Lysis of *E. coli* for protein recovery using chicken and human lysozyme

**Introduction**

The extraction and recovery of active proteins from *E. coli* cells is a crucial step in purification. During extraction, lysozyme from hen egg white is often used to hydrolyze the bacterial cell wall. As a muramidase enzyme (Salazar and Asenjo, 2007), lysozyme splits the linkages between N-acetylmuramic acid and N-acetyl-D-glucosamine residues in peptidoglycan and between N-acetyl-D-glucosamine residues in chitodextrin. Because it is readily available, chicken lysozyme (cLZ) is often used to prepare the initial cell free extract. The objective of this study is to compare the lysis activity between human and chicken lysozyme, and to measure the amount of protein recovered after lysis in an *E. coli* expression system.

**Material and Method**

Chicken lysozyme (cLZ) from hen egg-white was obtained from a commercial source (Calbiochem, CA). Recombinant human lysozyme (Lysobac) was produced in rice seeds (Huang et al., 2002) and purified by following the protocol as described by Wilken and Nikolov (2006).

Lysozyme activity was measured following a standard cell lysis protocol (Sigma, MO). In this assay, Lysobac and chicken lysozyme were diluted to 2ug/ml in analysis buffer containing 66mM Potassium Phosphate, pH 6.24. The substrate, *Micrococcus lysodeikticus* (ATCC 4698 lyophilized cells) was prepared at 0.015% in analysis buffer. Following sample preparation, 100µl of lysozyme was added to 2.5ml of *Micrococcus* cells and mixed thoroughly. Lysis activity was measured by monitoring the absorbance at 450nm for 3 minutes at 20 second intervals. Units per ml of enzyme were calculated using the following formula: U/ml = (mOD/min sample – mOD/min blank) (Dilution factor) / (0.001) (0.1), where 0.001 accounts for a change in absorbance at A450 nm per unit definition and 0.1 is the volume of enzyme used (Sigma, MO).

In a separate assay, protein recovery was measured using an *E. coli* strain DH108 carrying an ampicillin resistance marker. In this assay, a single colony of *E. coli* was inoculated into 1ml of LB with 100µg/ml Ampicillin and incubated at 37°C overnight. Following overnight incubation, the culture was transferred to 200ml of LB with 100µg/ml Ampicillin. The cells were incubated at 37°C until log phase growth was reached. The cells were centrifuged at 7,400Xg for 10 minutes, and the pellet was re-suspended in 100mM Tris-Cl, 2mM EDTA, pH 8.0 (Coligan et al., 1998). The suspension was re-centrifuged at 7,400Xg for 10 minutes, followed by re-suspension in lysis buffer (100mM Tris-Cl, 2mM EDTA, 0.025% TritonX-100.). The cells were divided into 1ml aliquots and treated with increasing amounts of lysozyme for 5 minutes. Following lysis, the cells were centrifuged for five minutes at 12,000Xg. Total soluble protein was collected and assayed by using Bradford method (Bio-Rad, CA).

**The Lysobac™ is over three times more potent than chicken lysozyme**

Lysobac’s bactericidal activity, at 118,000 U/mg dry mass, is equivalent to native human lysozyme and over three times potent than chicken lysozyme (35,000 U/mg). The assay was performed with a *Micrococcus lysodeikticus* suspension following a standard lysis protocol (Sigma, MO).
Lysobac™ reduces the amount of enzyme needed to lyse bacterial cells and increases the total protein yield.

Results
The comparison of lysis activity between Lysobac™ and chicken lysozyme was shown in Figure 1 on the previous page. The Lysobac activity was at 118,000 units/mg solid, and over three times more potent than chicken lysozyme (35,000 units/mg solid). To quantify the amount of protein recovered after cell lysis, we lysed E. coli cells with lysozyme, and then measured the total soluble protein (Figure 2a and 2b). Figure 2a indicated that more proteins were recovered as more chicken or human lysozyme (Lysobac) was added to the cell suspension. It was shown that using 1.32 µg Lysobac/ml E. coli or 5.29 µg cLZ/ml E. coli and essentially yielded approximately 400µg of total soluble protein per milliliter of E. coli. This was the minimum amount of enzyme needed to lyze equal amounts of bacteria.

Summary
A comparison of activity assays indicates that Lysobac (recombinant human lysozyme) is over three times more active than chicken lysozyme (cLZ). Protein recovery data shows that 4-fold lower concentration of Lysobac is needed in relative to cLZ that lyze equal amounts of bacteria with similar amounts of recovered total soluble protein. These data suggest that using Lysobac reduces the amount of enzyme needed to lyse bacterial cells and increases the total protein yield.

References:

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