Comparative purification of hen egg lysozyme using sephadex G-75 and Amberlite CG-50

David Martín and Gianfranco Paccione

Student laboratory, Departement of Biochemistry and Molecular Biology I, Faculty of Chemistry, UCM

INTRODUCTION

Lysozyme (EC 3.2.1.17) is an enzyme that catalyzes the hydrolysis of β(1→4) glycosidic bonds, in particular that of the bacteria cell wall peptidoglycan: between the N-acetyl muramic acid and the N-acetyl–D-glucosamine. It is a basic protein with a low molecular mass. It can be found in multiple organisms, such as human secretions, other vertebrates, invertebrates, plants, bacteria and even viruses. Its bacteriolytic effect was first described by Fleming[1] and it is the first enzyme of which its three dimensional structure has been determined. This enzyme has multiple applications, such as in eye drops, in the treatment of intestinal infections[2] or as a substitute of sulfites in vines, etc. Due to its great utility, this experiments' purpose is to propose a new purification method for the hen egg white lysozyme.

MATERIALS AND METHODS

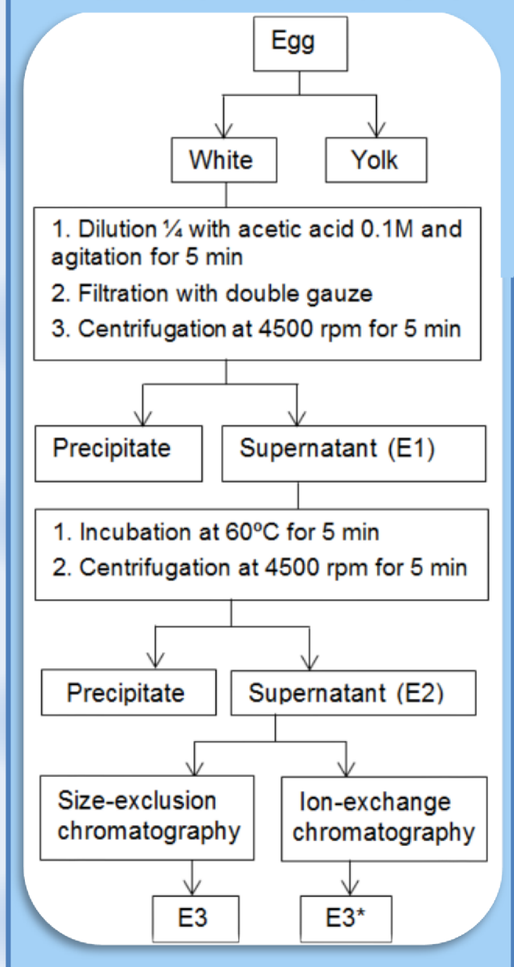

Figure 1. Lysozyme purification method followed.

Materials used: Brown hen egg (*Gallus gallus domesticus*) bought at a local supermarket. *Micrococcus lysodeikticus* cell wall suspension, Sephadex G-75, Amberlite CG-50, BSA solution, Coomasie Blue, Polyethylene glycol 35000, SDS/Glycine/Tris bought from Sigma-Aldrich. Acrylamide and bisacrylamide from SERVA. TEMED from Nzy-Tech. Ammonium persulfate and patron solution of known molecular mass for PAGE-SDS from BIO-RAD. Bromophenol Blue from Fluka.

Methods followed: The purification was followed according to the diagram (Figure 1). Moreover, each samples' enzyme activity was determined through Morsky's method[3], and the protein concentration through Bradford's method[4]. Finally, the protein composition of each sample was studied by electrophoresis in denaturalizing conditions (PAGE-SDS) following Laemli's method[5].

RESULTS

Part 1 – Chromatography and activity of eluted fractions

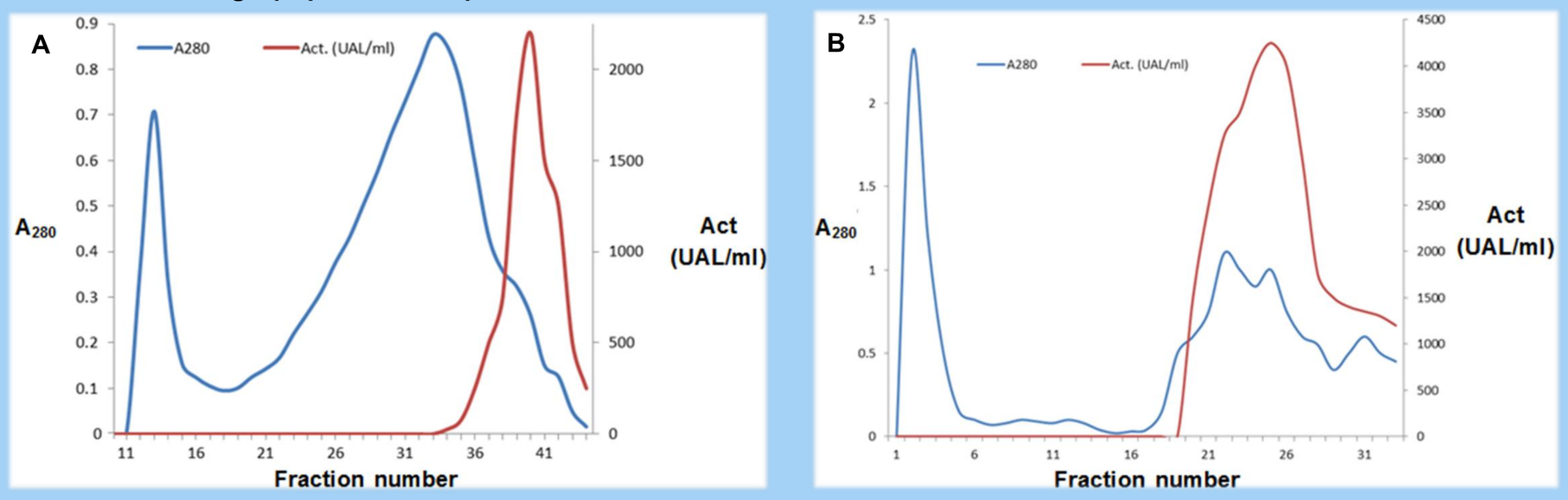

Figure 2. Elution profile for **(A)** size-exclusion chromatography and **(B)** ion-exchange chromatography.

Part 2 – Electrophoresis (SDS-PAGE)

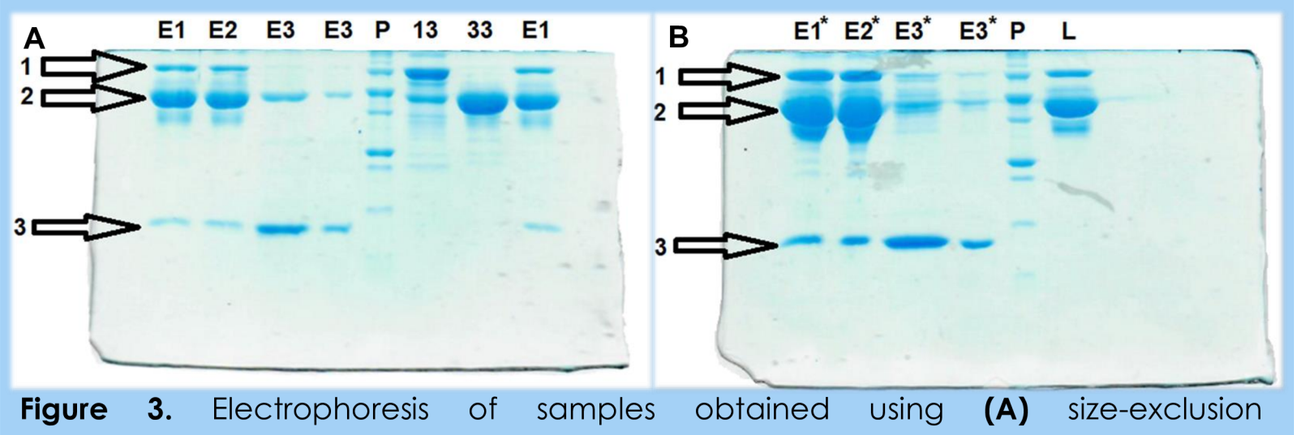

Figure 3. Electrophoresis of samples obtained using **(A)** size-exclusion chromatography and **(B)** ion-exchange chromatography.

Part 3 – Final comparative purification table

Step	Total volume (ml)	[Protein] (mg/ml)	Total protein (mg)	Total activity (UAL)	Specific activity (UAL/mg)	Yield (%)	Purification (fold)
E1	15	20.1	302	380000	1260	100	1
E2	14	19.5	273	348000	1270	92	1
E3	10	0.02	2.8	66300	24000	17	19
E3*	22	0.42	9.2	63100	6800	38	11

Table 1. Purification table for the hen egg white lysozyme. Yields and purifications are shown for samples of E1, E2, E3 (obtained using size-exclusion chromatography) and E3* (obtained using ion-exchange chromatography.)

CONCLUSIONS

These results obtained following the initial comparative purification method allow us to conclude a series of ideas about the utility of the different steps followed:

- The heat treatment is not efficient, the protein concentration and specific activity do not vary significantly before and after the heat treatment. Furthermore, the electrophoresis results show that the samples before and after the heat treatment are almost identical.
- During the size-exclusion chromatography, a purification of 19 fold and a yield of 17% was obtained, we can see that it shows a high purification but low yield. Through electrophoresis, we can see that the only protein contamination this sample has after the chromatography is a protein of approximately 41 kDa, probably the ovoalbumin, which is the most abundant protein in the hen egg white.
- The ion-exchange chromatography provides a lower purification, approximately, half the purification, while it shows double the yield. Meanwhile, after the electrophoresis, apart from contamination due to ovoalbumin, there is a protein of approximately 70 kDa which is most likely the conalbumin.
- Finally a new purification method is proposed: the heat treatment should be eliminated, while the two chromatographies should be used together to increase the efficiency of the process. After the acidic treatment, an ion-exchange chromatography in Amberlite should be followed, due to its high yield, and then, to refine the sample obtained, a size-exclusion chromatography in Sephadex should be followed, obtaining a high purity.

REFERENCES

1. Fleming A. & Allison V. D. (1922). "Observation on a bacteriolytic substance (lysozyme) found in secretions and tissues." *Br. J. Exp. Pathol.* **13**: 252-260.
2. Islam R., Kite J., Baker A. S., Ching Jr. A. & Islam M. R. (2006). "Affinity purification of hen egg lysozyme using Sephadex G75." *Afr. J. Biotechnol.* **5**: 1902-1908.
3. Morsky P. (1983). "Turbidimetric determintion of lysozyme with *Micrococcus lysodeikticus* cells: reexamination of reation conditions." *Anal.Biochem.* **128**: 77-85.
4. Bradford M. (1976). "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding." *Anal. Biochem.* **72**: 248-254.
5. Laemmli U. K. (1970). "Cleavage of structural proteins during the assembly of the head of bacteriophage T4." *Nature* **277**: 680-685.