Enzyme Purification: Hen Egg White Lysozyme

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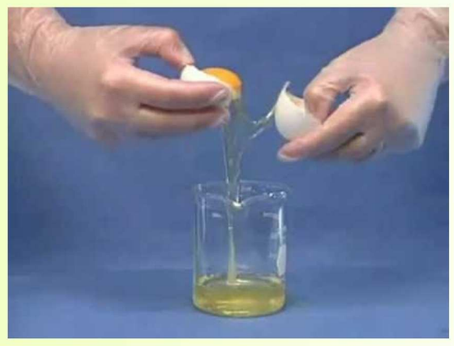

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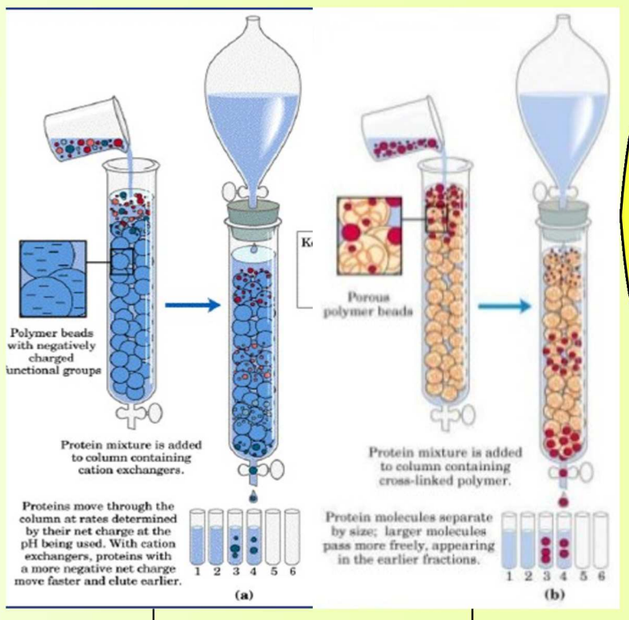
1. Abstract

Some purification and isolation methods have certain advantages in respect of other methods. Lysozyme or muramidase is an enzyme that can hydrolyze glycosidic bonds β 1-4 of bacterial cell wall polysaccharides, such us the one on this experiment, *Micrococcus lysodeikticus*. It is widespread in tears, saliva, blood serum, human and cow milk and avian egg whites, it has a basic pI, stable at acid pH, and low molecular weight. This characteristic of the enzyme has allowed us to propose a method that we can use to isolate and purify the lysozyme from chicken egg white. They were used an acid and heat treatment before the two methods to compare: ion exchange and molecular exclusion chromatography. After this process, it is needed to determine the activity and the amount of protein of the different fractions obtained in the purification process to establish with precision that with the ion exchange chromatography we obtained a better purified lysozyme than with the molecular exclusion chromatography. Finally, results were confirmed in a SDS-PAGE electrophoresis.

2. Introduction

Lysozyme or muramidase (peptidoglycan N-acetylmuramyl hydrolase) is an enzyme that catalyzed the hydrolysis of β 1-4 glycosides linkages of bacterial cell wall polysaccharides, specifically of N-acetylglucosamine (NAG) and N-acetylmuramic (MUR). It was the first sequenced enzyme, the first of which a three-dimensional model was arranged by X-ray crystallography and from which a catalytic mechanism was proposed. It has a crucial ability against bacterial, virus and fungi infections. Its molecular weight is 14-15kDa and the isoelectric point is around 10-11, and the methods for the lysozyme purification are based in these characteristics. Fleming and Wolff found some substances and matrices capable to absorb the enzyme, and the elution efficiency is affected by pH. On one hand, the molecular exclusión chromatography allows different size proteins to pull apart by passing through a cylindrical column containing as stationary phase retained liquid inside a matrix formed by a gel with pores of controlled diameter. On the other hand, ion exchange chromatography has a stationary phase with charged groups that interact electrostatically with ions of the opposite sign in the mobile phase. In this experiment, we are going to use the one with a negative stationary phase and the lysozyme with positive charge, so the enzyme stays attached at the stationary phase. The main objective in this experiment is to compare which of the two methods proposed, ion exchange and molecular exclusion chromatography, in better to carry out optimally the isolation and purification of the lysozyme.



Ion Exchange Chromatography

VS

Molecular Exclusion Chromatography

E3

E3'

WHICH IS A BETTER METHOD TO PURIFY THE LYSOZYME?

3. Materials and methods

As biological source a hen egg white from a supermarket was used. First, an acid treatment was executed with acetic acid 0,1M to remove not-resistant proteins to acidity and we obteined E1. Secondly, we realized a heat treatment at a thermostat during 5 minutes at 60ºC to remove not-resistant proteins, and we obtained E2. At the end of both treatments, samples were spined-dry at 4500rpm during 5 minutes. Next, two chromatographies were realized, so we could obtained E3 and E3', one for each chromatography. Lysozyme activity in UAL/ml, concentration in molarity and an electrophoresis to compare results were realized.

3.1 Ion exchange Chromatography

Amberlita C6-50 column was used, equilibrated with 100ml of 0,1M potassium phosphate buffer pH 6,6. We prepared sample E2 with 1M potassium phosphate buffer. To wish 120ml of 0,1M potassium phosphate buffer pH 6,6 were needed and 10ml fractions were collected. To the elution state 60ml of 0,6M potassium phosphate buffer pH 6,6 so salt concentration increses and 3ml fractions were collected. We measured absorbance at 280nm of the fractions and E3 was obtained and dialyzed.

3.2 Molecular exclusion chromatography

Sephadex G-75 (fractionation interval 3000-80000Da), equilibrated with acetic acid 0,1M, calibrated with dextran blue and potassium ferricyanide. We collected fractions of 2ml and its absorbance at 280nm was measured. We obtained E3' and it was dialyzed.

3.3 Activity Assay

It is known that lysozyme can lyse bacterial cell walls, so a cell wall dissolution prepared of *Micrococcus lysodeikticus* was used to determine the enzyme activity in UAL/ml, lysozyme activity units per mililitre. Abosrbance at 450nm was measured and it is proportional to the activity of the enzyme.

3.4 Protein concentration

The concentration of protein was stablish by Bradford method.

3.5 SDS-PAGE electrophoresis.

SDS-PAGE electrophoresis (denatured conditions) was realized using Laemmli method with the fractions obtained: E1, E2 and E3/E3' dialyzed, the peaks from each chromatography and with the molecular weight pattern. To see the results and compare, the gel was dyed with Coomassie R-250.

4. Results and discussion

4.1 Purification

After acidic and thermal treatments, lysozyme was recuperated intact due to its basic character and its great thermal stability. E1 showed a volume of 15,5ml and E2, of 12ml. Figures 1 and 2 represent both chromatographic elution profiles and enzimatic activity profiles, from ion exchange and size exclusión chromatographies, respectively. En figure 1, fractions from 159ml to 207ml displayed the máximum activity, which leads to conclude that lysozyme is in those fractions, collected to form E3 (V=55ml). From figure 2, it may be concluded that lysozyme is in fractions from 46ml to 56ml (VE3'=12ml).

4.2 Lysozyme Activity

Once E1, E2 and E3/ E3' were obtained, their enzimatic activity was measured. Results for each sample are shown in the purification table 1.

4.3 Protein Concentration

Based on a patron elaborated with BSA, protein concentración was determined for each sample E1, E2, E3, E3', resulting in the values shown in the table 1 below.

4.4 Electrophoresis

Electroforesis (Figures 3 and 4) were run with a sample of E1 (lanes 3.1, 4.1), E2 (lanes 3.2, 4.2), E3 (lane 3.3), E3' (lane 4.3) and a maximum of absorbance from chromatographies with no enzimatic activity (lanes 3.5, 4.6). Lanes with maximum absorbance and no enzimatic activity do not contain lysozyme, but other proteins found in hen egg– white. It was certified that E3 and E3' were much more purified than E1 and E2 and lysozyme had a molecular mass of 12,023kDa, aprox. It was also concluded that ion exchange chromatogrphy offered a higher purification degree, with less contamination from other proteins.

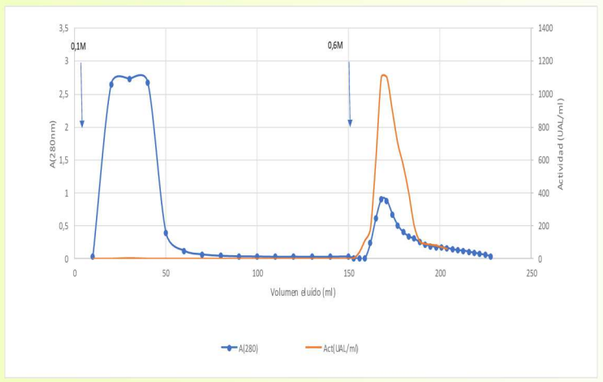


Figure 1. Ion exchange chromatography elution profile and enzimatic activity profile.

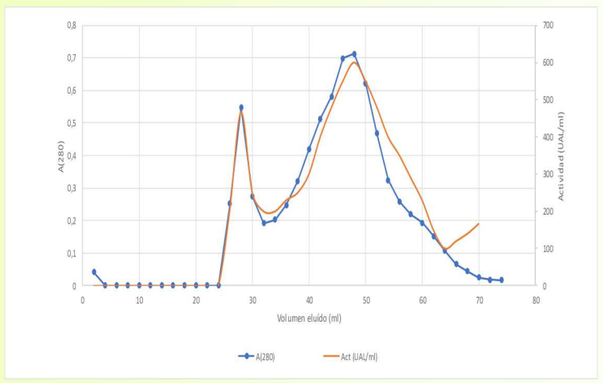


Figure 2. Size exclusion chromatography elution profile and enzimatic activity profile.

5. Conclusions

The explained methods allow us to purify lysozyme from hen egg– white and compare the degree of purification obtained for each of them, although further experiments could be developed to obtain a higher purification degree.

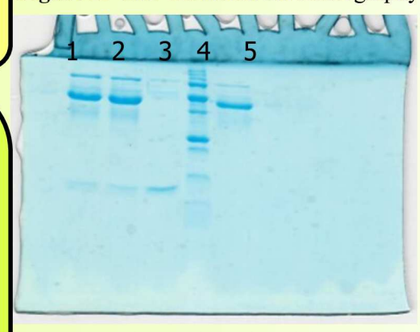


Figure 3. Electrophoresis gel (IE)

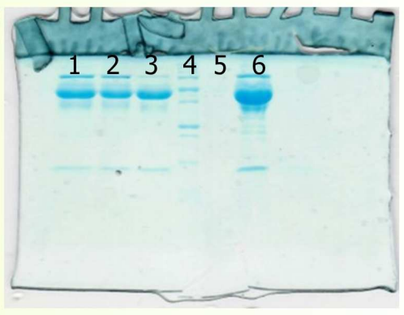


Figure 4. Electrophoresis gel (SE)

6. References

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Table 1. Purification table

	Steps	Total volumen (ml)	[Protein] (mg/ml)	Total prot. (mg)	Total Act. (UAL)	Act. (UAL/ml)	E.A. (UAL/mg)	Efficiency (%)	Purif. (no. times)
Ion Exchange chrom.	**E1**	15,5	11,2	173,6	164300	10600	946,43	100	1
	E2	12	14	168	117199,98	9766,66	697,62	71,33	0,7371
	E3	55	0,22	12,1	22916,3	416,66	1893,909	13,95	2,001
Size exclusión chrom.	**E1**	15,7	16,2	254,34	52332,81	3333,3	205,76	100	1
	E2	15,5	19,4	300,7	51666,15	3333,3	171,82	98,73	0,835
	E3'	12	0,55	95,7	81235,38	466,87	848,85	10,71	4,125