LYSOZYME PURIFICATION FROM HEN EGG WHITE

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2. INTRODUCTION

Lysozyme is an enzyme which catalyzes the hydrolysis of β glycosidic bonds (1-4) in polysaccharides of bacterial cellular walls. More specifically, it has β (1-4) glucosaminidase activity on *N*-Acetylglucosamine and *N*-Acetylmuramic acid. It was discovered by Alexander Fleming[1] in 1922 and it can be found in human secretions like saliva, tears, mucus, swear,etc. It is a part of the human innate immunity system. It is also present in other vertebrates, invertebrates, bacteria, virus and plants.

In this experiment the enzyme has been purified using different methods: ion exchange and size exclusion chromatography, enzymatic assay, Bradford's method[3] (to determine protein concentration) and electrophoresis. The two chromatographies were compared to see which one was the most efficient way to purify lysozyme.

Lysozyme's main traits are: its basicity (pI: 10.5-11), its low molecular mass (14-30 KDa), its stability in acid pH and its activity on *Micrococcus lysodeikticus*[2].

Finally, the objective is to propose a purification method of lysozyme.

1. METHODS

2.1 Purification of lysozyme

- Preparation of enzyme extract (E1): Separate yolk from egg white and dilute it in acetic acid 0.1 M. Filter the result and centrifuge it. Separate sediment from supernatant (the supernatant is E1).
- Thermal treatment (E2): Incubate E1 in a 60ºC water bath for 5 minutes and cool it in ice for another 5 minutes. Centrifuge and keep the supernatant, which is E2.
- Ion Exchange chromatography (E3): Amberlite CG-50 is the resine used. Potassium phosphate buffer 0.1 M pH 6.6, potassium phosphate buffer 1 M pH 7.0 and potassium phosphate buffer 0.6 M pH 6.6 are used as the buffers.
- Size exclusión chromatography (E3): Sephadex G-75 and acetic acid 0.1 M is used.

2.2 Enzyme Assay

The absorbance at 450 nm during the enzyme's activity on Micrococcus lysodeikticus is measured every 30 seconds for 3 minutes.

2.3 Protein concentration determination (Bradford's method)

0.3 mL of E1, E2 and E3 are prepared. Absorbance is measured at 595 nm. A standard curve is drawn using different concentrations of BSA (within a range of 0.02-0.2 mg/mL).

2.4 Electrophoresis (Enzyme's molecular mass determination)

Following Laemmli's method[4], an electrophoresis is performed. A standard curve is drawn to determine the molecular mass.

3. RESULTS

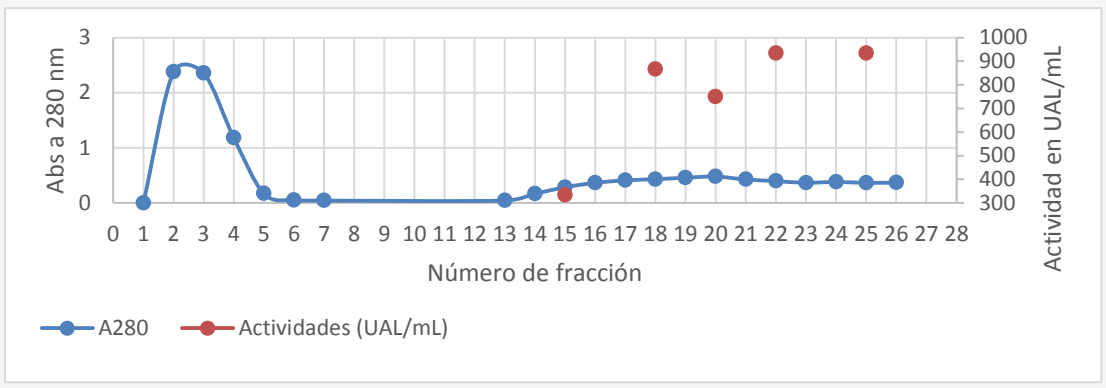

FIGURE 1. Chromatographic profile and enzyme activity for ion exchange chromatography. E3 has been obtained by putting together those fractions with the highest activity and quantity of protein (same protocol for size exclusion chromatography).

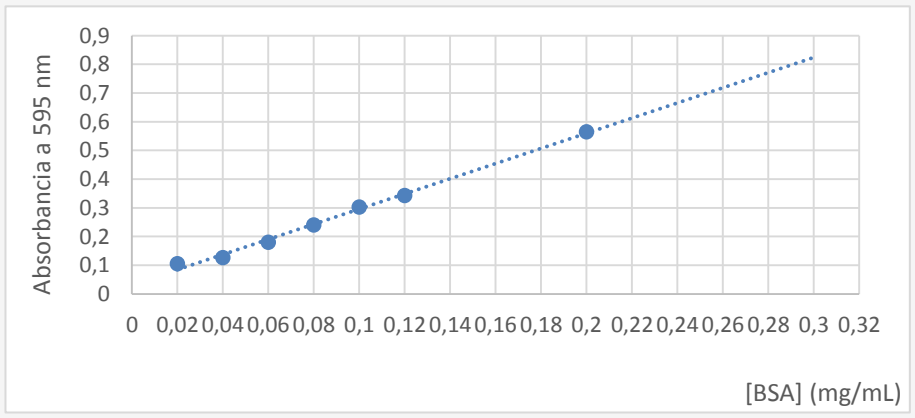

FIGURE 2. Standard curve of BSA. The values obtained by interpolation are: E1=95.2 mg/ml, E2= 42.8 mg/ml and E3= 0.062 mg/ml.

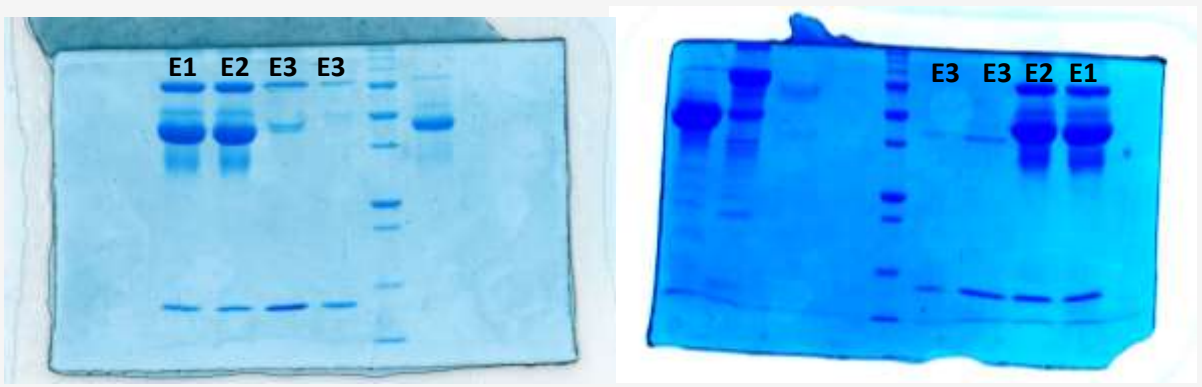

FIGURE 3. Resulting gels from ion exchange (right) and size exclusion chromatographies (left). Between E1 and E2 there's no difference of composition. E1 and E2 present three principal stains: the heaviest is ovotransferrin, the medium-heavy is ovalbumin and the lightest is the lysozyme. E3 present similar stains in both gels, similar level of purification.

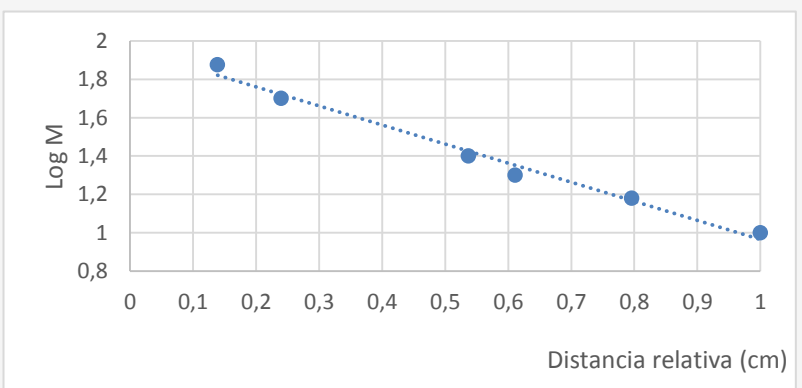

FIGURE 4. Standard curve for electrophoresis, used to calculate the molecular weight of: lysozyme (12.23 kDa), ovotransferrin (47.31 kDa) and ovalbumin (63.06 kDa).

	Tot Act E1 (UAL)	Tot Act E2 (UAL)	Tot Act E3 (UAL)	Tot Prot E1 (mg)	Tot Prot E2 (mg)	Tot Prot E3 (mg)	Specific activity E1 (UAL/mg)	Specific activity E2 (UAL/mg)	Specific activity E3 (UAL/mg)	Yield E1 (%)	Yield E2 (%)	Yield E3 (%)	Purific. E1	Purific. E2	Purific. E3
Ion exchange	154667	93600	24650	258	154	3	599	606	7302	100	61	16	1	1	2
Size exclusion	116000	105170	26400	171	150	5	678	703	5280	100	91	23	1	1	8

FIGURE 5. Purification table of both types of chromatographies.

4. CONCLUSIONS

- Thermal treatment has no effect on the enzyme's purification.
- No chromatography is better than the other one. Maybe if both of them are put together, starting with ion exchange to have more enzyme quantity and then size exclusion to separate it from other components, is a solution.
- One way to purify lysozyme is extracting the enzyme from the shell instead of the egg with since there are less proteins in the shell. The shell's membrane has to be dissolved carefully and then with a centrifugation, supernatants are obtained and dialyzed to separate the enzyme from other components[5].

(1)Fleming A. On a remarkable bacteriolytic element found in tissues and secretions. Proc Roy Soc Ser B 1922;93:306-17, (2) Morsky P. (1983). Turbidimetric determination of lysozym with Micrococcus lysodeikticus cells: reexamination of reaction conditions. Anal. Biochem. 128, 77-85, (3) Bradford M (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72. 248-254, (4) Laemmli U.K. (1970) Cleavage of structural proteins during the assenbly of the head of bacteriophage T4. Nature 277, 680-685, (5) Ariga H et al. (2014) Rapid and Simply Purification of Lysozyme from Egg Shell Membrane. J Nutri Sci Vitaminol, 61, 101-103.