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Programme: M.Sc., Biochemistry

Course Title : Enzymology
Course Code : BC102CR

Unit-5
Multienzyme system

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Multifunctional enzymes are a class of enzymes capable of catalyzing multiple, distinct reactions or performing diverse functions within a single protein structure. These enzymes are of significant interest in biological research, biotechnology, and medicine due to their efficiency and versatility.

Key Features of Multifunctional Enzymes:

1. **Single Polypeptide Chain with Multiple Active Sites**:
 They have distinct catalytic domains for different reactions within the same protein.
2. **Catalytic Efficiency**:
By performing multiple reactions sequentially, they reduce the need for intermediates to diffuse between different enzymes, enhancing reaction efficiency.
3. **Structural Integration**:
 The various domains may be connected via linker regions, ensuring coordinated functionality.
4. **Biological Advantage**:
 These enzymes play essential roles in pathways where intermediate stability or rapid processing is crucial.

Examples:

1.Fatty Acid Synthase (FAS):
A large enzyme complex involved in fatty acid biosynthesis. It contains multiple domains, each catalyzing a specific step in the fatty acid elongation process.

2.DNA Polymerase I:
It has both polymerase activity (adding nucleotides) and exonuclease activity (proofreading or removing primers).

3.Dihydrofolate Reductase-Thymidylate Synthase (DHFR-TS):
Found in some protozoa, this enzyme combines folate metabolism and nucleotide synthesis functions in one molecule.

4.Pyruvate Dehydrogenase Complex:
1.Though technically a multi-enzyme complex, it behaves like a multifunctional enzyme by catalyzing several steps in the decarboxylation of pyruvate.

Applications:

- **Biotechnology**: Used in designing more efficient synthetic biology systems.
- **Therapeutics**: Multifunctional enzymes are often drug targets because inhibiting a single enzyme can disrupt multiple pathways (e.g., cancer and infectious diseases).
- **Industrial Enzymes**: Ideal for processes requiring multiple reactions with fewer components, such as biofuel production or pharmaceutical synthesis.

Multienzyme Complex

A **multienzyme complex** is an assembly of multiple enzymes that work together to catalyze sequential reactions in a metabolic pathway. These complexes enhance metabolic efficiency by minimizing the diffusion of intermediates between reactions and providing better regulation of the pathway.

1. Pyruvate Dehydrogenase Complex (PDC)

Function: Converts pyruvate into acetyl-CoA, a critical step in linking glycolysis to the citric acid cycle (TCA cycle).

Structure:

- Comprised of three enzyme subunits:
  - **E1**: Pyruvate dehydrogenase (decarboxylation of pyruvate).
  - **E2**: Dihydrolipoyl transacetylase (transfers acetyl group to CoA).
  - **E3**: Dihydrolipoyl dehydrogenase (regenerates oxidized lipoamide).
- Contains tightly bound cofactors like TPP (thiamine pyrophosphate), FAD, and NAD^+.

Biological Importance:

- Ensures efficient conversion of pyruvate to acetyl-CoA, avoiding loss of intermediates.
- Regulated by feedback inhibition (e.g., by acetyl-CoA and NADH) and covalent modification (phosphorylation).

2. Fatty Acid Synthase (FAS)

Function:
Catalyzes the synthesis of long-chain fatty acids from acetyl-CoA and malonyl-CoA.

Structure:

- A multifunctional enzyme complex with multiple catalytic domains for distinct steps in fatty acid elongation:
  - Acetyl transferase.
  - Malonyl transferase.
  - Ketoacyl synthase.
  - Ketoacyl reductase.
  - Enoyl reductase.
  - Thioesterase (for chain termination).
- In mammals, FAS is a single multifunctional polypeptide, while in prokaryotes, it consists of discrete enzymes.

Biological Importance:

- Plays a central role in lipid metabolism and energy storage.
- Targeted in cancer and metabolic disorders for therapeutic interventions

3. Na^+/K^+-ATPase

Function:

Maintains the electrochemical gradient across the plasma membrane by actively transporting Na^+ out of and K^+ into the cell using ATP.

Structure:

- A heterodimer with three main subunits:
  1. **α-subunit**: Contains ATPase activity and binding sites for Na^+, K^+, and ATP.
  2. **β-subunit**: Assists in membrane insertion and stabilization of the complex.
  3. **γ-subunit** (optional in some cells): Modulates activity.

Mechanism:

- Binds 3 Na^+ ions inside the cell and hydrolyzes ATP.
- Phosphorylation causes conformational change, releasing Na^+ outside.
- Binds 2 K^+ ions from outside, leading to dephosphorylation and transport of K^+ inside.

Biological Importance:

- Crucial for nerve impulse transmission, muscle contraction, and maintaining osmotic balance.
- Target for drugs like **cardiac glycosides** (e.g., digoxin) to treat heart failure

1. Oligomeric Enzymes

Oligomeric enzymes are proteins composed of multiple subunits, which can be identical or different. These subunits are often held together by non-covalent interactions. Their activity is typically regulated by **allosteric mechanisms**.

Key Features:

- **Quaternary Structure**: Made up of multiple polypeptide chains (subunits).
- **Cooperativity**: Binding of a substrate to one subunit can influence the activity of other subunits.
- **Allosteric Regulation**: Effector molecules can bind at sites other than the active site, modulating activity.

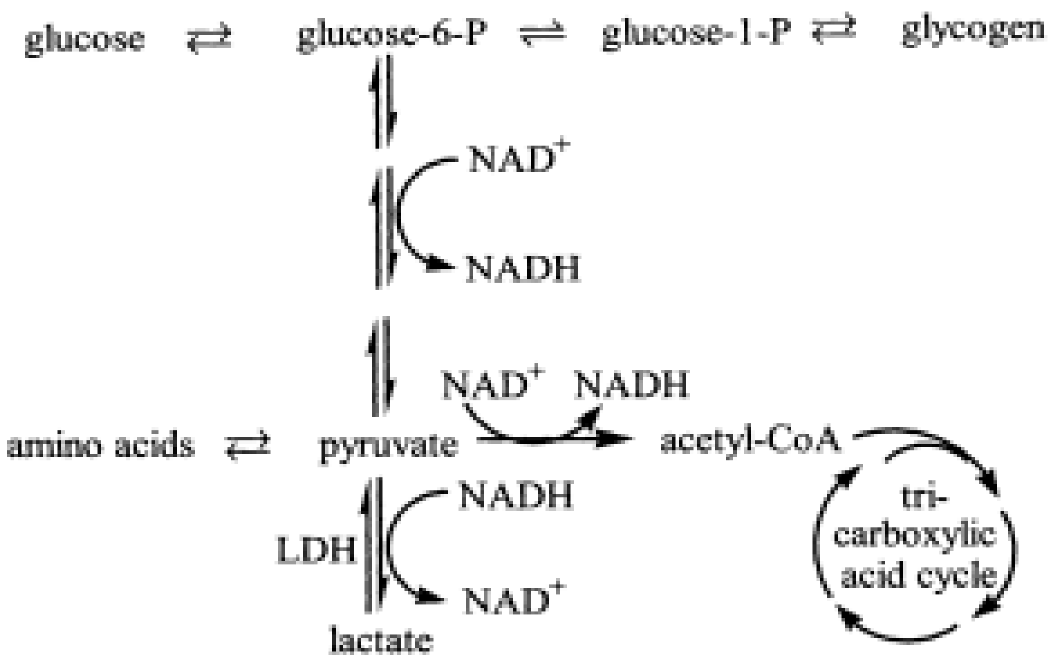
Examples:

- **Hemoglobin**: Though not an enzyme, it exhibits classic oligomeric cooperative behavior.
- **Aspartate Transcarbamoylase (ATCase)**: An enzyme regulated by feedback inhibition in pyrimidine biosynthesis.
- **Lactate Dehydrogenase (LDH)**: Exists as oligomers with different subunit compositions depending on tissue type.

Importance:

- Oligomerization can provide stability, facilitate regulation, and allow coordinated activity.

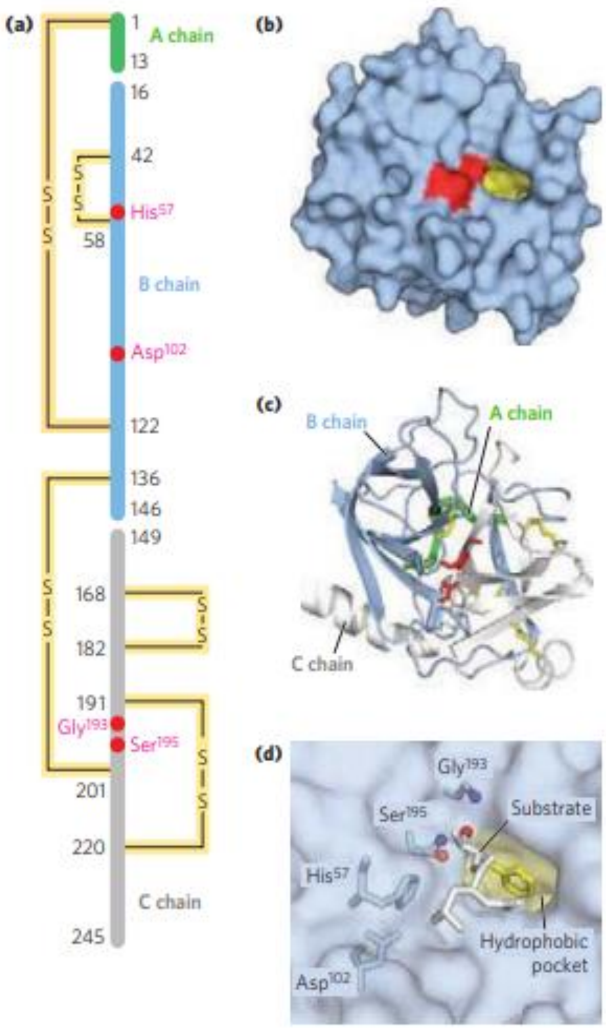
OLIGOMERIC ENZYME



-The main features ofpyruvate metabolism in vertebrates (simplified for clarity).

ENZYMES – TREVOR PALMER

chymotrypsin



Structure of chymotrypsin. (PDB ID 7GCH) (a) A representation of primary structure, showing disulfide bonds and the amino acid residues crucial to catalysis. The protein consists of three polypeptide chains linked by disulfide bonds. (The numbering of residues in chymotrypsin, with "missing" residues 14, 15, 147, and 148, is explained in Fig. 6–38.) The active-site amino acid residues are grouped together in the three-dimensional structure. (b) A depiction of the enzyme emphasizing its surface. The hydrophobic pocket in which the aromatic amino acid side chain of the substrate is bound is shown in yellow. Key activesite residues, including Ser195, His57, and Asp102, are red. The roles of these residues in catalysis are illustrated in Figure 6–22. (c) The polypeptide backbone as a ribbon structure. Disulfide bonds are yellow; the three chains are colored as in part (a). (d) A close-up of the active site with a substrate (white and yellow) bound. The hydroxyl of Ser195 attacks the carbonyl group of the substrate (the oxygens are red); the developing negative charge on the oxygen is stabilized by the oxyanion hole (amide nitrogens from Ser195 and Gly193, in blue), as explained in Figure 6–22. The aromatic amino acid side chain of the substrate (yellow) sits in the hydrophobic pocket. The amide nitrogen of the peptide bond to be cleaved (protruding toward the viewer and projecting the path of the rest of the substrate polypeptide chain) is shown in white

LEHNINGER-PRINCIPLES OF BIOCHEMISTRY

2. Ribozymes

Ribozymes (ribonucleic acid enzymes) are RNA molecules with catalytic activity. They are unique because they act as both genetic material and biocatalysts, challenging the traditional "protein-centric" view of enzymatic catalysis.

Key Features:

- Made entirely of RNA.
- Catalyze reactions like cleavage, ligation, or polymerization of RNA and other molecules.
- Rely on complex 3D structures for activity.

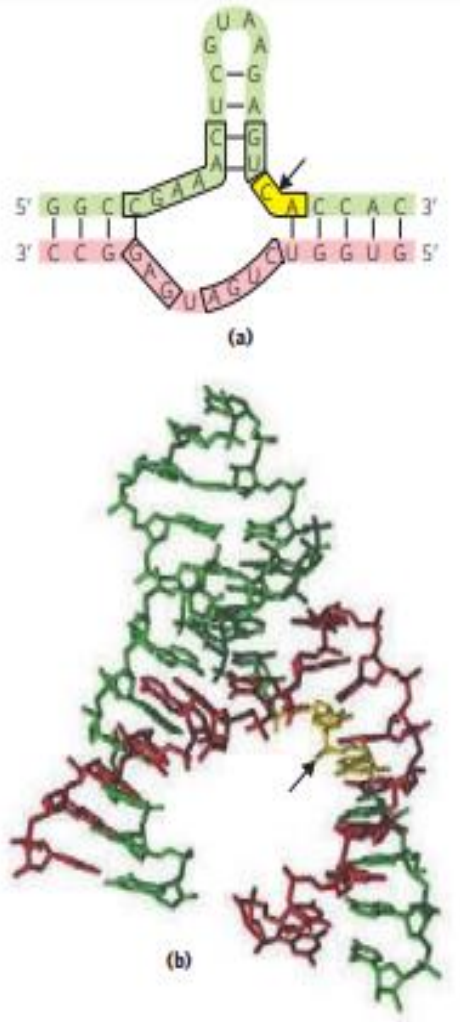
Examples:

- **Self-splicing Introns**: Catalyze their own excision from RNA.
- **Hammerhead Ribozyme**: Found in certain RNA viruses; performs site-specific RNA cleavage.
- **Ribonuclease P**: Processes tRNA precursors in bacteria.
- **Ribosome**: Contains rRNA that catalyzes peptide bond formation during translation.

Importance:

- Support the "RNA World Hypothesis," suggesting early life forms relied on RNA for both genetic information and catalysis.
- Potential applications in molecular biology and therapeutics (e.g., targeted RNA cleavage for gene silencing).

Ribozyme



Hammerhead ribozyme. Certain viruslike elements, or virusoids, have small RNA genomes and usually require another virus to assist in their replication or packaging or both. Some virusoid RNAs include small segments that promote site-specific RNA cleavage reactions associated with replication. These segments are called hammerhead ribozymes, because their secondary structures are shaped like the head of a hammer. Hammerhead ribozymes have been defined and studied separately from the much larger viral RNAs. (a) The minimal sequences required for catalysis by the ribozyme. The boxed nucleotides are highly conserved and are required for catalytic function. The arrow indicates the site of self-cleavage. (b) Three-dimensional structure (PDB ID 1MME; see Fig. 8–25b for a space-filling view). The strands are colored as in (a). The hammerhead ribozyme is a metalloenzyme; Mg2 ions are required for activity in vivo. The phosphodiester bond at the site of self-cleavage is indicated by an arrow. Hammerhead Ribozyme

ENZYMES –TREVOR PALMER

3. Abzymes

Abzymes (antibody enzymes) are antibodies engineered to have catalytic activity. These are synthetic catalysts created by combining the specificity of antibodies with enzymatic function.

Key Features:

- Derived from antibodies that bind to a **transition state analogue** of a reaction.
- Mimic enzymatic catalysis by stabilizing transition states and lowering activation energy.

Examples:

- Abzymes have been developed for reactions such as ester hydrolysis and amide bond cleavage.
- Natural abzymes have been observed in autoimmune diseases, such as lupus, where autoantibodies can cleave DNA or proteins.

Applications:

- **Therapeutics**: Abzymes are being explored as tools for drug delivery or for selectively degrading harmful biomolecules.
- **Research**: Provide insights into enzyme mechanisms and transition state theory.

Metalloenzymes

Metalloenzymes are enzymes that require one or more metal ions as integral components for their structure or catalytic function. These metal ions are often tightly bound to the enzyme and play a crucial role in stabilizing structures, activating substrates, or directly participating in redox reactions.

Key Features of Metalloenzymes

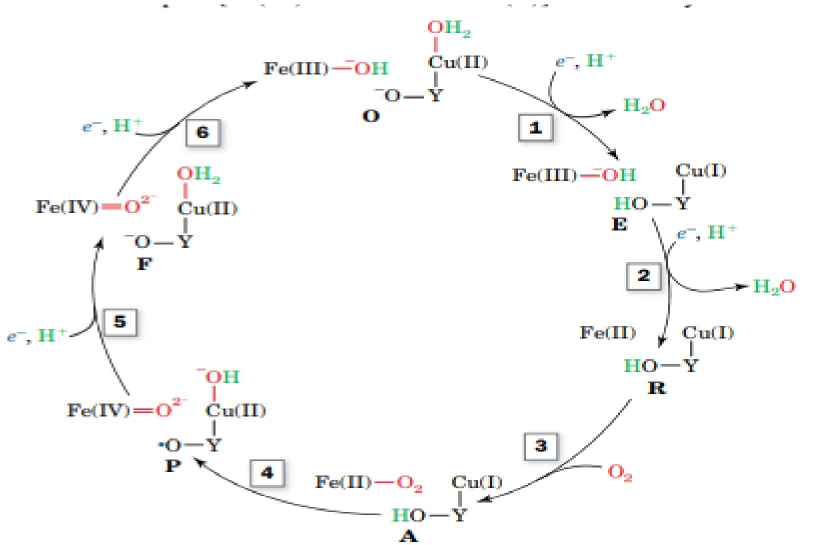
1.Metal Ion Requirement:
Contain metal ions such as **Fe^{2+}/Fe^{3+}, Zn^{2+}, Cu^{2+}/Cu^{+}, Mn^{2+}, Mg^{2+}, Co^{2+}, Mo^{6+}, Ni^{2+},** or others.
2.Roles of Metals:
1. Act as a **catalytic center** by stabilizing transition states.
2. Facilitate **electron transfer** in redox reactions.
3. Stabilize enzyme conformation by forming coordination complexes.

3.Active Site Structure: Metals are often coordinated with amino acid residues like **histidine, cysteine, glutamate, aspartate,** or water molecules

Examples of Metalloenzymes

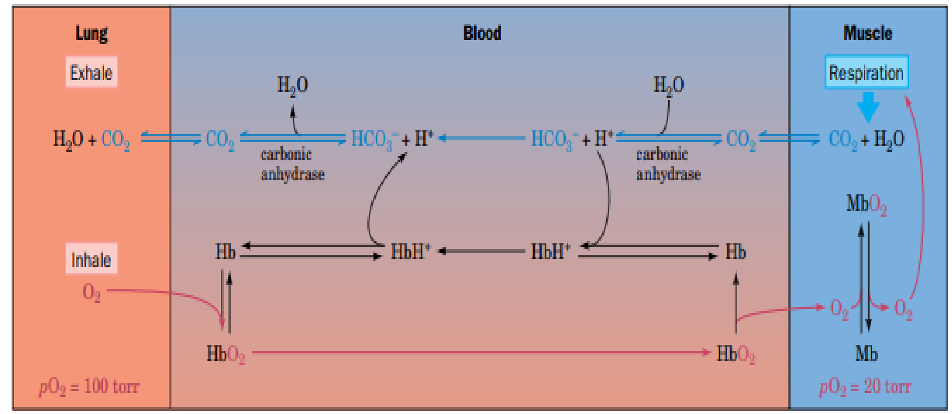
1. **Cytochrome c Oxidase**:
   1. **Metal Ions**: Copper (Cu) and Iron (Fe).
   2. **Function**: Catalyzes the reduction of oxygen to water in the electron transport chain.
   3. **Significance**: Essential for ATP production during oxidative phosphorylation.
2. **Carbonic Anhydrase**:
   1. **Metal Ion**: Zinc (Zn^{2+}).
   2. **Function**: Catalyzes the reversible hydration of carbon dioxide to bicarbonate and protons.
   3. **Significance**: Critical in maintaining pH balance and CO_2 transport in blood.
3. **Superoxide Dismutase (SOD)**:
   1. **Metal Ions**: Cu/Zn, Mn, or Fe (depending on the isoform).
   2. **Function**: Converts superoxide radicals (O_2^-) into oxygen and hydrogen peroxide, protecting cells from oxidative damage.
   3. **Significance**: Antioxidant defense mechanism.

Metalloenzyme



Cytochrome c

Proposed reaction sequence for cytochrome c oxidase. A total of four electrons ultimately donated by four cytochrome c molecules, together with four protons, are required to reduce O2 to H2O at the cytochrome a3–CuB binuclear complex. The numbered steps are discussed in the text. The entire reaction is extremely fast; it goes to completion in ~1 ms at room temperature. [Modifi ed from Babcock, G.T., Proc. Natl. Acad. Sci. 96, 12971 (1999).]



Carbonic anhydrase

The roles of hemoglobin and myoglobin in O2 and CO2 transport. Oxygen is inhaled into the lungs at high pO2, where it binds to hemoglobin in the blood. The O2 is then transported to respiring tissue, where the pO2 is low. The O2 therefore dissociates from the Hb and diffuses into the tissues, where it is used to oxidize metabolic fuels to CO2 and H2O. In rapidly respiring muscle tissue, the O2 fi rst binds to myoglobin (whose oxygen affi nity is higher than that of hemoglobin).

TREVOR PALMER - ENZYMES

1. Industrial Applications of Enzymes

Enzymes are widely used in various industries due to their high specificity, efficiency, and eco-friendly nature. Their applications span sectors such as food production, pharmaceuticals, textiles, biofuels, and environmental management.

Food and Beverage Industry

Enzymes improve the production processes, enhance flavors, and increase the shelf life of products.

•**Amylases**: Break down starch into sugar syrups (e.g., glucose and fructose) used in confectionery and soft drinks.
•**Proteases**: Hydrolyze proteins in cheese-making (rennet), meat tenderization, and beer production to prevent haze formation.
•**Lactase**: Converts lactose into glucose and galactose, producing lactose-free milk for lactose-intolerant consumers.
•**Lipases**: Enhance flavor in dairy products like cheese and butter by breaking down fats.
•**Pectinases**: Used in juice clarification and extraction to increase yield and reduce turbidity

2. Detergent Industry
Enzymes are essential components in modern detergents, enabling effective cleaning at lower temperatures.

- **Proteases**: Break down protein stains (e.g., blood, sweat, and food stains).
- **Lipases**: Remove fat-based stains (e.g., grease, oils).
- **Amylases**: Degrade starch-based stains (e.g., sauces, ice cream).
- **Cellulases**: Brighten colors and soften fabrics by breaking down microfibrils on cotton surfaces.

3. Textile Industry
Enzymes are used for fabric finishing and improving textile processing efficiency
.
- **Amylases**: Remove starch-based sizing agents from fabrics after weaving.
- **Cellulases**: Provide bio-polishing, improving fabric smoothness and brightness.
- **Proteases**: Remove protein-based impurities like wool scouring.
- **Laccases**: Aid in denim bleaching as a safer alternative to chemical treatments.

4. Paper and Pulp Industry
Enzymes help reduce chemical use and energy consumption in paper production .

- **Xylanases**: Improve bleaching processes by breaking down hemicellulose.
- **Cellulases**: Assist in fiber modification and deinking recycled paper.
- **Lipases**: Prevent pitch deposits caused by fat and resin accumulation during pulp processing.

5. Pharmaceutical and Healthcare Industry
Enzymes are used in drug production, diagnostics, and therapeutics.

- **Streptokinase**: Dissolves blood clots in patients with cardiovascular conditions.
- **L-Asparaginase**: Used in cancer therapy to deplete asparagine levels for leukemia treatment.
- **DNA Polymerases**: Essential for DNA amplification in polymerase chain reactions (PCR) for diagnostics and research.
- **Proteases**: Aid in wound debridement by removing dead tissue.
- **Enzyme-based Diagnostics**: Glucose oxidase for blood glucose monitoring in diabetes

6. Biofuel Production
Enzymes are vital for converting biomass into renewable energy sources like bioethanol and biodiesel.

- **Cellulases and Hemicellulases**: Break down plant biomass into fermentable sugars.
- **Amylases**: Hydrolyze starch into sugars for fermentation in bioethanol production.
- **Lipases**: Catalyze biodiesel production via transesterification of fats and oils.

7. Environmental Applications
Enzymes are employed in eco-friendly processes for pollution control and waste management.

- **Proteases and Lipases**: Treat industrial effluents by breaking down organic pollutants.
- **Amylases**: Assist in biodegradation of food waste.
- **Laccases and Peroxidases**: Remove phenolic compounds and dyes from wastewater in textile and paper industries.
- **Ureases**: Aid in removing nitrogen waste from urea-based effluents.

8. Cosmetic Industry
Enzymes are used for skin and hair care formulations.

- **Proteases**: Exfoliate dead skin cells in chemical peels.
- **Lipases**: Aid in fat metabolism for slimming products.
- **Collagenases**: Used in anti-aging products to promote collagen remodeling

9. Leather Industry
Enzymes improve leather processing by reducing chemical usage.

- **Proteases**: Remove hair and other protein residues during hide preparation.
- **Lipases**: Degrease hides by breaking down fat deposits.
- **Amylases**: Assist in softening and cleaning leather.

10. Research and Biotechnology
Enzymes play a central role in various biotechnological applications.

- **Restriction Enzymes**: Cut DNA at specific sequences for genetic engineering.
- **Ligases**: Join DNA fragments during cloning.
- **Reverse Transcriptase**: Synthesize cDNA from RNA for molecular studies

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and Philip Bonner
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