ENZYME PURIFICATION: LYSOZYME FROM HEN'S EGG WHITE

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INTRODUCTION

The lysozyme (N-acetylmuramide glycanhydrolase; EC 3.2.1.17), which was discovered by Alexander Fleming [1] in 1922, is an enzyme present in the hen's egg white. It is a globular, basic protein composed by a single polypeptide chain with a molecular weight between 14-30 kDa. This enzyme shows capacity to lyse the bacterial walls by catalyzing hydrolysis of 1,4-beta-linkages between N-acetylmuramic acid and N-acetyl-D-glucosamine, components of peptidoglycan.
This article describes a full purification method for lysozyme from hen's egg white, using the main protein characteristics.

METHODS

1. **Acid treatment (E1):** Hen's egg white was diluted with acetic acid and then filtrated. After that, it was centrifuged, obtaining the supernatant E1.
2. **Heat treatment (E2):** E1 was incubated at 60ºC and then centrifuged, obtaining the supernatant E2.
3. **Size exclusion chromatography in Sephadex G-75:** E2 was subjected to a size exclusion chromatography, picking fractions of 2 mL and measuring their A_{280}.
4. **Enzymatic assay (E3):** The lysozyme activity was measured using a solution of *Micrococcus lysodeikticus* walls [2]. This assay was done in order to establish the lysozyme position in the chromatogram, determining the enzymatic activity of those fractions with high values of A_{280}. E3 was obtained by mixing the fractions wich showed high activity. After that, the enzymatic activities of E1, E2 and E3 were determined.
5. **Protein concentration determination:** The protein concentration of E1, E2 and E3 was resolved following the Bradford method [3].
6. **SDS-PAGE electrophoresis:** The purity grade achieved in E1, E2 and E3 was evaluated following the Laemmli method [4].

RESULTS

Isolation of lysozyme: After the acid treatment, 15 mL of supernatant E1 were obtained. 13,5 mL of supernatant E2 were obtained after the heat treatment.

Lysozyme position in the chromatogram: With the values of A_{280} of the fractions, a chromatogram was done. The lysozyme activity was plotted in the same graphic (**figure 1**). The fractions 25-28, which showed high activity, were mixed to obtain E3. The final volume of E3 was 5,7 mL.

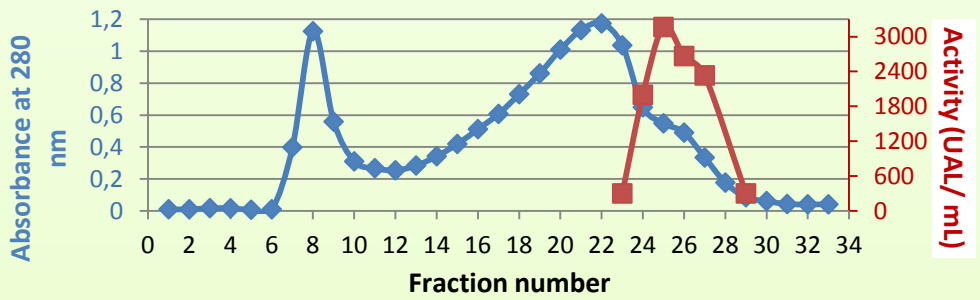

Figure 1: Lysozyme position in the chromatogram

Enzymatic activity of E1, E2, E3: The activities of E1, E2, E3 were calculated in UAL/ mL, obtaining 24166,67 UAL/mL for E1, 25833,33 UAL/mL for E2 and 2333,33 UAL/mL for E3.

Protein concentration of E1, E2, E3: The protein concentration was determined by interpolating the A_{595} of E1, E2, E3 in a calibration curve (**figure 3**) which was previously done with known BSA concentrations.

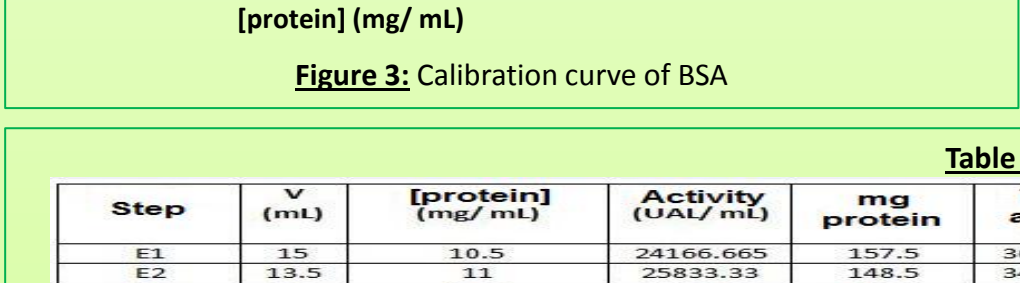

Figure 3: Calibration curve of BSA

Molecular weight of lysozyme and other proteins: A calibration curve was done using the logarithm of the molecular weights and the migration distance of protein markers. The molecular weight of the principal proteins presents were obtained by interpolating the migration distances of the most significant bands.

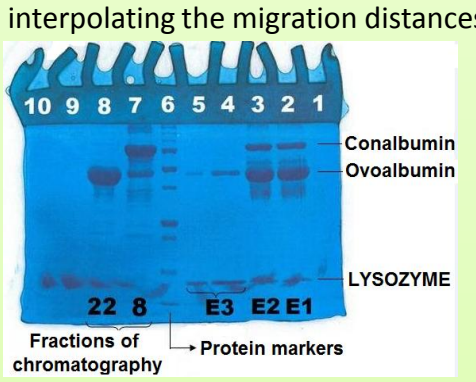

Figure 4: Electrophoresis gel

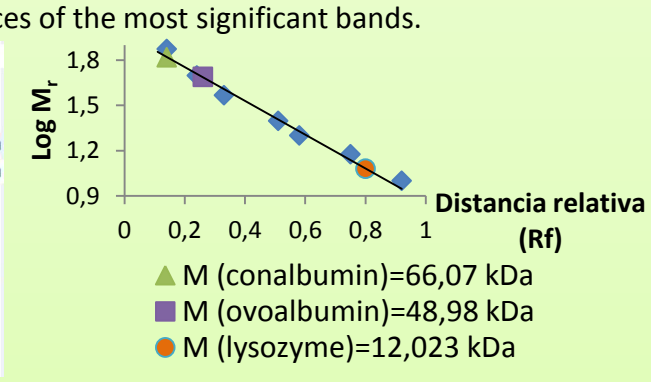

Figure 5: Calibration curve done with the protein markers

Table of purification

Step	V (mL)	[protein] (mg/ mL)	Activity (UAL/ mL)	mg protein	Total activity (UAL)	Specific activity (UAL/ mg)
E1	15	10.5	24166.665	157.5	362499.98	2301.59
E2	13.5	11	25833.33	148.5	348749.95	2348.48
E3	5.7	0.03	2333.33	0.171	13299.98	77777.66
E3*	57	0.03	2333.33	17.1	132999.8	77777.66

Step	Yield (%)	Purification (fold)
E1	100	1
E2	96.21	1
E3	3.67	34
E3*	36.7	34

E3*: This line was introduced to compare the results with those obtained by ionic interchange chromatography

CONCLUSION

Although, as can be seen in the table, the purification process has been successful (lysozyme has been purified 34 times), some changes could be introduced in order to improve this method and obtain better results:

- Heat treatment (E2) could be eliminated because this step does not purify the lysozyme: E1 and E2 show similar results, and the purification does not increase.
- With the size exclusion cromatography, the major part of proteins have been eliminated, obtaining the lysozyme purified 34 times and a high value of specific activity in E3. The problems of this step are its low yield and its incapacity to eliminate all the ovoalbumin.
- Comparing the results with those obtained by ionic interchange chromatography, the purification and activity are better in the first, but the second provides a higher yield and more amount of protein. Furthermore, ionic interchange chromatography eliminates basically all the ovoalbumin but not conalbumin.
- Combination of both types of cromatography could be another improvement that could be introduced in the purification process.

REFERENCES

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