LIBM1 — January 17, 2017 · Scientific Congress of Biology

Purification of lysozyme from hen egg white

Guillermo Fernández Milán, Óscar Herranz Varea

Department of Biochemistry and Molecular Biology I, School of Biology, Complutense University, Madrid, Spain

Introduction

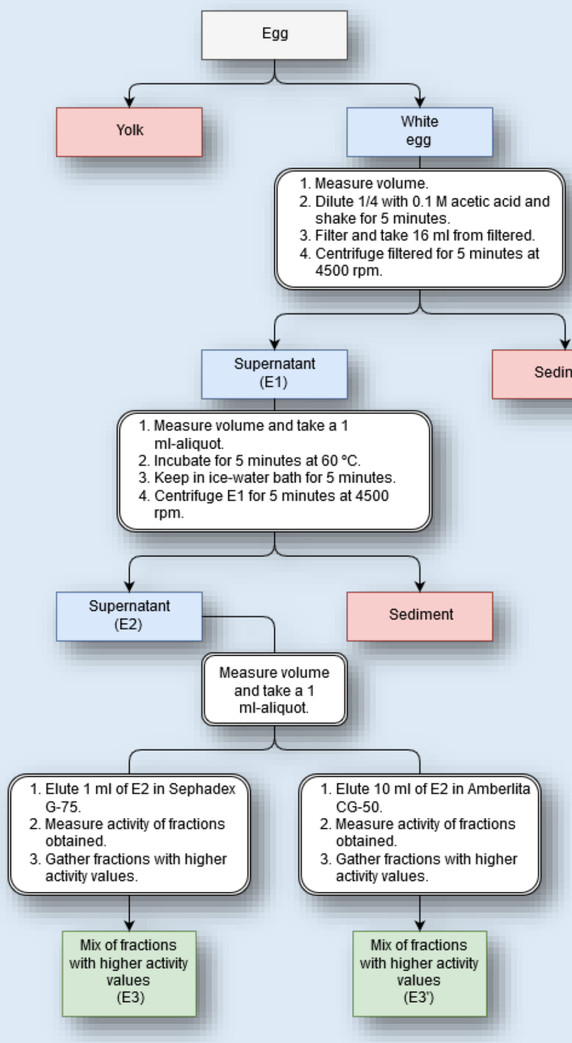
Lysozyme or muramidase (EC 3.2.1.17) is a globular protein that catalyzes the hydrolysis of β-glycosidic bonds (1→4) from bacterial wall polysaccharides. The enzyme can be found in different human secretions, as well as in other vertebrates and invertebrates. The main objective of this research is the proposal of a purification and isolation method for lysozyme from hen egg white. Firstly, acid and heat treatments were used, followed by a chromatographic process: ion-exchange chromatography (IEC) and size-exclusion chromatography (SEC) were assessed. Once the purification process was performed, the results of each chromatography were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and gathered in the purification table, including the scale of purity and enzyme recovery (yield). This way, after the study some suggestions will be included to improve further studies involved in this subject.

Objective

Tune up a method for purifying lysozyme from hen egg white.

Material and Methods

Fresh brown hen egg purchased at a local market. 0.1 M acetic acid. 0.6 M potassium phosphaye buffer, pH 6.6. 0.1 M potassium phosphate buffer, pH 6.6. 1 M potassium phosphate buffer, pH 7.0. 0.3 mg/ml solution of cell walls from *Micrococcus lysodeikticus*. Sephadex G-75 column. Amberlita CG-50 column. Bradford reagent. 0.2 mg/ml solution of BSA.



Scheme 1: Purification of lysozyme. The steps to follow are indicated in double squared boxes. In red, fractions discarded. In blue, fractions utilized. In green, final fractions that are supposed to have the lysozyme purified.

Finally, enzymatic activity of each fraction was assessed by Mörsky method. Protein concentration of each fraction was determined using Bradford method. Purity of E3 and E3' was evaluated using gel electrophoresis (SDS-PAGE; 15 %).

Conclusion

After the developed studies, it has been proved that ion exchange chromatography shows a more accurate procedure for lysozyme purification. However, it is clearly noticed that this method alone is not enough for a complete purification and isolation, as the PAGE-SDS shows a considerable contamination from other egg white proteins in the different purification steps. Therefore, omitting the heat treatment which resulted useless as there was not purification between E1 and E2, a three-step protocol for lysozyme purification could be considered. Beginning again with an acid treatment followed by the combination of ion exchange chromatography and gel filtration chromatography obtaining greater yield and purity.

Results

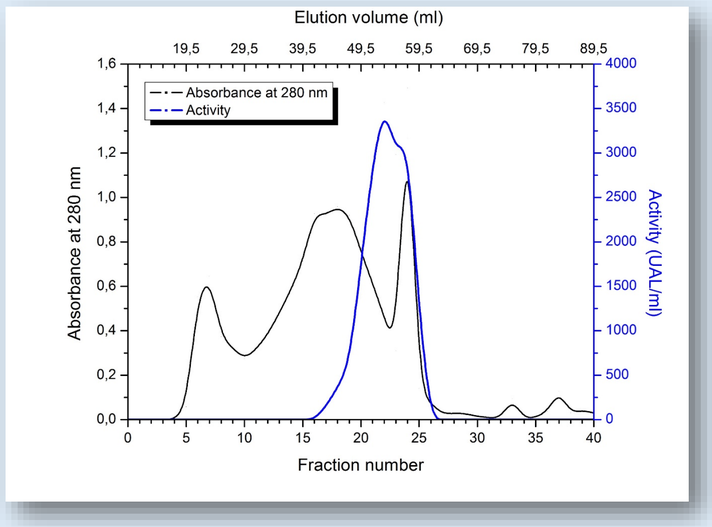
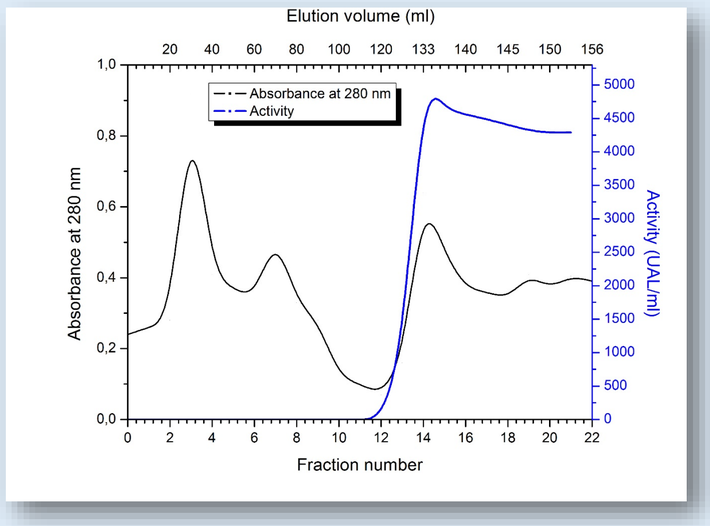


Figure 1: Chromatogram obtained from SEC for supernatant E2. 1 ml applied. Elution profile is showed in black. Enzymatic activity of fractions obtained after enzymatic assay with cell walls from *Micrococcus lysodeikticus* is overlapped in blue. The volume eluted so far (in milliliters) is shown on top axis. The bottom axis shows the fraction number utilized. It will be used to refer a specific fraction throughout the investigation.

Figure 2: Chromatogram obtained from IEC for supernatant E2. 1 ml applied. Elution profile is showed in black, Enzymatic activity of fractions obtained after enzymatic assay with cell walls from *Micrococcus lysodeikticus* is overlapped in blue. The volume eluted so far (in milliliters) is shown on top axis. The bottom axis shows the fraction number utilized. It will be used to refer a specific fraction throughout the investigation.



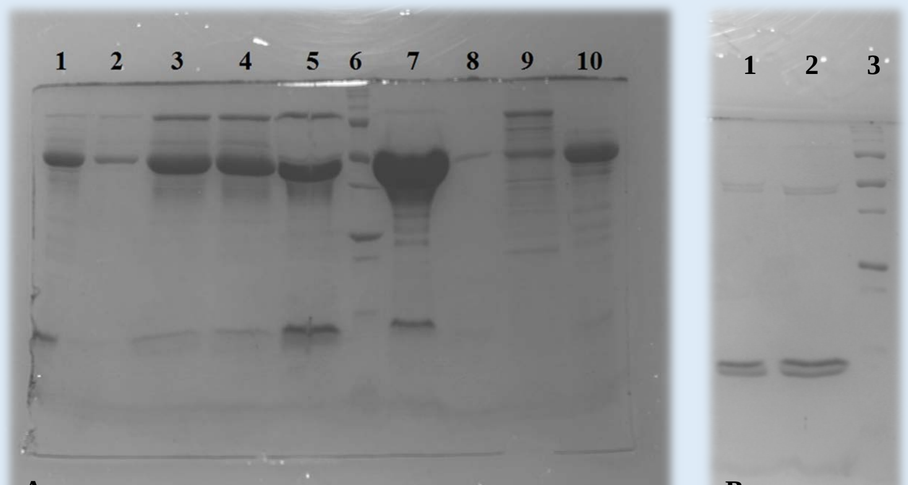


Figure 3: SDS-PAGE pattern of different purification steps of hen egg white. Coomassie blue staining. Protein markers used had a molecular weight of 10, 15, 20, 25, 37, 50, 75, 100, 150 and 250 kDa. **A) Sephadex G-75.** 1) E3. 2) Fraction 24. (dilution 1/10). 3) E1 (dilution 1/10). 4) E2 (dilution 1/10). 5) E3 dialyzed and concentrated. 6) Protein markers. 7) E4 dialyzed and concentrated. 8) Fraction 22 (dilution 1/10). 9) Fraction 7. 10) E4 **. B) Amberlita CG-50.** 1) E3' (dilution 1/10). 2) E3'. 3) Protein markers.

Table 1: Purification table. Yield and number of times lysozyme has been purified in each step is shown. Besides, mean values for E1, E2, E3 (Sephadex G-75) and E3' (Amberlita CG-50) are shown. Each data point is the mean of at least four and in most cases six or more independent experiments.

Step	Total protein (mg)	Total activity (UAL)	Specific activity (UAL/mg)	Yield (%)	Purification (fold)
E1	229.3	313999.95	1369.38	100	1
E2	456.3	210600	461.54	67.07	0.34
E3	10.29	167400	16268.22	53.31	11.90
E4	14.03	85400	6086.96	27.20	4.45
E1 (mean values)	155	224250	1418	100	1
E2 (mean values)	155	145646	1193	75	1
E3-SEC (mean values)	174	97520	81020	20	14
E3-IEC (mean values)	142	94367	41961	52	15