Purification and Characterization of Lysozyme From the Egg Of Japanese Quail
*Coturnix coturnix japonica*

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Abstract
The present study, the quail egg lysozyme was extracted, purified and characterized for its secondary structure. The protein’s concentration was estimated by colourimetrically using BSA as a standard. Chromatography was performed with G-100 column to get the purest form of the enzyme. The extent of purity was confirmed by running native PAGE. The molecular weight of purified lysozyme was also determined by SDS-PAGE. Similarly, the sample was used for its secondary structure conformational studies using infra red (IR), the spectrum gave a peak at 1643.2 cm⁻¹ showing the existence of a helix. Also the same was further confirmed by standard plot. The conformational investigations have been carried out on lysozyme, in which measurements of the reduced molar ellipticities showed the presence of lysozyme, and observed negative circular dichroism (CD) band at 207 nm and a shoulder around 221 nm. Results were in agreement with standard ellipticity data. The conformational changes revealed by CD data measurements showed alpha helix conformations. All these studies were carried only after the protein was purified from quail egg white.

Keywords: *C. japonica*; Lysozyme; Sephadex G-100; IR and CD spectrum.

Introduction
Development of efficient and scalable strategies for separation of proteins particularly enzymes is especially relevant for those molecules that are used in the health and pharmaceutical sectors[1-4]. Such proteins need to be purified to a higher level of purity. This invariably means that chromatographic methods, with their inherently high resolution, are generally used. Lysozyme is a globular protein containing 129 amino acids that was discovered in 1922 by Alexander Flemming by accident. It was the first enzyme to have its entire three-dimensional structure determined with the aid of X-ray crystallography in the 1950’s[5]. There are three lysozymes in which the tertiary structures have been solved by X-ray crystallography[6-9]. Lysozyme constitutes approximately 3.5% of hen egg white[10]. Lysozyme is a part of innate immunity, which is a non-adaptive defence mechanism and acts as an early barrier to infectious diseases. It occurs in saliva, sweat and tears and acts by dissolving the cell walls of some Gram-positive bacteria[11-13]. This anti-infectious activity has been exploited for preparing tablets and capsules containing hen’s egg-white lysozyme[14]. It is also an important constituent of eye drops[15]. The shape of the amide I band of globular proteins is characteristic of their secondary structure. With a publication by the determination of secondary structures in proteins from FTIR spectra really started[16]. The CD spectra between 260 and approximately 180 nm can be analyzed for the different secondary structural types: alpha helix, parallel and antiparallel beta sheet, turn, and other. A number of review articles are available describing the technique and its application[17]. Modern secondary structure determination by CD are reported to achieve accuracies of 0.97 for helices, 0.75 for beta sheet, 0.50 for turns, and 0.89 for other structure types[18].

From the various studies undertaken on the enzyme lysozyme, almost always the source had been hen’s egg. Hence an attempt has been made to characterize the enzyme purified from quail egg white for finding the possible conformations of its secondary structure.

Materials and Methods
Quail eggs were purchased from Roselin Proteins, Tambaram. Lysozyme, the antibacterial enzyme was purified from quail egg white according to the protocol[19]. The concentration of purified lysozyme was determined by Bradford method[20]. The purified lysozyme is run for native PAGE and SDS-PAGE with marker protein (standard lysozyme was purchased from SRL, India) to determine the molecular weight of the purified sample. The purified sample is lyophilized in a Model LG-5 lyophilize and

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subjected to IR spectrophotometer to characterize the protein. To further characterize the protein its CD spectrum over a range of 180 to 240 nm determined using JASCO 715 model CD spectrophotometer.

**Results and Discussions**

The lysozyme purified from crude egg white extract from eggs of Japanese quail (*Coturnix coturnix japonica*) was chromatographed on a G-100 sephadex column. Among the collected 40 fractions, 10 fractions (23 to 33) showed maximum absorbance (Figure 1), which were pooled (active fractions), dialysed and freeze-dried. The dried powder was used for further study. The yield of lysozyme in the purified sample was found to be 12 µg/ml (Figure 2).

The native PAGE was performed for the active fractions. A single band is observed showing the presence of a single protein which was compared with the standard (Lane 1 & 2 - Standard, Lane 3 – Isolated lysozyme), which is shown in Figure 3.

The same active fraction was used to run a SDS-PAGE, which is shown in Figure 4. The lane 1 (L-1) and lane 2 (L-2) in the protein gel corresponds to marker proteins (standard lysozyme) and its corresponding molecular weight is predicted to be 14 kDa.

Lane 3 (L-3) and Lane 4 (L-4) correspond to the protein eluted from the column, the sample. The single band in lane 3 and lane 4 being equal in position to lane1 and lane 2 shows that the sample indeed is lysozyme and also a monomer.

The fraction was concentrated and obtained as a powder after lyophilization. The powdered sample produced IR absorption spectra, Proteins secondary structure determined from their IR data which indicates alpha helix, a peak at 1643.2, which is in accordance with standard data [22]. This was depicted in Figure 5.
The fractionated and purified sample was subjected to CD spectrometer at wavelengths ranging from 180 to 240 nm respectively. A plot of ellipticity with wavelength showed that at 207 nm and 221 nm there were two inverted peaks confirming the presence of alpha helix in the structure. The inverted peaks are represented in Figure 6.

Figure 5. Infra red spectrum of purified lysozyme

Figure 6. CD Spectrum of purified lysozyme
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