1 Introduction

Hydrolases are the group of enzymes that catalyze bond cleavage by reaction with water. The natural function of most hydrolases is digestive – to break down nutrients into smaller units for digestion. For example, proteases hydrolyze proteins to smaller peptides and then to amino acids and lipases hydrolyze lipids (triglycerides) to glycerol and fatty acids (Fig. 1). Because of the need to break down a wide range of nutrients, hydrolases usually have a broad substrate specificity.

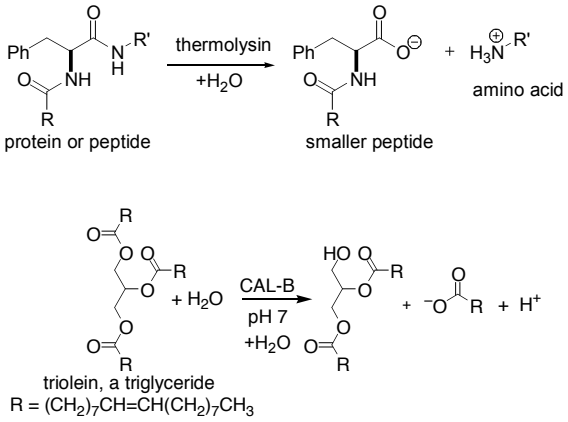

Fig. 1. The natural role of most hydrolases is digestive – to break down nutrients into smaller units. Thermolysin, a protease secreted by thermophilic bacteria, catalyzes the hydrolysis of proteins to peptides and then further to amino acids. Lipase B from *Candida antarctica* (CAL-B) catalyzes the stepwise hydrolysis of triglycerides (e.g., triolein) to fatty acids and glycerol. The reaction shows only the first step from a triglyceride to a diglyceride.

Several characteristics make hydrolases useful to the organic chemist. First, because of their broad substrate specificity, hydrolases often accept as substrates various synthetic intermediates. Second, hydrolases often show high stereoselectivity, even toward unnatural substrates. Third, besides hydrolysis, hydrolases also catalyze several related reactions – condensations (reversal of hydrolysis) and alcoholysis (a cleavage using an alcohol in place of water). Two examples from industry are shown in Fig. 2. Thermolysin catalyzes the condensation of two amino acid derivatives to make an aspartame derivative (Isowa et al., 1979). The reaction proceeds in the condensation direction because the product precipitates from solution. The high enantioselectivity permits using racemic starting materials and the high regioselectivity of thermolysin eliminates the need to protect the β-carboxyl group of the aspartic acid derivative. The second example is an alcoholysis reaction (Morgan et al., 1997a). The ester, vinyl acetate, is cleaved not by water, but by the substrate alcohol. The liberated vinyl alcohol (not shown) tautomerizes to acetaldehyde. These alcoholysis reactions are also called transesterification reactions.

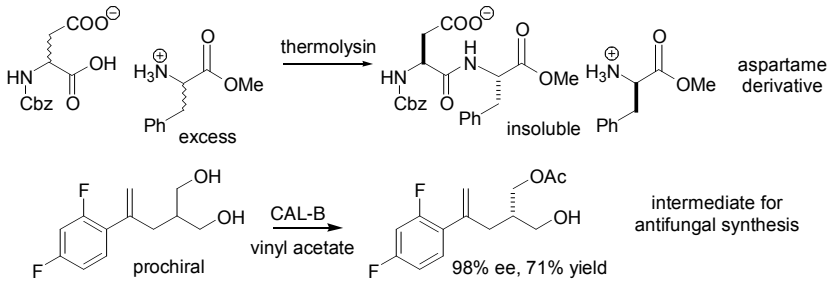

Fig. 2. Several unnatural, synthetically useful reactions catalyzed by hydrolases. Thermolysin catalyzes the regio- and enantioselective coupling of *N*-benzyloxycarbonyl-L-aspartate with L-phenylalanine methyl ester. Precipitation of the product drives this reaction in the condensation direction instead of the normal hydrolysis direction. This condensation is a key step in the manufacture of aspartame, a low-calorie sweetener. Because of the high enantioselectivity of thermolysin, racemic substrates may be used. Because of the high regioselectivity of thermolysin for the α-carboxyl group, the β-carboxyl group in the aspartic acid derivative needs no protection. Lipase B from *Candida antarctica* (CAL-B) catalyzes the enantioselective acetylation of a prochiral diol yielding an intermediate for the synthesis of antifungal agents. This example is an alcoholysis where the ester, vinyl acetate, is cleaved not by water, but by the substrate alcohol. This reaction is run in an organic solvent to avoid the competing hydrolysis.

Several other features make hydrolases convenient to use as synthetic reagents. Many hydrolases (approximately several hundred) are commercially available. They do not require cofactors and they tolerate the addition of water-miscible solvents (e.g., DMSO, DMF). Lipases, esterases and some proteases are also stable and active in neat organic solvents.

Enzymes are often classified according to the reaction catalyzed using an Enzyme Commission (EC) number. According to this classification, hydrolases form group 3 and are further classified according to the type of bond hydrolyzed. For example enzymes in the group 3.1 hydrolyze ester bonds (Tab. 1). Further classification into subcategories yields a four digit EC number. For example, lipases have the number EC 3.1.1.3. Classification of the more useful enzymes for organic synthesis is given in Tab. 1. A convenient web site to look up numbers and classification is at *http://www.expasy.ch/enzyme*. One disadvantage of this classification is that all enzymes catalyzing the same reaction have the same number, even though they may have very different structures, properties and other characteristics. For example, all lipases have the same number even though there are more than one hundred different lipases.

Tab. 1. Selected Hydrolases Useful in Organic Synthesis.

EC Number	Type of bond hydrolyzed	Examples
3.1	**Ester**	
3.1.1	in carboxylic acid esters	triacylglycerol lipase, acetylcholine esterase, phospholipase A_1, phospholipase A_2, gluconolactonase, lipoprotein lipase
3.1.3–4	in phosphoric acid mono- or diesters	phospholipase C, phospholipase D
3.2	**Glycosidic**	
3.2.1	in *O*-glycosides	α-amylase, oligo-1,6-glucosidase, lysozyme, neuraminidase, α-glucosidase, β-galactosidase, α-mannosidase, N-acetyl-β-glucosaminidase, sucrose α-glucosidase, nucleosidases
3.3	**Ether**	
3.3.2	in epoxides	epoxide hydrolase
3.4	**Peptide**	
3.4.11	aminopeptidase	leucine aminopeptidase
3.4.16, 21	serine proteinase	subtilisin, chymotrypsin, thermitase
3.4.18, 22	cysteine proteinase	papain
3.4.17, 24	metalloproteinase	thermolysin
3.5	**Other amides**	
3.5.1	in linear amides	penicillin amidase (penicillin G acylase)
3.5.2	in cyclic amides	hydantoinase
3.5.5	in nitriles	nitrilase[a]
3.8	**Halide bonds**	
3.8.1	carbon-halide bonds	haloalkane dehalogenase

[a]Nitrile hydratase (EC 4.2.1.84), which catalyzes addition of water to a nitrile yielding an amide, is not a hydrolase, but a lyase.

This book describes the application of lipases and proteases in organic syntheses, but also surveys esterases, epoxide hydrolases, nitrile hydrolyzing enzymes and glycosidases. The emphasis is on examples that are synthetically useful, especially those that exploit the regio- and stereoselectivity of hydrolases.