

Short Communication

Studies on isolation and partial purification of lysozyme from egg white of the lovebird (*Agapornis* species)

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Lysozyme was extracted from the egg white of the eggs of lovebird (*Agapornis* sp.) and partially purified using the dialysis membrane. The amount of lysozyme extracted was determined through absorbance assays using *Micrococcus lysodeikticus* cell walls as substrate. The total protein content in the crude (0.5 mg/ml) and partially purified (0.01 mg/ml) enzyme extract was estimated. The presence of lysozyme enzyme in the egg white was confirmed and its molecular weight (14.3 kDa) was determined through SDS-PAGE.

Key words: Lysozyme, *Agapornis*, *Micrococcus lysodeikticus*, SDS-PAGE.

INTRODUCTION

Lysozyme is one of the best-characterized hydrolases, which cleaves β -1,4 linkages of N-acetylglucosamine (Glc Nac) homopolymer and Glc Nac-N-acetyl muramic acid heteropolymer, which causes lysis of the bacteria containing that polymer in their cell walls (Araki et al., 2003). The demand for lysozyme is great in the pharmaceutical as well as food market (Elli et al., 1999). Egg white is a natural source of proteins of proved and potential nutritional, biological interest. Three major portions, i.e. lysozyme, ovotransferrin and ovalbumin are of particular significance. Because of its antibacterial activity, lysozyme is widely used for food preservation and in pharmaceutical industry (Vachier et al., 1995).

Lysozyme is a commercially important enzyme with different applications, e.g. (a) cell disrupting agent for extraction of bacterial intracellular products, (b) antibacterial agent in ophthalmologic preparations, (c) food additive in milk products, (d) antibacterial agent in wine production process, and (e) drug for the treatment of ulcers and infections.

It has recently been reported that lysozyme boosts

antibody production in hybridoma reactors (Murakami et al., 1997). It exists broadly in animal tissues and sera, as well as in tears, human and cow's milk etc. (Chi Hou and Lin, 1997). The lysozyme is highly stable in acidic solution and helps its activity even after 100°C for 1 – 2 min. The thermal stability is due to the four-disulfide bonds (Abraham, 1939).

Lysozyme is used for the treatment of HIV infections and as an anti-cancer drug (Ghosh, 2003), as well as a germinative agent of bacterial spores (Ando, 1975). It is used for lysing *Escherichia coli* and *Streptomyces* species for the extraction of specific antigen and to treat ophthalmic ulcers and infections as well as in ophthalmologic preparations (Ghosh et al., 2000). Lysozyme was the first enzyme to have its entire 3D structure determined with the aid of X-ray crystallography (Philips, 1967).

Lovebirds are smaller members of the parrot family. They are averagely about six inches in length. There are nine species of lovebirds, and many different colour varieties. Their scientific name *Agapornis* comes from the Greek *Agapa* meaning, "Love" and *ornis* meaning "bird". This paper describes the isolation, partial purification and determination of molecular weight of lysozyme of *Agapornis* sp. egg white.

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MATERIALS AND METHODS

Collection of sample and separation of egg white

Freshly laid 10 eggs of lovebirds were collected in and around of Perungalathur, Kancheepuram (District), Tamil Nadu, India. The weight of the eggs was recorded apart from the circumference and diameter of the individual eggs. The surface of the egg was sterilized with 75% ethanol and the egg white was separated by breaking the shell using hands.

Culture of microorganism used

The strain *Micrococcus lysodeikiticus* (NCIM No.2170) was obtained from National Collection of Industrial Microorganisms, National Chemical Laboratory (NCL), Pune, India. Nutrient agar medium (Hi Media) and Nutrient broth (Hi Media) were used to culture the microorganism.

Estimation of total protein in crude and dialysed sample of egg white

Total protein in crude and dialysed sample of egg white was estimated using Lowry et al. (1951) method.

Isolation of lysozyme

The method adopted for isolation of lysozyme was from Alderton et al. (1944). Isolation of lysozyme is by using active adsorbent morilonite clay (bentonite), which was found to be most efficient for lysozyme separation from native egg white.

Ammonium sulphate precipitation

This was carried out at 4°C. The crude enzyme was precipitated from 100 ml of the filtrate, obtained from the induction medium with 2.6 M ammonium sulphate and was dissolved in 10 ml of 0.2 M sodium phosphate buffer (pH 7.2).

Determining the activity of the lysozyme by lysoplate assay

The lysozyme is sensitive to the cell wall of *M. lysodeikiticus*. The rate of breakdown of cell wall of the organism is directly proportional to the enzyme concentration.

SDS-PAGE for the determination of molecular weight of the enzyme

The molecular weight of lysozyme was determined by SDS-PAGE using standard lysozyme (Hi Media).

RESULTS AND DISCUSSION

Lysozyme is present in various body fluids and change in the lysozyme content in these fluids is indicative of certain disease states (Jolles and Jolles, 1984). The weights of the collected *Agapornis* sp. egg were between 2 and 3 g and the content of the egg white varied from 0.4 to 1.2 ml. The circumference and diameter of the egg was found to be 6.57 and 5.4 cm. The total amount of

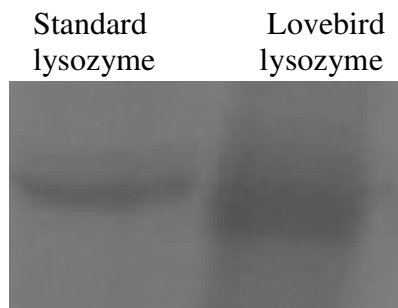


Figure 1. SDS-PAGE of lovebird (*Agapornis* sp.) egg white lysozyme with standard lysozyme.

protein present in the crude extract (egg white) of 10 eggs freshly laid of lovebirds was found to be 0.5 mg/ml.

Lysozyme's basic character is tending to bind to other proteins in the egg white. As lysozyme is highly stable in acidic medium, elution was done at pH 5.0 with 5% pyridine solution with concentrated sulphuric acid from the active adsorbent of bentonite. The lysozyme was finally eluted in the supernatant and collected. Enzyme activity was estimated by lysoplate assay (Li-Chan et al., 1986). Formation of zone of clearance was observed. The enzyme activity was estimated by using *M. lysodeikiticus* (cell wall) as substrate. Lysozyme catalyses the hydrolysis of N-acetylglucosamine homopolymer and N-acetyl muramic acid heteropolymer in the cell wall of the bacteria. This hydrolysis causes the lysis of the bacterial cell wall. The standard lysozyme and the isolated lysozyme in the wells diffuse in the medium and the lysis of the bacterial cell wall is observed as a zone of clearance around the well. The total amount of protein present in the dialysed sample was found to be 0.01 mg/ml (from 10 eggs freshly laid of lovebirds). The enzyme isolated was confirmed to be lysozyme, which is similar to the molecular weight of standard lysozyme 14.3 kDa (Figure 1).

This work was aimed to isolate lysozyme from egg and to compare the concentration, activity of the isolate with the standard (hen's egg white) lysozyme (Roy et al., 2003). The isolation was performed by Alderton et al. (1944) method and the elution of the lysozyme was high in acidic medium using 5% aqueous pyridine at pH 5.0. The activity of the isolated extract was determined by lysoplate assay where the substrate was incorporated in the nutrient agar medium. The sensitivity of lysozyme was determined by its ability to lyse the cell wall of substrate, which was observed as zone of clearance around the well. The diameter of the zone of clearance is directly proportional to the enzyme concentration. This assay gives a rough estimation about the activity of the enzyme, as the substrate used as live culture (Ghosh, 2003). The isolated extract was subjected to concentration by ammonium sulphate precipitation and partial purification by conventional dialysis method. After partial purification of the enzyme, it was subjected to sodium dodecyl poly-

acrylamide electrophoresis for the confirmation and determination of molecular weight of the isolated enzyme with the standard lysozyme as marker and molecular weight was determined 14.3 kDa.

The extraction of lysozyme from lovebird (*Agapornis* sp.) proves to be promising as it has tremendous application in various industrial sectors. This work of isolation, concentration, partial purification of the enzyme suggests that the activity of the enzyme was found to be effective using the cell wall of *M. lysodeikitus* as substrate. The molecular weight of the isolated enzyme (Lysozyme) was also determined as 14.3 kDa by SDS-PAGE along with protein marker (standard lysozyme).

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