Course: PGPathshala-Biophysics

Paper 5: Molecular enzymology and protein engineering

Module 6: Nomenclature & classification, Hydrolases & Transferases, Peptidases, Esterases, Kinase, ATPases, Oxidoreductases, Lyases

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Introduction:

Enzymes are catalysts that increase the velocity of reactions without undergoing any permanent change. These are being utilized to accelerate the rate of reactions in various industrial processes. Very few enzymes were known to biochemists years ago and enzymes were given 'trivial' names before any attempt was made to develop a rational system of nomenclature. The 'trivial' names generally give no idea about the source, function or reaction catalyzed by the enzyme, e.g. Old yellow enzyme, catalase, trypsin, papain, rhodenese. Only those who are directly involved can have an idea about the reaction catalyzed by rhodenese and old yellow enzyme. Rapid growth in the rate of discovery of enzymes led to the development of cogent and specified nomenclature rules.

Malcolm Dixon and Edwin Webb (1958) tried to bring an order to the anarchic situation of enzyme nomenclature by classifying enzymes in terms of the reactions catalyzed, rather than by their structures. This system has been adopted and developed by the International Union of Biochemistry and Molecular Biology (IUBMB), through its Joint Nomenclature Committee with the International Union of Pure and Applied Chemistry (IUPAC). In 1958, the first edition following the same nomenclature rules was out and listed 659 enzymes. This has been through several editions and the most recent version was published in 1992 contained 3196 enzymes. This number has further grown up to 5588 and will continue to increase. This material can also be accessed through the Swissprot Enzyme on-line database.

Objectives:

- Enzyme nomenclature structure
    - Recommended names
    - Systematic names
    - EC number
- Enzyme classification
    - Oxidoreductases
    - Transferases
    - Hydrolases
    - Lyases
    - Isomerases
    - Ligases

1.1 Enzyme nomenclature structure

1.1.1 Recommended names

The commonly used name for the enzyme is frequently used. Generally, recommended names are based on the same general principles as the systematic names, but with a minimum of detail to produce a short name for convenient use. A number of generic words indicating reaction types may be used in recommended names, e.g. *dehydrogenase, reductase, oxidase, peroxidase, kinase, tautomerase, deaminase, dehydratase* etc. The prefix D and L should be omitted for common sugars and amino acids. Commonly used abbreviations for substrates (eg. ATP) can be used in enzyme names but use of chemical formula instead of substrate names is discouraged. Substrate name composed of two nouns eg. glucose phosphate should be written hyphenated when they form part of enzyme names eg. glucose-6-phosphate dehydrogenase. Direct addition of *–ase* to the substrate name indicates that enzyme brings about hydrolysis. The name *dehydrogenase* and *dehydratase* should be used for used for dehydrogenating and dehydrating enzymes in place of *dehydrase*, which was previously in use for both.

1.1.2 Systematic names

The system attempts to describe in unambiguous terms what the enzyme actually catalyzes. Systematic names consist of two parts. The first contains the name of the substrate or, in the case of a bimolecular reaction, of the two substrates separated by a colon. The second part, ending in *-ase*, indicates the nature of the reaction. A number of generic words indicating a type of reaction may be used in either recommended or systematic names: oxidoreductase, oxygenase, transferase (with a prefix indicating the nature of the group transferred), hydrolase, lyase, racemase, epimerase, isomerase, mutase, ligase. Where additional information is needed to make the reaction clear, a phrase indicating the reaction or a product should be added in parentheses after the second part of the name, e.g. (ADP-forming), (dimerizing), (CoA-acylating).

1.1.3 EC number

Enzymes can be further classified on the basis of a number given by enzyme commission. Enzyme commission came in existence in 1956 by IUB. The commission presented its first report in 1961, in which enzymes have been classified into six groups according to the reaction catalyzed. The IUB standing committee on enzymes replaced the enzyme commission in 1961, and its work is now the responsibility of the Nomenclature Committee of the IUBMB. Despite these changes in responsibility, however, the original classification has been maintained and the prefix "EC" is still used in enzyme numbers.

The EC number consists of four parts (a, b, c, d). The first number (a) indicates the type of reaction catalyzed and can take values from 1 to 6 according to the classification of reaction types, described above. The second number (b) defines the subclass, which specifies the type of substrate or the bond cleaved more precisely. The third number (c) indicates the sub-subclass, gives a more precise definition of the reaction catalyzed in terms of the type of electron acceptor or the type of group removed. The fourth number (d) denotes the serial number of the enzyme in its sub-subclass. The digit (d) is generally used to distinguish between different enzymes of the same function based on the actual substrate in the reaction. This system does not take into account amino acid sequence, protein structure and chemical mechanism.

1.2 Enzyme classes:
Broadly, enzymes can be divided into six classes according to the reaction catalyzed.

1.2.1 Oxidoreductases

This class consists of the enzymes catalyzing oxidation-reduction reactions. Since the oxidation of one group must be accompanied by the reduction of another, they are categorized as the oxidoreductases.

$$A_{red} + B_{ox} \longrightarrow A_{ox} + B_{red}$$

The systematic enzyme name should be in the form *donor:acceptor oxidoreductase*. The substrate that is being oxidized is the hydrogen donor, hence the recommended name is usually *donor dehydrogenase*. The term *donor oxidase* is used when O_2 is the acceptor. Sometimes the term '*reductase*' can also be used as an alternative. The recommended name does not define the equilibrium position of the reaction or the net direction of flux through the enzyme. Furthermore, in some cases, an enzyme within a metabolic pathway can proceed in a thermodynamically unfavoured direction.

The first figure is for class oxidoreductase and the second figure in the code number denotes the type of group in the hydrogen-donor substrate that is being oxidized or reduced. The third number designates the hydrogen acceptor: 1, denotes NAD(P); 2, cytochrome; 3, molecular oxygen; 4, disulfide; 5, quinone or similar compound; 6, nitrogenous group; 7, iron– sulfur protein and 8, flavin. Number 99 denotes all other acceptors.

A different classification scheme is used for the subclasses 1.13 and 1.14, since these enzymes catalyze the incorporation of oxygen into the substrate. The recommended names of these enzymes are *monooxygenase* or *dioxygenase*, depending on whether one or two atoms of oxygen are incorporated into the substance oxidized.

Example: EC 1.1.1.27
Recommended name: Lactate dehydrogenase
Systematic name: (*S*)-lactate:NAD^+ oxidoreductase
Reaction: (*S*)-Lactate + NAD^+ = Pyruvate + NADH

Table 1: Oxidoreductases (1.-.-.-)

1.1.-.- *(sub-subclass 1,2 3,4, 5, 99)*	Acting on CH-OH group of donors
1.2.-.- *(sub-subclass 1,2 3,4, 7, 99)*	Acting on aldehyde or oxo group of donors
1.3.-.- *(sub-subclass 1,2 3,5, 7, 99)*	Acting on CH-CH group of donors
1.4.-.- *(sub-subclass 1,2 3,4, 7, 99)*	Acting on CH-NH_2 group of donors
1.5.-.- *(sub-subclass 1, 3, 4, 5, 99)*	Acting on CH-NH group of donors
1.6.-.- *(sub-subclass 1,2 ,4, 5, 6, 8, 99)*	Acting on $NADH_2$ or $NADPH_2$

1.7.-.- *(sub-subclass 1, 3, 7, 99)*	Acting on other nitrogenous compounds as donors
1.8.-.- *(sub-subclass 1, 2, 3, 4, 5, 7, 99)*	Acting on sulfur group of donors
1.9.-.- *(sub-subclass 3, 6, 99)*	Acting on haem group of donors
1.10.-.- *(sub-subclass 1, 2, 3, 99)*	Acting on diphenols and related substances of donors
1.11.-.-	*Acting on peroxide as acceptor*
1.11.1.-	Peroxidases
1.12.-.- *(sub-subclass 1, 2, 99)*	Acting on hydrogen as donor
1.13.-.-	*Acting on single donors with incorporation of molecular O_2*
1.13.11.-	Incorporation of two atoms of oxygen
1.13.12.-	Incorporation of one atom of oxygen
1.13.99.-	Miscellaneous
1.14.-.-	*Acting on paired donors with incorporation of molecular O_2*
1.14.11.-	2-oxoglutarate as one donor
1.14.12.-	$NADH_2$ or $NADPH_2$ as one donor
1.14.13.-	$NADH_2$ or $NADPH_2$ as one donor
1.14.14.-	Reduced flavin or flavoprotein as one donor
1.14.15.-	Reduced iron-sulfur protein as one donor
1.14.16.-	Reduced pteridine as one donor
1.14.17.-	Ascorbate as one donor
1.14.18.-	Other compounds as one donor
1.14.99.-	Miscellaneous
1.15.-.-	Acting on superoxide radicals as acceptor
1.16.-.- *(sub-subclass 1, 3)*	Oxidizing metal ions
1.17.-.- *(sub-subclass 1, 3, 4, 99)*	Acting on $-CH_2-$ groups
1.18.-.- *(sub-subclass 1, 6, 99)*	Acting on reduced ferredoxin as donor
1.19.-.-	*Acting on reduced flavodoxin as donor*
1.19.6.-	Dinitrogen as acceptor
1.97.-.-	Other oxidoreductases

1.2.2 Transferases

These enzymes catalyze the transfer of a group from one substrate (the donor) to another (the acceptor).

A-B + C ------ A + B-C

The systematic name for the class is in the form *donor:acceptor group- transferase*, while the recommended names are formed according to *acceptor grouptransferase* or *donor grouptransferase*. Sometimes transferase reactions can be considered in different ways; for example, in the reaction shown above there may be transfer of the group B from A to C, and would therefore be termed a *B-transferase*. However, it could also be considered as a breaking of the A–B bond by the introduction of C. If C represents phosphate, the enzyme catalyzing the reaction would be *phosphorylase*. However for systematic purposes, these enzymes are classified as phosphotransferases. Similarly, aminotransferases might be classified as oxidoreductases as the reaction involves the transfer of a $-NH_2$ group and H to a compound containing a carbonyl group, in exchange for the =O of that group. Thus, in the reaction oxidative deamination of the donor (e.g. an amino acid) is linked to the reductive amination of the acceptor (e.g. oxo acid). Still, since the characteristic feature of the reaction is the transfer of the amino group, hence the enzymes are classified as amino transferases (subclass 2.6.1.)

In the EC number for the class, the second figure denotes the general nature of the group transferred (one- carbon group, 2.1; aldehydic or ketonic group, 2.2; acyl group, 2.3, etc.) and the third number specifies that group (methyltransferase, 2.1.1; formyltransferase, 2.1.2, etc.) with an exception of subclass 2.7, where, the third number specifies the nature of the acceptor group (Table 2).

Example: EC 2.7.1.1
Recommended name: Hexokinase
Systematic name: ATP:D-hexose 6-phosphotransferase
Reaction: ATP + D-hexose = ADP + D-hexose 6-phosphate

Table 2: Transferases (2.-.-.-)

2.1.-.-	*Transferring one-carbon groups*
2.1.1.-	Methyltransferases
2.1.2.-	Hydroxymethyl-, formyl- and related transferases
2.1.3.-	Carboxyl- and carbamoyltransferases
2.1.4.-	Amidinotransferases
2.2.-.-	*Transferring aldehyde or ketone residues*
2.2.1.-	Transaldolases and transketolases
2.3.-.-	*Acyltransferases*
2.3.1.-	Acyltransferases
2.3.2.-	Aminoacyltransferases
2.4.-.-	*Glycosyltransferases*
2.4.1.-	Hexosyltransferases
2.4.2.-	Pentosyltransferases
2.4.99.-	Other glycosyl groups
2.5.-.-	*Transferring alkyl or aryl groups, other than methyl groups*
2.5.1.-	Mixed enzymes
2.6.-.-	*Transferring nitrogenous groups*
2.6.1.-	Transaminases
2.6.3.-	Oximinotransferases
2.6.99.-	Other groups

2.7.-.-	*Transferring phosphorous-containing groups*
2.7.1.-	Phosphotransferases with an alcohol group as acceptor
2.7.2.-	Phosphotransferases with an carboxyl group as acceptor
2.7.3.-	Phosphotransferases with an nitrogenous group as acceptor
2.7.4.-	Phosphotransferases with an phosphate group as acceptor
2.7.6.-	Diphosphotransferases
2.7.7.-	Nucleotidyltransferases
2.7.8.-	Transferases for other substituted phosphate groups
2.7.9.-	Phosphotransferases with paired acceptors
2.8.-.-	*Transferring sulfur-containing groups*
2.8.1.-	Sufurtransferases
2.8.2.-	Sulfotransferases
2.8.3.-	CoA-transferases
2.9.-.-	*Transferring selenium-containing groups*

1.2.3 Hydrolases

These enzymes catalyze the hydrolytic cleavage of bonds such as C–O, C–N, C–C and some other bonds, including phosphoric anhydride bonds. It difficult to formulate general rules for the enzymes belong to this class due to overlapping specificities.

A-B + H_2O ----- A-H + B-OH

The systematic names are usually in the form of *substrate X-hydrolase*, where X is the group removed by hydrolysis. The recommended names 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 ester, glycosyl, peptide, amide or other bonds are known to catalyze not only hydrolytic removal of a particular group from their substrates, but also the transfer of this group to suitable acceptor molecules. Hence, all hydrolytic enzymes might be classified as transferase. Since, the reaction with water as the acceptor was discovered earlier and is considered as the main physiological function of the enzyme, hence such enzymes are classified as hydrolases rather than transferases.

The second number designates the nature of the bond hydrolyzed. 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.

Example: EC 3.6.3.14
Recommended name: H^+-transporting two-sector ATPase
Systematic name: ATP phosphohydrolase (H^+-transporting)
Other name: Adenosinetriphosphatase
Reaction: ATP + H_2O + H^+_{in} = ADP + Phosphate + H^+_{out}

Table 3: Hydrolases (3.-.-.-)

3.1.-.-	*Acting on ester bonds*
3.1.1.-	Carboxylic ester hydrolases
3.1.2.-	Thiolester hydrolases
3.1.3.-	Phosphoric monoester hydrolases
3.1.4.-	Phosphoric diester hydrolases
3.1.5.-	Triphosphoric monoester hydrolases
3.1.6.-	Sulfuric ester hydrolases
3.1.7.-	Diphosphoric monoester hydrolases
3.1.8.-	Phosphoric triester hydrolases
3.1.11.-	Exodeoxyribonucleases producing 5'-phosphomonoesters
3.1.13.-	Exoribonucleases producing 5'-phosphomonoesters
3.1.14.-	Exoribonucleases producing other than 5'-phosphomonoesters
3.1.15.-	Exonucleases active with either ribo- or deoxyribonucleic acids
3.1.16.-	Exonucleases active with either ribo- or deoxyribonucleic acids
3.1.21.-	Endodeoxyribonucleases producing 5'-phosphomonoesters
3.1.22.-	Endodeoxyribonucleases producing other than 5'-phosphomonoesters
3.1.25.-	Site-specific endodeoxyribonucleases specific for altered bases
3.1.26.-	Endoribonucleases producing 5'-phosphomonoesters
3.1.27.-	Endoribonucleases producing other than 5'-phosphomonoesters
3.1.30.-	Endonucleases active with either ribo- or deoxyribonucleic acids
3.1.31.-	Endonucleases active with either ribo- or deoxyribonucleic acids
3.2.-.-	*Glycosidases*
3.2.1.-	Hydrolyzing *O*-glycosyl compounds
3.2.2.-	Hydrolyzing *N*-glycosyl compounds
3.2.3.-	Hydrolyzing *S*-glycosyl compounds
3.3.-.-	*Acting on ether bonds*
3.3.1.-	Thioether hydrolases
3.3.2.-	Ether hydrolases
3.4.-.-	*Peptidase*
3.4.11.-	Aminopeptidases
3.4.13.-	Dipeptidases
3.4.14.-	Dipeptidyl and tripeptidyl peptidases
3.4.15.-	Peptidyl dipeptidases
3.4.16.-	Serine-type carboxypeptidases
3.4.17.-	Metallocarboxypeptidases
3.4.18.-	Cysteine-type carboxypeptidases
3.4.19.-	Omega peptidases
3.4.21.-	Serine endopeptidases
3.4.22.-	Cysteine endopeptidases
3.4.23.-	Aspartic endopeptidases
3.4.24.-	Metalloendopeptidases
3.4.99.-	Other endopeptidases
3.5.-.-	*Acting on C-N bonds, other than peptide bonds*
3.5.1.-	In linear amides
3.5.2.-	In cyclic amides
3.5.3.-	In linear amidines
3.5.4.-	In cyclic amidines
3.5.5.-	In nitriles
3.5.99.-	In other compounds

3.6.-.-	*Acting on acid anhydrides*
3.6.1.-	In phosphorous- containing anhydrides
3.6.2.-	In sulfonyl- containing anhydrides
3.7.-.-	*Acting on C-C bonds*
3.7.1.-	In ketonic substances
3.8.-.-	*Acting on halide bonds*
3.8.1.-	In C-halide compounds
3.9.-.-	*Acting on P-N bonds*
3.10.-.-	*Acting on S-N bonds*
3.11.-.-	*Acting on C-P bonds*
3.12.-.-	*Acting on S-S bonds*

1.2.4 Lyases

These enzymes catalyze the cleavage of C–C, C–O, C–N and other bonds by elimination, leaving double bonds or rings.

A (XH)-B ------ A-X + B-H

The systematic name is formed according to the pattern *substrate group-lyase*. The hyphen is an important part of the name and, to avoid confusion, should not be omitted, e.g. *hydro-lyase* not 'hydrolyase'. In the recommended names, expressions like *decarboxylase* or *aldolase* are used. *Dehydratase* is used for those enzymes catalyzing the elimination of water. In cases where the reverse reaction is much more important, or the only one demonstrated, synthase (not synthetase) may be used in the name.

The second figure gives information about the bond broken: 4.1 are carbon–carbon lyases, 4.2 are carbon–oxygen lyases, 4.3 are carbon-nitrogen lyases and 4.4 are carbon-sulfur lyases. The third figure gives indicates the eliminated group (Table 4).

Example: EC 4.1.2.13
Recommended name: Fructose-bisphosphate aldolase
Systematic name: D-fructose-1,6-bisphosphate D-glyceraldehyde-3-phosphate-lyase (glycerone-phosphate-forming)
Reaction: D-fructose 1,6-bisphosphate = Glycerone phosphate + D-glyceraldehyde 3-phosphate

Table 4: Lyases (4.-.-.-)

4.1.-.-	*C-C lyases*
4.1.1.-	Carboxy-lyases
4.1.2.-	Aldehyde-lyases
4.1.3.-	Oxo-acid-lyases
4.1.99.-	Other C-C lyases
4.2.-.-	*C-O lyases*
4.2.1.-	Hydro-lyases
4.2.2.-	Acting on polysaccharides
4.2.99.-	Other C-O lyases

4.3.-.-	*C-N lyases*
4.3.1.-	Ammonia-lyases
4.3.2.-	Amidine-lyases
4.3.3.-	Amine-lyases
4.3.99.-	Other C-N lyases
4.4.-.-	*C-S lyases*
4.5.-.-	*Carbon-halide lyases*
4.6.-.-	*Phosphorus-oxygen lyases*
4.99.-.-	Other lyases

1.2.5 Isomerases

These enzymes catalyze geometric or structural transformations within one molecule. According to the type of isomerism involved, they may be called *racemases, epimerases, cis–trans-isomerases, isomerases, tautomerases, mutases* or *cycloisomerases*. The subclass (second number) denotes the type of isomerism involved and the sub-subclass (third figure) indicates the type of substrate (Table 5).

Sometimes, the interconversion in the substrate involves an intramolecular oxidoreduction (EC 5.3.-.-) and can be classified as oxidoreductases. But, since the hydrogen donor and acceptor groups are in the same molecule and no oxidized product appears, the more appropriate class for these enzymes is isomerases, even though they may have tightly bound $NADP^+$.

Example: EC 5.3.1.1
Recommended name: Triose-phosphate isomerase
Systematic name: D-glyceraldehyde-3-phosphate aldose-ketose-isomerase
Reaction: D-glyceraldehyde 3-phosphate = Glycerone phosphate

Table 5: Isomerases (5.-.-.-)

5.1.-.-	*Racemases and epimerases*
5.1.1.-	Acting on amino acids and derivatives
5.1.2.-	Acting on hydroxy acids and derivatives
5.1.3.-	Acting on carbohydrates and derivatives
5.1.99.-	Acting on other compounds
5.2.-.-	*cis-trans isomerases*
5.3.-.-	*Intramolecular oxidoreductases*
5.3.1.-	Intraconverting aldoses and ketoses
5.3.2.-	Intraconverting keto and enol groups
5.3.3.-	Transposing C=C bonds
5.3.4.-	Transposing S-S bonds
5.3.99.-	Others

5.4.-.-	*Intramolecular transferses (mutases)*
5.4.1.-	Transferring acyl groups
5.4.2.-	Phosphotransferases
5.4.3.-	Transferring amino groups
5.4.99.-	Transferring other groups
5.5.-.-	*Intramolecular lyases*
5.99.-.-	Other isomerases

1.2.6 Ligases

These enzymes catalyze the joining of two molecules coupled with the hydrolysis of a diphosphate bond in ATP or a similar triphosphate. The systematic name of the enzyme is in the form *A:B ligase* (XDP- or XMP-forming). The recommended name should be written as *A–B ligase*. Sometimes the name synthase or synthetase is used for the recommended name to emphasize the synthetic nature of the reaction.

The second figure in the code number denotes the bond formed: 6.1 for C–O bonds (enzymes acylating tRNA), 6.2 for C–S bonds (acyl-CoA derivatives), etc. Sub-subclasses are only in use in the C–N ligases (6.3), which include the amide synthases (6.3.1), the peptide synthases (6.3.2), enzymes forming heterocyclic rings (6.3.3), etc (Table 6).

Example: EC 6.1.1.5
Recommended name: Isoleucine—tRNA ligase
Systematic name: L-isoleucine:tRNA^{Ile} ligase (AMP-forming)
Reaction: ATP + L-isoleucine + tRNA^{Ile} = AMP + Diphosphate + L-isoleucyl-tRNA^{Ile}

Table 6: Ligases (6.-.-.-)

6.1.-.-	*Forming C-O bonds*
6.1.1.-	Ligases forming aminoacyl-tRNA and related compounds
6.2.-.-	*Forming C-S bonds*
6.2.1.-	Acid–thiol ligases
6.3.-.-	*Forming C-N bonds*
6.3.1.-	Acid–ammonia (or amine) ligases (amide synthases)
6.3.2.-	Acid–amino-acid ligases (peptide synthases)
6.3.3.-	Cyclo-ligases
6.3.4.-	Other carbon–nitrogen ligases
6.3.5.-	Carbon–nitrogen ligases with glutamine as amido-*N*-donor
6.4.-.-	*Forming C-C bonds*
6.5.-.-	*Forming phosphoric ester bonds*

Summary

Enzyme classification and nomenclature system provides explicit identification of enzymes according to the reaction catalyzed. It does not take into the structural aspects of enzymes. All the enzymes have systematic names based on the reaction involved. Enzyme commission (EC) gives a numerical system to classify enzymes. Enzymes can be divided into six classes and various sub-classes that offer more precise view of the reaction and the components involved within.