Preparation And Research Progress of Biomimetic Selenase

Mingyu Wu, Hanjiao Hu, Ruirui Zhang, Shuming Zhong, Zijie Liu, Min Liu, Yanzhen Yin*

Qinzhou Key Laboratory of Biowaste Resources for Selenium-enriched Functional Utilization, Beibu Gulf University, Qinzhou 535011, China.

*Corresponding author: yinyanzhen@bbgu.edu.cn

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Abstract: Simulating some special life processes to prepare bionic materials is an important source for scientists to study nature and make original innovations. In view of the important physiological functions of natural glutathione peroxidase, researchers have carried out a series of studies on the preparation and catalytic mechanism of bionic selenase. In this paper, the methods of chemical synthesis, semi synthesis and molecular imprinting for the preparation of biomimetic selenase are summarized, and the problems and solutions in the preparation of biomimetic selenase materials are prospected, which can provide support for the preparation process optimization of biomimetic selenase and the development and application of new antioxidant materials.

1. General situation of glutathione peroxidase

Glutathione peroxidase (GPx) is an important selenium-containing enzyme in human body, which was first discovered by Mills et al in 1957, and its crystal structure was first reported by Epp et al. GPx uses glutathione as reducing agent to decompose excessive free radicals in human body, which plays an important role in defense against oxidative diseases and has broad prospects for medicinal development. The research shows that the selenium element in the catalytic center of selenocysteine ammonia, the hydrophobic microenvironment composed of hydrophobic amino acid residues such as 150phe, 148trp and 34leu, and the substrate recognition sites based on the hydrogen bond between 40arg, 130arg and 167arg arginine and the substrate are three important catalytic factors to maintain the high catalytic activity of GPX [3]. Due to the high cost, low yield and complex process of natural GPX preparation, exploring a new efficient preparation method of GPX is of great significance for the development of antioxidant drugs and the treatment of oxidative diseases.

2. Significance of Biomimetic Selenase Preparation

Nature not only endows all things in the world that human beings depend on for survival, but also contains many wonderful biological and chemical laws in the process of everything cycle. Simulating some special life processes and creating bionic materials with similar functions is an important source for scientists to study nature and make original innovation. Inspired by nature, the preparation of biomimetic materials has greatly enriched the research connotation of functional materials [4]. Based on the understanding of GPX structure and the preparation concept of biomimetic materials, a series of biomimetic selenium enzymes were prepared by scientists represented by Zhang Xi [5], Liu Junqiu [6], Luo Guimin [7], Mugesh [8], Engman [9], Professor Hilbert [10].

3. Preparation and research progress of bionic selenase

Due to the important biological role of natural GPx in the treatment of antioxidant diseases, scientists have invested considerable energy in simulating its enzymatic behavior, and a large number of enzyme models have been reported. At present, the main methods to construct bionic glutathione peroxidase (bionic selenase) include chemical synthesis, semi-synthesis, molecular imprinting, antibody-antigen technology and genetic engineering.

3.1 Chemical synthesis

The GPx enzyme model constructed by chemical method is mainly based on the interaction among selenium-nitrogen, selenium-oxygen and nitrogen bonds, chemically synthesizing a series of organic micromolecule selenium-sulfur enzyme simulants, and designing and constructing compounds with substrate recognition ability,Such as cyclodextrin enzyme model [11], dendrimer enzyme model [5], etc.

(1) Small molecule selenase model

Among the organic small molecule selenase simulants, 2-phenyl-1,2-benzoisoselenide-3 (2H) - one (ebselen, PZ51) is a famous GPX simulant, which is called "small molecule selenase", and has been listed as an antioxidant drug in Japan. The discovery of ebselen bioactivity opened the prelude to the simulation of GPx by small molecular compounds.Scientists have constructed many small molecular selenides [3]. It was found that when the oxidation-reduction microenvironment of selenium in the catalytic center was adjusted, the small molecules containing selenium at this time had strong biological activity of GPx. Up to now, the methods of constructing small molecule simulants mainly focus on the modification of the short-range or long-range interaction forces of Se-N, Se…N, Se-O, Se…O, etc., which are based on the simulation of catalytic microenvironment of natural GPx active center [12]. Through these methods, scientists have obtained many excellent GPx simulators. In the study of Se. O interaction, it is found that the small molecule selenase model containing Se... O interaction shows high catalytic ability.

(2) Small molecule phosphoenzyme model

The success of small molecule selenase model makes people think that it belongs to the same main group as selenium. The research shows that compared with selenium; it has stronger redox ability. Engman et al reported that diphenyl diselenide compounds can catalyze the reduction of organic hydroperoxides by mercaptan compounds, and proved that dilute can act as a catalytic group like selenium to simulate GPx [13]. This study paves the way for simulating GPx with broken organic compounds. Similar to GPx simulated by small molecule selenide, scientists have also built many organic small molecule enzyme models of interaction between Te and N/O. It is proved that most tellurise models have higher catalytic ability than their corresponding selenase models. This may be related to the more active redox properties of dilute elements, which has been confirmed by the vigorous development of double organic dilute compounds simulating GPx.

(3) Cyclodextrin selenase model

Organic selenium molecules lack substrate binding sites, so their catalytic activity is generally not high. Therefore, it is a good technical route for the preparation of bionic selenase to select the skeleton with binding ability to GPx substrate and then modify and introduce catalytic center. Cyclodextrin has a hydrophobic cavity, which has a good binding ability to hydrophobic substrates. By modifying it at 2- position or 6- position, an enzyme model with substrate binding ability was constructed [14]. Luo Guimin [15], Liu Junqiu [11] and Engman [13] have made important contributions in this regard. Their cyclodextrin GPX enzyme model can accurately simulate the hydrophobic cavity structure of natural GPx. Compared with small molecule biomimetic selenases, cyclodextrin derivatives can further simulate the hydrophobic cavity of GPX, while most small molecule biomimetic selenases are difficult to simulate the synergistic catalytic process of GPX as a whole.

(4) Dendrimer enzyme model

Using the binding of the substrate by the hydrophobic cavity in the dendrimer, the catalytic center was introduced into it. Zhang Xi and Liu Junqiu constructed the dendrimer enzyme model. Different generations of dendrimers were designed and synthesized. The experiments show that the different hydrophobic microenvironment provided by different generations of dendrimers has a great impact on the catalytic activity. In addition, Zhang Xi et al constructed hyperbranched selenium containing polymer [5] as GPx mimic enzyme.Although the activity of this simulated enzyme is not high, the method designed by them has strong universality and operability, which is expected to provide an enzyme model with higher activity.

3.2 Semi synthetic method

The chemical modification of proteins is called semi synthesis. Generally speaking, chemical modification can be used to change the catalytic function of natural enzymes and give them new catalytic activity, or give proteins that usually have no enzyme activity a certain catalytic activity.Therefore, it is one of the simplest choices to construct GPx mimetic enzyme with GSH binding ability by using naturally existing binding sites and introducing catalytic centers at correct positions [6]. Hilvert constructed seleno subtilisin by this method, and explained its enzymatic mechanism well. Luo Guimin et al. constructed seleno glutathione transferase model [10]. Recently, Liu Junqiu and others successfully introduced more active chemical properties into it, constructed dilute Bacillus subtilis protease, and showed high catalytic activity for the reaction of TNB and peroxide [16].

(1) Molecular imprinting

Molecular imprinting can construct substrate binding sites and modify them again. It is an effective method to construct GPX artificial simulant [17]. Luo Guimin and others prepared derivatives of substrate GSH as template molecules, denatured natural proteins to produce binding sites through the process of biological imprinting, and obtained catalytic imprinting enzymes after introducing them into the catalytic center through chemical modification.Different from traditional imprinting, the main processes of molecular imprinting are as follows: ① Firstly, prepare selenium protein with catalytic activity; (2) template molecules are added, so that the template molecules are fully combined with the protein and then partially denatured, thus disturbing the conformation of the initial protein; ③ adjusting pH value, renaturating protein, and crosslinking imprinted protein with crosslinking agent.Due to the full interaction and denaturation of the starting protein with the template molecule, a substrate binding site similar to the enzyme is generated, giving it new enzyme activity [18].

(2) Antibody-antigen technology

Luo Guimin and others first prepared several analogues of substrate GSH as hapten by using antibody antigen technology, and then introduced the catalytic center sec into the generated binding site through chemical modification, so as to obtain selenium containing antibody enzyme. These antibody enzymes show high GPx activity, which is on the same order of magnitude as the catalytic efficiency of natural enzymes [19]. They found that the size and hydrophobicity of hapten have regular effects on the substrate binding ability and catalytic activity of the model. Considering that the monoclonal antibody 2F3 has the highest activity potential, the active site of the prepared single-chain antibody has a specific microenvironment, and the function of glutathione peroxidase can be played normally if the microenvironment features are retained.

(3) Genetic engineering method

Gene engineering can selectively mutate designated sites, which is one of the important means to construct GPx model. This method lays a foundation for the elaboration of enzyme catalytic mechanism. Liu Junqiu and others have done a lot of outstanding work in this field. Selenocysteine was introduced into different positions of subtilisin by using the auxotrophic expression of Escherichia coli, and the catalytic mechanism of enzyme was studied in detail. Experiments proved that the hydrophobic pocket of subtilisin played an important role in catalysis [6]. Recently, they reported the introduction of identified cysteine sites into glutathione thiotransferase. Seleno glutathione thiotransferase, as a GPX simulant, shows high catalytic activity, which is the first report of introducing confirmed cysteine into protein by genetic engineering [18].

Generally speaking, the biomimetic selenases prepared based on micromolecule and macromolecular skeleton are easy to realize industrial application and popularization in terms of material source, preparation cost, structural stability and operability. In terms of whether it can better simulate the catalytic nature of natural GPx and clarify the catalytic mechanism of natural GPx, macromolecular bionic selenase and protein bionic selenase have more advantages.

4. Development trend of preparation methods of biomimetic selenase

The research shows that catalytic center, hydrophobic cavity and recognition site are the important catalytic units for constructing efficient bionic selenase, and their synergistic catalytic mechanism is the key factor to enhance the catalytic activity of bionic selenase [2,20]. Therefore, based on the research on the catalytic mechanism of natural GPx and the experience in the preparation of bionic selenase, researchers have recently devoted themselves to the study of bionic selenase with multiple catalytic units.Among them, Professor Liu Junqiu's research group [21,22] and our research group [23,24] prepared intelligent response biomimetic selenium enzyme by using stimulus response macromolecular skeleton. Based on the characteristics that the structure of stimulus response polymer can change dynamically; we realized the dynamic simulation of GPX synergistic catalysis mechanism. The results show that compared with small molecule biomimetic selenase, macromolecular biomimetic selenase is easier to realize the effective matching and synergistic catalysis of multiple catalytic units, and is an ideal skeleton material for constructing biomimetic selenase.At present, for the breakthrough from the theoretical research of macromolecular bionic selenase to the industrial application of antioxidant bionic selenase materials. Degradation of traditional biomimetic selenase skeleton is one of the key scientific problems that need to be solved urgently. The solution and breakthrough of related problems will effectively promote the development of selenium-containing antioxidant enzymes and other selenium-containing functional materials industries.

5. Conclusion

Due to the high cost, low yield and complex process of natural GPx preparation, it is urgent to explore new efficient preparation methods of GPx. The main methods to construct bionic glutathione peroxidase (bionic selenase) include chemical synthesis, semi-synthesis, molecular imprinting, antibody-antigen technology and genetic engineering.From the aspects of preparation material source, preparation cost, structural stability and operability, the biomimetic selenium enzyme prepared based on small molecule and macromolecular skeleton is easy to realize industrial application and popularization. Macromolecular biomimetic selenase and protein biomimetic selenase can better simulate the catalytic essence of natural GPX. In the future, the preparation of degradable biomimetic selenase based on macromolecular skeleton materials will be the research direction of biomimetic selenase with important industrial significance.

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