Research Progress on Improving the Performance of Natural Enzymes with Catalytic Activity

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Abstract: Enzyme modification technology can improve the catalytic activity and stability of enzymes, making them more suitable for use in the medical testing industry. This article focuses on seven molecular chemical modification methods, including small molecule chemical modification, polymer modification, enzyme cross-linking modification, and cofactor modification, as well as three genetic engineering modification methods, including directed evolution and site-specific mutation modification. The development prospects of enzyme modification technology are also discussed.

Keywords: Enzyme; Chemical Modification; Genetic Engineering; Cofactor

1. Introduction

Enzymes are bioactive substances that can catalyze specific chemical reactions, with advantages such as high catalytic efficiency, strong specificity, and mild reaction conditions[1]. However, most enzymes are proteins that are prone to losing their activity in special environments (such as strong acids, strong bases, high temperatures, etc.) and cannot meet the current needs in fields such as medicine, food, and chemical engineering. Therefore, researchers must continuously conduct research and use chemical or molecular biology methods to modify enzyme molecules to overcome the shortcomings of natural enzymes and meet market demand[2]. Domestic research mainly focuses on modifying the amino acid side chains of enzymes, while there are few reports on modifying and improving enzyme properties, especially in the application of cofactor introduction and site-specific mutagenesis combined with chemical modification[3].

Herr et al improved the reaction conditions of a hydrogenase with broad application prospects by covalent modification, from the original temperature of 35 ℃, pH 8.0, and maximum half-life of 5.3 hours, to a half-life of 161 hours at 29 ℃ and 32 hours at 35 ℃, greatly improving the stability and half-life of the enzyme[4]. Through appropriate enzyme modification techniques, the stability of enzymes can be improved, catalytic efficiency can be enhanced, and the half-life of enzymes can be extended. Therefore, enzyme modification technology has broad application prospects. This article introduces seven chemical modification methods, including small molecule chemical modification, polymer modification[5], and cofactor modification[6], as well as three genetic engineering modification methods, including directed evolution and site-specific mutation modification. At the same time, it looks forward to the future development prospects of enzyme modification technology[7]. These studies have played an important foundational role in the rapid development of disciplines such as chemistry, biology, bioengineering, medicine, and life sciences.

2. Chemical Modification

2.1 Definition of Chemical Modification

Enzyme chemical modification refers to the use of chemical methods to cleave or modify the main or side chains of enzymes, in order to improve their stability, biological activity, or extend their half-life, in order to obtain enzymes with wider application value[8].

2.2 Chemical Modification Methods

2.2.1 Small molecule chemical modification

The technology of small molecule modification of enzymes is also one of the most widely used techniques at present. Small molecule

modification technology is simple and has obvious effects. Xiong et al used phthalic anhydride (PA), succinic anhydride (SA), and maleic anhydride (MA) to chemically modify laccase[9]. After modification, the enzyme activity of laccase can be increased by 50% and its thermal stability can be improved by 15%. A study has modified alkaline protease with decanoyl chloride, and the solubility of the modified protease in chloroform has been increased to 44mg/L, and its activity has been increased by 4~22 times in different organic solvents[10]. Common small molecule modifiers include glucosamine, acetic anhydride, stearic acid, phthalic anhydride, adipic acid di-N-hydroxysuccinimide ester, dimethyl adipic acid imine ester, succinic anhydride, acetic acid N-succinimide ester, etc.

2.2.2 Polymer modification

By modifying with a single functional polymer, the structure of enzyme molecules is altered, and the characteristics and functions of enzymes are also altered, which can enhance enzyme activity, increase enzyme stability, and reduce or eliminate enzyme antigenicity[11]. If N, O-carboxymethyl chitosan, O-carboxymethyl chitosan, methyl PEG grafted vinylpyrrolidone maleic acid copolymer (comb shaped polymer) is used to modify Escherichia coli L-aspartate aminotransferase, immunogenicity can be eliminated, half-life can be increased, and treatment efficacy can be improved through modification. Research has shown that lysozyme, A-chymotrypsin, and Candida rugosa lipase are glycosylated and PEGylated, respectively[12]. The PEGylation of enzymes significantly improves their thermal stability in the aqueous phase by increasing their surface hydrophobicity. On the contrary, glycosylation increases the hydrophilicity of the enzyme surface, causing a decrease in the enzyme's thermal stability.

2.2.3 Enzyme cross-linking modification

Enzyme cross-linking is the use of bifunctional reagents through covalent cross-linking to reinforce the molecular active structure, improve its stability, and expand the range of enzyme use in non-aqueous solvents. Research has shown that using serine protease and chymotrypsin for cross-linking modification can transform room temperature enzymes into thermophilic enzymes. The optimal reaction temperature has been increased from 45 ℃ to 76 ℃, and the chain breaking temperature has been increased by 22 ℃. The combination of glycosylation and cross-linking technology was applied to penicillin G acylase, resulting in a 9-fold increase in its half-life at 55 ℃. Barboursville et al. found that the addition of 30% ethanol significantly improved the stability of Antarctic Candida lipase B by covalently crosslinking with glutaraldehyde[13]. Glutaraldehyde, hexanediamine, glucose diacetaldehyde, and other commonly used bifunctional group reagents.

2.2.4 Surface active agent modification

Surfactant modification is also a commonly used modification method. Research has shown that using surfactants to modify a-amylase, if all other ions are replaced with Ca^{2+} through ion displacement, the enzyme's activity is increased by three times and its stability is greatly increased. Bogie et al found that the HLB value of surfactants and functional groups of fatty acids significantly affect enzyme activity and product quantity[14]. Zeng et al used Span and Tween to modify porcine pancreatic lipase in ethanol solution[15]. Compared with the original enzyme, the modified enzyme activity was significantly enhanced and the thermal stability was also improved; However, the amount of ester exchange catalyzed by modified enzymes is greatly affected by temperature.

2.2.5 Cofactor modification

Many enzymes contain coenzyme or coenzyme active groups. The limited variety of these functional groups in natural enzymes limits their function. If chemical methods can be used to add other functional groups to these cofactors, and then the modified cofactors can replace the original cofactors of natural enzymes, a variety of new enzymes can be produced. Research has shown that using calcium ions to replace zinc type protease with calcium type protease can increase its activity by more than 20%[7].

3. Genetic Engineering Modification

3.1 Definition of Genetic Engineering Modification

Genetic engineering modification refers to the modification of enzyme protein molecules through genetic engineering technology, in order to modify the characteristics of enzymes and obtain enzymes with the desired characteristics[7].

3.2 Genetic Engineering Modification

3.2.1 Mutation

On the basis of knowing the structure and function of the enzyme, purposefully changing a certain active group or amino acid residue of the enzyme to obtain an enzyme with new characteristics. Usually, the substrate specificity, catalytic properties, and thermal stability of modified enzymes can be altered through gene recombination and site-specific mutations. Site directed mutagenesis technology is a commonly used method for amino acid and nucleotide substitution modifications. Research has shown that converting the 51st position of threonine in T4 lysozyme to proline enhances the enzyme's affinity for ATP and increases enzyme activity by 25 times[16].

3.2.2 Directed Evolution

Directed evolution refers to the PCR amplification reaction of protein genes to be evolved, utilizing the properties of Tanana polymerase that do not have 3-5 proofreading function, combined with appropriate conditions, to introduce mutations into the target gene at a very low rate, construct a mutation library, and select the desired protein quality through directed selection methods. Hall et al[17] reported that they altered the substrate specificity of the second galactosidase in Escherichia coli K12 through directed evolution, and developed enzymes with hydrolysis ability for several glycosidic bonds. Molecular directed modification has gradually become an important technique for enzyme modification.

3.2.3 Comprehensive methods

By combining directed mutagenesis with chemical modification, a novel chemically modified mutant enzyme can be obtained. This technology can improve the catalytic properties, substrate specificity, and thermal stability of Bacillus subtilis protease[18].

4. Conclusion

The application of enzyme preparations in the field of medical testing is becoming increasingly widespread, and current research is mainly limited to the modification of enzyme side chains. There is relatively little research on cofactor introduction methods, chemical mutations, and modifications after peptide chain unfolding. Chemical modification plays an important role in studying the structure of enzyme activity centers and revealing the biological functions of enzyme activity. Therefore, in order to better apply enzyme modification technology, it cannot be limited to enzyme side chain modification. The development and application of other modification methods will be the focus of future research.

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