Purification of lysozyme from hen white egg

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INTRODUCTION

Lysozyme (EC 3.2.1.17) is a hydrolytic enzyme that cleaves the β-(1,4)-glycosidic bond between N-acetylmuramic acid and N-acetylglucosamine in peptidoglycan, a major bacterial cell wall polymer (1). Lysozyme occurs in almost all body fluids, and tissues of animal organisms. Lysozyme from hen egg white is a small (14KDa) and strongly basic protein, that is stable at low pH and at high temperatures (75ºC) (2).

OBJECTIVE

To determine an effective method to purify the lysozyme from the hen egg white.

MATERIALS & METHODS

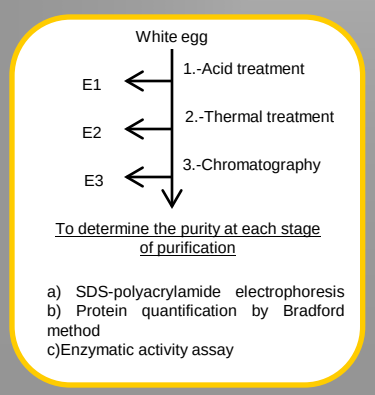
The egg white was separated from the yolk, and then:

1. Protein precipitation: An acid treatment followed of a thermal one was applied to the egg white.

2. Chromatography: Supernatant from 1, was injected (A) on an Amberlite CG-50 filled column, ion-exchange chromatography, or (B) on a Sephadex G-75 filled column, gel-filtration chromatography; with the aim to determine the efficiency of each method.

The purity of the samples at the different steps were measured using an enzymatic activity assay (3), and an analysis by SDS-PAGE electrophoresis. Protein was quantified by the Bradford method

PURIFICATION DIAGRAM



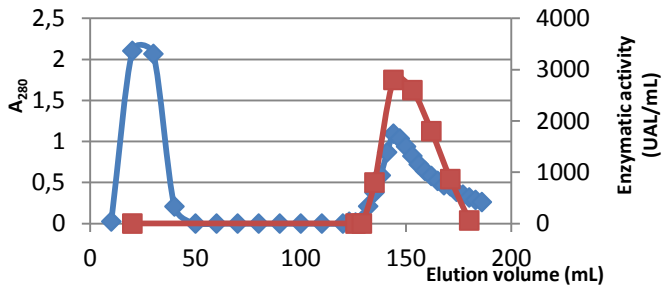
RESULTS

1.- Acid and thermal treatment

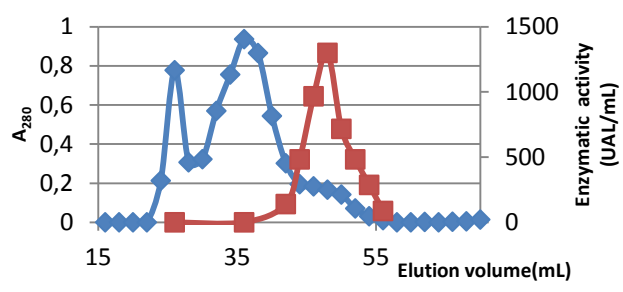
Egg white was treated with 0.1M acetic acid, for 5 min at room temperature. Proteins that weren't stable at acid pH precipitated. Thus, after centrifugation (in duplicated) a white sediment and supernatants (E1a = 16 mL and E1b = 15 mL) were obtained. Then, thermal treatment of these supernatants (40ºC for 10 min) also produced a white sediment due to protein denaturalization and the supernatants: E2a = 15 mL and E2b = 14 mL.

2.- Representations of the chromatographies

A. Ion-exchange chromatography (Amberlite CG-50)



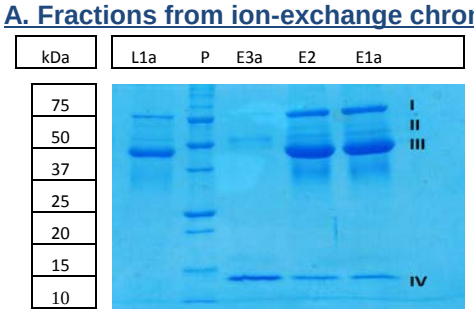
B. Gel-filtration chromatography (Sephadex G-75)



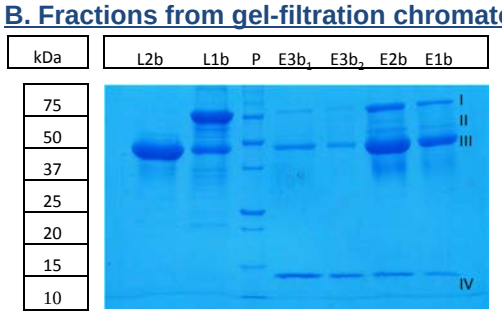
1.-In A, the sample E2a (15 mL) was eluted with 0.6 M phosphate potassium buffer. **In B**, E2b (2 mL) was eluted with 0.1M acetic acid. In both, the protein amount was represented by $Absorbance_{280nm}$ (blue diamonds) and the enzymatic activity was determined by using *Micrococcus lisodeikticus.* The unit of the enzyme activity (UAL) was calculated by the equation: [UAL/mL=(ΔAbsorbance$_{450nm}$/min)*1UAL/-(0.001/min) *1/3mL * Dilution factor] (red squares).
In A, the fraction with highest enzymatic activity value were found to coincide with the second protein peak (E3a, 144 mL). In B, the fraction with the highest enzymatic activity value were found to coincide with the shoulder of the second protein peak (E3b, 47 mL).

3.- SDS-PAGE electrophoresis

A. Fractions from ion-exchange chromatography



B. Fractions from gel-filtration chromatography



2.- In gel A: Fraction L1a (first maximum of absorbance; 10uL), P (standarts; 10uL), E3a (5.4ug), E2a (8.7ug) and E1a (9.2ug). **In gel B**: Fraction L2b (second maximum of absorbance; 20uL), L1b (first maximum of absorbance; 20uL), P (standarts; 8uL), $E3b_1$ (2.2ug), $E3b_2$ (0.7ug), E2b (7.8ug) and E1b (7ug). E3a and E3b were not pure. E3a was constituted by two proteins, avidine and lysozyme, and E3b was consituted by ovotransferine, ovoalbumine and lysozyme.

4.- Purification data

Stage	Vtot(mL)	Prot tot(mg)	Act tot(UAL)	Specific act(UAL/mg)	Yield %	Purification
E1a	16	196	160000	816.32	100	1
E1b	15	140	150000	1075	100	1
E2a	15	173	145000	838.15	90.63	1.026
E2b	14	147	130662	889	87	0.83
E3a	33	5.94	74799.99	12592.59	46.75	15.42
E3b	13	8.1	81900	10227	51.6	9.5

Values of E1a and E1b were similar, as well as E2a and E2b, meaning that the firsts stages of purification were reproducible. E3a (15.42 times purified) was purer than E3b (9.5 times purified), confirming that Amberlite CG-50 was better than Sephadex G-75 to purify lysozyme.

CONCLUSION

According to our data, neither affinity chromatography nor gel filtration chromatography yielded pure lysozyme. We suggest that an effective method for lysozyme purification from hen egg white will consist in a three-step protocol, beginning with an acid treatment, followed by ion exchanged chromatography and gel-filtration chromatography.

REFERENCES

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3.-Morsky P. (1983). Turbidimetric determination of lysozyme with Micrococcus lysodeikticus cells: reexamination of reaction conditions. Anal. Biochem. 128, 77-85.