockets for aromatic (chymotrypsin),

Table 7.1 **Catalytic tricks in four different protease groups**

Feature	Serine protease	Cysteine protease	Aspartyl protease	Metallo-protease
Proximity and orientation				
	Yes	Yes	Yes	Yes
Electrostatics				
	Oxyanion hole	Oxyanion hole	Asp-COOH to polarize carbonyl	Zn^{2+} to polarize carbonyl
Acid–base catalysis				
	Yes	Yes	Yes	Yes
Nucleophile used				
	DHS catalytic triad; Ser-OH	His-Cys-SH	H-OH activated by asp-COO^-	H-OH activated by Zn^{2+} (or Glu-COO^-)
Covalent catalysis				
	Acyl enzyme	Acyl enzyme	No	No (Yes)
Transition state binding				
	Tetrahedral intermediate	Tetrahedral intermediate	Tetrahedral intermediate	Tetrahedral intermediate
Examples	Chymotrypsin, subtilisin, carboxypeptidase II	Papain, caspase, cathepsin C	Pepsin, renin, HIV-1 protease	Carboxypeptidase A, thermolysin, leucine aminopeptidase

positively charged (trypsin), small R (elastase) groups or C-terminal (carboxypeptidase A) and N-terminal (leucine aminopeptidase) amino acid residues. Protease active sites typically include features that allow for the activation of water or another nucleophile, polarization of the peptide carbonyl group, and subsequent stabilization of a tetrahedral intermediate. Table 7.1 lists the different catalytic forces acting at the active sites of four major protease classes.

The problem of peptide bond hydrolysis has been solved ingeniously by nature. The result is different protease classes, namely, serine proteases, cysteine proteases, metallo-proteases, and acid proteases. The non-isolable high-energy intermediate – the tetrahedral transition state – is generated and stabilized in different ways in these enzymes. The catalytic triad (Asp→His→Ser-OH) of serine proteases must be an effective apparatus to hydrolyze peptide bonds (Hedstrom 2002). It has been independently selected, three different times throughout evolution. Chymotrypsin, subtilisin, and carboxypeptidase II exhibit very different protein architecture. But they all contain the active site catalytic triad – an excellent example of convergent evolution (the intrinsic chemical constraints to build a catalyst has led evolution to converge on equivalent solutions independently and repeatedly). Serine proteases like chymotrypsin, trypsin, and elastase, on the other hand, represent similar ancestry and are examples of divergent evolution (all three derived from a common ancestral homologous gene but diverged to perform different functions).

References

Hedstrom L (2002) Serine protease mechanism and specificity. Chem Rev 102:4501–4523
Kirby AJ (2001) The lysozyme mechanism sorted - after 50 years. Nat Struct Biol 8:737–739
Vocadlo DJ, Davies GJ, Laine R, Withers SG (2001) Catalysis by hen egg-white lysozyme proceeds via a covalent intermediate. Nature 412:835–838