METHOD FOR THE PURIFICATION OF EGG-WHITE LYSOZYME

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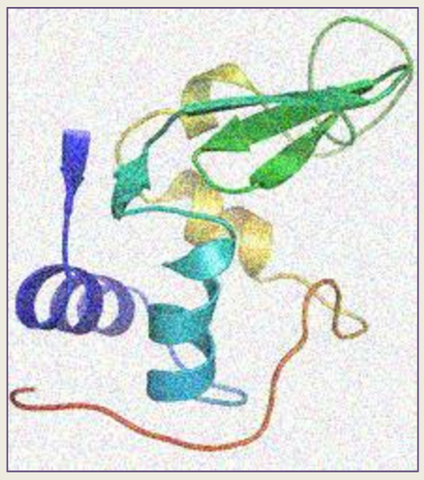


INTRODUCTION

The name of lysozyme, also know as muramidase (N-acetylmuramide glycanhydrolase, EC 3.2.1.17) is applied to the different components of a group of enzymes that catalyze the hydrolysis of β (1-4) glycosidic linkages of between N-acetylmuramic acid and N-acetyl-D-glucosamine residues in peptidoglycan, which is the major component of gram-positive bacterial cell wall. This enzyme is abundant not only in human secretions, such as tears, saliva and milk, but also in other vertebrates, invertebrates, bacteria, viruses and plants.

Among all the proteins that are included in the group of lysozymes, the most studied is that of chicken egg white. Egg white, also known as albumen, has a large amount of proteins of which about 50% is ovalbumin and only 3.4% of the remaining half is lysozyme.

Therefore, the objective of this study is the implementation of an effective method for the isolation and purification of lysozyme from the white of a mature egg.

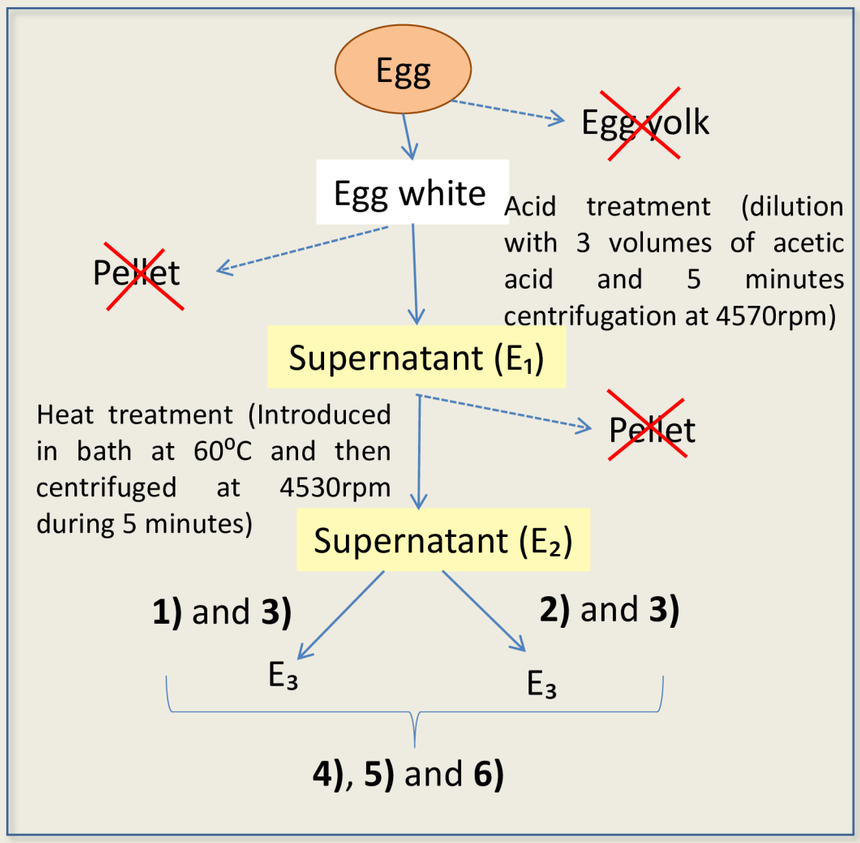
MATERIALS AND METHODS

1) SIZE EXCLUSION CHROMATOGRAPHY, SEC (Sephadex G-75). After calibration with acetic acid 0.1M, 1mL of E_2 was applied to the column and fractions of 3mL were collected. The absorbance at 280nm (A_{280}) of each fraction was then measured.

2) ION EXCHANGE CHROMATOGRAPHY, IEC (Amberlite CG-50). The E_2 sample was first prepared with the addition of 5 volumes of phosphate buffer 1M, pH7.0. It was then applied to the calibrated column (in phosphate buffer 0.1M),fractions of 10mL were collected and their absorbances (A_{280}) measured. When the values got close to 0, the buffer solution was changed to one 0.6M. Fractions of 3mL were collected and their absorbances (A_{280}) measured.

3) ENZYME ASSAYS. The ability of the enzyme to hydrolyze cell walls from *Micrococcus lysodeiktikus* was used to measure the activity of different fractions. Such activity was calculated with the formula:

$$\text{Activity (UAL/mL)} = (\Delta Abs_{450}/min) \times (1UAL/-0.001(\Delta Abs_{450}/min)) \times (1/0.3mL) \times \text{Dilution Factor}$$



4) DIALYSIS. E_3 fractions were dialyzed to change their solvent to phosphate buffer, and later concentrated using polyethilenglycol.

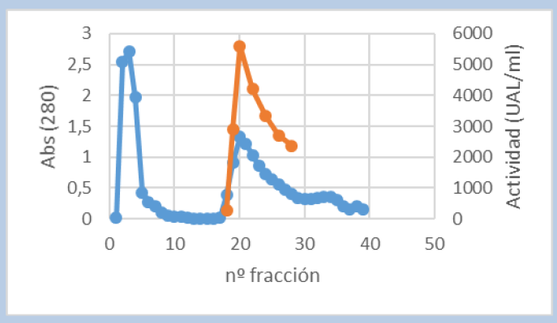
5) PROTEIN CONCENTRATION DETERMINATION. A calibration curve was drawn using the Bradford method [1]. The concentration of protein in samples E_1, E_2 and E_3 was later determined by interpolation.

6) ELECTROPHORESIS SDS-PAGE. Carried out following Laemmli´s method [2]. Used to determine lysozime´s molecular weight and the purity reached after the whole purification process.

RESULTS

1. ISOLATION OF LYSOZYME: ACID AND THERMAL TREATMENT. After submitting the egg white to acid treatment and thermal treatment, 15.5 mL of E1 and 14.0 mL of E2 samples were obtained, respectively. White precipitate was obtained by both of the methods.

2. SIZE-EXCLUSION AND ION EXCHANGE CHROMATOGRAPHIES. Figures show (in blue line) the elution profiles obtained for the same sample using by both methods. The orange lines correspond to the activity values obtained for the different fractions during the enzymatic assay.



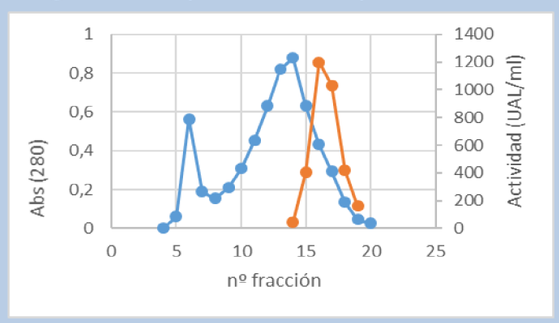


Figure 1. elution profiles for SEC (left) and IEC (right). Results show that the buffer with the lower ionic strength eliminates the contaminating proteins for IEC . The first peak in SEC corresponds to proteins of higher molecular mass than lysozyme.

3. ENZYMATIC ASSAY. For the SEC (left) E3 was formed by uniting 19-28 fractions showing a maximum lysozyme activity of 5600 UAL/mL. For the IEC (right) selected fractions were 17-19 containing a lysozyme activity of 1200 UAL/mL.

4. DIALYSIS. A total volumen of 2 mL of E3 sample was dialyzed for both methods, providing a final volumen of 600 µL of dialyzed E3.

5. PROTEIN CONCENTRATION DETERMINATION. BSA calibration curve determined a protein concentration of 14.4, 13.2 and 0.46 for E1, E2 and E3 respectively.

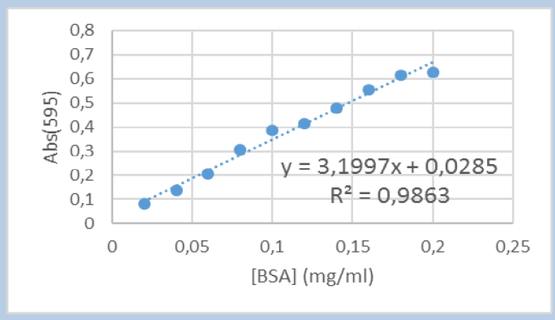


Figure 2. BSA calibration curve used to determinate protein concentration of E1, E2 and E3 fractions.

6. ELECTROPHORESIS.

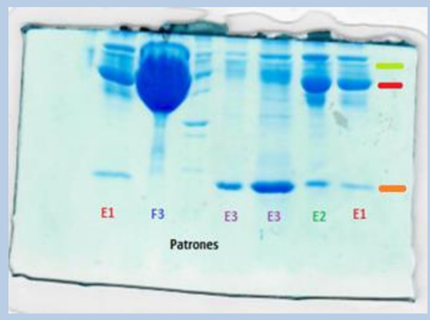


Figure 3. SDS-PAGE SEC electrophoresis gel

Table 1. Purification table for both types of chromatographies

E3	Act. (UAL)	Prot. (mg)	Sp. Act (UAL/mg)	Yield (%)	Purif. number
IEC	132 750	13.54	9 804	37	6.1
SEC	56 810	5.29	10 740	22	6.6

CONCLUSION

- Eliminating acid treatment from the purification method seemed to be a good way to improve the yield of the whole process. Furhtermore, since none of the chromatographies provided a fully purified lysozyme, combining both could allow us to obtain the highest purification possible with this procedure.
- Other measures that would enable getting a more purified lysozyme would be:
  -Making the fractions eluded from both chromatographies pass through cellulose beads before measuring their absorbances (A_{280}) [3].
  -Treating the lysozyme sample (E_2) with Sephadex G-75 and a basic solution of NaCl (pH 8,0) before the chromatography, then carrying it out using a more basic and concentrated solution for elution[4].

REFERENCES

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[2]- U.K. Laemmli (1970). Cleavage of structural proteins during the assembly of the head of Bacteriophage T4. Nature Vol.227, pp. 680-685.

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