DESING OF THE PURIFICATION OF HEN EGG WHITE LYSOZYME

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Abstract.
This study sought the best purification method for the lysozyme enzyme (EC 3.2.1.17.). This enzyme hydrolyzes the β (1, 4) glycosidic bond present in the bacterial cell wall, and is therefore essential in antibacterial processes. The enzyme is present in hen egg white, which was the starting material for this study. Several experiments were performed, and their effectiveness was later determined with protein concentration and enzymatic activity assays. Among those experiments were acid and heat treatments, and two different kinds of chromatography: size exclusion in a Sephadex G-75 column and cation exchange in an Amberlite G-50 column. Two major conclusions were drawn. First, the heat treatment was mostly ineffective. Second, while the latter two were reliable ways of separating the enzyme, the cation exchange chromatography was the most precise method. Finally, a purification method was proposed: the heat treatment would be overlooked, and only the acid treatment and the chromatographies would be used. These two should happen in a certain order: first amberlite and then Sephadex.

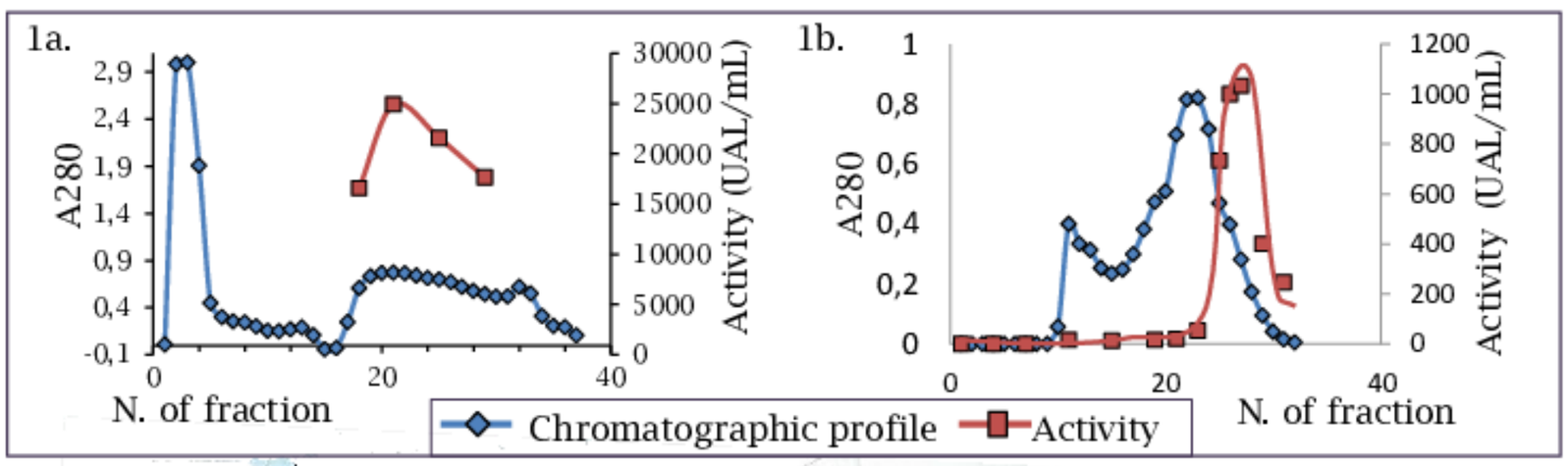
Materials and methods.

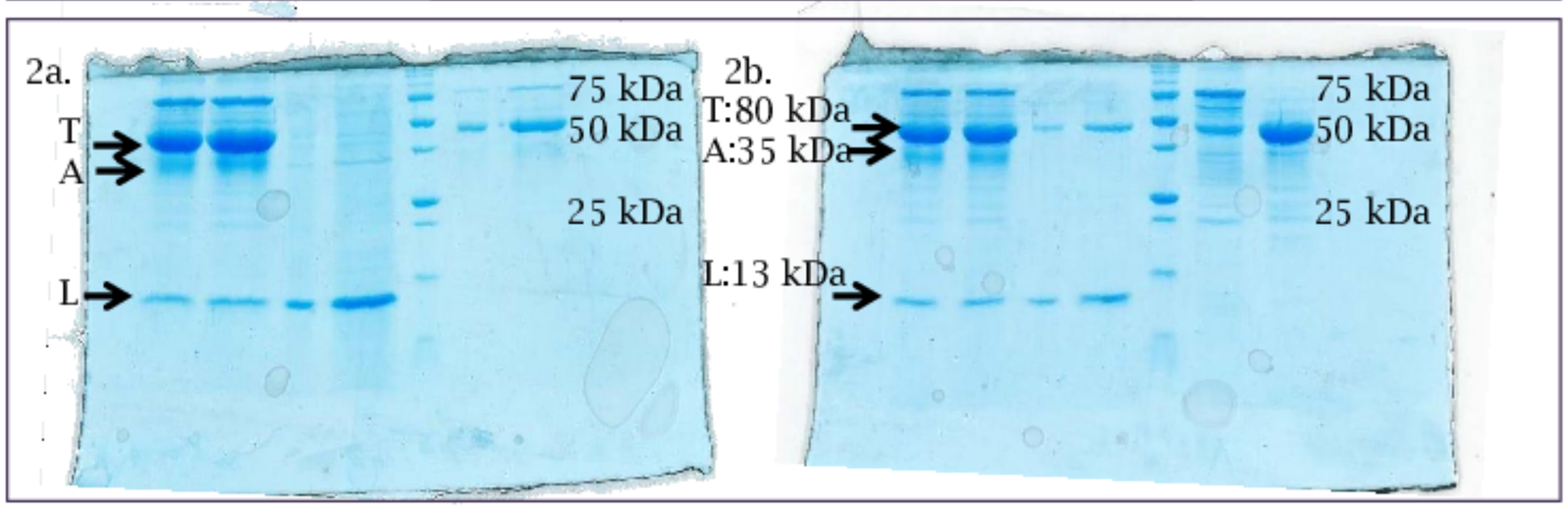
1. Acid treatment. Acetic acid 0.1 M was added on a 3:1 ratio to egg white. The result was filtered and then centrifugated at 4500 rpm for 5 min. The sediment was discarded and the supernatant saved, and labeled E1.
2. Heat treatment. E1 was incubated in a 60º water bath for five minutes and then centrifuged at the same conditions. Again, the sediment was discarded and the supernatant saved, and labeled E2.
3. Cation exchange chromatography. The resin was Amberlite G-50, and two elution buffers were used: first, phosphate buffer 0.1 M and pH 6.6; and second, phosphate buffer 0.6 M and pH 6.6.
4. Size exclusion chromatography. The resin was Sephadex G-75 and the elution buffer was acetic acid 0.1 M.
5. Enzymatic assay. The resulting fractions from the chromatographies, as well as samples of E1 and E2 were assayed. The study observed the decrease in absorbance (280 nm) of a *Micrococcus luteus* cell wall preparation when the sample was added. The blank used was phosphate buffer 0.1 M, pH 6.6. Absorbance measurements were taken every 30 seconds for 3 minutes. A graph was plotted from those data points, of absorbance vs. time, and it resulted on different straight lines, whose slope was used to find the enzymatic activity:

$$\frac{UAL}{mL} = n * \frac{1UAL * min}{-0{,}001} * \frac{1}{0{,}3\,mL} * F.D.$$

6. Protein concentration. The Bradford method was used [1].
7. Electrophoresis. It was conducted by the Laemli method [2]. Stacking gel: polyacrylamide 5%. Running gel: polyacrylamide 15%.

Results:





1. Chromatographic and activity profiles. 1a: Amberlite. The enzyme appeared along with the change of buffer. 1b: Sephadex. The enzyme appeared at the end of the albumin peak.
2. Electrophoresis. The purification was approximately the same on E1 and E2, but more lysozyme (L) appeared on E3. 2a. Amberlite. It didn't completely eliminate transferrin (T). 2b. Sephadex. Albumin (A) appears as a significant band.
3. Purification table. E3 appears as the best purification step, although Amberlite is slightly better than Sephadex.

3.

Phase	Total volume (ml)	Protein concentration (mg/ml)	Total protein (mg)	Total activity (UAL)	Specific activity (UAL/mg)	Effinciency (%)	Purification factor
E1	16	14,40	230,40	160000	694,44	100,00	1,00
E2	14	14,25	199,50	140000	701,75	87,50	1,01
E3 Amberlite	29	0,34	9,93	38657	382,95	24,16	5,60
E3 Sephadex	11,5	17,94	17,6	9619	5449	41	4,7

Bibliography.
[1] Brafdord, Marion M. (1976) "A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding". Anal. Biochem., 72: 248-245
[2] Laemmli U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4.Nature 277, 680-685.

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