CHAPTER 2

ENZYME CLASSIFICATION

2.1 INTRODUCTION

2.1.1 Enzymes

Enzymes are biological catalysts that increase the rate of chemical reactions by lowering the activation energy. The molecules involved in the enzyme mediated reactions are known as substrates and the outcome of the reaction or yield is termed product. Generally, the chemical nature of most of the enzymes are proteins and rarely of other types (e.g., RNA). The enzymes are too specific towards their substrates to which they react and thereby the reaction will also be so specific. Sometimes the enzyme needs the presence of a non-protein component (co-enzyme, if it is a vitamin derived organic compound or co-factor, if it is a metal ion) for accomplishing the reaction. In this case, the whole enzyme may be called a holoenzyme, the protein part as apoenzyme and the non-protein constituent a prosthetic group.

2.1.2 Enzyme Nomenclature Principles

The sixth complete edition of *Enzyme Nomenclature*, was published under the patronage of the International Union of Biochemistry and Molecular Biology (formerly the International Union of Biochemistry). By the late 1950's it had become evident that the nomenclature of enzymology was not following the guidelines formulated owing to an increase in the number of enzymes. The naming of enzymes by individual workers had proved far from satisfactory in practice. In many cases the same enzymes were known by several different names, while conversely the same name was sometimes coined to different enzymes. Many of the names conveyed little or no idea about the nature of the reactions catalyzed. To solve this problem, various attempts have been made to bring an order into the general nomenclature of enzymes. Because of their close interdependence, it is convenient to deal with the classification and nomenclature together.

group that serves to catalyze transfer from a donor to an acceptor (*e.g.* flavin, biotin, or pyridoxal-phosphate enzymes) the name of the prosthetic group is not normally included in the name of the enzyme.

A consequence of the adoption of the chemical reaction as the basis for naming enzymes is that a systematic name cannot be given to an enzyme until it is known what chemical reaction it catalyzes. This applies, for example, to a few enzymes that have so far not been shown to catalyze any chemical reaction, but only isotopic exchanges; the isotopic exchange gives some idea of one step in the overall chemical reaction, but the reaction as a whole remains unknown.

A second consequence of this concept is that a certain name assigned to enzymes is not applicable to single enzyme protein but a group of proteins with the same catalytic property. Enzymes from different sources (various bacterial, plant or animal species) are also classified as one entry. The same even applies to isoenzymes. However, there are exceptions to this general rule. Some are justified because the mechanism of the reaction or the substrate specificity is so different as it ought to have different entries in the enzyme list. This applies, for example, to the two cholinesterases, EC 3.1.1.7 and 3.1.1.8, the two citrate hydrolyases, EC 4.2.1.3 and 4.2.1.4, and the two amine oxidases, EC 1.4.3.4 and 1.4.3.6).

A *third general principle* approved is that the enzymes are divided into groups on the basis of the type of reaction catalyzed, and this, together with the name(s) of the substrate(s) provides a basis for naming individual enzymes. It is also the basis for classification and code numbers.

2.1.3 Common and Systematic Names

The first enzyme commission gave much thought to the question of a systematic and logical nomenclature for enzymes and finally recommended that there should be two nomenclatures for enzymes, one systematic and the other trivial. The systematic name of an enzyme, formed in agreement with definite set of rules, exposing its action exactly, thus identifying the enzyme precisely. The trivial name was amply short enough for general use.

The commission for revision of enzyme nomenclature discussed this problem at length and breadth and a change in emphasis was made. It was decided to give the trivial names more prominence in the enzyme list; they now follow immediately after the code number and are described as common name. However, it was decided to retain the systematic names as the basis for classification for the following reasons:

(i) The code number alone is only useful for identification of an enzyme, whereas the systematic name is self-explanatory.

(ii) The systematic name reveals the type of reaction catalyzed by the enzyme.

(iii) Systematic names can be formed for new enzymes by the discoverer, by application of the rules, but individuals should not allot the code numbers.

(iv) Common names for new enzymes are frequently formed as a condensed version of the systematic name; therefore, the systematic names are helpful in finding common names that are in accordance with the general pattern.

It is recommended that for enzymes that are not the main subject of a paper or abstract, the common names can be used, but their code numbers and source should be mentioned in their first indication. Where an enzyme is the main subject of a paper or abstract, its code number, systematic name, or, alternatively, the reaction equation and source should be given at its first mention, then the common name should be used. When a paper deals with an enzyme that is not yet in the Enzyme List, the author may introduce a new name and, if desired, a new systematic name, both formed according to the recommended rules. Only the nomenclature committee of IUBMB should assign a number.

2.1.4 Scheme for the Classification of Enzymes and the Generation of EC Numbers

The first Enzyme Commission, in its report in 1961, devised a system for classification of enzymes that also serves as a basis for assigning code numbers to them, which was approved by the last commission in 1992. These code numbers are prefixed by EC, which are now widely in use, contain four elements separated by points, with the following meaning:

(i) The first number shows to the main class that an enzyme belongs.
(ii) The second figure indicates the subclass.
(iii) The third figure gives the sub-subclass.
(iv) The fourth figure is the serial number of the enzyme in its sub-subclass.

2.2 OXIDO REDUCTASES

In this class all enzymes catalyzing oxidation/reduction reactions will be categorized. The substrate that is oxidized is regarded as hydrogen/ electron donor and the compound that is reduced will be considered a hydrogen/ electron acceptor. The systematic name is based on *donor: acceptor oxidoreductase*. The common name will be *dehydrogenase*, as an alternative, *reductase* can be also used. *Oxidase* is only used in cases where O_2 is the acceptor.

The second figure in the code number of the oxidoreductases, unless it is 11, 13, 14 or 15, indicates the group in the hydrogen (or electron) donor that undergoes oxidation: 1 denotes a -CHOH- group, 2 a -CHO or -CO-COOH group or carbon monoxide, and so on.

The third figure, except in subclasses EC 1.11, EC 1.13, EC 1.14 and EC 1.15, indicates the type of acceptor involved: 1 denotes $NAD(P)^+$, 2 a cytochrome, 3 molecular oxygen, 4 a disulfide, 5 a quinone or similar compound, 6 a nitrogenous group, 7 an iron-sulfur protein and 8 a flavin. In subclasses EC

1.13 and EC 1.14 a different classification scheme is used and sub-subclasses are numbered from 11 onwards.

EC 1.1 Acting on the CH-OH group of donors

EC 1.1.1 With NAD^+ or $NADP^+$ as acceptor

EC 1.1.1.1 alcohol dehydrogenase
EC 1.1.1.2 alcohol dehydrogenase ($NADP^+$)
EC 1.1.1.3 homoserine dehydrogenase
EC 1.1.1.4 (*R,R*)-butanediol dehydrogenase
EC 1.1.1.5 acetoin dehydrogenase
EC 1.1.1.6 glycerol dehydrogenase
EC 1.1.1.7 propanediol-phosphate dehydrogenase
EC 1.1.1.8 glycerol-3-phosphate dehydrogenase (NAD^+)
EC 1.1.1.9 D-xylulose reductase
EC 1.1.1.10 L-xylulose reductase
EC 1.1.1.11 D-arabinitol 4-dehydrogenase
EC 1.1.1.12 L-arabinitol 4-dehydrogenase
EC 1.1.1.13 L-arabinitol 2-dehydrogenase
EC 1.1.1.14 L-iditol 2-dehydrogenase
EC 1.1.1.15 D-iditol 2-dehydrogenase
EC 1.1.1.16 galactitol 2-dehydrogenase
EC 1.1.1.17 mannitol-1-phosphate 5-dehydrogenase
EC 1.1.1.18 inositol 2-dehydrogenase
EC 1.1.1.19 glucuronate reductase
EC 1.1.1.20 glucuronolactone reductase
EC 1.1.1.21 aldehyde reductase
EC 1.1.1.22 UDP-glucose 6-dehydrogenase
EC 1.1.1.23 histidinol dehydrogenase
EC 1.1.1.24 quinate dehydrogenase
EC 1.1.1.25 shikimate dehydrogenase AND so on.

EC 1.1.2 With a cytochrome as acceptor

EC 1.1.2.1 now EC 1.1.99.5 glycerol-3-phosphate dehydrogenase
EC 1.1.2.2 mannitol dehydrogenase (cytochrome)
EC 1.1.2.3 L-lactate dehydrogenase (cytochrome)
EC 1.1.2.4 D-lactate dehydrogenase (cytochrome)
EC 1.1.2.5 D-lactate dehydrogenase (cytochrome *c*-553)

EC 1.1.3 With oxygen as acceptor

EC 1.1.3.1 deleted, included in EC 1.1.3.15 (*S*)-2-hydroxy-acid oxidase
EC 1.1.3.2 now EC 1.13.12.4 lactate 2-monooxygenase
EC 1.1.3.3 malate oxidase
EC 1.1.3.4 glucose oxidase
EC 1.1.3.5 hexose oxidase
EC 1.1.3.6 cholesterol oxidase
EC 1.1.3.7 aryl-alcohol oxidase
EC 1.1.3.8 L-gulonolactone oxidase
EC 1.1.3.9 galactose oxidase
EC 1.1.3.10 pyranose oxidase
EC 1.1.3.11 L-sorbose oxidase
EC 1.1.3.12 pyridoxine 4-oxidase
EC 1.1.3.13 alcohol oxidase
EC 1.1.3.14 catechol oxidase (dimerizing)
EC 1.1.3.15 (*S*)-2-hydroxy-acid oxidase AND so on

EC 1.1.4 With a disulfide as acceptor

EC 1.1.4.1 vitamin-K-epoxide reductase (warfarin-sensitive)
EC 1.1.4.2 vitamin-K-epoxide reductase (warfarin-insensitive)

EC 1.1.5 With a quinone or similar compound as acceptor

EC 1.1.5.1 Deleted, see EC 1.1.99.18 cellobiose dehydrogenase (acceptor)
EC 1.1.5.2 quinoprotein glucose dehydrogenase

EC 1.1.99 With other acceptors

EC 1.1.99.1 choline dehydrogenase
EC 1.1.99.2 2-hydroxyglutarate dehydrogenase
EC 1.1.99.3 gluconate 2-dehydrogenase (acceptor)
EC 1.1.99.4 dehydrogluconate dehydrogenase
EC 1.1.99.5 glycerol-3-phosphate dehydrogenase
EC 1.1.99.6 D-2-hydroxy-acid dehydrogenase
EC 1.1.99.7 lactate—malate transhydrogenase
EC 1.1.99.8 alcohol dehydrogenase (acceptor)
EC 1.1.99.9 pyridoxine 5-dehydrogenase
EC 1.1.99.10 glucose dehydrogenase (acceptor)
EC 1.1.99.11 fructose 5-dehydrogenase
EC 1.1.99.12 sorbose dehydrogenase
EC 1.1.99.13 glucoside 3-dehydrogenase

EC 1.1.99.14 glycolate dehydrogenase
EC 1.1.99.15 now EC 1.7.99.5
EC 1.1.99.16 malate dehydrogenase (acceptor)
EC 1.1.99.17 now EC 1.1.5.2
EC 1.1.99.18 cellobiose dehydrogenase (acceptor)
EC 1.1.99.19 deleted
EC 1.1.99.20 alkan-1-ol dehydrogenase (acceptor)
EC 1.1.99.21 D-sorbitol dehydrogenase (acceptor)
EC 1.1.99.22 glycerol dehydrogenase (acceptor)
EC 1.1.99.23 polyvinyl-alcohol dehydrogenase (acceptor)
EC 1.1.99.24 hydroxyacid-oxoacid transhydrogenase
EC 1.1.99.25 quinate dehydrogenase (pyrroloquinoline-quinone) AND so on

EC 1.2 Acting on the aldehyde or oxo group of donors

EC 1.2.1 With NAD^+ or $NADP^+$ as acceptor

EC 1.2.1.1 deleted, replaced by EC 1.1.1.284 and EC 4.4.1.22
S-(hydroxymethyl)glutathione synthase
EC 1.2.1.2 formate dehydrogenase
EC 1.2.1.3 aldehyde dehydrogenase (NAD^+)
EC 1.2.1.4 aldehyde dehydrogenase ($NADP^+$
EC 1.2.1.5 aldehyde dehydrogenase [$NAD(P)^+$]
EC 1.2.1.6 deleted
EC 1.2.1.7 benzaldehyde dehydrogenase ($NADP^+$)
EC 1.2.1.8 betaine-aldehyde dehydrogenase
EC 1.2.1.9 glyceraldehyde-3-phosphate dehydrogenase ($NADP^+$)
EC 1.2.1.10 acetaldehyde dehydrogenase (acetylating) AND so on

EC 1.2.2 With a cytochrome as acceptor

EC 1.2.2.1 formate dehydrogenase (cytochrome)
EC 1.2.2.2 pyruvate dehydrogenase (cytochrome)
EC 1.2.2.3 formate dehydrogenase (cytochrome-*c*-553)
EC 1.2.2.4 carbon-monoxide dehydrogenase (cytochrome-*b*-561)

EC 1.2.3 With oxygen as acceptor

EC 1.2.3.1 aldehyde oxidase
EC 1.2.3.2 now EC 1.1.3.22 xanthine oxidase
EC 1.2.3.3 pyruvate oxidase
EC 1.2.3.4 oxalate oxidase
EC 1.2.3.5 glyoxylate oxidase

EC 1.3.3 With oxygen as acceptor

EC 1.3.3.1 dihydroorotate oxidase

EC 1.3.5 With a quinone or related compound as acceptor

EC 1.3.5.1 succinate dehydrogenase (ubiquinone)

EC 1.3.7 With an iron-sulfur protein as acceptor

EC 1.3.7.1 6-hydroxynicotinate reductase
EC 1.3.7.2 15,16-dihydrobiliverdin:ferredoxin oxidoreductase

EC 1.3.99 With other acceptors

EC 1.3.99.1 succinate dehydrogenase
EC 1.3.99.2 butyryl-CoA dehydrogenase
EC 1.3.99.3 acyl-CoA dehydrogenase
EC 1.3.99.4 3-oxosteroid 1-dehydrogenase
EC 1.3.99.5 3-oxo-5α-steroid 4-dehydrogenase

2.3 TRANSFERASES

Transferases are enzymes transferring a group, *e.g.,* a methyl group, a sulphate group, a phosphate group or a glycosyl group, from one compound (generally considered as donor) to another compound (generally considered as acceptor). The systematic names are formed according to the scheme *donor:acceptor group transferase*. The common names are normally formed according to *acceptor group transferase* or *donor group transferase.* In many cases, the donor may be a cofactor or coenzyme, charged with the group to be transferred. A special case is that of the transaminases or amino transferases.

Some transferase reactions can be viewed in different ways. For example, the enzyme-catalyzed reaction

$$A–X + C \leftrightarrow A + C–X$$

may be regarded either as a transfer of the group X from A to C, or as a breaking of the A-X bond by the introduction of C. Where C represents phosphate, sulphate or arsenate, the process is often referred as 'phosphorolysis', sulphatolysis or 'arsenolysis', respectively, and a number of enzyme names based on the pattern of *phosphorylase* have come into use. These names are not suitable for a systematic nomenclature, because there is no reason to single out these particular enzymes from the other transferases, and it is better to regard them simply as *X-transferases*.

In the above reaction, the group transferred is usually exchanged, at least formally, for hydrogen, so that the equation could more strictly be written as:

$$A–X + C–H \leftrightarrow A–H + C–X$$

hydrolase, the common name is, in many cases, formed by the name of the substrate with the suffix *-ase*. It is understood that the name of the substrate with this suffix means a hydrolytic enzyme.

A number of hydrolases acting on esters of thiol and carboxyl, glycosyl, peptide, amide, fatty acid esters or phosphodiester bonds are known to catalyze not only hydrolytic removal of a particular group from their substrates, but likewise the transfer of this group to suitable acceptor molecules. In principle, all hydrolytic enzymes might be classified as transferases, since hydrolysis itself can be regarded as transfer of a specific group to water acting as an acceptor. So far, in most cases, the reaction with water as the acceptor was discovered earlier and is considered the main physiological function of the enzyme. That is why such enzymes are classified as hydrolases rather than as transferases.

Some hydrolases (especially the esterases and glycosidases) pose problems because they have a very wide variety of specificity towards the substrate molecules. An example is *vitamin A esterase* (formerly EC 3.1.1.12, now believed to be identical with EC 3.1.1.1). To some extent the choice must be random and however, separate entries should be given only when the specificities are sufficiently different.

Another problem is that proteinases have 'esterolytic' action; they usually hydrolyze ester bonds in appropriate substrates even more rapidly than natural peptide bonds. In this case, classification among the peptide hydrolases is based on historical priority and presumed physiological function.

The second figure in the code number of the hydrolases indicates the nature of the bond hydrolyzed; EC 3.1 are the *esterases*; EC 3.2 the *glycosylases*, and so on.

The third figure normally specifies the nature of the substrate, *e.g.* in the esterases the *carboxylic ester hydrolases* (EC 3.1.1), *thiolester hydrolases* (EC 3.1.2), *phosphoric monoester hydrolases* (EC 3.1.3); in the glycosylases the *O-glycosidases* (EC 3.2.1), *N-glycosylases* (EC 3.2.2), *etc*. Exceptionally, in the case of the peptidyl-peptide hydrolases the third figure is based on the catalytic mechanism as shown by active centre studies or the effect of pH.

EC 3.1 Acting on Ester Bonds

EC 3.1.1 Carboxylic Ester Hydrolases

EC 3.1.1.1 carboxylesterase

EC 3.1.1.2 arylesterase

EC 3.1.1.3 triacylglycerol

EC 3.2 Glycosylases

EC 3.2.1 Glycosidases, i.e. enzymes hydrolysing *O*- and *S*-glycosyl compounds

EC 3.2.1.1 α-amylase

EC 3.2.1.2 β-amylase

EC 4.2 Carbon-Oxygen Lyases

EC 4.2.1 Hydro-Lyases

EC 4.2.1.1 carbonate dehydratase
EC 4.2.1.2 fumarate hydratase
EC 4.2.1.3 aconitate hydratase
EC 4.2.1.4 citrate dehydratase

EC 4.3 Carbon-Nitrogen Lyases

EC 4.3.1 Ammonia-Lyases

EC 4.3.1.1 aspartate ammonia-lyase
EC 4.3.1.2 methylaspartate ammonia-lyase
EC 4.3.1.3 histidine ammonia-lyase
EC 4.3.1.4 formiminotetrahydrofolate cyclodeaminase
EC 4.3.1.5 phenylalanine ammonia-lyase

EC 4.4 Carbon-Sulfur Lyases

EC 4.4.1.1 cystathionine γ-lyase
EC 4.4.1.2 homocysteine desulfhydrase
EC 4.4.1.3 dimethylpropiothetin dethiomethylase
EC 4.4.1.4 alliin lyase

EC 4.5 Carbon-Halide Lyases

EC 4.5.1.1 DDT-dehydrochlorinase
EC 4.5.1.2 3-chloro-D-alanine dehydrochlorinase

EC 4.6 Phosphorus-Oxygen Lyases

EC 4.6.1.1 adenylate cyclase
EC 4.6.1.2 guanylate cyclase

2.6 ISOMERASES

The enzymes that catalyze the geometric or structural changes within one molecule are termed as isomerases. According to the type of isomerism, they may be entitled as *racemases, epimerases, cis-trans-isomerases, isomerases, tautomerases, mutases, cycloisomerases*, etc.,

In some cases, the interconversion in the substrate is brought about by an intramolecular oxidoreduction (EC 5.3), i.e, the hydrogen donor and acceptor are the same molecule and no oxidized product appears, they are not classified as oxidoreductases, even though they may contain firmly bound $NAD(P)^+$.

The subclasses are formed according to the type of isomerism, the sub-subclasses to the type of substrates.

EC 6.1 Forming Carbon-Oxygen Bonds

EC 6.1.1 Ligases Forming Aminoacyl-tRNA and Related Compounds

EC 6.1.1.1 tyrosine—tRNA ligase
EC 6.1.1.2 tryptophan—tRNA ligase
EC 6.1.1.3 threonine—tRNA ligase
EC 6.1.1.4 leucine—tRNA ligase

EC 6.2 Forming Carbon-Sulfur Bonds

EC 6.2.1 Acid-Thiol Ligases

EC 6.2.1.1 acetate—CoA ligase
EC 6.2.1.2 butyrate—CoA ligase
EC 6.2.1.3 long-chain-fatty-acid—CoA ligase

EC 6.3 Forming Carbon-Nitrogen Bonds

EC 6.3.1 Acid-Ammonia (or Amine) Ligases (Amide Synthases)

EC 6.3.1.1 aspartate—ammonia ligase
EC 6.3.1.2 glutamate—ammonia ligase

EC 6.4 Forming Carbon-Carbon Bonds

EC 6.4.1.1 pyruvate carboxylase
EC 6.4.1.2 acetyl-CoA carboxylase
EC 6.4.1.3 propionyl-CoA carboxylase

EC 6.5 Forming Phosphoric Ester Bonds

EC 6.5.1.1 DNA ligase (ATP)
EC 6.5.1.2 DNA ligase (NAD^+)

EC 6.6 Forming Nitrogen—Metal Bonds

EC 6.6.1 Forming Coordination Complexes

EC 6.6.1.1 magnesium chelatase
EC 6.6.1.2 cobaltochelatase

2.8 SPECIFICITY

2.8.1 Definition

Perhaps the most distinctive feature of enzyme-based catalysis is its specificity. Chemical catalysts display only limited selectivity and specificity, whereas enzymes shows several levels of specificity like, for the reactants, susceptible bond involved in the reaction, substrate groups and the type of spatial orientation