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BIOCHEMICAL REACTIONS OF ENZYMES COMPARED WITH ELECTROCHEMICAL REACTIONS

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ABSTRACT

Many biochemical reactions, especially enzyme catalyses, are similar to electrochemical reactions. In electrochemical reactions the substrate is either oxidized or reduced on the electrodes by electron transfers. Developments in electroorganic chemistry showed that many of the chemical bonds can be cleaved electrochemically. Especically R—O, C—X, Ar—O, N—H, C≡N, C—S, C=O, C=N, C=S and C=C bonds and some other heteroatom bonds are known to be cleaved electrochemically. As a result of those cleaveges dimerizations, polymerizations, rearrengements, substitutions, remo valof functional groups, etc. occur.

When we examine the biochemical reactions of the enzymes they result in reduction, oxydation, cleavage, addition, rearrengement and polymerization, similar to electrochemical reactions.

In the present work, enzymes which are classified as oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases are compared with electrochemical reactions.

INTRODUCTION

Enzymes are macromolecular biochemicals which catalyze various reactions that are essencial for life. The catalytic activity of enzymes can be atributed to special geometric area present in the structure.

In electrochemical systems since the electron transfer occurs on the surface of electrode, the geometry of the adsorbed substrate plays an important role on the final product.

Catalytic activity of enzymes were suggested to be related to geometry of protein by Kunitz et. al (1936). As an example carboxypepsidase-A mechanism can be examined. Between the substrate and the enzyme various types of hydrogen bonding takes place. When all the interactions occur suitably, then the substrate-enzyme complex can have low activation energy. The final reaction can proceed (Figure–1).

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Similar to interactions between substrate an enzyme, on electrode surface the substrate must be adsorbed properly. Following the adsorbment, the electrode potential must suit to substrates molecular orbital energy levels. Then the electron transfer occurs either from substrate to anode or from cathode to substrate (Figure–1).

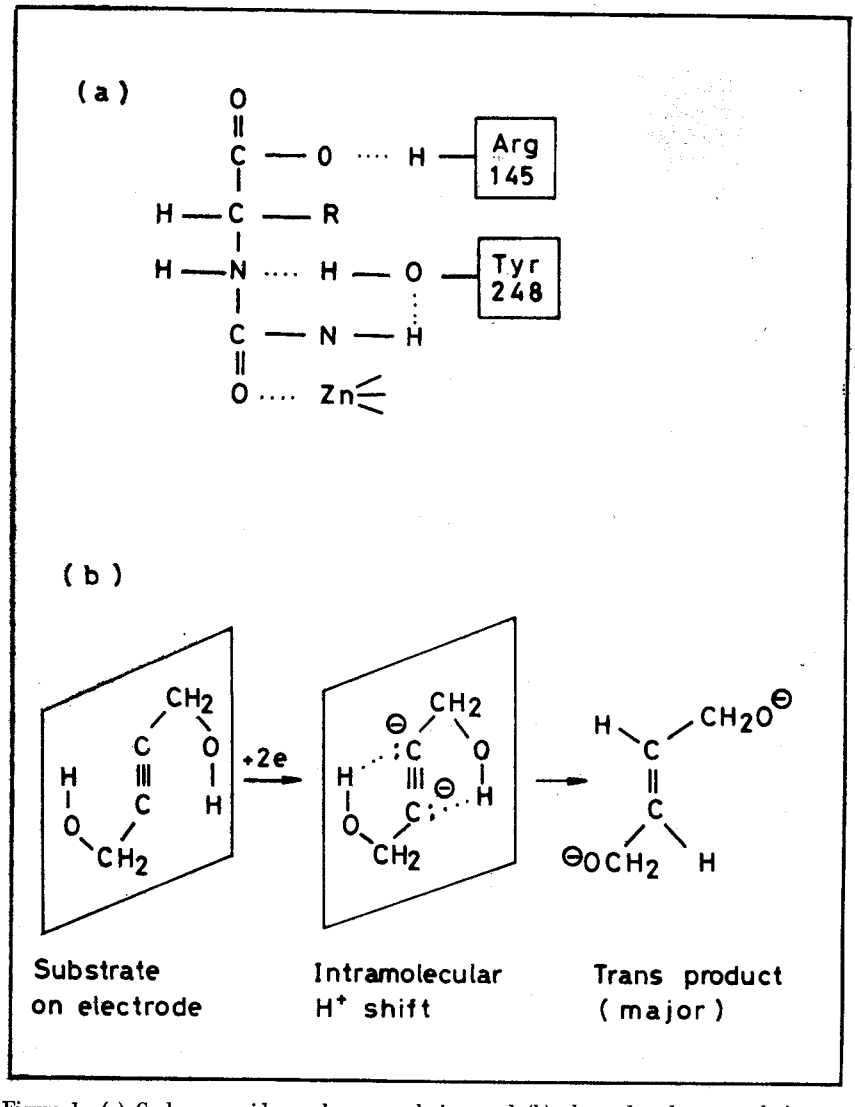

Figure 1. (a) Carboxypepsidase-substrate relation and (b) electrode-substrate relation.

More than 2000 enzymes have been isolated and characterized (Barman, 1974; C.B. Nomencl, 1978). All the enzymes can be classified according to their six-basic functions.

1. Oxydation-reduction
2. Functional group transfers
3. Hydrolysis
4. Lysis
5. Isomerization
6. Ligation

There are many examples for the above functions of enzymes accomplished by electrochemical procedures for organic substrates (Akbulut, 1975; 1979; 1982; 1983; 1984; 1986).

Many enzymes require a coenzyme for their activity. Most of the coenzymes have a function of accepting or donating a group involved in enzyme catalysis. Some of the coenzymes are consumed but some are regenerated.

In electro-organic syntheses sometimes a second substrate, similar to coenzyme can be required. Especially when the reduction or the oxidation potential of the substrate is very high, the secondary substrate is required. The solvent, electrolyte, complexing agents or the electrode itself can function such as coenzyme for electron transfer to occur.

Enzyme denaturation is an important phenomenon in enzyme activity. Prevention of the various interactions which is responsible for biochemical activity is called denaturation. The electrode, plays an important role on electrochemistry. Platinized platinium, smooth platinium electrodes, and platinum poisoned upon oxidation, all have different behaviours. Electrodes made out of same metal can be totally inactive for the same substrate at the same potential when they have different surface properties. We may therefore compare the electrode surface properties with coenzymes or inhibitors in enzyme catalysis.

Electrochemically it is possible to reduce, double bonds, nitro, carbonyl, —CN, carboxyl and similar functional groups. Electrochemical removal of $—CO_2$, $—NH_3$, halogens, —OH, —OR, —SH etc. are well known. Rearrengements, ring expansions, ring contractions, isomerization, and substitutions along with addition reactions have been accomplished electrochemically. Many types of reactions catalysed by enzymes have been, and can be realysed electrochemically.

DISCUSSION

Six basic functions of enzymes, along with effects of coenzymes and inhibitors can be related to electrochemical reactiones.

Oxyreductases:

This class of enzymes are dehydrogenases, reductases, oxidases, peroxidases and oxygenases. Table–1 lists some of these enzymes according to related reaction types.

Table 1. Some Oxidoreductases and Related Typical Reactions.

Common Name	Reaction Type	Oxidant	Reductant	Substrate
Dehyrogenase, reductase	$S+NAD \rightleftarrows P—NADH$	NAD or NADP	CHOH group	alcohols, carbohydrates, carboxylic acids, and stroids
Dehydrogenase	$S+NAD+H_2O \rightleftarrows P+NADH$	NAD	aldehyde and keto group	formaldehyde, aldehydes carboxylates
Oxidase	$S+HcO+O_2 \rightleftarrows P+NH_3+H_2O_2$	O_2	$CH—NH_2$ group	L-amino acids, D-amino acids, amines
Oxidase	$2S+O_2 \rightleftarrows 2P+2H_2O$	O_2	diphenols, ascorbate	catechol, diphenols, ascorbate
Oxidase	$S+O_2 \rightleftarrows P+H_2O_2$	O_2		galactose, arylalcohols, lactic acid, glucose

As seen in Table–1, oxidoreductases can reduce or oxidize various functional groups of the substrates. Functional groups such as, OH, COOH, COOR, C=O, CHO, NH_2, $CONH_2$, NO, NO_2, H_2S, SO_2, SH etc. can be oxidized or reduced by enzymes.

Transferases:

Theese types of enzymes are responsible for transfer of functional groups. Transferases such as, methyltransferase, formyltransferase, carboxytransferase, hydroxymethyltranferase, transketolase, tran-sal dolase, acetyltransferase, hexosyl transferase, aminotransferase, hexokinase, phosphotransferase, sulfurtransferase etc. are some of the well known enzymes. Table–2 gives some of the transferases and their functions.

Hydrolases:

Hydrolases have various types such as esterases, peptidases, lipases etc. Hydrolysis of various esters are cathalysed to yield related acids and alcohols by hydrolases. In Table–2 some of the well known hydrolases are listed.

Table 2. Some Transferases, Hydrolases, Lyses, Isomerases and Ligases and Related Reactions.

Common Name	Reaction Type	Substrate
Methyltransferase, formyltransferase, hydroxymethyltransferase	transfer of one methyl, hydroxymethyl or formyl group	S-adenosylmethion+catechol (or nicotinate, aspartate, ornithine, histamine, thiols)
Transketolase, transaldolase-acetyltransferase	transfer of carbonyl or acetyl groups	Acetyl-CoA + L-aspartate (or L-glutamate, imidazole, arylamines glycine,diglycerides)
Aminotransferase	transfer of amino group	aspartate, glutarate, L-alanine, glycine
Sulfurtransferases, Sulphotransferase, coenzyme A-transferase	transfer of sulfate to phenols, arylamines, steroids	3'-phosphoadenyl-sulfate, $S_2O_3^-$
Esterases, hydrolases, peptidase, phosphoesterase, lipase, nucleotidase	hydrolysis of esters to acid and alcohol	carboxylic esters, glycerol esters cholesterol esters, orthophosphoric acid monoesters, phosphate sugars
Glycosyl hydrolases, S and N-glycosyl hydrolases	hydrolysis of polysaccarides to small carbobydrates	oligo and polysaccarides
Aminopeptidases, carboxypeptidase, pepsin, rennin, chymotrypsin, thrombin etc.	hydrolysis of peptides (but not cleaving the chain)	dipeptides, proteins, aminoacids
Asparaginase, glutaminase, amidase, urease, deacylase	hydrolysis of C–N bonds op peptides and yield NH_2 or CO_2	L-asparagine, L-glutamine, urea, N-acyl-aspartate
Carboxy-lyases, decarboxylase, carboxylase	S $\rightleftarrows$ p+CO_2	Z-dxd acids, oxalate, acetoacetate, L-valine, L-aspartate, p-on-0-aminobenzoate etc
Aldolases, ketolases	S $\rightleftarrows$ p_2 + p_2	L-isocitrate, L-malate, citrate
Dehydratases, hydratases	S $\rightleftarrows$ p + H_2O	carbonic acid, malate, citrate, L-threonine
Lyses, deaminases	S $\rightleftarrows$ p+NH_3+H_2S	L-aspartate, L-histidine, L-phehylalinine, L-arginosuccinate
Amino acid racemase	L-amino asid $\rightleftarrows$ D-amino acid	alanine, methionine glutamate, proline, lysine, threonine
Epimerase, mutarotase	α-D-glucose β-D-glucose	phosphate sugars, nucleosides, -D-glucose
Isomerase, tautomerase, mutase	cis $\rightleftarrows$ trans aldose $\rightleftarrows$ ketose keto $\rightleftarrows$ enol shift in —C=C—	maleate, D-glyceraldehyde 3–phosphate, D-erythrose, D-fructose, D-arabinose etc.
Synthetase	ATP+NH_3+acid $\rightleftarrows$ AMP—PO_4^{-3}+amine	L-aspartate, L-glutamate, glycine
Carboxylase	ATP+S+CO_2+H_2O $\rightleftarrows$ ADP+PO_4^{-3}+P	pyruvate, acetyl-CoA, propionyl-CoA

Lyases:

Catalytic role of lyases are on cleavege of various bonds of the substrate. Removal of CO_2, CHO, OH, NH_2 and SH groups of substrates can be accomplished by lyases (Table–2).

Isomareses:

Various isomerases such as racemase, epimerase, mutarotase etc. are known. These enzymes are responsible for geometrical or optical isomerization of the substrates. In Table–2 representative isomerases are listed with their related functions.

Ligases:

Ligases are enzymes similar to lyses, their basic function however is to, activate the reactions in which addition of functional groups to substrate take place. CO_2, H_2O or NH_2 can be incorprated into substrate by ligasec (Table–2).

When electro-oxidation or electro reduction of an organic compound is studied, various factors affect the final product. Anodic oxidation or cathodic reduction potentials of a functional groups of an organic substrate can be measured easily by cyclic voltammetry. Other methods like polarography, chronopotentiometry, chronoamperometry, thin layer voltammetry, rotating ring disk voltammetry etc. can also be employed for organic electrochemistry.

The potential of anode (E_a) in electro-oxidation acts just like an oxidase enzyme. The role of oxidase is to reduce the activation energy in the reaction profile, and thus allow the oxidation of the substrate. In other words the enzyme allows the loss of electrons from the substrate, which is not possible (or very slow) in the absence of enzyme. If we examine the electro-oxidation of an organic substrate we observe a similar behaviour of electrode potential of anode. Figure–2 represents the roles of enzyme and electrode potential during oxidation of substrate. As seen in Figure–2(a), when anode potential is zero the energyband of anode is higher than energy of substrate for HOMO level. When the anode potential is made more anodic, and reaches to E_a of substrate electron can be transferred from HOMO to anode. Therefore the energy band of anode can be made lower than the energy of HOMO of the substrate, by adjustment of potential of anode. For different substrates, HOMO energy level will be different. In order to oxidize various subst-

rates, the anode potential can be adjusted to related HOMO energy levels. As a result it becomes possible to oxidize all types of substrates at a suitable anode potential.

Figure–2 (b), represents the molecular orbital energy diagrams of substrate (S) and oxidant (O), and that of substrateenzyme complex. As seen in Figure–2 (b), without the enzyme, the energy level of LUMO of oxidant is higher than energy level of HOMO of substrate. Electron transfer can not take place from HOMO of substrate to oxidant in asbsence of enzyme. When substrate-enzyme complex (ES)* is formed however, energy level of HOMO of ES* bescome higher than LUMO of substrate. Electron transfer from ES* to oxidant thus becomes possible. In electrooxidation, the energy band of electrode is made lower than HOMO of substrate. In enzyme activation, similarly HOMO of anode potential is required for oxidation of different substrates.

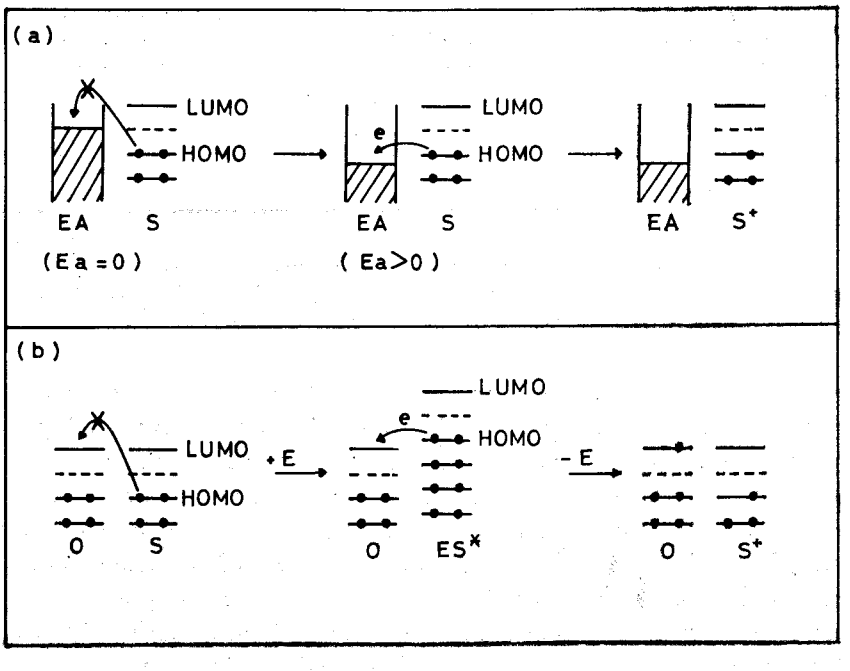

Figure 2. Schematic representation of oxidation, (a) by electro-oxidation and (b) by enzyme (oxidase). (EA: Energy band of anode, E_0: Anode potential, S: Substrate, S^-: Oxidized substrate, E: Enzyme, O: Oxidant, ES*: Substrate-enzyme complex.)

The roles of reductase enzymes, and cathodic reduction potentials have similarities as in case of oxidation. In electro-reduction, electron

should be transferred from cathode to the LUMO of substrate as shown in Figure 3.

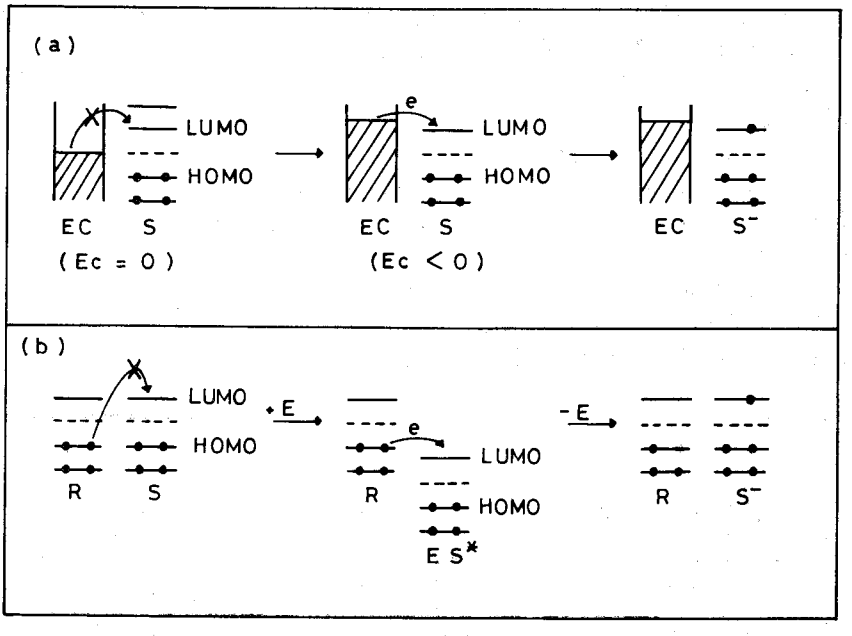

Figure 3. Schematic representation of reduction, (a) by electro-reduction and (b) by enzyme (reductase). EC: Energy band of cathode, E_o: Cathode potential, S: Substrate, S^-: Reduced substrate, E: Enzyme, R: Reductant, ES*: Substrate-enzyme complex.

To attain this the energy band of cathode must be brought to a higher level than energy level of LUMO of substrate. By making cathode potential (E_c) negative enough, the energy level can be brought to the suitable level. When reductase enzyme is not present, the LUMO of substrate is higher than HOMO of reductant. Electron can not be transferred from HOMO of reductant to LUMO of substrate. As a result, reduction of substrate does not take place. In the presence of reductase enzyme, substrate-reductase complex forms. LUMO of substrateenzyme complex becomes lower than HOMO of reductant. As a erresult, electron can be easily transferred from HOMO of reductant to substrate-enzyme complex. Then enzyme leaves the reduced substrate. A suitable cathode potential is required for each substrate. On the other hand a suitable type of reductase must be used for reduction of different substrates.

Following the electron transfer S^- or S^+ will have a rearrengement in molecular energy levels which will be different than shown in Figures 2 and 3. Depending on the thermodynamic stability of S^- or S^+, a suitable product will be produced. The pH of solution temperature etc. can affect the type of final product in electrolytic or enzyme activated reactions. Transfer of a group, hydrolysis of the related bond, cleavage of a group, isomerization, addition of a group or polymerization can take place.

Table–2 gives some examples of electrochemical products obtained from various substrates. As seen in the table, the products obtained at specific anodic or cathodic potentials are similar to products of enzyenzymes. Table–1 gives enzyme activated products, some of which can be accomplished by electrolysis, as seen in Table–3.

It should be speculative to claim that all the enzyme reactions can be accomplished by electrolysis. Thousands of specific enzyme reactions are known, it is interesting to see that many of those reactions

Table 3. Electrochemical Reactions of Some Substrates and Related Enzyme Equivalents.

Name of substrate (and functional groups)	Products	Type of reaction	Enzyme equivalent	Reference
Phenylisocyanate (–N=C=O)	Radical anion interme diate	$S+e \rightarrow S \rightarrow p$	Oxydoreductase	Akbulut, 1979
Epoxydes 	Radical cation intermediate 	$S \rightarrow S^- + e \rightarrow p$	Oxydoreductase	Akbulut, 1983
Cycloadducts of pyridinium–3–olates 	Radical cation intermediate 	$S \rightarrow S^- + e \rightarrow p$	Oxydoreductase	Akbulut, 1984
p-Nitrotoluene (–CH_3)	m-Methyl substproduct	Methyl Schift $S+e \rightarrow p_1 \overset{o}{\rightarrow} p_2$	Transferase	Guayeder,1966
–Nitrostyrene –(CH=$CHNO_2$)	Hydroxylamine (–CH_2CH=NOH)	Double bond shift $S+e \rightarrow p_1 \overset{o}{\rightarrow} p_2$	Transferase	Masui, 1956
Aryl tetrazoliyl ether (Ar–o–T_2)	Hydrolysis product Ar–OH (major)	$S+e+H^- \rightarrow p$	Hydrolase	Akbulut, 1982
Uric acid (amide)	Alloxan (Hydrolysis of amide)	$S \rightarrow p_1 + e^- \overset{o}{\rightarrow} p_2$	Hydrolase	Vetterl, 1966

Table 3. (continued)

Name of substrate (and functional groups)	Products	Type of reaction	Enzyme equivalent	Reference
Acetic acid-sodium acetate	Methane and CO_2	$S \rightarrow p + e^- + CO_2$	Lyase	Baizer, 1973
Uric acid (amide)	Urea and CO_2	$S \rightarrow e^- + \text{urea} + CO_2$	Lyase	Dryhurst, 1972
Amino acids	$RCHO—NH_3—CO_2$	$S \rightarrow e^- + RCHO + NH_3 + CO_2$	Lyase	Baizer, 1973
cis-Stilbene trans-Stilbene ($_H > C=C < ^H$)	trans-stilbene Phenyl ethylene carbonate ($PhCH(CO_2)CH(CO_2)ph$)	$S \rightarrow e^- + p_1 \overset{o}{\rightarrow} p_2$ $S + 2e^- + 2CO_2 \rightarrow p$	Lygase	Baizer, 1973
1,3-Cyclohexadine	1,2 and 1,4 addition	$S + 2OAc^- \rightarrow p + 2e^-$	Lygase	Baizer, 1973
olefins ($>C=C<$) or ($—N=C=O$)	Trimers or $(\sim C(\mid)C(\mid)\sim)$	$S \rightarrow \dot{S}^+ — e^-$ $S^- + s \rightarrow$ polymer	Lygase	Akbulut, 1979

can be repeated electrochemically. Table-3 gives few of the representative examples of electrochemical reactions of substrates. As seen in the table, there are examples of electrochemical reactions similar to all classes of enzyme reactions. Thousands of electroreduction and electrooxidation reactions are known and many of them yield the same or similar products as oxidoreductase enzymes. The least number of examples, of electro organic reactions, duplicating enzymes, are isomerase and transferase type reactions. There are various electro organic reactions yielding lyase and lygase type products. Most of the hydrolase and oxidoreductase type reactions have been and can be duplicated electrochemically.

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