ENZYME PURIFICATION: Lysozyme from hen egg

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INTRODUCTION

This study describes and compares two different methods for the purification of lysozyme from hen egg. For the enzyme isolation, the experimental procedures which have been used are: acid and thermic treatments and two different chomatographies, in order to find the most effective one. In one purification it was used a size-exclusion chromatography with G-75 Sephadex while, in the other one, it was used an ion-exchange chromatography with CG-50 Amberlite. For the purpose of checking the efficiency of both proceedings, there were performed enzymatic essays, the Bradford method and PAGE-SDS electrophoresis. The results showed that the highest purification coefficient was obtained by the ion-exchange chromatography, so this is the most effective purification way.

METHODS

Egg white

1. Acid treatment
- ¼ dilution with acetic acid 0.1M
- Filtrate through doublé gauze
- Centrifugate 5 min, 4500 rpm

E1

2. Heat treatment
- Incubate the supernatant 5 min,60ºC
- Cool down 5 min, ice bath
- Centrifugate 5 min, 4500 rpm

E2

3. Chromatography

- Size exclusion chromatography → E3 → PAGE-SDS
- Ion Exchange chromatography → E3 → PAGE-SDS

Bradford method and enzimatic assay were done in order to determine the protein concentration of each extract and its enzymatic activity.

RESULTS

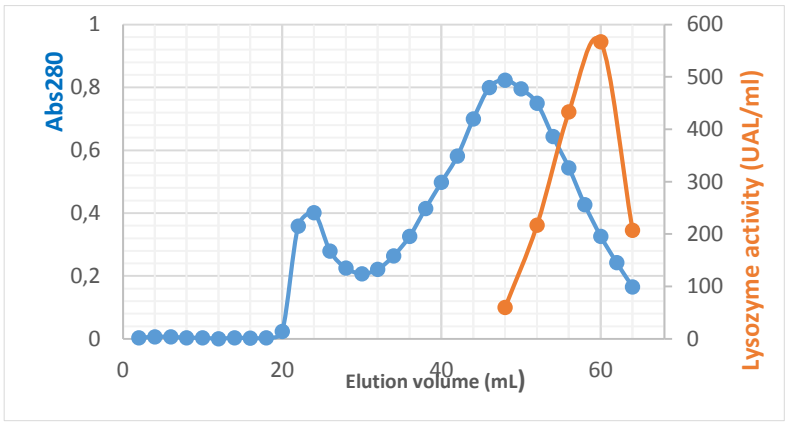

Fig. 1: Sephadex molecular exclusion chromatography profile: absorbance at 280nm and enzymatic activity vs. elution volume (ml). The elution profile shows two peaks that correspond to the elution volumen of proteins with a higher molecular mass tan the lysozyme. This peaks were used to form L1. Using an activity assay, lysozyme activity is found between 48 and 62 mL and the peak of ativity is found at 60 mL. Lysozyme's elution volumen has been found between 54 and 62mL.

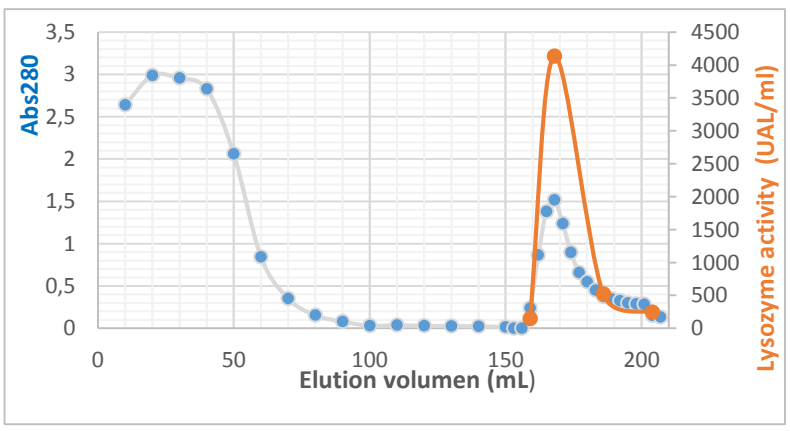

Fig. 2: Elution profile of Amberlite ion exchange chromatography: absorbance at 280 nm and enzymatic activity as a function of elution volume (ml). The elution profile shows two peaks: the first one (elution volume=20mL) shows no lysozyme activity; the second one shows lysozyme activity and its elution volume is found at 170mL.

Ion Exchange chromatography is a better method for lysozyme purification than size exclusion chromatography. This is shown in the PAGE-SDS gels as the contaminant band in E3 is weaker in ion exchange chromatography. In both cases the L1 fraction does not contain lysozyme. Therefore, lysozyme has not been lost in that stage of purification.

Lysozyme molecular mass has been determined: 13,8kDa.

It can be concluded that ovotransferrin and ovoalbumin are contained in E1 and E2, apart from lysozyme. The differences between both tables, 1 and 2, are not significant. However, it is relevant that the specific activity of E3 is higher in table 2 as well as lysozyme purification times.

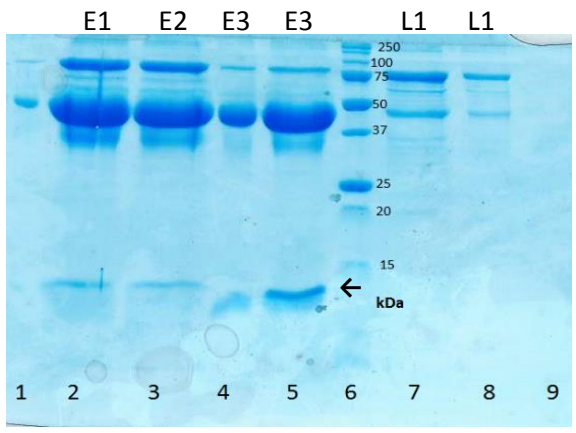

Fig.3: PAGE-SDS of E1, E2, E3 and L1 after size exclusión chromatography.

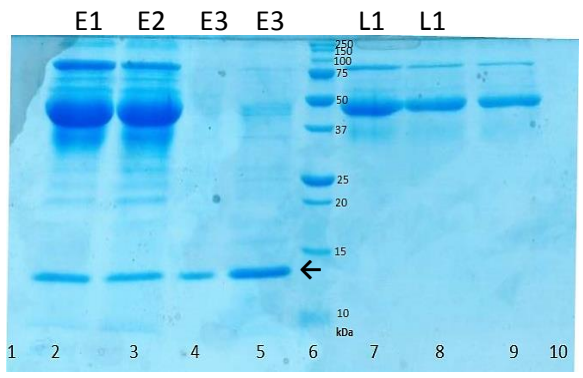

Fig. 4: PAGE-SDS of E1, E2, E3 and L1 after ion exchange chromatography.

Table 1. Purification table of size exclusion chromatography

Estage	Volume (ml)	Protein concentration (mg/ml)	Activity (UAL/ml)	Total protein (mg)	Total activity (UAL)	Specific activity (UAL/mg)	Yield (%)	Purification (nº times)
1	16	18	10000	288	160000	556	100	1
2	15	20	8000	300	120000	400	75	0.7
3	8	0,3	500	36	60000	1667	37.5	3

Table 2. Purification table of ion exchange chromatography

Estage	Volume (ml)	Protein concentration (mg/ml)	Activity (UAL/ml)	Total protein (mg)	Total activity (UAL)	Specific activity (UAL/mg)	Yield (%)	Purification (nº times)
1	15	28	8333	421	125000	297	100	1
2	13	24	7000	316	91000	288	72.8	0,97
3	38	0.87	2267	33	86100	2605	68.9	8.77

CONCLUSION

The chromatography with the best results, from a purification point of view, is ion Exchange chromatography. Therefore, this technique is more effective than the one of molecular exclusion.

However, the purification method by which a further purification of the lysozyme would be achieved would be a three-step procedure: first, an acid treatment, then an ion exchange chromatography and, finally, a molecular exclusion chromatography .