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Research Paper

Review of Collagenase From Microbes

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Enzymes are biocatalysts that make chemical reactions faster in living cells. Cells can carry out their tasks effectively because enzymes support a variety of chemical reactions in them through highly specialized and efficient mechanisms. The following features provide insight into how enzymes work.

a) Acts as a catalyst
Enzymes act as catalysts, which means they speed up chemical reactions without being utilized or replaced by the reaction. Enzymes allow reactions to be established in the cell very quickly since under normal circumstances they would take hours or even years [1] .

b) Specificity
Enzymes have great specificity to their substrates. Substrates are molecules or ions that are used and replaced by enzymes. Each enzyme generally only reacts with one or more specific substrates[2].

c) Key-Lock Model
This model describes how enzymes interact with their substrates. The special structure of the enzyme acts as a "key" that can only enter the "key lock" or the matching substrate [3].

d) Active site
The active side is the part of the enzyme molecule where the chemical response occurs. It is a special position in the enzyme where the substrate binds to and is replaced by the product [4].

e) Mechanism of Action
When the substrate binds to the active side, the enzyme undergoes a change of form known as "induced bugs". This facilitates the chemical response by lowering the activation energy required for the reaction. After the reaction ends, the product is released from the enzyme, which returns to its original form and is ready to catalyze the next response [5].

f) Area Influence
Aspects such as pH, temperature, and ion concentration can affect enzyme activity. Different optimum conditions can increase or decrease enzyme activity.

g) Cofactors and Coenzymes
Certain enzymes need additional molecules in order to work properly. Cofactors are ions or small organic molecules that help the enzyme in catalysis. Coenzymes, on the other hand, are non-protein organic molecules that help the enzyme perform its role by transferring functional groups between substrates [6].

h) Inhibitors
There are molecules that can limit enzyme activity, known as inhibitors. Inhibitors can be reversible or irreversible[7].

Collagenase enzyme is generally defined as an enzyme capable of degrading the polypeptide bonds of collagen[8]. The substrate used in the formation of collagenase enzyme is a substrate containing collagen. Collagen is a major component of the extracellular matrix and provides strength, elasticity, and structure to various body tissues. Collagenase enzyme is also called metallocollagenase which uses metal ions as its catalytic cofactor. These metal ions help in breaking the bonds in the collagen structure. Collagenase enzyme activity is influenced by several factors such as temperature, pH and cofactors.

Temperature

Temperature affects enzyme activity by affecting the molecular structure and function of the enzyme. Changes in temperature can affect reaction speed and enzyme stability. Every enzyme has an optimum temperature, which is the temperature at which the enzyme reaches its maximum activity. At this temperature, the enzyme has the highest catalytic speed because it has the most optimal structural conformation. When the temperature exceeds the optimum temperature, the enzyme may denature or lose its three-dimensional structure that is critical for enzyme function. As a result, the active side of the enzyme can be damaged, so the enzyme loses its ability to bind to its substrate and catalyze the reaction. Enzymes that have undergone denaturation usually cannot be restored to their original functionalform. Within the optimal temperature range, an increase in temperature will increase the reaction speed as it increases the kinetic energy of the molecules. Molecules move faster, thus increasing the possibility of interaction between the enzyme and substrate, as well as the speed of the reaction. If the temperature drops below the optimum temperature, the enzyme activity will usually decrease as the molecules move more slowly. Although enzymes may not denature at low temperatures, they may not be efficient in catalyzing reactions[9]. Some optimum temperatures for collagenase enzyme activity produced by microbes can be seen in Table 1 below.

Table 1: Optimum temperature of collagenase in some microbes

No.	Microorganisms	Optimum temperature	Reference
1	*Nocardiopsis dassonvillei NRC2aza*	60 C^{o}	[10]
2	*Bacillus subtilis WB600*	60 C^{o}	[11]
3	*Clostridium histolytium*	55 C^{o}	[8]
4	*Pseudoalteromonas mariniglutinosa* 7-246-6	50 C^{o}	[12]
5	*Rhizocotonia solani*	40 C^{o}	[13]
6	*Lysinibacillus sphaericus VN3*	37 C^{o}	[14]
7	*Bacillus cereus MBL13*	33.8 C^{o}	[15]

Metal ions

Metal ions can affect enzyme activity through various mechanisms, either as cofactors or as allosthetic regulators. Some enzymes require metal ions to function effectively. These metal ions usually bind covalently to the enzyme and help in catalyzing the reaction. Some metal ions function as allostheric effectors, which are molecules that can bind to allosteric sites on the enzyme and affect its activity. Metal ions can form coordination bonds with the amino acid side chains of enzymes, which can affect the structure and function of the enzyme. These coordination bonds can stabilize the active site of the enzyme or substrate. Metal ions can contribute to the structural stability of enzymes by maintaining a proper 3D structure, metal ions can ensure that enzymes remain in optimal conditions to interact with their substrates[16]. There are several metal ions that affect the collagenase activity produced by bacteria (Table 2).

Table 2: Metal ions that affect the collagenase activity

No	Microorganisms	Effect of metal ions	Reference
1	*Bacillus cereus strain Soc 67*	Enzyme activity is increased by Ca^{2+} and increased Cu2+ levels decrease the enzyme's affinity for the substrate PZ-PLGPA	[17]
2	*Lysinibacillus sphaericus VN3*	Enzyme activity is inhibited by Cu^{2+} and enhanced by Zn^{2+}	[14]
3	*Bacillus subtilis WB600*	Enzyme activity is inhibited by Fe^{3+} and Cu^{2+}. Enzyme activity was increased by the presence of Co^{2+} , Ca^{2+} , Zn^{2+} and Mg^{2+}	[11]
4	*Pseudoalteromonas mariniglutinosa* 7-246-6	Enzyme activity is enhanced by Ca^{2+} and inhibited by Hg^{2+} , Pb^{2+}, Zn^{2+} , Ni^{2+} , Fe^{2+}	[12]
5	*Bacillus cereus MBL13*	Enzyme activity is enhanced by Ca^{2+} and Cu^{2+}	[15]
6	*Pseudoaltero monas Agarivoran NW4327*	Enzyme activity is enhanced by Ca^{2+} and Zn^{2+}	[18]

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7	*Rhizocotonia solani*	Enzyme activity is affected by Ca^{2+}, Co^{2+}, Cu^{2+}, K^{+}, Mg^{2+}, Na^{+},	[13]
8	*Bacillus licheniformis F11.4*	Enzyme activity is enhanced by Ca^{2+} and Cu^{2+}	[19]

Optimum pH

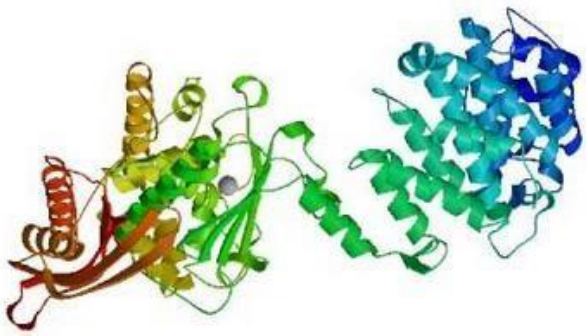
Changes in pH can alter the charge and structure of the enzyme, which in turn affects the enzyme activity. The active side of an enzyme usually has several amino acids that have a certain charge at a certain pH. A change in pH can change the charge on the amino acids, which can affect the interaction with the substrate or cofactor, thus affecting the catalytic activity of the enzyme. Enzymes have an optimal pH at which they are most stable in terms of conformation and activity. Outside this pH range, the enzyme structure may become more unstable, reducing its catalytic efficiency. Changes in pH can affect the ionization of the substrate, which in turn affects the ability of the substrate to interact with the active site of the enzyme. If the substrate is in a form that is not suitable for interacting with the enzyme at a certain pH, enzyme activity may be inhibited. Changes in pH can disrupt stable hydrogen bonds and ionic interactions in the secondary and tertiary structures of enzymes. This can lead to significant conformational changes and reduce or eliminate catalytic activity. In Table 3, it can be seen that the collagenase enzyme has different optimum pH [20].

Table 3: Optimum pH of collagenase enzyme

No	Microorganisms	Optimum pH	Reference
1	*Bacillus subtilis WB600*	9.0	[11]
2	*Pseudoalteromonas mariniglutinosa* 7-246-6	8.0	[12]
3	*Nocardiopsis dassonvillei NRC2aza*	8.0	[10]
4	*Clostridium histolytium*	7.5	[8]
5	*Lysinibacillus sphaericus VN3*	7.0	[14]
6	*Bacillus cereus MBL13*	6.8	[15]
7	*Rhizocotonia solani*	5.0	[13]

Protein Size

Collagenase enzymes also have different protein sizes. The size of a protein affects many aspects of its function and characteristics. The size of a protein can affect its structural stability. Larger proteins usually have more intramolecular bonds, which can increase their structural stability. However, larger proteins may also be more susceptible to denaturation in the event of environmental changes such as changes in pH or temperature. Table 4 displays collagenase data from various sources[21].



Bacillus cereus MH19 110 kDa[22]

Clostridium histolyticum 27.77 kDa[23]

Table 4: Size of collagenase from various sources

No.	Source	Microbes	Size (kDa)	Ref
1	Mediterranean sea water	*Nocardiopsis dassonvillei NRC2aza*	150	[10]
2	Exploration of chitinase and protease from Palembang,Indonesia	*Bacillus licheniformis F11.4*	124	[19]
3	Marine sponge	*Pseudoalteromonas Agarivoran NW4327*	116.25	[18]
4	Soil and wastewater	*Lysinibacillus sphaericus Vnn*	110	[14]
5	Bone waste (gelatin)	*Bacillus cereus MBL13*	52.0	[15]
6	Recombinant	*Bacillus subtilis*	35.4	[11]
7	Internal organs of a mackerel	*Clostridium histolytium*	14.8	[8]

Collagenase application :

1. **Food Industry**:
   Collagenase enzymes are used in food processing, especially in the meat industry, to break down collagen in meat which makes it softer and easier to digest [24].
2. **Textile**:
   Collagenase enzyme can be used in dyeing process in leather industry[25].
3. **Beverage Industry**:
   In the production of beverages such as beer, collagenase enzymes can be used in the clarification process to remove floating particles.
4. **Applications in pharmaceutical and medical**
   The utilization of collagenase enzyme in the medical field is the repair of inflammation in tissues, clinical transplantation, cellular function in blood clotting, fibrinolysis and fertilization and accelerating wound healing. Collagenase enzyme is used as an active ingredient in topical medications that help in the treatment of burns, cuts, or minor surgical wounds[26].

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