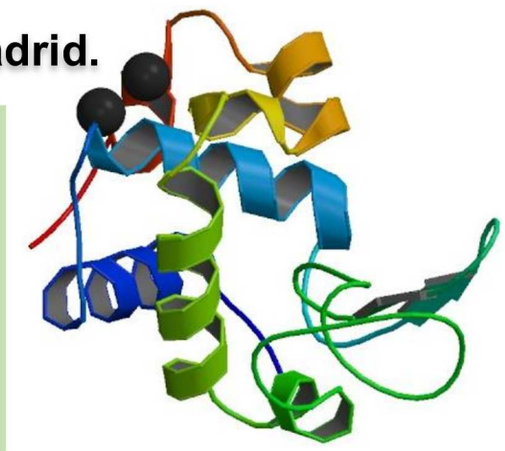
HEN EGG WHITE LYSOZYME PURIFICATION METHOD.

Paula Polonio, Icíar Polo

Laboratory of biochemistry and molecular biology. Universidad Complutense of Madrid.



ABSTRACT

Lysozyme catalizes the hydrolysis of the β (1-4) bond between N-acetylglucosamine and N-acetylmuramic, main components of gram positive bacterial wall. Due to its antibacterial properties it has several applications and must be purified. We try to propose a method of purifying hen egg white lysozyme. We carry out a thermal treatment and precipitation, and two different cromatographies, size exclusion and ion exchange. After an enzime assay and a PAGE-SDS we conclude that thermal treatment should be eliminated, and that none of the methodes purifies lysozyme by its own. Ion exchange must be carried out first, followed by size exclusion in order to obtain a better purification.

INTRODUCTION

Lysozyme is characterized by carrying out the hydrolysis of peptidoglycan, main component of gram positive bacterial wall. Because of its antibacterial properties, this enzyme has been employed in medicines, in food preservatives etc.

Henn egg white lysozyme has 129 aminoacids, a molecular mass of 14 kDa and an isoelectric point of 9.32. At physiological pH it is positively charged, which means that it has basic character. This enzyme makes up only a 2-3% of the total protein amount of the hen egg white.

The objective of this experiment is to propose a purification method for hen egg white lysozyme, based on the results obtained in the isolation of this enzyme that was carried out.

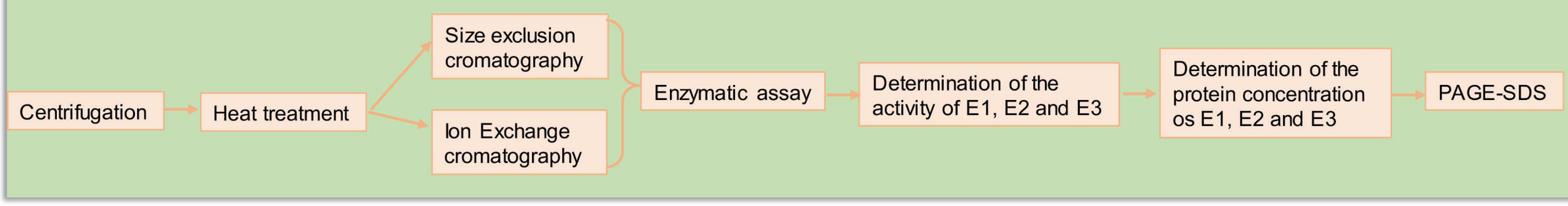
MATERIALS AND METHODS

Materials: Brown hen egg, bacterial walls suspension (Micrococcus lysodeikticus), Bradford reagent, bovine serum albumine (BSA) solution, CG-50 amberlite and Sephadex G-75.

Methods: Hen egg white was diluted 1/4 in acetic acid and then centrifugated, obtaining a sediment and supernatant (E1). E1 was incubated for 5 minutes at 60ºC and centrifugated again, obtaining a sedment and a supernatant (E2). E2 was employed in an ion exchange cromatography and a size exclusion cromatography (Figures A and B). The fractions collected from each cromatography were subjected to an enzymatic assay to determine their activity. The enzymatic assay was based on the capacity of lisozyme to hydrolyze Micrococcus lysodeikticus walls. As lysozyme hydrolyzed the walls, the aparent absorbance (turbidity) decreased. For each cromatography, the fractions with higher activity were put together to form E3 (one E3 for each cromatography). In the Sephadex cromatography, E3 was formed following a purity criterion, where only the less contaminated fractions were put toghether. After that, the enzymatic assay was carried out again to determine the activity of aliquots of E1, E2 and both E3 (Figure C).

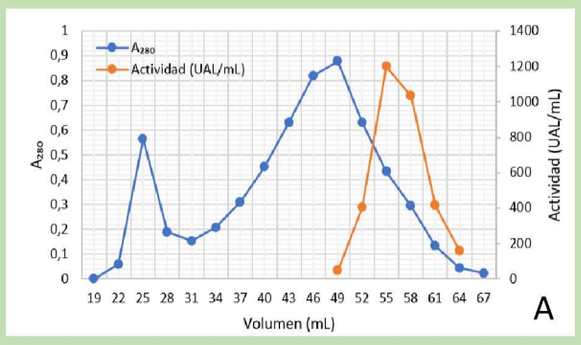
To determine the protein concentration of aliquots of E1, E2 and E3, a BSA calibration curve was built using the Bradford method (Figure G), and the concentration of each aliquot was interpolated.

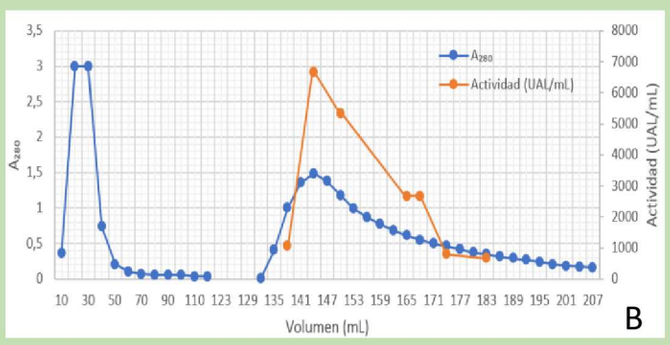
Eventually, to determine the purity of E1, E2 and E3, a PAGE-SDS was performed.



RESULTS

1. Purification and enzymatic assay





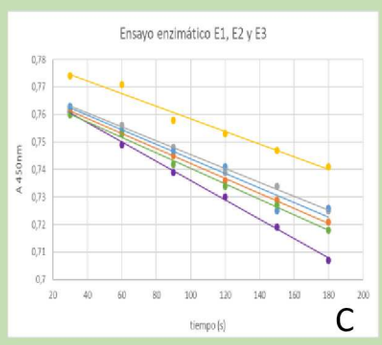


Figure A: chromatogram of the size exclusion chromatography together with the corresponding activity variation. There are two absorbance peaks and a displaced peak of activity. peak that coincides with the maximum activity.

Figure B: chromatogram of the ion exchange chromatography again with the corresponding activity variation. In this case, it is shown an initial peak of absorbance generated by washing the column, and a second peak, corresponding to lysozyme and the proteins that have a similar isoelectric point.

Figure C: Representation of the absorbance slopes for the calculation of the enzymatic extracts activities.

2. Determination of protein concentration

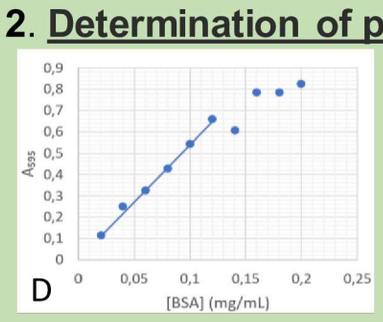


Figure D: Calibration curve with BSA as standard. The linear zone is represented by a line.

3 Purification table (Table 1)

In the following table, the progress in the purification process is shown. The results obtained for E3 can be compared for the two chromatographic methods.

Step	V total (ml)	Total protein (mg)	Total activity (UAL)	Specific activity (UAL/ml)	Yield (%)	Purification (nº of times)
E1	16	158.4	256000	16000	100	1
E2	14.5	165.3	141133.6	9733.3	55.13	0.53
E3 sephadex	7.3	5.29	56809.7	536.7	22.2	6.64
E3 amberlita	29	18.97	90866.6	4791	37.91	7.21

4. Electrophoresis

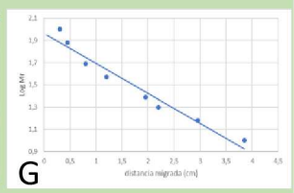


Figure E: Gel of the Sephadex column. Application in rails: 2(E1), 3(2º max absorbance), 4(1º max absorbance), 5(standards), 6(E3,1 µg), 7(E3, 0.25 µg), 8(E2), 9(E1).

Figure F: Gel of the Amberlita column. Application in rails: 2(E1), 3(E2), 4(E3, 9.6 µg), 5(E3, 2.4 µg), 6(standars), 7(peak of the chromatographic wash).

Figure G: Calibration curve from molecular masses and migration patterns.

DISCUSSION AND CONCLUSION

The data that offer an idea of the degree of purification obtained in the different enzymatic extracts can be compared in the purification table (Table 1). It shows that, in the case of both Amberlite and Sephadex, there is a purification of E3 respect to the initial extracts. However E2, result of the heat treatment, shows no improvement on the degree of purification, moreover it decreases performance.

On the other hand, the electrophoresis (PAGE-SDS) gels (Figures E and F) show some initial extracts in which several protein bands are presented, from which the lysozyme band can be distinguished as the one that is intensified when going from initial E3 to a more concentrated E3.

Observing the gels obtained by SDS-PAGE, it can be concluded that, through the two chromatographic methods is possible to eliminate most of the contaminating bands, thus purifying the lysozyme. However, none of the cromatographies completely purificates lysozyme.

Sephadex completely eliminates one of the two main contaminants and a higher degree of purification is obtained. However, the yield is much lower than the one obtained with Amberlite because of the purity criterion followed to form E3 for Sephadex. In the Amberlite cromtography, the purification was worse as we can se in the gel (Figure F), where there are more contaminant bands than for Sephadex, however, the yield was higher.

These conclusions lead to the proposal of a more efficient method that omits the thermal treatment and combines the two chromatographic methods, starting with the Amberlite to get a higher yield, and then the Sephadex to obtain a better purification.

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