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Enhancing Stability and Purity of Crude Chitinase of *Achatina fulica* by Crystallization

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Abstract

A crystallization method was developed to enhance the purity and stability of hydrolase mixtures from the digestive gland of the snail *Achatina fulica*, as demonstrated by chitinase activity. Crude chitinase was concentrated by freeze drying and then crystallized at 10°C. Crystal formation was observed under the microscope. The best concentration for crystallization was obtained with 1.5-fold concentrated crude chitinase. Crystallization enhanced the chitinase specific activity from 0.87 U mg^{-1} to 0.95 U mg^{-1}. The loss of chitinase activity from liquid and crystals of crude chitinase on four days storage at 10°C was 83.0% and 17.7%, respectively. It was concluded that the crude chitinase crystals showed a significant increase in stability and purity.

Peer-review under responsibility of the organizing committee of the Molecular and Cellular Life Sciences: Infectious Diseases, Biochemistry and Structural Biology 2015 (MCLS 2015)

Keywords: Chitinase; crystallization; digestive gland; *Achatina fulica*; purification; stability

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1. Introduction

Snail meat is a food with a high nutritional value and has been consumed by humans worldwide since prehistoric times[1]. Many people in developing and under-developed countries use snail meat in their diet as a source of protein to raise the defense mechanisms of the body[2]. It means that snail meat is a source of nutrition, including enzymes produced in the digestive gland. The digestive gland of *Achatina fulica* produces a mixture of hydrolases, which effectively degrade the extracellular matrix polymer of fungal biofilms, especially of *Candida*. This enzyme mixture is being developed as an antifungal drug to eradicate *Candida* biofilm in all types of candidiasis pathologies, where nearly all current antifungals cannot penetrate and do not work well.

Candida species are major human fungal pathogens that contribute to human morbidity and mortality through both mucosal and deep tissue infections. As with other microbiomes[4,5], the majority of *Candida* biofilms contributes to human diseases, like autism[3] and degenerative diseases. Biofilms are biological communities with a high degree of organization, in which microorganisms form structured, coordinated and functional biological communities. These communities are embedded in a self-created extracellular matrix polymer[6]. The fungal cell walls are predominantly composed of β-1,3-glucan, β-1,6-glucan, chitin, and mannoprotein. These components increase considerably in the biofilm matrix, and become a barrier to any drug to penetrate the cell membrane and cytoplasm[7]. However, a variety of polysaccharide degrading enzymes occurs in snails. In particular, *Achatina fulica* has a mixture of enzymes, which potentially degrade the extracellular matrix polymer of fungal biofilms, especially *Candida*[8].

These mixtures of enzymes are unstable during room temperature storage. It is known that many enzymes show a higher stability in the crystalline form. Crystallization of proteins and enzymes is usually used to obtain a pure solid for X-ray diffraction analysis for structure determination. However, the novelty and the long-term goal of the current research is the large-scale partial purification of snail crude enzymes by crystallization for treatment of candidiasis. Crystallization is widely used in the final steps of chemical industry processing, to provide higher-purity solids that are easier to handle and store compared to liquids. Like the crystallization process of small inorganic molecules, protein crystallization goes through the three distinct stages of nucleation, crystal growth, and termination. Unlike inorganic small molecules, however, protein crystallization is very sensitive to environmental conditions due to the conformational flexibility of proteins. Protein crystallization is influenced by biological parameters such as the presence of contaminating biomolecules. Protein crystallization is also influenced by protein concentration, pH, temperature, ionic strength, purity, and viscosity. From the large-scale processing point of view, operational parameters such as protein concentration, salt type and concentration, pH, and temperature are considered critical. Salt concentration, pH, and temperature can directly alter a protein's solubility characteristics. This article reports a crystallization process at optimum protein concentration at 5-10°C.

2. Methods

2.1. Materials

The digestive enzymes of *Achatina fulica*, here in after referred to as crude chitinase, were harvested from the *digestive* gland of *Achatina fulica*, which had been incubated in a room with controlled humidity for a week. The snail shell was cleaned and broken down to shed the digestive gland liquid, and then centrifuged at 4000 rpm. The supernatant was the crude chitinase.

2.2. Preparation of supersaturated solution of crude chitinase

The crude chitinase was concentrated by *freeze-drying.* The harvested crude enzyme was directly frozen by dipping it in liquid nitrogen. It was then concentrated with a freeze dryer until we have 1.5x, 2x and 3x crude enzyme solutions. That means that we concentrated the crude enzyme to 65%, 50% and 33% of the original volume, here in after respectively called the 1.5x, 2x and 3x crude enzyme solutions.