MILK ENZYMES

WHAT ARE ENZYMES?

- Enzymes are group of proteins that catalyze as well as speed up many of the functional reactions.

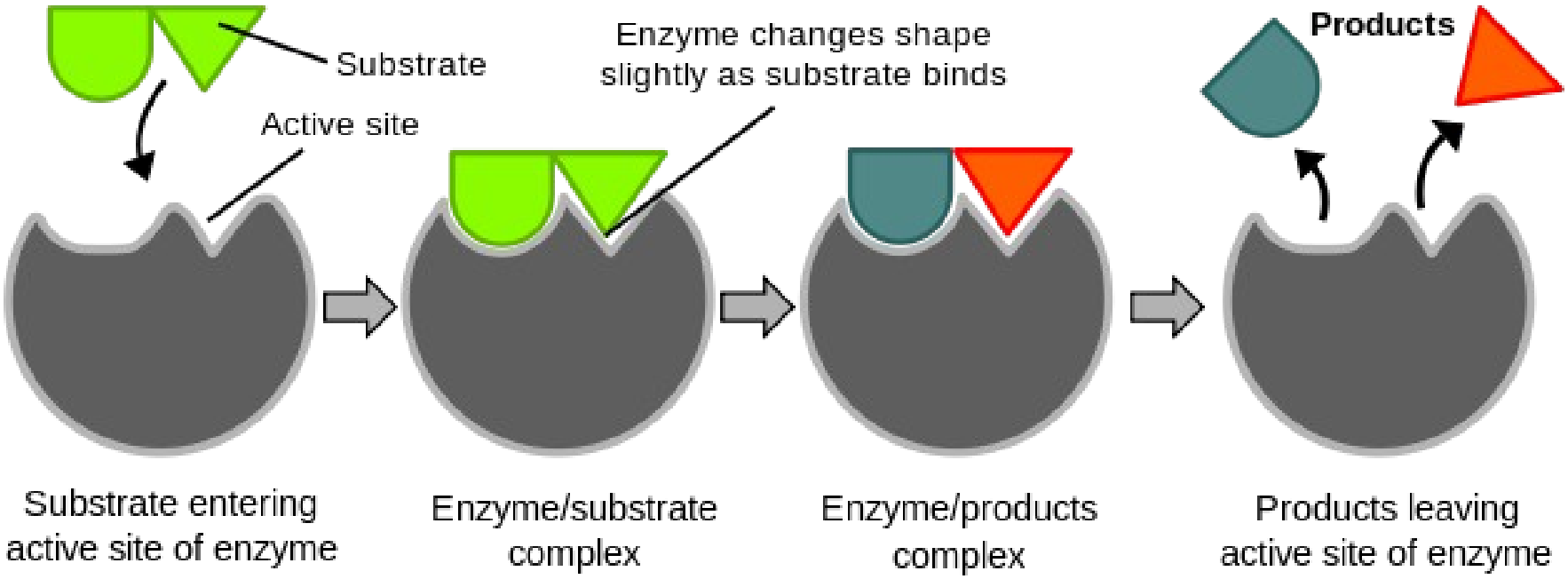
- Some non-protein molecules (prosthetic groups, coenzymes) which are essential to their catalytic activity.

- Most enzymes exhibit a high degree of specificity acting only on certain compounds (substrates) or linkages.

- Enzyme activity usually depends strongly on pH.

- Mostly inactivated by exposure to elevated temperatures, which denature proteins. Individual enzymes differ a great deal in their stability to heat treatment.

ACTION OF ENZYMES



The enzyme fits into the particular spot in the substrate where it weakens the bond and splits the substrate

CHARACTERISTIC FEATURES

- Some enzymes have a very fast catalytic activity eg., Conversion up to 10,00,000 mols of reactant in one minute whereas; some enzymes catalyze only few hundred molecules of reactant in one min to the product.

- Rate of catalysis per molecule of enzyme is expressed as kcat (turnover number for the enzyme). The kcat is a function of the efficiency of the enzyme and the chemistry of the reaction (The number of times each enzyme site converts substrate to product per unit time).

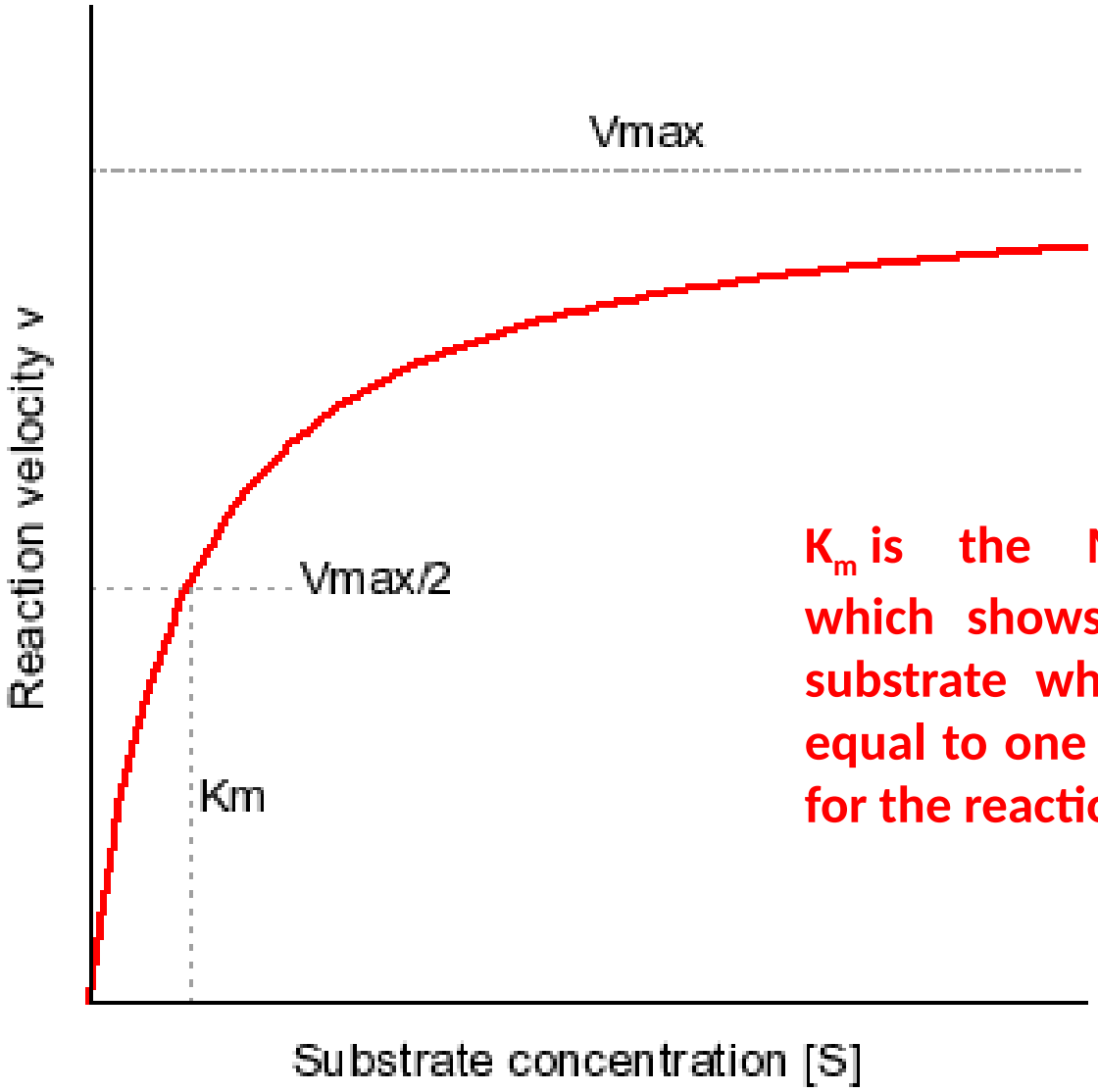
- Enzyme activity is frequently expressed in units. One unit of enzyme is the quantity that will catalyze the transformation of one micromole of substrate to product(s) per minute under standard conditions.

- **For comparative purposes and quantization, initial velocities are preferred.**
- **In order to employ the relation between reaction velocity and enzyme concentration, three other parameters viz. substrate concentration, temperature and pH-must be recognized and controlled.**
- **A plot of initial velocity against substrate concentration is a section of a rectangular hyperbola. Such a relation is rationalized and explained on the basis of the well-known Michaelis-Menten equation.**

$$V_i = V_{max}[S]/(K_m+[S])$$

where Vi = initial velocity of reaction, Vmax = maximum velocity (or activity),Km = Michaelis constant, and [S] = substrate concentration.

MICHAELIS-MENTEN GRAPH



K_m is the Michaelis-Menten constant which shows the concentration of the substrate when the reaction velocity is equal to one half of the maximal velocity for the reaction

MILK ENZYMES

- **Milk is a biological secretion of epithelial cells of the mammary gland has to undergo several biochemical reactions during its secretion.**

- **Some of the enzymes enter the milk which have not been utilized during milk biosynthesis. Apparently these are constituents and products of the mammary tissue that gain entrance to the milk accidentally or unavoidably during the secretory process.**

- **Some enzymes naturally found in milk play a very significant role not only during the processing but also during their storage.**

- **Some of the milk enzymes such as lipase and protease, act upon substrates present in milk, others only upon substrates foreign to milk.**

TYPES OF MILK ENZYMES

Indigenous enzymes

- **Hydrolases (alkaline phosphatase, lipase, acid phosphomonoesterase, lysozyme, plasmin)**

- **Oxidases (catalase, xanthine oxidase, lactoperoxidase, superoxide dismutase, sulfhydryl)**

- **Transferases**

(Xanthine oxidase, lactoperoxidase and ribonuclease are especially prominent in bovine milk whereas; lysozyme predominates quantitatively in human milk)

Exogenous enzymes

Lipases, proteinases

- Catalase is a constituent of leukocytes
- Plasmin may gain entrance to milk from blood
- Galactosyl transferase (component of lactose synthase) are constituents of Golgi membranes
- Alkaline phosphatase of the cell membrane

(Individual milk enzymes are associated with casein micelles, fat globules or leukocytes or are dispersed in the serum)

Indigenous enzymes

➢ **Sources: The blood via. defective mammary cell membranes**

Secretory cell cytoplasm, some of which is occasionally entrapped within fat globules by the encircling fat globule membrane (MFGM)

The MFGM (outer layers of which are derived from the apical membrane of the secretory cell, which, in turn, originates from the Golgi membranes

- **Deterioration: Lipase, proteinase, acid phosphatase, xanthine oxidoreductase**
- **Preservation of milk quality: Sulphydryl oxidase, superoxide dismutase**
- **Thermal history of milk: Alkaline phosphatase, γ-glutamyl transferase, lactoperoxidase**
- **Mastitic infection: Catalase, N-acetyl-β-D-glucosaminidase, acid phosphatase**
- **Antimicrobial activity: Lysozyme, lactoperoxidase (lactoperoxidase-thiocyanate system for the cold pasteurization of milk)**
- **Potential commercial source of enzymes: Ribonuclease, lactoperoxidase**

Exogenous enzymes

- The principal constituents of milk (lactose, lipids and proteins) can be modified by exogenous enzymes, added to induce specific changes and being a liquid, milk is more amenable to enzyme.

- Some of the microbial enzymes may cause undesirable changes, e.g., hydrolytic rancidity in milk and dairy products, bitterness and/or age gelation of UHT milk, bittiness in cream, malty flavours or bitterness in fluid milk, or they may cause desirable flavours, e.g., in ripened cheese.

1. LIPASES (EC 3.1.1.34)

Lipoprotein lipase liberate monoglycerides and fatty acids from core triglycerides of lipoproteins and chylomicrons (blood). Fatty acids are then resorbed by the secretory cells of the mammary gland. Monoglycerides are further hydrolysed to glycerol and fatty acids.

A low molecular weight apoprotein cofactor (present in blood serum) is necessary for the enzyme to attack its acylglycerol substrate.

In bovine milk, the lipoprotein lipase (> 90%) is bound largely to casein micelles while, the triglyceride substrates are in fat globules surrounded and protected by the fat globule membrane (MFGM). When the MFGM is damaged by agitation or homogenization, may bring the lipase into contact with the milk fat resulting in fat degradation and off-flavors (soapy, rancid off-flavor due to butyric acid). Milk contains an adequate level of lipase for rapid lipolysis, but become rancid only after the fat globule membrane has been damaged.

Inactivated at pH 4.6 for 1 hr/ pasteurization to increase shelf-life. Optimum pH and temperature are ~9 and 37 C.

Flavour enhancement in cheese products (e.g. enzyme-modified cheese flavour), acceleration of cheese ripening and off-flavours (hydrolytic rancidity) in milk.

Cow's colostrum contains little of the lipoprotein lipase but has a different lipase that is not bound to casein, does not bind to heparin-Sepharose, is not activated by blood serum, and is stable at pH 4.6 for 1 h.

It disappears after the first few milking following calving.

The colostral lipase probably categorized under triacylglycerol lipase but does not appear to be homologous to the bile-salt stimulated lipase of human milk.

2. ALKALINE PHOSPHATASE (EC 3.1.3.1)

Scientific name: Orthophosphomonoester phosphohydrolase

Two major isozymes have been identified, α and β phosphatase

β phosphatase has been highly purified from bovine milk and found to be a dimer of two identical or very similar subunits each of MW ~ 85,000. It contains about four atoms of zinc per dimeric molecule. Its optimal pH is 10.5 and optimum temperature is ~37 C.

Alkaline phosphatase catalyzes hydrolysis of phosphoric monoesters in milk slowly.

Index or indicator of HTST pasteurization: Time-temperature combinations required for the thermal inactivation of alkaline phosphatase are greater than those required to kill *Mycobacterium tuberculosis* (pathogen).

Phosphatase Test: Heat treatment to milk – Chilled to 5C – Analysis

AlP is concentrated in cream and released into buttermilk, where it occurs in lipoprotein particles, on churning (about 50 % of AlP is in the skimmed milk but the specific activity is higher in cream).

AlP is released from the lipoprotein particles by treatment with n-butanol, which, combined with salting-out and ion-exchange or gel permeation chromatography.

3. ACID PHOSPHOMONOESTERASE

A second phosphatase present in milk is more active at pH optimum at about 4.0.

It is primarily present in low quantity in the milk plasma and higher in colostrum. About 80 % of the enzyme is in the skimmed milk but the specific activity is higher in cream; it is strongly attached to the MFGM and is not released by non- ionic detergents.

Reduce heat stability of milk; cheese ripening

Heat stable: 88 C for 10 min

4. LYSOZYME

Important proteins of human milk possessing bactericidal activity.

It hydrolyses the β(1 → 4)-linkage between muramic acid and N-acetylglucosamine of mucopolysaccharides in the cell wall of certain bacteria, resulting in cell lysis.

Lysozyme content in bovine milk contains only about 0.1 mg. liter-1.

It is a polypeptide of 129 residues with MW 14,602. Relatively stable to heat at pH values (3–4) but are relatively labile at pH >7. >75 % of the lysozyme activity in bovine milk survives heating at 75 C for 15 min or 80 C for 15 s and is less affected by HTST pasteurization.

Lysozyme in milk is usually isolated from whey.

α -lactalbumin and lysozyme have different residues at 81 of 129 positions having four disulfide bridges identically placed.

5. PLASMIN

Plasmin is a well characterized proteolytic enzyme

Milk contains the complete plasmin system: plasmin, plasminogen, plasminogen activators (PAs) and inhibitors of PAs and of plasmin. This system enters milk from blood and plasmin activity increases during a mastitic infection and in late lactation, when there is an increased influx of blood constituents into milk.

Most of the plasmin in milk is present as inactive plasminogen and associated with casein micelles. Milk contains one or more promoters (especially urokinase) that catalyze the hydrolysis of plasminogen to yield plasmin. Plasminogen is converted to plasmin by cleavage of the Arg557-Ile558 bond by specific proteinases, of which there are two types, urokinase-type and tissue-type plasminogen activators.

Plasmin is heat stable, partially inactivated by heating at 72 C for 15 s but its activity in milk increases following HTST pasteurization, probably through inactivation of the indigenous inhibitors of plasmin or of plasminogen activators. It partly survives UHT sterilization but is inactivated by heating at 80 C for 10 min at pH 6.8; its stability decreases with increasing pH in the range 3.5–9.2.

Plasmin can hydrolyze proteins to yield large degradation products as well as γ-casein and proteose peptone from β casein (5 C). Can solubilize casein micelles (140 C for 15 mins).

Responsible for the development of bitterness in pasteurized and UHT processed milk.

Plasmin and plasminogen accompany the casein micelles on the rennet-induced coagulation of milk and are concentrated in cheese in which plasmin contributes to primary proteolysis of the caseins, especially in high-cooked cheeses, e.g., Swiss and some Italian varieties.

Contribute to age gelation in UHT milk, contributes to the poor cheese making properties of late-lactation milk.

6. LACTOPEROXIDASE

It is a glycoprotein (1% of the total serum proteins of milk) catalyzes oxidation of thiocyanate by H_2O_2 to OSCN (hypothiocyanite) that inhibits certain bacteria and increase the raw milk shelf-life.

Milk is rich in lactoperoxidase (about 0.4 µM) but thiocyanate concentration in milk varies depending on cyanoglucoside content of feed.

Its activity in milk increases with advancing lactation to a maximum about 40 days postpartum and subsequently slow down.

Thiocyanate is a natural constituent of milk and H_2O_2 is produced by some bacteria themselves. Thus, in milk such bacteria exhibit self inhibition.

7. SUPEROXIDE DISMUTASE

This enzyme catalyzes the dismutation of superoxide anion O2 to H2O2 and triplet oxygen.

$$2O_2\cdot^- + 2H^+ \rightarrow H_2O_2 + O_2$$

The enzyme consists of two identical subunits of MW 16,000, each containing one Cu and one Zn per mole with one free thiol and one disulfide bond.

Bovine milk serum contains a superoxide dismutase that is similar if indeed not identical to that of the erythrocytes.

Heat-stable, SOD is stable at 71 C for 30 min but loses activity rapidly at even slightly higher temperatures.

SOD occurs in many animal and bacterial cells; its biological function is to protect tissue against oxygen free radicals in anaerobic systems.

8. CATALASE

This haem containing enzyme catalyzes the decomposition of H_2O_2 to H_2O and O_2. ~70 % of the catalase in milk is in the skimmed milk but the specific activity in the cream is 12-fold higher than in skimmed milk.

It is particularly prominent in liver, erythrocytes and kidneys of animals.

In milk, its activity parallels leukocyte count and is higher in mastitic milk and colostrum than in normal milk.

Catalase increases with the multiplication of bacteria and helps in the detection of diseased condition of the udder.

Catalase is relatively heat-labile; heating at 70 °C for 1 h causes complete inactivation and inhibited by Hg^{+2},Fe^{+2},Cu^{+2},Sn^{+2},CN^{-} and NO_3^{-}

9. XANTHINE OXIDASE

Prominent in bovine milk (goat's milk: one-tenth of the activity) and mostly associated with the fat globule membrane.

Activity of the enzyme depends on the molybdenum content of the feed consumed in cows milk.

It can oxidize 12 moles of xanthine per mole per second. Although hypoxanthine and xanthine are its normal substrates in the pathway of purine metabolism, it catalyzes oxidation of many other substrates, including various aldehydes.

The activity in fresh milk is increased about fourfold by storing at 4 C, by heating at 70 C for 5 min, by homogenization or by incubation with commercial proteinase or lipase preparations. Such treatments also transfer much of the enzyme from fat globules to plasma.

Reduction of nitrate in cheese: The enzyme reduces nitrate to nitrite, which is bactericidal, and then to NO.

Production of H2O2: The produced H2O2 can serve as a substrate for lactoperoxidase in its action as a bactericidal agent.

Purine catabolism: It catalyses the catabolism of purines and may be involved in the regulation of blood pressure.

Bactericidal activity: XOR has strong antibacterial activity in the human intestine.

Secretion of milk fat: Secretes milk fat globules from the mammary secretory cells.

Index of heat treatment: It has been considered as a suitable indicator of milk heated in the temperature range 80–90 C

10. TRANSFERASES

Galactosyl transferase, component of lactose synthase found in the Golgi membrane of many tissues.

The binding of regulatory protein, α-lactalbumin to galactosyl transferase increases the affinity of enzyme for glucose, thus enabling lactose synthesis at physiological glucose concentration.

In the absence of α-lactalbumin, this enzyme transfers a galactosyl residue from UDP-galactose to an N-acetylglucosamine residue either free or in a protein bound oligomer.

The transferase contains 12-13% carbohydrate consisting of about 8% neutral sugars, 1% glucosamine,1% galactosamine, and 2% sialic acid.

11. AMYLASES

The principal amylase in milk is α-amylase, with a lesser amount of β-amylase.

Highly concentrated preparation of α-amylase obtained from whey.

Amylase is quite heat-labile.

Human milk and colostrum contain 25–40 times more α-amylase than bovine milk; α-amylase has been purified from human milk by gel permeation chromatography.

Since milk contains no starch, the function of amylase in milk is unclear.