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Purification and characterization of a high-salt-resistant microbial transglutaminase from *Streptomyces mobaraensis*

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ABSTRACT

A novel microbial transglutaminase (MTG-TX) was obtained from *Streptomyces mobaraensis* by fermentation and purification. The enzyme was purified by ethanol precipitation via a two-step purification method, with a 44.0% yield and a specific activity of 39.2 U mg^{-1}. The purified enzyme exhibited stable performance over a range of pH 5.0–pH 10.0 and displayed maximal activity at pH 6 and 48 °C. We verified through biochemical analyses that the enzyme is a novel MTG variant possessing the same zymogen characteristics as that of another reported MTG from *Streptoverticillium ladakanum* B1. Furthermore, the loss of enzyme activity by MTG-TX in the presence of high salt was only 79.8% that observed in a control MTG from *S. mobaraensis* DSM40847. On the basis of salt resistance, the novel MTG-TX presented here also performed well in food-related applications by successfully crosslinking proteins in high-salt environments, thereby enhancing the cohesiveness of bacon.

1. Introduction

Transglutaminases (EC 2.3.2.13) are a class of transferases that exist in animals and microorganisms [1]. These enzymes catalyze transfer reactions between the γ-carboxylamide groups of glutamine residues in various proteins, peptides, and primary amines, and have been used to improve the texture, elasticity, appearance, taste, flavor, and shelf-life of food [1]. Various microbial transglutaminases (MTGs) from *Streptomyces* have been reported since 1989 [2–5], resulting in decreased costs associated with transglutaminase production by fermentation of *Streptomyces*.

To obtain pure MTGs, purification processes involving two or more steps are common [2–5], and gel-filtration chromatography as a high-performance purification method has been used in most laboratory-scale processes [2,4,5]. Given the variations in valid purification methods, many reports over the last decade focused on characterization of the effects of MTG inhibitors and thermal stability [2–5].

Although MTGs play important roles in the meat and dairy industry [6–8], few types of transglutaminases have been used in food-related applications [9,10]. One classic use of MTGs is in the production of restructured meat [11]; however, there are few reports addressing the use of MTGs in harsh environments, specifically high-salt foods (seafood, bacon, and ham) [12,13]. To obtain insight into the application of MTGs in high-salt foods, we purified a novel MTG from *Streptomyces mobaraensis*.

2. Materials and methods

2.1. Microorganisms and culture conditions

S. mobaraensis TX was collected from soil, identified in our laboratory, and used throughout this study. Another strain of *S. mobaraensis* (type strain, collection number: DSM40847) was purchased from DSMZ (Braunschweig, Germany) for use as a control. Both strains were cultivated on Gauze's synthetic medium number one (20 g L^{-1} soluble starch, 1 g L^{-1} KNO_3, 0.5 g L^{-1} NaCl, 0.5 g L^{-1} $MgSO_4 \cdot 7H_2O$, 0.5 g L^{-1} K_2HPO_4, 0.01 g L^{-1} $FeSO_4 \times 7H_2O$, and 15 g L^{-1} agar [pH 7.4]) at 28 °C.

2.2. Analytical determinations

2.2.1. MTG activity

Activity was assessed by colorimetry according to a procedure described by Grossowicz et al. [14] and using *N*-α-CBZ-Gln-Gly and hydroxylamine hydrochloride (Sinopharm, Beijing, China) as substrates. One unit of MTG-TX activity was defined as the formation

Abbreviations: MTG, microbial transglutaminase; MTG-TX, novel microbial transglutaminase (this study); PMSF, phenylmethanesulfonyl fluoride.

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of 1.0 μmol L-glutamic acid gamma-monohydroxamate per minute at 37 °C and pH 6.0.

2.2.2. Protein concentration

Protein concentration was determined using the BCA method and the enhanced BCA protein assay kit (Beyotime, Shanghai, China).

2.2.3. SDS-PAGE

Purified protein was analyzed by SDS-PAGE according to previously reported methods [15]. Gel separation (12% acrylamide) was performed at 120 V for 70 min, and gels were stained with Coomassie Brilliant Blue R-250. Bands were visualized using a ChemiDoc XRS+ (Bio-Rad, Hercules, CA, USA).

2.3. MTG production

Spores were collected by scratching from Gauze's synthetic medium number one after cultivating at 28 °C for 7 days, followed by cultivation for activation in fermentation medium (25 g L^{-1} peptone, 20 g L^{-1} glycerol, 6 g L^{-1} yeast extract, 2 g L^{-1} $MgSO_4 \cdot 7H_2O$, and 2 g L^{-1} K_2HPO_4 [pH 7.4]) at 30 °C for 24 h while shaking at 200 rpm. Activated fermentation broth (5 mL) was transferred to 50 mL of the fermentation medium and cultivated at 30 °C while shaking at 200 rpm. After the specific activity of the supernatant reached a maximum of 1.75 U mg^{-1} protein for 30 h, the fermentation broth was harvested by centrifugation at 8000*g* for 10 min at 4 °C (Fig. S1).

2.4. MTG purification

2.4.1. Ethanol precipitation

Microorganisms were removed by centrifugation at 8000*g* for 10 min at 4 °C, followed addition of the fermentation supernatant to chilled ethanol to a final concentration of 50% (w/v) as previously described [5]. The precipitated protein was harvested by centrifugation at 8000*g* for 10 min at 4 °C, followed by immediate resuspension in 50 mM sodium acetate buffer (pH 6.0).

2.4.2. Cation-exchange chromatography

All chromatography experiments were performed using an AKTA Purifier (GE Healthcare, Beijing, China). The enzyme solution was applied to a SP sepharose fast-flow chromatography column (1.6 cm × 10 cm; GE Healthcare, Beijing, China) equilibrated with 50 mM sodium acetate buffer (pH 6.0). Elution of MTG-TX was performed using an increasing gradient of NaCl, which was obtained by mixing 50 mM sodium acetate buffer (pH 6.0) with increasing NaCl concentrations, beginning with 0.5 M NaCl. The flow rate was 5 mL min^{-1}, and fractions were collected and assayed for MTG activity.

2.4.3. Hydrophobic chromatography

NaCl was added to the active fractions to a final concentration of 2 M, followed by application of the solution to a phenyl-sepharose high-performance column (1.6 cm × 10 cm; GE Healthcare) equilibrated with 50 mM sodium acetate buffer (pH 6.0) and 2 M NaCl. Elution of MTG-TX was obtained using a decreasing gradient of NaCl by mixing 50 mM sodium acetate buffer (pH 6.0) with decreasing NaCl concentrations, beginning with 2 M NaCl. The flow rate was 5 mL min^{-1}, and fractions were collected and assayed for MTG activity.

2.5. MTG-TX characterization

2.5.1. Determination of molecular weight and amino acid sequences

The molecular weight was measured by LC–MS (Fig. S2), and N-terminal amino acid sequence analysis was determined by using Edman degradation method. Measurement of the molecular weight and N-terminal amino acid sequence analysis was all determined by Shanghai Applied Protein Technology, Ltd. (Shanghai, China). The whole genome was extracted using a rapid bacterial genomic DNA isolation kit purchased from Sangon Biotech, Inc. (Shanghai, China), and DNA-sequence analysis was used Chain Termination Method and performed by Major Biosystem Corp., Ltd. (Shanghai, China). The amino acid sequence of MTG zymogens were determined according to the DNA sequences.

2.5.2. Kinetic parameters

The MTG transfer reaction follows a ping-pong mechanism [11]. Hydroxylamine hydrochloride was used as the acyl acceptor, with *N*-α-CBZ-Gln-Gly acting as the acyl donor. We varied the concentration of *N*-α-CBZ-Gln-Gly between 3.6 mM and 30 mM to acquire three groups of Lineweaver-Burk plots for acyl acceptor concentrations of 10 mM, 6 mM, and 2 mM. All reactions were performed in 0.2 M Tris-HCl buffer (pH 6.0), and kinetic parameters were determined at pH 6.0 and 37 °C.

2.5.3. Effects of pH and temperature

MTG-TX activity was determined under different pH and temperature conditions to determine the optimal pH and temperature. Sodium citrate buffer was used at pH ranges of 3.0–6.0, phosphate buffer was used at pH ranges of 6.0–8.0, and Tris-HCl buffer was used at pH ranges of 8.0–10.0. MTG-TX pH stability was determined following incubation at different pH levels at 30 °C for 30 min. MTG-TX thermal stability was determined following incubation at different temperatures at pH 6.0 for various times.

2.5.4. Effects of ions and inhibitors

MTG-TX activity was determined following incubation in the presence of various metal ions and three inhibitors at concentrations of 2.5 mM and 5 mM for 30 min at 37 °C.

2.6. Applications in bacon

Pork belly was cleaned, cut into small pieces, and separated into 100-g experimental groups. Each group received injections of 10 mL 10% (w/v) NaCl solution and was tumbled for 5 min. This was followed by an additional injection of 1 mL of 2.5 U mL^{-1} MTG-TX solution, followed by inserting the pork into molds measuring 10 × 5 × 5 cm^3 and incubating at room temperature for 1.5 h. The shaped pork was then boiled at 78 °C for 1 h to initiate transformation into bacon, followed by quick-freezing and cutting into 0.5-cm thick pieces for analysis. The control MTG from *S. mobaraensis* DSM40847 was collected by the same two-step purification method described in this study (Table S1). TG-B was purchased from Ajinomoto Inc. (Shanghai, China). Commercial bacon samples were purchased from Nanjing Yurun Food, Ltd. (Nanjing, China). All bacon samples were analyzed using a TA.XTplus texture analyzer (Stable Microsystems, Godalming, UK).

3. Results and discussion

3.1. MTG purification

Ethanol precipitation resulted in a loss of yield during the purification process. Previous reports used ultrafiltration to reduce the volume of fermentation supernatant [2]. Although ultrafiltration