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Study on Catalytic Mechanism of Microbial Transglutaminase in Protein Substrate

Yunbin Dai*, Hongchen Li

Heriot-Watt University, Edinburgh EH14 4AS, Scotland, UK

*** Corresponding author**

Abstract: Transglutaminase (abbreviated as TG,EC2.3.2.13) can catalyze the acyl transfer reaction of peptides in protein, but it can only catalyze a certain acyl transfer reaction instead of every acyl group. Protein is widely used to modify protein in food industry because it is safe and non-toxic and the catalyzed product can be digested and absorbed by human body. In this paper, the catalytic mechanism of protein substrate for MTG (Microbial TG) was studied in order to provide theoretical basis for the follow-up research and application of MTG. The results show that within 1%~4%, the total amount of biopolymer produced increases linearly, and then it remains basically unchanged. The catalytic amount of MTG to substrate and catalytic efficiency are contradictory. Under the condition of fixed MTG concentration, the higher the substrate concentration, the smaller the catalytic efficiency, and it shows a linear downward trend. When the concentration is 10%, the gel strength is the highest, reaching 68.09g. When the concentration of protein is low, protein-solvent interaction is dominant, and the system is not easy to form gel. The lower the hydrophobicity of protein surface, the easier it is to be catalyzed by MTG. However, the surface hydrophobicity of soybean globulin is in the middle, and MTG has moderate catalytic activity for it.

Keywords: Microbial transglutaminase, Protein, Biopolymer.

1. Introduction

Except for a few protein whose linear pairs are compliant, most food protein are globular proteins, which makes hydrophobic groups contained in protein through certain nonpolar hydrophobic action or disulfide bond action, thus showing a certain spatial structure. Transglutaminase (abbreviated as TG,EC2.3.2.13) can catalyze the acyl transfer reaction of peptides in protein, but it can only catalyze a certain acyl transfer reaction instead of every acyl group. Because it is safe and nontoxic, and the catalyzed protein product can be digested and absorbed by human body, it is widely used to modify protein in food industry, which can make protein cross-link intermolecular or intramolecular or make protein deamidate and glycosylate, thus improving the physical and chemical properties of food [1-2].

TG can catalyze the amido transfer reaction between γ-carboxamide group of glutamine residue in protein peptide chain and various acyl receptors to form ε-(γ-glutamyl) lysine covalent bond. The covalent bond of ε-(γ-glutamyl) lysine formed by catalysis leads to intramolecular or intermolecular crosslinking of protein, forming a network structure connection reaction. TG widely exists in all kinds of natural organisms, including animals [3-4], plants [5] and microorganisms [6-7]. Among them, TG from microorganisms has the advantages of being independent of Ca 2+, low substrate specificity, fast catalysis speed and low production cost, and is more widely used in industry [8-9]. MTG (Microbial TG) has attracted the attention of researchers because of its application value in industry [10]. It is one of the research focuses on MTG to construct MTG producing strains with high enzyme activity and high yield by molecular modification and enzymatic modification.

In two or more different protein systems, there is no clear conclusion as to which protein is polymerized first by MTG, or to the same extent, or even cross-linking between them. Therefore, it is of great practical significance to study and develop TG. Based on this, this paper studied the protein substrate catalytic mechanism of MTG by consulting the literature in recent years, in order to provide theoretical basis for the follow-up research and application of MTG.

2. Materials and Methods

2.1. Materials and instruments

Transglutaminase commercial enzyme, whey protein concentrate, UVP gel imaging system, SHZ-88 desktop water bath thermostat oscillator, glycinin, and β -conglycinin. Other electrophoresis reagents were purchased from Shanghai Boao Biological Company. All other chemical reagents are analytical grade.

2.2. Determination of optimum pH for enzyme catalysis

Take 50 mL of 5 sample solutions, adjust them to different pH values: 6.0, 6.5, 7.0, 7.5 and 8.0, take 5 mL of each solution, add 5 mL 1 U/mL of MTG to each labeled test tube, cover the test tube plug, and put it in a water bath at 40℃ for 2 h.. After taking it out, adjust its pH to the vicinity of the isoelectric point of whey protein for acid precipitation, and then centrifuge it for 0.5 h at 4000 r/min. After centrifugation, take the precipitate to 10 mL and measure its protein content.

2.3. Purification of MTG

15gMTG enzyme powder was dissolved in 100 ml of ice bath, 20 mol/L, sodium acetate buffer (pH = 6.0), and centrifuged at 5℃ to remove insoluble substances. Ammonium sulfate was added until it was 50% saturated, and most of the impurities were removed. After that, the same buffer was dialyzed at 5℃ for 3 times, each time at 1:220. Finally, the dialyzed enzyme solution was concentrated with polyethylene glycol. The impurity protein content of the enzyme solution is almost negligible, and the active concentration of the enzyme solution is about 10U/mL.

2.4. MTG Catalyzed Heterogeneous protein Polymerization

Two kinds of protein were dissolved in 0.1mol/L Tris-HCl buffer (pH7.5) at a ratio of 1:1, with a concentration of 1%, including 20mmol/LDTT, and the total protein concentration was 1%. The purified MTG was added to make the ratio of enzyme activity equivalent to substrate protein 25U/g protein. A certain amount of reaction mixture was taken out and directly mixed with SDS- polypropylene gel sample buffer. The reaction mixture reacted at 37℃ for 41h, and after the reaction, the enzyme reaction was stopped by adding electrophoresis sample buffer directly.

3. Result

3.1. Effect of different substrate concentrations on polymerization of protein by MTG

Generally speaking, the larger the ratio of enzyme amount to substrate, the more favorable it is for the enzyme to completely catalyze the substrate. When the amount of enzyme is constant, there will be a "critical" concentration in the substrate concentration, and the enzyme is just saturated. In this study, 1%~10% level was selected for the experiment. The total amount of biopolymers at different substrate concentrations and the total optical density of biopolymers in protein are shown in Figure 1:

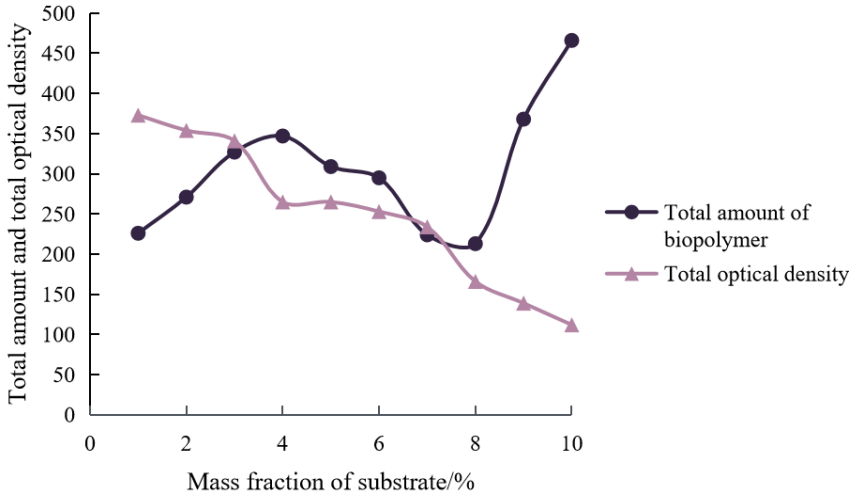

Figure 1. The total amount of biopolymers and the total optical density of protein

It can be clearly seen that within 1%~4%, the total amount of biopolymers produced increased linearly, and then remained basically unchanged. The catalytic amount of MTG to substrate and catalytic efficiency are contradictory. Under the condition of fixed MTG concentration, the higher the substrate concentration, the smaller the catalytic efficiency (that is, the amount of synthesized biopolymer), and it shows a linear downward trend. For an MTG catalytic reaction, it is necessary to comprehensively consider the relationship between the two, and improve the catalytic efficiency as much as possible on the premise of ensuring the maximum polymer content.

3.2. Effect of substrate concentration on the strength of protein gel catalyzed by MTG

MTG can catalyze the formation of ε-lysine covalent bonds between protein molecules of soybean, which strengthens the intermolecular network and enables protein solution to form gel without high-temperature heating treatment, as shown in Figure 2:

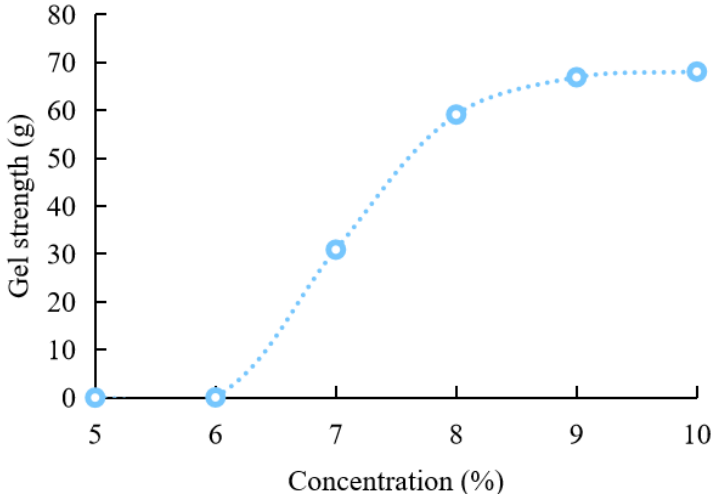

Figure 2. Effect of substrate concentration on the strength of protein gel catalyzed by MTG

The minimum substrate concentration for protein solution to form gel by MTG is 7%. With the increase of substrate concentration, the gel strength gradually increases. When the concentration is 10%, the gel strength is the highest, reaching 68.09g. The formation of protein gel is the result of the interaction between protein-protein and protein-solvent and the balance of attraction and repulsion between adjacent peptide chains. When the concentration of protein is low, protein-solvent interaction is dominant, and the system is not easy to form gel.

3.3. Effect of protein hydrophobicity on catalytic activity of MTG

Non-globular proteins is more susceptible to MTG catalysis than globular proteins. That is to say, the spatial structure of non-globular proteins in solution is more flexible, which makes it easier to expose the substrate to the catalytic part of the enzyme. The surface hydrophobicity of some protein is known. Without reducing agent, we found that the lower the surface hydrophobicity of protein, the easier it is to be catalyzed by MTG. However, the surface hydrophobicity of soybean globulin is in the middle, and MTG has moderate catalytic activity for it. It is certain that MTG can only catalyze the protein polymerization in water phase, which also explains that some conditions such as pH and organic solvents have great influence on its catalytic activity.

4. Discussion

TG widely exists in animals, plants and microorganisms, and is closely related to biological phenomena such as blood coagulation, wound healing, epidermal keratinization and erythrocyte membrane sclerosis. It can participate in signal transduction and regulate cell growth, differentiation and proliferation [11], and has a wide application prospect in food, biopharmaceuticals, cosmetics, textiles and other fields. The tertiary structure molecules of MTG are discoid as a whole, with a deep crack at the edge, which is highly hydrophilic, but some aromatic amino acids and hydrophobic amino acids are scattered around the active site, which plays a role in specifically recognizing the substrate of enzyme action [5]; The sulfhydryl group of Cys64, the catalytic active center of MTG, is fully exposed in the solvent, so MTG can react with the substrate quickly and its catalytic efficiency is higher [6].

Cys64 residue at the bottom of MTG molecular crack is MTG as the catalytic activity center of enzyme, and His274 and Asp255 next to it form catalytic triad Cys64-His274-Asp255, which have extremely important influence on MTG's catalytic action. The experiment shows that [5], the hydrophobic residues and aromatic residues of MTG active site directly interact with the substrate through hydrophobic interaction and π -π bond interaction. The experiment of studying the activity of MTG enzyme by nuclear magnetic resonance technology shows that the N-terminal region of the peptide chain in the active site of MTG has the ability to recognize and bind to the substrate. At the same time, this study shows that when the N-terminal region has too much negative charge, it will hinder its binding with the substrate. This study shows that within 1%~4%, the total amount of biopolymer produced increases linearly, and then it remains basically the same. The catalytic amount of MTG to substrate and catalytic efficiency are contradictory. Under the condition of fixed MTG concentration, the higher the substrate concentration, the smaller the catalytic efficiency (that is, the amount of synthesized biopolymer), and it shows a linear downward trend.

MTG can change many nutritional and functional properties of protein, such as gelation, emulsification, foaming, viscosity, water holding capacity and so on, by catalyzing cross-linking, deamidation and glycosylation, and improve the taste and quality of products. Gel is an intermediate state between solid and liquid. Macromolecules are crosslinked by covalent bonds or non-covalent bonds to form a highly ordered three-dimensional network structure, which can retain water and some small molecules. This property is extremely important for meat products, dairy products and some bean products, and can effectively improve the properties of products. The principle of improving emulsion stability by MTG is related to the matrix. When casein is treated by MTG, it is found that although crosslinking will lead to the decrease of solubility, it may be due to the conformational change of protein and the increase of negative charge, which will improve the balance of amphiphilic properties of protein and help to form a more elastic protein film in oil and water, thus increasing emulsion stability [3].

At present, the research shows that the mutant MTG strain with high enzyme activity is mainly obtained by biological transformation of amino acid residues near Cys64 residue of MTG molecular active center and its N-terminal amino acid residues. Molecular docking experiments have simulated the binding mode of TG and substrate [2]. The synthetic small molecular substrate CBZ-Gln-Gly is stretched in the crack of TG molecule, and the active site is bound to the substrate through hydrophobic interaction. When TG binds to macromolecular substrates (casein, etc.), the substrate recognition site of TG will further expand on the surface of molecular cracks, which reflects the adaptability of microbial TG to substrates. This experiment shows that the lower the hydrophobicity of protein surface, the easier it is to be catalyzed by MTG. However, the surface hydrophobicity of soybean globulin is in the middle, and MTG has moderate catalytic activity for it.

5. Conclusions

Within 1%~4%, the total amount of biopolymers produced increased linearly, and then remained basically unchanged. The catalytic amount of MTG to substrate and catalytic efficiency are contradictory. Under the condition of fixed MTG concentration, the higher the substrate concentration, the smaller the catalytic efficiency, and it shows a linear downward trend. When the concentration is 10%, the gel strength is the highest, reaching 68.09g. The formation of protein gel is the result of the interaction between protein-protein and protein-solvent and the balance of attraction and repulsion between adjacent peptide chains. When the concentration of protein is low, protein-solvent interaction is dominant, and the system is not easy to form gel. The lower the hydrophobicity of protein surface, the easier it is to be catalyzed by MTG. However, the surface hydrophobicity of soybean globulin is in the middle, and MTG has moderate catalytic activity for it.

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