PURIFICATION OF HEN EGG-WHITE LYSOZYME

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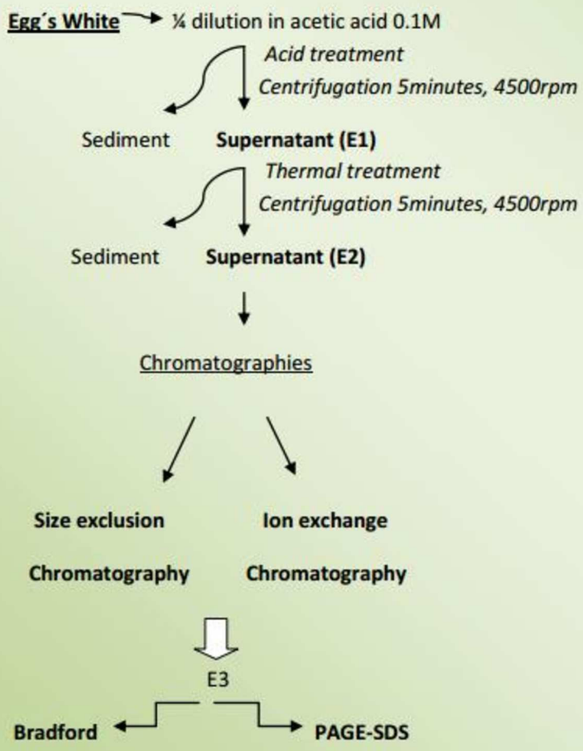
1.INTRODUCTION

The objective of this study is to find an efficient method of purification of lysozyme, an enzyme which biological function is to catalyze the hydrolysis of glycosidic bonds β(1-4) in polysaccharides of bacterial cell walls. This enzyme was discovered by Alexander Fleming (1) in 1922 and it is found in animals and plants. It can be found too in human secretions like saliva, tears or swear.

The purification of egg´s white lysozyme was achieved using different methods: first, an acid treatment followed by a thermal treatment. Then, an exclusion chromatography with G-75 Sephadex and an ionic exchange chromatography were carried out in order to compare its results. To determinate the activity of lysozyme an enzymatic assay was carried out, and to determinate its concentration Bradford´s method (2) was used. Finally, a PAGE-SDS electrophoresis was accomplished to analyze the composition of proteins in the samples obtained after both chromatographies. A purification table was elaborated in order to see if the process followed is an efficient way to purify lysozyme.

2.METHODOLOGY

Starting from a hen egg and separating the yolk from the white, the procedure was next:



2.1 PURIFICATION STAGE: *dilute the egg´s white ¼ in acetic acid 0.1M. Filter and centrifuge it. Supernatant is E1, that is incubated in a 60ºC water bath for 5 minutes and then cooled in ice. Centrifugation. Supernatant is E2.*

2.2 CHROMATOGRAPHIES: *Size exclusion- G-75 Sephadex is the support and buffer is acetic acid 0.1M. Ion exchange-Amberlite CG-50 is used as resine. Buffers needed are: potassium phosphate buffer 0.1M and 0.6M, pH 6.6 and potassium buffer 1M, pH 7.0.*

2.3 ENZYMATIC ASSAY: *measuring absorbance of the sample at 450nm every 30 seconds for 3 minutes. Knowing that the activity of Micrococcus lysodeikticus is 0% of absorbance.*

2.4 BRADFORD´S METHOD (2): *absorbance at 595 nm of 0.3 mL of E1, E2 and E3 is measured and interpolated in the previous drawn standard curve using different concentrations of BSA (between 0.02-0.2 mg/mL)*

2.5 ELECTROPHORESIS: *using Laemmli´s method (3) it is performed. A standard curve is built to determinate the molecular mass of the sample.*

3. RESULTS

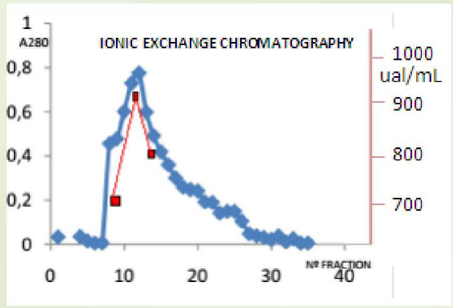


FIGURE 1. Elution profile of ionic exchange chromatography. The peaks that correspond to fractions 13, 14 and 15 (E3) were used for Bradford's method and electrophoresis.
In red, activity obtained in the enzymatic assay.

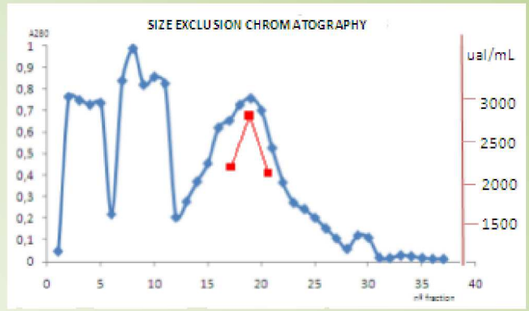


FIGURE 2. Elution profile of size exclusion chromatography. The peak that corresponds to purified lysozyme is fraction 20. In red, activity obtained in the enzymatic assay.

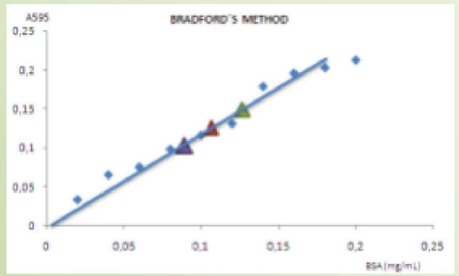


FIGURE 3. Standard calibration curve to determinate protein concentration on each extract. In green, E1= 5.24 mg/mL, (10µL). in red E2= 6.85 mg/mL, in purple E3= 0,09 mg/mL.

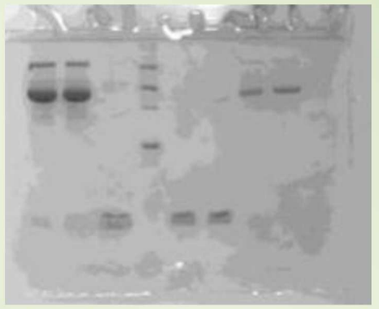


FIGURE 4. SDS-PAGE gel electrophoresis (15% acrilamide). E3 in wells 4, 6 and 7 (15µL), in well 5 MW standard proteins E1 in wells 2 and 8, E2 in wells 3 and 9 (15µL).

TABLE 1. Purification table of both types of chromatographies made.

Chrom.	UAL E1	UAL E2	UAL E3	mg E1	mg E2	mg E3	UAL/mg E1	UAL/mg E2	UAL/mg E3	Y% E1	Y% E2	Y% E3	Purif E1	Purif E2	Purif E3	MW (KDa)
IONIC	117200	115800	57600	83.84	102.75	0.58	1398	1127	99310	100	99	49	1	0.8	71	13.18
SIZE	127500	116100	105000	147	141	7	867	826	15556	100	91	82	1	0.95	18	12.02

4.CONCLUSIONS

The results show that thermal treatment has no effect on the purification, so it could be eliminated.
Both chromatographies are effective, but in ionic Exchange chromatography the purification is higher, so it is better for our study.
The process could be improved by connecting both chromatographies to obtain better results, both in acid conditions. It could also be improved by taking lysozyme from the shell instead of the egg white, so that we find less contaminant proteins (4).

5.REFERENCES

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