A review Article on Enzymes and Their Role in Resist and Discharge Printing Styles

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Abstract : The current review summarizes textile styles and printing methods. Where, there are three basic styles to print a color on fabric namely: **(a)** direct, **(b)** discharge and **(c)** resist. While printing methods comprise: **(a)** block, (b) roller, **(c)** screem, **(d)** rotary, **(e)** transfer, **(f)** foam and digital "ink jet" printing. The theory of discharge printing involves the degradation, by chemical reagents; of the chromophore system of dyestuffs applied to the textile material, most of them are hazardous chemicals. However, recently, the environmental and industrial safety conditions increased the potential for use of enzymes in textile processing to ensure ecofriendly production. The present review fulfils the following items: **(a)** application of enzymes in textiles, **(b)** factors affecting enzyme activity and **(c)** enzyme inhibitors.
[K. Haggag, A.A. Ragheb, I. Abd EL-Thalouth, S.H. Nassar and H.EL- Sayed. **A review Article on Enzyme and Their Role in Resist and Discharge Printing Styles.** *Life Sci J* 2013;10(1):1646-1654] (ISSN: 1097-8135). http://www.lifesciencesite.com. 242

Keywords : enzyme, discharge printing, textile , callulse, silk, wool.

1.Introduction:

Textile printing is the most versatile and important of the methods used for introducing colour and design to textile fabrics. It is a process of bringing together a design idea, one or more colorants, with a substrate (usually a fabric), using a method for applying the colorants with some precision. Several methods have been used and the colorants available have multiplied (1 – 3).

In principle, any dyes used to produce plain coloured fabric could be used to print that fabric. Since the same forces which are operating between dye and the fiber in dyed cloth are operating between the dye and the fibre in a printed cloth. Textile printing, then, may be looked upon as a form of dyeing; but, whereas in dyeing proper the whole fabric is uniformly covered with one colour, in printing one or more colours are applied to it in certain parts only, and in sharply defined patterns. In dyeing, for instance, it is sufficient, for the most part, to immerse the fabric in an aqueous solution of the dyestuff, stirring it about constantly or otherwise manipulating it to prevent unevenness.

In printing, however, the colour must be applied by special means, either by a wooden block, a stencil or engraved plates, or rollers and thickened to prevent it from spreading, by capillary attraction, beyond the limits of the pattern or design. Many colours also contain, besides the colouring matter and thickening, all the substances necessary for their proper fixation on the cloth when the latter is simply passed through a subsequent process of steaming, and others again require to be subjected to many after treatments before they are thoroughly developed and rendered fast to light and washing (4).

Printing Styles

There are three basic styles to printing a colour on fabric namely: direct, discharge, and resist (5).

Direct Printing

The most common style for applying a colour pattern is direct printing. It may be done on white fabric or over a previously dyed fabric, in which case it is called over printing. The dyes are usually dissolved in a limited amount of water to which a thickening agent has been added to give the necessary viscosity to the print paste. All pastes or printing materials contact the fabric surface with no subsequent processing alterations (6).

Discharge Printing

The discharge style depends on dyeing the fabric first and then printed with a chemicals that will destroy the colour in designed areas. Sometimes the base colour is removed and another colour printed in its place but usually a white area is desirable to brighten the over all design. Thus in discharge style of printing, a readily reducible dye, say, azo dye, is dyed on a cloth and a reducing agent, is printed and the fabric steamed, when the dye at the printed portion is destroyed by the reducing agents. The final washing removes the dye decomposition products there by producing a white printed effect on a coloured ground. This is known as the white discharge style of printing. By incorporating a dye, which is not dischargeable by reducing agent, in the discharge printing paste and printing of the dyed cloth followed by steaming, a coloured printed effect on a differently coloured ground can be produced (coloured discharge style) (4).

Resist Printing

The resist printing style, as its name implies, comes from printing the material with a substance which will resist dyeing later. The dye will affect only the parts that are not covered by resist paste and hence produce a pattern on a coloured ground. Resist style are divided into chemical (those that employ agents such as glyoxal-bisulfite adducts or stannous chloride) and physical (those that use wax to block the fibre from being dyed) (1).

Each of these printing styles used one or more methods of application described below:

2. Printing Methods

• Traditional:

- Block printing (wooden blocks with printing paste imprints fabric) (7).
- Roller printing (fabric passes over engraved copper rollers, each containing a different colour) (2, 8).

• Screen printing:

- Hand, semi-automated and fully automatic screen printing; (silk fabric in a wooden frame serves as stencil through which the colourant is selectively transformed to a fabric in contact with the screen) (9 – 11).
- Rotary screen printing (continuous movement of the fabric has been achieved by moving the screen along with the fabric while printing (2, 12, 13).

• Transfer printing:

- Colour is transferred from the surface of paper to surface of fabric (14).

• Foam printing:

- Low wet pick-up method in which the dye is part of foam and applied to fabrics (15, 16).

• Digital "ink jet" printing:

- New printing method that is being evaluated for its commercial potential (17 – 24).

Discharging agents

Clearly, the most important methods of discharging are based on reduction. This general method can be varied and adapted to give discharges with most classes of dye in use and on most types of fibre. Indeed, to many printers the term "reducing agent" and "discharge agent" are synonymous.

The most widely used reducing agents are the formaldehyde sulphoxylates. The stability of these compounds is such that only limited losses of sulphoxylates occur during printing and prior to steaming. The use of sodium formaldehyde sulphoxylate was established as long ago as 1905, when it was recognied that methods based on this reducing agent offer many advantages. Other products are the insoluble zinc formaldehyde sulphoxylate, water-soluble zinc formaldehyde sulphoxylate, and the water soluble calcium formaldehyde sulphoxylate.

Considerable amount of work has been carried out to show the manner in which the formaldehyde sulphoxylate decompose when used for discharge printing and on the effect of different conditions on the resulting prints. A much simplified explanation of the process is shown in scheme 1–A.

Thiourea dioxide, sold as Manofast, has been used successfully in certain sectors. Although chemically inert to many reagents, an irreversible rearrangement takes place when it is heated with alkali and water with the formation of formamidine sulphonic acid. Decomposition then takes place; one of the products being sulphoxylic acid, and it is this which is the active reducing (Scheme 1–B). Another reducing agent, which has been used since the earliest times, is tin II chloride. It is a readily soluble compound which reacts with an azo dye as shown in scheme 1–C. It is important that tin II chloride solutions are used quickly since hydrolysis, which gives a turbid solution, occurs on standing.

The importance of tin II chloride diminished considerable on the introduction of the sulphoxylates, but it has now regained some significance in the discharge printing of synthetic fibres. It should be noted, however, that tin salts are undesirable in effluents.

The choice of reducing agent is determined largely by the fibre to be printed and, to some extent, by the dye used. The soluble sulphoxylate can give haloing problems on the synthetic fibres, caused by capillary movement of solution along the yarns. This problem can be overcome by using the insoluble formaldehyde sulphoxylates or thiourea dioxide. The latter product has had a considerable success in the discharge printing of acetate and triacetate, due to its low tendency to haloing and also because it is effective under acid conditions, which do not saponify the fibres as an alkaline reducing, system can do.

Printing pastes containing a high proportion of insoluble matters can, however, give rise to the difficulties of 'sticking in', scratching of copper rollers and blocking of screens unless finely ground powders with soft particles are used. In this respect, certain forms of calcium formaldehyde sulphoxylates are considered to be better than the insoluble zinc formaldehyde sulphoxylate, as they have a softer, 'talc-like' consistency as well as being more stable.

Generally, the sulphoxylates are stronger reducing agents than tin II chloride, and can be used to discharge a greater range of dyes. On the other hand, since very few dyes are absolutely resistant to reducing agents, tin II chloride is preferred with illuminating dyes. It would, therefore, seem logical to use both types of reducing agent for a pattern with white and coloured discharges, but most printers prefer to use only one reducing agent for simplicity of operation.

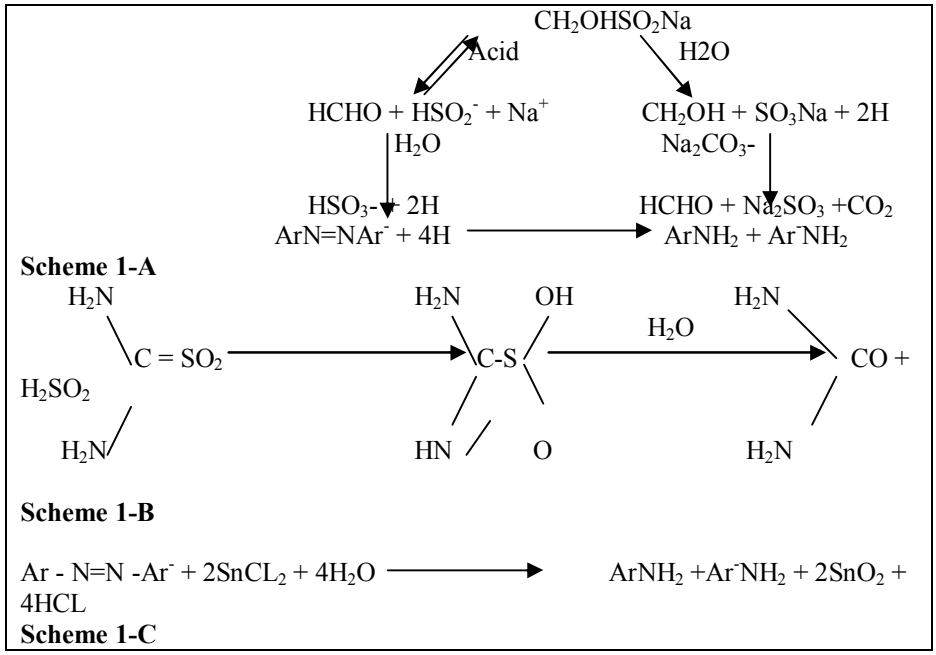
The actual amount of reducing agent required for optimum discharge will depend upon:

- the dye to be discharge,
- the depth of the ground, and
- the fabric being printed

The use of insufficient reducing agent will, of course, give an incomplete discharge, whereas too much results in flushing or haloing during steaming, as well as being wasteful and uneconomic. Flushing of a white discharge results in blurred edges and a loss of fine detail, whilst in coloured discharges it is usually seen as a white halo around the printed areas. This is due to migration of the soluble reducing agent which, if the ground fabric has been dyes with a mixture of dyes with different discharge-ability, may produce a coloured halo. That is not to say that haloing and flushing must be avoided at all costs, since some styles actually depend upon the various effects which can be achieved in this manner – the so called – 'bleeder styles'.

Enzymes and their Role in Discharge Resist Printing Style

The use of biotechnology in textile processing has been known for more than 2000 years. The first application known is the retting of bast fibres with the use of microorganisms (25). Other early examples are the removal of starch by soaking starch-sized cloth with water liquor containing barely (1857) and those of amylases in the same desizing process in 1912 (26). Since ca. 1990, the use of enzyme technology has increased substantially, especially in the processing of natural fibres. A major reason for embracing this technology is the fact that application of enzymes is regarded as environmentally friendly and the reactions catalyzed are very specific with a focused performance as a consequence. In contrast, chemical processes are less specific and often results in side effects, mostly undesired, for example, a reduction in the degree of polymerization of cellulose. Other potential benefits of enzyme technology include cost reduction, energy and water saving, improved product quality and potential process integration. Application and potential of enzyme technology have been reviewed (27 – 31).



The term enzyme was appeared in the literature in 1881 (32) as a modification of the German word *"enzym"* that has been introduced in 1877 (33). The term "enzyme" is derived from the Greek words *"en zyme"* meaning 'in leaven', leaven being the substance (especially yeast, or fermenting dough to produce fermentation) (34).

Enzymes are biocatalysts – or activators – meaning that by their mere presence, and without being consumed in the process, enzymes can speed up chemical processes that otherwise run slowly, if at all.

Enzymes are present in all living cells, where they perform a vital function by controlling the metabolic processes. All enzymes are proteins, composed of amino acid residues linked together in a specific sequence to form macromolecules with a high molecular weight.

What are enzymes?

The building blocks that produce enzymes are various amino acids, and can be written in the

following general form: NH_2–R–COOH. These simple molecules condense with the elimination of water, to produce longer polypeptide chains, [–NH–R–CONH–R–CO–]$_n$. As the polymeric chain length increases more and more, ionic and other interactions eventually cause the complex molecule to assume certain three-dimensional configurations. When this orientation happens, the intertwined polymeric mass becomes a protein, and some of these proteins function as *enzymes*.

Protein molecules that function as enzymes are biological catalysts. They do not eat anything. In fact, these compounds cannot cause a reaction to occur. These compounds only accelerate reactions at substrate surfaces that would naturally occur at much slower rates in the absence of enzymes.

Many enzyme proteins lack catalytic activity until a cofactor is present (35). The inactive protein component of an enzyme is called apoenzyme while the active enzyme composed of the apoenzyme plus cofactor is termed haloenzyme. The cofactor may be a metal ion, or an inorganic molecule known as a coenzyme.

Why do we use enzyme in textile industry?

Enzymes are used in many industries because they:

1- Accelerate reactions;
2- Act only on specific substrates by lowering the activation energy for the reaction
3- Operate under mild conditions;
4- Are safe and easy to control;
5- Can replace harsh chemicals;
6- Are biologically degradable i.e. biodegradable.

Table I: Enzyme classes according to the Enzyme Commission classification system

1st digit	Enzyme Class	Type of reaction catalysed
1	Oxidoreducatse	Oxidation/reduction reaction
2	Transferase	Transfer of an atom or group between molecules (excluding reactions in other classes)
3	Hydrolase	Hydrolysis reaction
4	Lyase	Removal of group from a substrate (not by hydrolysis)
5	Isomerase	Isomerisation reactions
6	Ligase	The synthetic joining of two molecules, coupled with the breakdown of the pyrophosphate bond in a nucleotide triphosphate

1.5. Application of enzymes in textiles (other than wool)

1.5.1. New fibres

The action of enzymes secreted by micro-organisms in fermentation processes to produce feedstock for polymer and fibre manufacture clearly has potential for new fibres. One example of a new fibre is the synthetic poly(lactic acid) (PLA) fibre (36).

1.5.2. Cellulosic fibres

1.5.2.1. Amylases for desizing of cotton (37)

1.5.2.2. Pictanases for bioscouring of cotton (38)

1.5.2.3. Cellulases for biopolishing of cotton (39)

1.5.2.4. Cellulases for biostoning of denim (a heavy grade cotton twill with indigo dyed warps) (40)

1.5.2.5. Catalase for reduction of hydrogen peroxide in the bleaching bath of cotton (41)

1.5.2.6. Zylanase, ligninase and pictinase to increase the crease recovery angle of jute after treated with crosslinking agents (42)

1.5.2.6. Special Cellulases to remove fibrillar fuzz from the surface (43)

1.5.2.7. Pectinases (in particular endopolygalacturonases) for retting of flax (44)

1.5.3. Synthetic and regenerated fibres

1.5.3.1. Polyesterase to improve pilling resistance, hydrophilicity and cationic dye binding of polyester (45)

1.5.3.2. Nitrile hydratase to improve hydrophilicity and impart antistatic ability and enhanced dyeability with acid dyes (46). **Nitrile hydratases** form amides from nitriles that can be subsequently hydrolyzed by amidases to the carbonic acid releasing ammonia (47).

1.5.3.3. Special Cellulases for depilling of lyocell (48)

1.5.4. Silk

Certain proteases were tried in degumming of silk to remove sericin gum that surrounds the silk filaments (49).

1.5.5. Degradation of wastewater dyes

Decolonization of wastewater from the dyeing process was effectively achieved by many enzymes (50, 51). Many biological based treatment systems are now available alone or in conjunction with physicochemical treatment systems, can offer cost effective decolourisation of textile effluent (52). Commercial azo, triarylmethane, anthraquinonic, and indigoid textile dyes are efficiently decolourised with enzyme preparation from various micro-organisms (53).

1.5.6. Degradation of synthetic fibres

Enzymes such as polyesterase, polypropylenase (54) and nitrilase (55) are used to decompose synthetic fibres which are not biodegradable.

1.5.7. Detergents enzymes

Since 1970s enzymes have been incorporated into washing powders and liquid detergents to boost the cleansing power, as the washing temperature and the liquor ratio in domestic washing machines has decreased (56, 57). Indeed, detergent enzymes probably account for about 30 % of total world-wide enzyme production. The enzymes incorporated in detergents are designed to operate in the temperature range 20 – 60 °C and within the pH range 7.5 – 10.5. Detergent enzymes are also used in stain removers and laundry prespotting treatments.

The main types of detergent enzymes in use include α-amylases, proteases, lipases, and Cellulases. Alpha-amylases solubilise starch-containing stains and alkaline proteases enhance the removal of protein-containing stains, such as blood, milk, and egg-stain. Lipases used in detergents remove difficult stains such as lipstick, cosmetics, animal fats, and butter and cellulose enzymes are utilized to enhance stain removal and to brighten the colour. In industrial and hospital laundries, detergent enzymes are especially important, boosting the detergent wash performance on stains that are difficult to remove, e.g. blood, body fluids, organic material, food, fat and oil stains. In this connection, prespotting or pre-soaking with an enzyme detergent prior to washing can markedly decrease the necessary to re-wash and amount of bleaching required (58).

Table II: Enzymes used in textile processing (Applications mentioned are industrially implemented or in the research phase)

Enzyme	Substrate	Application	Technical benefit	Performance
Amylase	amylose	desizing cotton	increased removal of starch	removal of starch from fibre
Cellulase	cellulose	1.biopolishing cotton 2. defibrillation Lyocell 3. stone washing of denim	removal of fuzz removal of micro fibres removal of indigo selectively	Maintaining the new look improve processibility/ generate peach-skin feel. Creation of ageing effects
Pectinase	pectin	1. scouring cotton 2. retting bast fibres	destabilization of outer cell layer destabilization of outer fibre layer	removal of noncellulo-sic Improve of fibre extraction
Catalase	hydrogen peroxide	bleaching	Peroxide breakdown	Neutralization of bleaching agent
Laccase	mediator indigo dye mediator	1. bleaching 2. bleaching dye 3. effluent treatment	creation of bleaching agent degradation of chromophore oxidation of dye	improvement of whiteness ageing of denim decolourization of effluent
Glucose oxidase	glucose	bleaching	creation of bleaching agent	Improvement of whiteness
Protease	protein sericin protein Cellulase protein	1. scouring wool 2. degumming silk 3. stone washing of denim	removal of scales degradation of sericin prevent indigo binding via protein	improve anti- shrinkage removal of sericin back staining
Hemi Cellulase	hemi-Cellulase	retting bast fibres	destabilization of outer fibre layer	improvement of fibre extraction

How do enzymes work?

Emil Fischer originally proposed the lock-and-key hypothesis for enzyme action, in1980, in which the active site was considered to have rigid structural features that were complementary to those of each substrate (35). As shown in figure 1, the enzyme forms a complex with the substrate in a lock and key fashion. After the reaction has taken place, the enzyme is released from the complex and functions on another substrate surface. In case of textiles and other insoluble substrates, the respective enzyme, before the complex is formed, is adsorbed by the material.

Enzyme Inhibitor

An enzyme inhibitor is a molecule that binds to an enzyme and decreases its rate of reaction. Many drugs are enzyme inhibitors and inhibitor discovery and improvement is an active area of biochemistry and pharmacology.

Some chemicals, such as anti-epileptic drugs alter enzyme activity by causing more or less of the enzyme to be produced. This process is not related to enzyme inhibition and is called enzyme induction or enzyme inhibition (35).

A competitive inhibitor (Figure 2) binds reversibly to the enzyme, preventing the binding of substrate. On the other hand, binding of substrate prevents binding of the inhibitor. Substrate and inhibitor compete for the enzyme.

Non-competitive inhibitors (Figure 3) do not bind at the same site as the substrate. Substrate and inhibitor do not compete

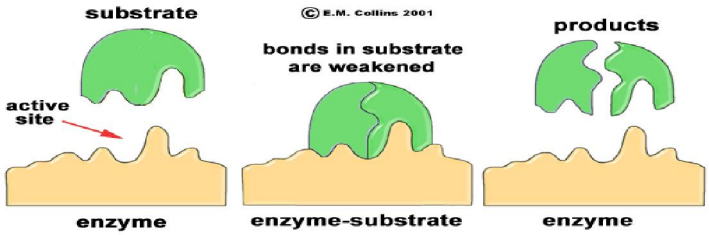


Fig. 1: Lock & Key Model of Enzyme Specificity

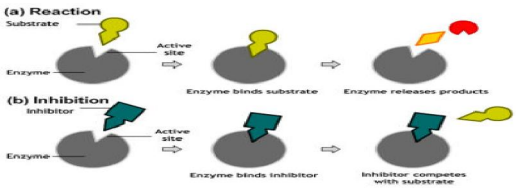


Fig. 2: A competitive inhibitor binds reversibly to the enzyme, preventing the binding of substrate

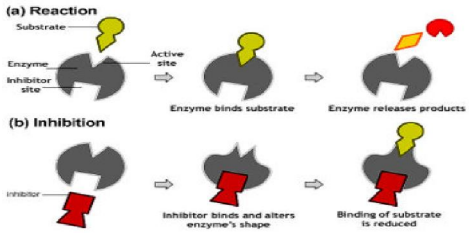


Fig. 3: Non-competitive inhibitors do not bind at the same site as the substrate Factors Affecting Enzyme Activity

Factors Affecting Enzyme Activity

Catalytic activities of enzymes depend upon a variety of external conditions such as, concentration of enzyme, the substrate concentration, the presence of activators and the temperature (59) which are briefly discussed in the following points.

Enzyme Concentration

Provided that the substrate concentration is maintained at a high level, and other conditions such as pH and temperature are kept constant. Thus as the enzyme concentration is increased, so will be the rate of the enzymatic reaction (60).

Substrate Concentration

At low substrate concentrations the rate of the reaction is control by the frequency of collision of the enzyme with its substrate. This factor will be directly proportional to the concentration of substrate molecules. As the [s] is increased, the collision frequency no longer becomes a factor in the reaction velocity. At this point the rate will be governed by one factor only, namely, the rate of conversion of substrate molecules to products molecules. Thus at low [s] the enzyme not only has to act on the substrate but must also seek it out in the medium (61).

The rate increases as the substrate concentration raised but eventually, it comes a point when any further increase in substrate concentration produces no significant change in reaction rate. This is because at high substrate concentration the active sites of the enzyme molecules at any given moment are virtually saturated with substrate. Thus any extra substrate has to wait until the enzyme/ substrate complex has dissociated into products and free enzyme before it may itself complex with the enzyme (60).

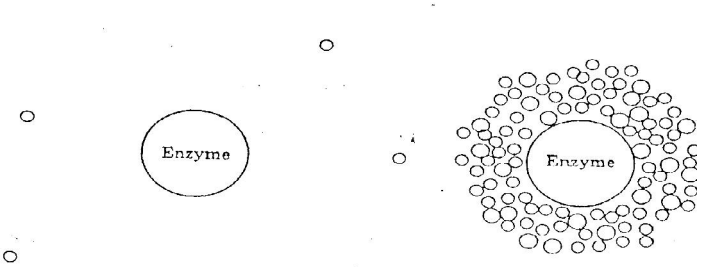


Fig 4: Diagrammatic representation of conditions at low and high substrate concentration

Effect of Temperature

The influence of temperature on enzymes and their activity has been reviewed by several scientists (62 – 64). At least over a certain range, enzymatic reactions behave like ordinary chemical reactions in that, as the temperature is increased, the rate increases. With enzymes, however, a "point of diminishing returns" is reached, since the enzyme itself will begin to suffer thermal inactivation at higher temperatures. High temperature denaturation is usually irreversible because essential weak bonding forces are broken by increased thermal vibration of the component atoms, a phenomenon that damages the three-dimensional structure.

Even under conditions where denaturation does not occur, most enzymes show an optimum temperature at which, other things being equal, activity is maximal. The changes in activity above or below this temperature are not always symmetric (65).

The optimum temperature depends on many factors such as the purity of enzyme and substrate and the presence of activators or inhibitors (61).

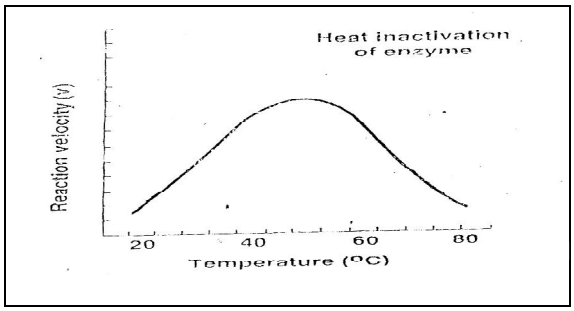


Fig 5: Effect of temperature on an enzyme catalyzed reaction

Effect of pH

The activity of an enzyme depends strongly on the pH of the medium for two major reasons (1) the presence of essential proton-accepting groups in the catalytic centre, and (2) maintenance of the overall structure of the enzyme. Proton-accepting groups can be directly titrated and the dependence of enzyme activity on pH often has the form of a bell-shaped titration curve, with a maximum usually in the neutral range (pH optimum) (66).

The pH optima shown by enzymes vary widely; pepsin, which exists in the acid environment of the stomach, has a pH optimum at about 1.5, whereas arginase, an enzyme that cleaves the amino acid arginine, has its optimum at 9.7. However, the large majority of enzymes have optima that fall between pH 4 and 8 (65).

Effect of Time

In the initial period of time the amount of substrate which has been transformed is directly proportional to the length of time for which the reaction has been proceeding after this initial period the rate of reaction starts to decrease and the amount of reaction is no longer directly proportional to the time.

Provided the substrate is present in excess the explanation of this phenomenon is the progressive loss of enzyme activity after a period of time. This may be due to the effect of heat on the tertiary structure of the enzyme or to the formation of some product or side product of the reaction which inhibits the enzyme (67).

Effect of Pressure

Enzymes are inactivated at very high pressures. Pressure inactivation has been studied extensively with trypsin and chymostrypsin solutions. It was found to be irreversible for exposures of 5 min; at least 6000 kg/cm2 were required for a measurable effect on the activity of trypsin. The reaction is time and temperature dependent [50]. The high pressure may cause changes in either the tertiary or the quaternary structure of enzyme (68).

Effect of Moisture

The rate of enzymatic reactions is strongly influenced by the nature and concentration of solvents. At a very low moisture levels, enzyme activity can be affected, for instance, at a moisture concentrations of 20% (or 4% free water) amylases formed mainly glucose and maltose. At higher moisture levels other oligosaccharides were formed besides glucose and maltose. It has been suggested that at very low levels of free water the rigidity of the medium prevents diffusion of enzyme or substrate. In this case hydrolysis is limited to those portions of the substrate which are in immediate contact with the enzyme (69).

Enzyme activators

A large number of enzymes require on additional component before the enzyme protein can carry out its catalytic functions. The general term co-factor encompasses this component. Cofactors may be divided into three groups which include (i) prosthetic groups, (ii) coenzymes, and (c) metal activators.

(i) Prosthetic group

A prosthetic group is usually considered a cofactor firmly bound to the enzyme protein; thus, for example, the porphyrin moiety of the hemoprotein peroxidise (70).

***(*ii) Coenzymes**

Coenzymes are a small, heat-stable, organic molecule which readily dissociates off an enzyme protein. Thus, NAD^+, $NADP^+$ and tetrahydrofolic acid, are examples of coenzyme (70). They serve as group transfer reagents that transport many substrates from their point of generation to their point of utilization (71).

***(*iii) Metal activators**

Some enzymes require metal ions as co-factors. In such enzymes the metal ion may serve as (1) the primary catalytic centre; (2) a bridging group, to bind substrate and enzyme together through formation of a coordination complex; or (3) an agent stabilizing the conformation of the enzyme in its catalytically active form. Enzymes requiring metal ions are sometimes called metallo-enzymes (72). These metals ions are mono-or divalent cations such as K^+, Mn^{++}, Mg^{++}, Ca^{++}, or Zn^{++} (70).

Enzyme inhibitors

The rate of enzyme reaction may be greatly reduced, or the reaction stopped, by the addition of

some reagent which may be without action on some other enzymes.

Two main types may be distinguished, non-competitive and competitive inhibition (73).

a)- Competitive inhibitors

In many cases an inhibitor resembles the substrate structurally and binds reversibly as the same site on the enzyme. Thus activity is called competitive inhibition because the inhibitor and the substrate compete for binding. The inhibitor is prevented from binding if the active site is **b)- Non-competitive inhibitors**

It is divided to: Non-competitive reversible inhibition:

The inhibitor binds at a site on the enzyme in such a manner that the binding of substrate to enzyme is not affected as shown in Fig. (8). However, the binding of the inhibitor to the enzyme molecule blocks its catalytic activity, thus the amount of enzyme appears to decrease.

$$E + I \leftrightarrow EI$$

$$ES + I \leftrightarrow ESI$$

This type of inhibition can be reversed, but not by adding more substrate. For example, the inhibition of an Mg^{++}-requiring enzyme by ethylenedi-aminetetraacetic acid (EDTA), a chelator of Mg^{++}, can be reversed by adding excess Mg^{++} (71).

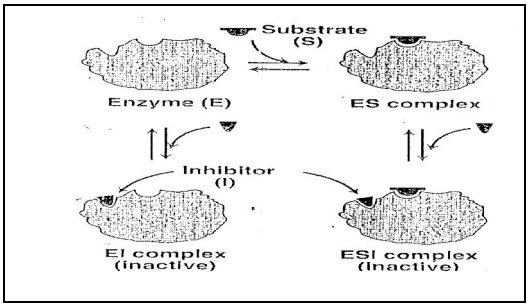


Fig 6: A non-competitive inhibitor binding to both free enzyme and enzyme-substrate complex

Non-competitive irreversible inhibition:

Since a non-competitive inhibitor may react chemically with the enzyme, the amount of inhibition can often be observed to increase with time (73).

Carbon monoxide is a good example of a nonionic compound that inhibits Fe^{++} or Cu^{++} containing enzymes by forming very stable bonds with the metal. Most of these noncompetitive inhibitors are highly toxic (poisonous) to living organisms and are usually lethal in low concentrations because of the irreversible nature of their inhibitory effects.

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