Introduction to Enzymes

Enzyme Engineering

What is enzymes?

- Life depends on well-orchestrated series of chemical reactions : *E. coli* has 4288 proteins, 2656 of which are characterized, and 64% (1701) of the characterized ones code enzymes
- Chemical reactions are far slow to maintain life
- Living system has designed catalysts to fasten the specific reactions

1.2 History of enzyme study

Table 1-1. Brief history of enzymes and their applications.

B C	Chymosin from the stomach of young cattle, sheeps and goats was used for cheese production in many ancient cultures for approximately 7000 years.	
1783	Hydrolysis of meat by gastric juice demonstrated.	Spallazani
1814	Starch degradation and sugar production by malted barley observed.	Kirchhoff
1833	The active principle of malt is called diastase and its application to industrial art described.	Payen and Persoz
1846	Invertase activity observed.	Dubonfout
1867	The term *enzymes* is coined to describe catalytic activity not bound to living cells (unorganised ferments). The name is extended later also to intracellular catalysts (organised ferments as defined by Pasteur).	Kühne
1893	Definition of a catalyst including enzymes is given.	Ostwald
1894	Enzyme stereospecificity anticipated.	E. Fischer
1894	"Taka diastase" produced commercially with *Aspergillus oryzae* by surface culture	Takamine
1897	The conversion of glucose to ethanol demonstrated by a cell free extract from yeast.	Buchner

Rock and key model
Fig. 1.1

Demonstrated that enzymes do not require a cell

1906	Preparative separation of L-leucine from the racemate carried out by hydrolysis of the propyl ester with liver extracts.	Warburg
1908	Synthesis of optically active cyanohydrins described, using D-oxynirerilase from almonds as catalyst.	Rosenberg
1908	Application of pancratic enzymes in the leather industry for the bating of hides.	Röhm
1911–1913	Glucoside synthesis in the presence of high concentration of ethanol or acetone described.	Bourquelot, Bridel and Verdon
1913–1915	Application of pancreatic enzymes to clean laundry introduced, first commercial product sold to the public: Burnus.	Röhm
1916	Immobilization of invertase on charcoal demonstrated with retention of activity.	Nelson and Griffin
1926	Urease from Jack beans crystallized.	Sumner
1936	Enzymatic ester synthesis improved using pancreatic lipase in the presence of benzene.	Sym
1953	The first primary sequence of a protein (Insulin) established, proving the chemical identity of proteins.	Sanger
1960	Cultivation of *Bacillus licheniformis* in submerged culture started for protease production on a large scale.	NOVO
after 1980	Application of genetic engineering techniques to improve enzyme production and to alter enzyme properties by protein engineering and evolutionary design.	many

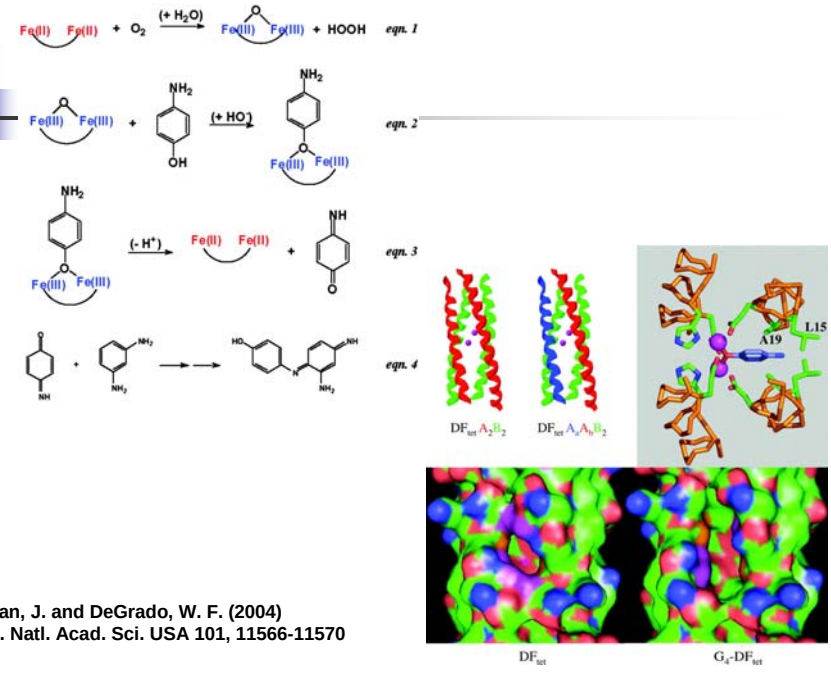
Enzyme is proved to be a protein

1957, Myoglobin structure was deduced by X-ray crystallography Kendrew

1963, The first aa sequence of enzyme, ribonuclease was reported
1965, The first enzyme structure of lysozyme was reported

1.2 History of enzyme study

- 1958, “Induced fit” model was proposed, Koshland
- 1965, “Allosteric model” of enzyme was porposed, Monod
- 1969, the first chemical synthesis of an enzyme was reported, proving an enzyme is a protein
- Mechanisms of thousands enzymes have been studied by X-ray crystallography and NMR
- DNA recombinant methods were used to overproduce enzymes and to pinpoint the important amino acids
- 2004, the first computer designed enzyme was reported



Kaplan, J. and DeGrado, W. F. (2004)
Proc. Natl. Acad. Sci. USA 101, 11566-11570

1.2 History of enzyme study

- Catalytic biological molecules other than conventional enzymes
  - Antibody
  - RNA (Ribozyme) : Usually involved in RNA processing (phosphate ester hydrolysis)–Cech, 1986
    - As short as 30 nucleotide (hammerhead ribozyme)–Fig. 1.3

1.3 Properties of enzymes
I. Catalytic power

- It increases the rate as much as 10^{17} fold
- It operates in moderate temperature and neutral pH (Enzymes from archeabacteria are exceptions)
- Extreme example is Nitrogen fixation (N_2 to ammonia)

  700 ~ 900K, 100 ~ 900atm with iron catalysts

  vs. 300K, neutral pH, 1atm with iron and molybdenum in nitrogenase

1.3 Properties of enzymes
I. Catalytic power

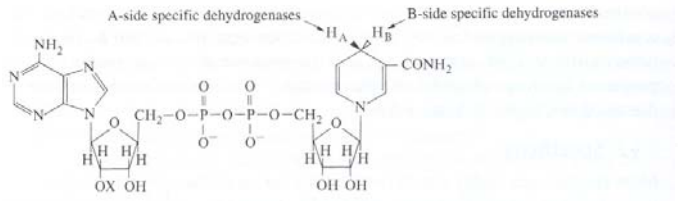
Table 1.1 Examples of the catalytic power of enzymes

Substrate	Catalyst	Temperature (K)	Rate constant k $(mol\ dm^{-3})^{-1}s^{-1}$
Amide (hydrolysis)			
benzamide	H^+	325	2.4×10^{-6}
benzamide	OH^-	326	8.5×10^{-6}
benzoyl-L-tyrosinamide	α-chymotrypsin	298	14.9
Urea (hydrolysis)	H^+	335	7.4×10^{-7}
	urease	294	5.0×10^{6}
$2H_2O_2 \rightarrow 2H_2O + O_2$	Fe^{2+}	295	56
	catalase	295	3.5×10^{7}

Data taken from reference 31.

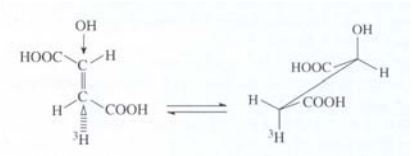
1.3 Properties of enzymes
2. Specificity

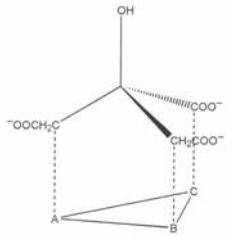
- Most enzymes are absolute or near-absolute specific to the substrates
  - Some enzymes that react with wide range of substrates are peptidises, phosphatases, esterases (bond specific), and hexokinases (group specific)
- Stereospecific
  - Dehydrogenases with NAD^+ or $NADP^+$ (Fig. 1.4)
  - React with only one chiral compound (Fig. 1.5)
- Recognizable accuracies in DNA/RNA polymerases and in protein synthesis



Reduced nicotinamide adenine dinucleotide (NADH), X = H, and reduced nicotinamide adenine dinucleotide phosphate (NADPH), X = phosphate.

A-side dehydrogenases	B-side dehydrogenases
Alcohol dehydrogenase (NAD^+)	Glycerol 3-phosphate dehydrogenase (NAD^+)
Lactate dehydrogenase (NAD^+)	3-Hydroxybutyrate dehydrogenase (NAD^+)
Malate dehydrogenase (NAD^+)	Glucose dehydrogenase (NAD^+)
Cytochrome b_5 reductase (NAD^+)	Glyceraldehyde-phosphate dehydrogenase (NAD^+)
Shikimate dehydrogenase ($NADP^+$)	Homoserine dehydrogenase ($NADP^+$)
3-Oxyacyl [acyl-carrier-protein] reductase ($NADP^+$)	Glucose 6-phosphate dehydrogenase ($NADP^+$)





1.3 Properties of enzymes
3. Regulation

- Enzyme activities are regulated by small ions or small molecules (effectors), such as phosphate or Ca^{2+}
- The regulations are mediated by changing covalent structure
- Feedback inhibition is common in many biosynthetic pathway enzymes

1.4 Cofactors

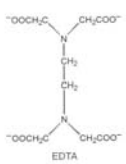
- Many enzymes, like chemotrypsin and triosephosphate isomerase, do not require additional factor
- Many others require non-protein component for enzyme activity
- Metal ions and organic cofactors (usually derived from B vitamins) are major groups of cofactors
- Tightly bound cofactors are called prosthetic groups (holoenzyme = enzyme + cofactor, apoenzyme = enzyme without cofator)
- Any other molecules that bind to an enzyme are called ligands (including substrates and effectors)

1.4 Cofactors

Table 1.2 Examples of the metal ion requirements of some enzymes

Metal	Enzyme
Na	Intestinal sucrose α-D-glucohydrolase
K	Pyruvate kinase (also requires Mg)
Mg	Kinases (e.g. hexokinase, pyruvate kinase), adenosinetriphosphatases (e.g. myosin adenosinetriphosphatase)
Fe	Catalase, peroxidase, nitrogenase
Zn	Alcohol dehydrogenase, carboxypeptidase, carbonic anhydrase
Mo	Xanthine oxidase, nitrogenase
Cu	Cytochrome c oxidase, amine oxidase
Ni	Urease, hydrogenase, carbon monoxide dehydrogenase, superoxide dismutase
Mn	Histidine ammonia lyase
V	Nitrogenase

Further information on the metal ions in enzymes can be found in references 40–44.



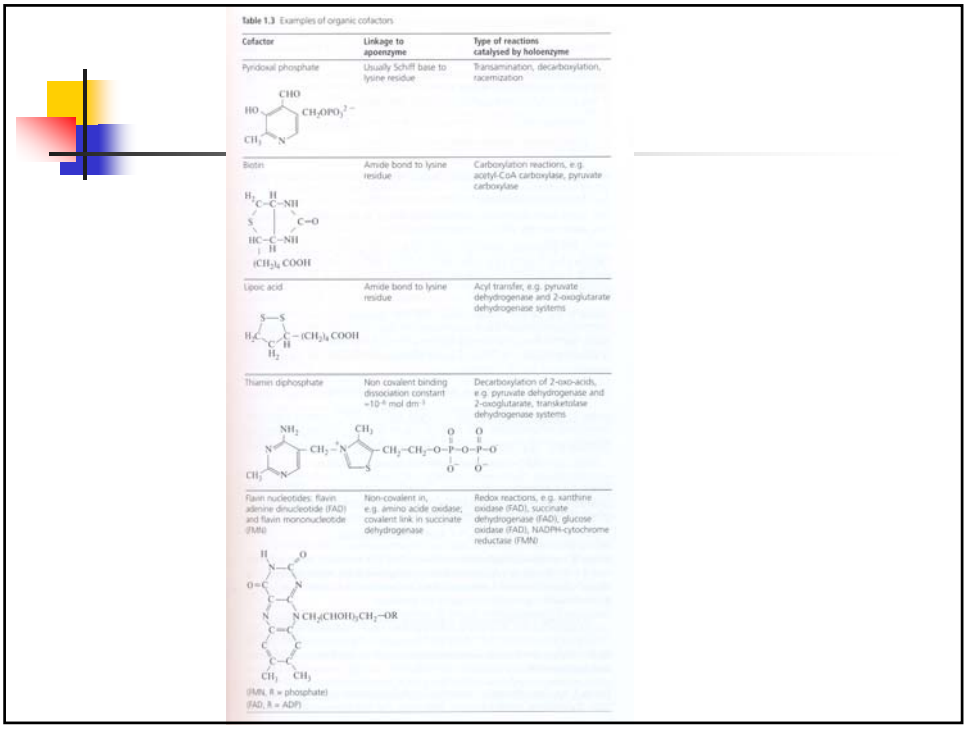


Table 1.3 Examples of organic cofactors

Cofactor	Linkage to apoenzyme	Type of reactions catalysed by holoenzyme
Pyridoxal phosphate	Usually Schiff base to lysine residue	Transamination, decarboxylation, racemization
Biotin	Amide bond to lysine residue	Carboxylation reactions, e.g. acetyl-CoA carboxylase, pyruvate carboxylase
Lipoic acid	Amide bond to lysine residue	Acyl transfer, e.g. pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase systems
Thiamin diphosphate	Non covalent binding dissociation constant $\sim 10^{-6}$ mol dm^{-3}	Decarboxylation of 2-oxo-acids, e.g. pyruvate dehydrogenase and 2-oxoglutarate, transketolase dehydrogenase systems
Flavin nucleotides: flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN)	Non-covalent in, e.g. amino acide oxidase; covalent link in succinate dehydrogenase	Redox reactions, e.g. xanthine oxidase (FAD), succinate dehydrogenase (FAD), glucose oxidase (FAD), NADPH-cytochrome reductase (FMN)

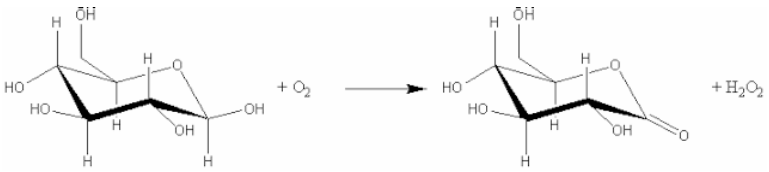
(FMN, R = phosphate)
(FAD, R = ADP)

1.5 Nomenclature of enzymes

- Some enzyme names are unhelpful to figure the reaction catalyzed, eg. catalase, trypsin, papain....
- IUBMB (International Union of Biochemistry and Molecular Biology) was set up in 1955
- Six classes of enzymes
  1. Oxidoreductase
  2. Transferase
  3. Hydrolase
  4. Lyase
  5. Isomerase
  6. Ligase

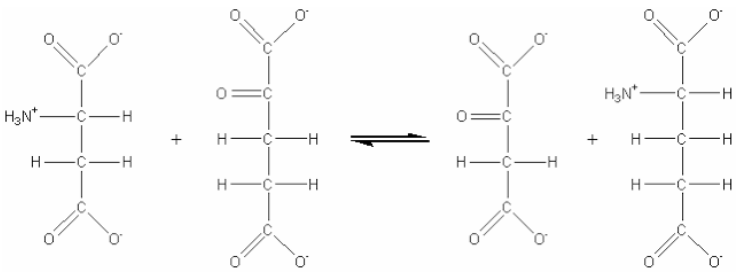
1. Oxidoreductases

- Involved redox reactions in which hydrogen or oxygen atoms or electrons are transferred between molecules.
- This extensive class includes the dehydrogenases (hydride transfer), oxidases (electron transfer to molecular oxygen), oxygenases (oxygen transfer from molecular oxygen) and peroxidases (electron transfer to peroxide).
- For example: glucose oxidase (EC 1.1.3.4, systematic name, b-D-glucose:oxygen 1-oxidoreductase).



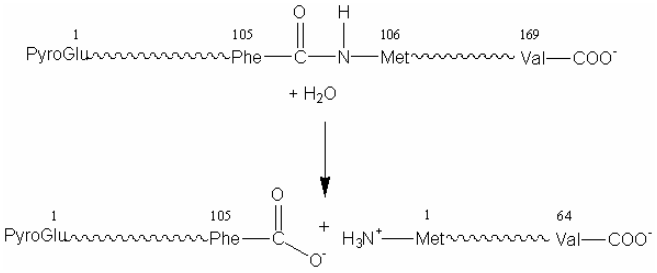
2. Transferases

- Catalysing the transfer of an atom or group of atoms (e.g. acyl-, alkyl- and glycosyl-), between two molecules, but excluding such transfers as are classified in the other groups (e.g. oxidoreductases and hydrolases).
- For example: aspartate aminotransferase (EC 2.6.1.1, L-aspartate:2-oxoglutarate aminotransferase; also called glutamic-oxaloacetic transaminase or simply GOT).



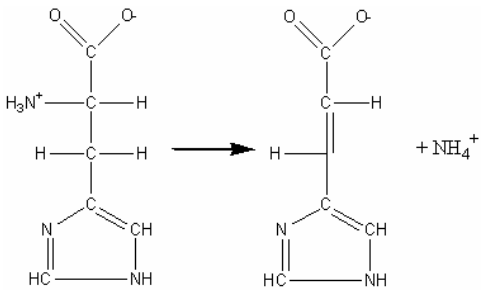
3. Hydrolases

- Involved hydrolytic reactions and their reversal. This is presently the most commonly encountered class of enzymes within the field of enzyme technology and includes the esterases, glycosidases, lipases and proteases.
- For example: chymosin (EC 3.4.23.4, no systematic name declared; also called rennin).



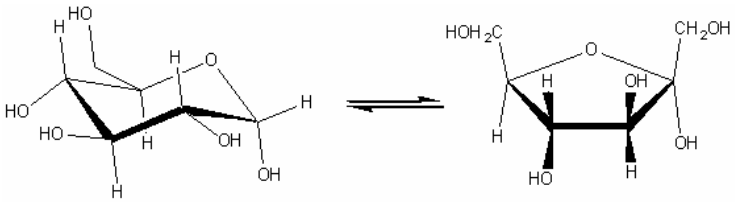
4. Lyases

- Involved elimination reactions in which a group of atoms is removed from the substrate. This includes the aldolases, decarboxylases, dehydratases and some pectinases but does not include hydrolases.
- For example: histidine ammonia-lyase (EC 4.3.1.3, L-histidine ammonia-lyase; also called histidase).



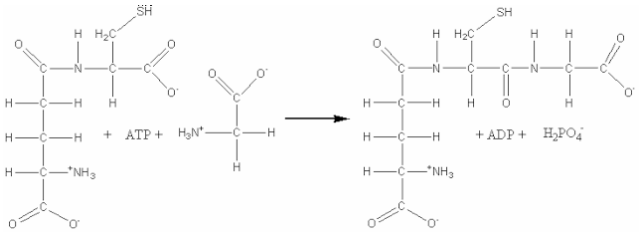
5. Isomerases

- Catalysing molecular isomerisations and includes the epimerases, racemases and intramolecular transferases.
- For example: xylose isomerase (EC 5.3.1.5, D-xylose ketol-isomerase; commonly called glucose isomerase).



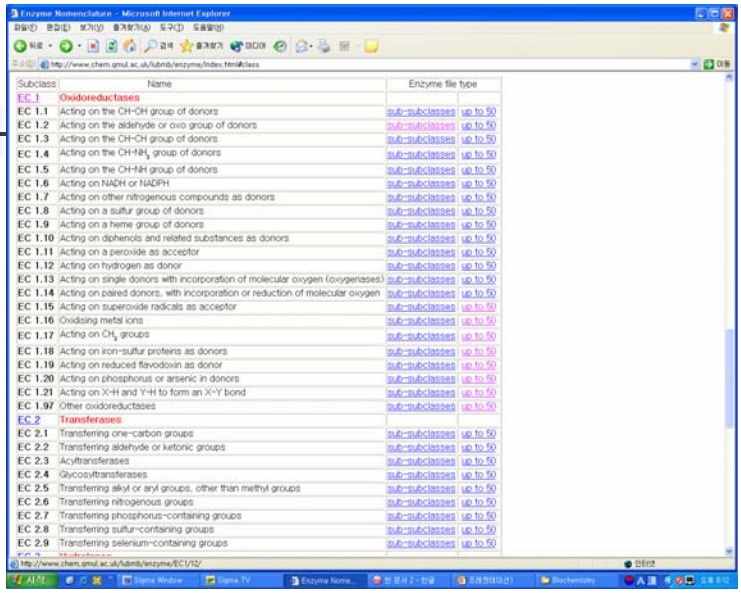
6. Ligases

- also known as synthetases, form a relatively small group of enzymes which involve the formation of a covalent bond joining two molecules together, coupled with the hydrolysis of a nucleoside triphosphate.
- For example: glutathione synthase (EC 6.3.2.3, g-L-glutamyl-L-cysteine:glycine ligase (ADP-forming); also called glutathione synthetase).

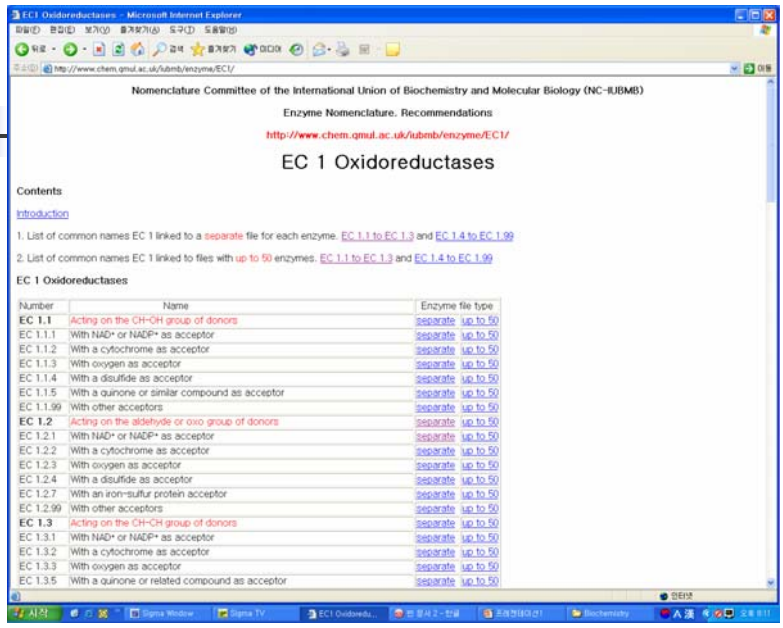


EC Number system

- 1st number : Class of the enzyme
- 2nd number : subclass by the type of substrate or the bond cleaved
- 3rd number : subclass by the electron acceptor or the type of group removed
- 4th number : serial number of enzyme found
- Classification based on the chemical reaction catalyzed not on the source (species or tissues) of the enzyme – Amino acid sequence may be very different



http://www.chem.qmul.ac.uk/iubmb/enzyme/index.html



Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB)

Enzyme Nomenclature. Recommendations

http://www.chem.qmul.ac.uk/iubmb/enzyme/EC1/

EC 1 Oxidoreductases

Contents

Introduction

1. List of common names EC 1 linked to a separate file for each enzyme. EC 1.1 to EC 1.3 and EC 1.4 to EC 1.99

2. List of common names EC 1 linked to files with up to 50 enzymes. EC 1.1 to EC 1.3 and EC 1.4 to EC 1.99

EC 1 Oxidoreductases

Number	Name	Enzyme file type
EC 1.1	Acting on the CH-OH group of donors	separate up to 50
EC 1.1.1	With NAD+ or NADP+ as acceptor	separate up to 50
EC 1.1.2	With a cytochrome as acceptor	separate up to 50
EC 1.1.3	With oxygen as acceptor	separate up to 50
EC 1.1.4	With a disulfide as acceptor	separate up to 50
EC 1.1.5	With a quinone or similar compound as acceptor	separate up to 50
EC 1.1.99	With other acceptors	separate up to 50
EC 1.2	Acting on the aldehyde or oxo group of donors	separate up to 50
EC 1.2.1	With NAD+ or NADP+ as acceptor	separate up to 50
EC 1.2.2	With a cytochrome as acceptor	separate up to 50
EC 1.2.3	With oxygen as acceptor	separate up to 50
EC 1.2.4	With a disulfide as acceptor	separate up to 50
EC 1.2.7	With an iron-sulfur protein acceptor	separate up to 50
EC 1.2.99	With other acceptors	separate up to 50
EC 1.3	Acting on the CH-CH group of donors	separate up to 50
EC 1.3.1	With NAD+ or NADP+ as acceptor	separate up to 50
EC 1.3.2	With a cytochrome as acceptor	separate up to 50
EC 1.3.3	With oxygen as acceptor	separate up to 50
EC 1.3.5	With a quinone or related compound as acceptor	separate up to 50

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
EC 1.2.3.3 pyruvate oxidase
EC 1.2.3.4 oxalate oxidase
EC 1.2.3.5 glyoxylate oxidase
EC 1.2.3.6 pyruvate oxidase (CoA-acetylating)
EC 1.2.3.7 indole-3-acetaldehyde oxidase
EC 1.2.3.8 pyridoxal oxidase
EC 1.2.3.9 aryl-aldehyde oxidase
EC 1.2.3.10 deleted
EC 1.2.3.11 retinal oxidase
EC 1.2.3.12 now EC 1.14.13.82
EC 1.2.3.13 4-hydroxyphenylpyruvate oxidase

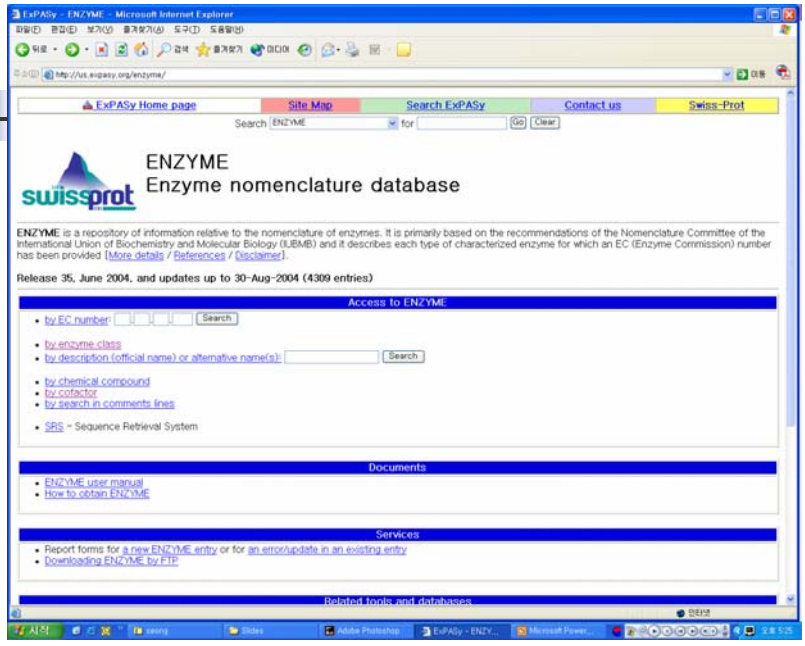
EC 1.2.4 With a disulfide as acceptor

EC 1.2.4.1 pyruvate dehydrogenase (acetyl-transferring)
EC 1.2.4.2 oxoglutarate dehydrogenase (succinyl-transferring)
EC 1.2.4.3 deleted, included in EC 1.2.4.4

EC 1.2.3.1

Common name: aldehyde oxidase
Reaction: an aldehyde + H2O + O2 = a carboxylic acid + H2O2
Other name(s): quinoline oxidase
Systematic name: aldehyde:oxygen oxidoreductase
Comments: A molybdenum flavohemoprotein. Also oxidizes quinoline and pyridine derivatives. May be identical with EC 1.2.3.11, retinal oxidase.
Links to other databases: BRENDA, EXPASY, KEGG, ERGO, CAS registry number: 9029-07-6
References:

1. Gordon, A.H., Green, D.E. and Subrahmanyan, V. Liver aldehyde oxidase. *Biochem. J.* 34 (1940) 764-774.
2. Knox, W.E. The quinine-oxidizing enzyme and liver aldehyde oxidase. *J. Biol. Chem.* 163 (1946) 699-711.
3. Mahler, H.R., Mackler, B., Green, D.E. and Bock, R.M. Studies on metalloflavoproteins. III. Aldehyde oxidase: a molybdoflavoprotein. *J. Biol. Chem.* 210 (1954) 465-480.
4. Huang D.-Y., Furukawa, A. and Ichikawa, Y. Molecular cloning of retinal oxidase/aldehyde oxidase cDNAs from rabbit and mouse livers and functional expression of recombinant mouse retinal oxidase cDNA in *Escherichia coli. Arch. Biochem. Biophys.* 364 (1999) 264-272. [PMID: 10190983]



1.5.2 Isoenzymes

- In a single species, more than one form of enzyme can catalyze the same reaction

  (1 SCD in mouse, 2 in human, 3 in rat)
- Differ in sequence, cofactor, and structure
- Electrophoresis is recommended as the basis of classification

1.5.3 Multienzyme systems

- One enzyme having more than one catalytic activities
- It can have more than one EC numbers
- 예)

1.6 Why studying enzymes?

- World Market : 6 billion $/yr in 1999
- 6~8% annual growth rate
- Korea Market in 2000
  - Production : 57억원
  - Export : 5억원
  - Import : 203억원

1.6 Why studying enzymes?

효소의 분류	효소의 종류	효소시장(5년간 증가율, %)		
		1999년	2004년	2009년
특수 효소	의약 및 진단용	870	1,274(46.6)	1,855(45.4)
	PCR 관련 효소	200	285(42.5)	370(29.8)
	제한효소	120	160(33.3)	210(31.3)
	다른 특수 효소	130	250(66.7)	450(80.0)
	소 계	1,340	1,970(47.0)	2,885(46.5)
산업용 효소	식품가공용 효소	168	230(36.9)	320(39.1)
	농업용 효소	130	170(30.8)	223(31.2)
	세제용 효소	109	148(35.8)	200()(35.1)
	화장품용 효소	0.31	0.50(61.3)	0.75(50.0)
	섬유용 효소	0.25	0.37(48.0)	0.55(48.7)
	기타 효소	0.27	0.25(-)	0.02(-)
	소 계	490	660(34.7)	875(32.6)
합계	-	1,830	2,630(43.7)	3,760(43.0)

(KISTI 산업동향, 미국시장, 단위 million$)