Fractionation of *Bovicola ovis* Homogenates Using the Mini Whole Gel Eluter—Effect of Buffer Composition on Elution Efficiency

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Introduction

Amino acid sequence and composition analyses of proteins can be performed on samples derived from polyacrylamide gels. Protein electro-elution, the most commonly used method of recovering proteins from gels, can be performed on "cut-out" gel slices or using the Whole Gel Eluter recently introduced by Bio-Rad.[1–4] The Mini Whole Gel Eluter was used here to fractionate crude homogenate of the common sheep louse with the aim of characterizing immunogenic components.

Methods

Common sheep lice, *Bovicola ovis*, were harvested from infested animals, homogenized by ultrasonication, and the homogenate was clarified by centrifugation on a benchtop microfuge. Homogenate samples (1 mg protein) were separated by SDS-PAGE in 12% preparative well Tris-HCl Ready Gels (Bio-Rad). Protein fractions were immediately electro-eluted at 100 mA for 25 min using the Mini-Whole Gel Eluter. Three elution buffers: (A) 100 mM NH_4HCO_3, 0.01% SDS, pH 8.5, (B) 10 mM phosphate buffer, 0.005% SDS, pH 7.8, and (C) 25 mM Tris, 19.2 mM glycine, 0.01% SDS, pH 8.3, were used to determine the elution efficacy. The fractions were analyzed in 12%, 15 well Tris-HCl Ready Gels (Bio-Rad) and proteins were visualized by Coomassie® blue staining. Bio-Rad's low molecular weight standards for SDS-PAGE are shown in Figure 1B, lane b.

Results and Discussion

Louse homogenate preparations are often rich in lipids and do not separate well on SDS-PAGE gels (Figure 1A, lane a). Individual proteins are poorly resolved and their extraction from individual "cut-out" bands is often inefficient. Several attempts were made to obtain amino acid sequence from various protein bands of louse homogenate using a variety of standard methods. This approach proved to be relatively ineffective and time-consuming.

The Mini Whole Gel Eluter was then used to fractionate crude louse homogenate. Samples of louse homogenate were separated on 12% preparative well gels and subjected to electro-elution. Two elution buffers, B and C, varying slightly in pH (7.8 v. 8.3) and in SDS content (0.005% v. 0.01%), were applied and in both experiments elution resulted in good recovery of a majority of proteins. It should be noted, however, that high molecular proteins did not elute well in buffer (B) containing low concentration of SDS (0.005%). The application of NH_4HCO_3/SDS buffer (A) resulted in virtually no protein elution, possibly due to the low stability of ammonium carbonate buffer.

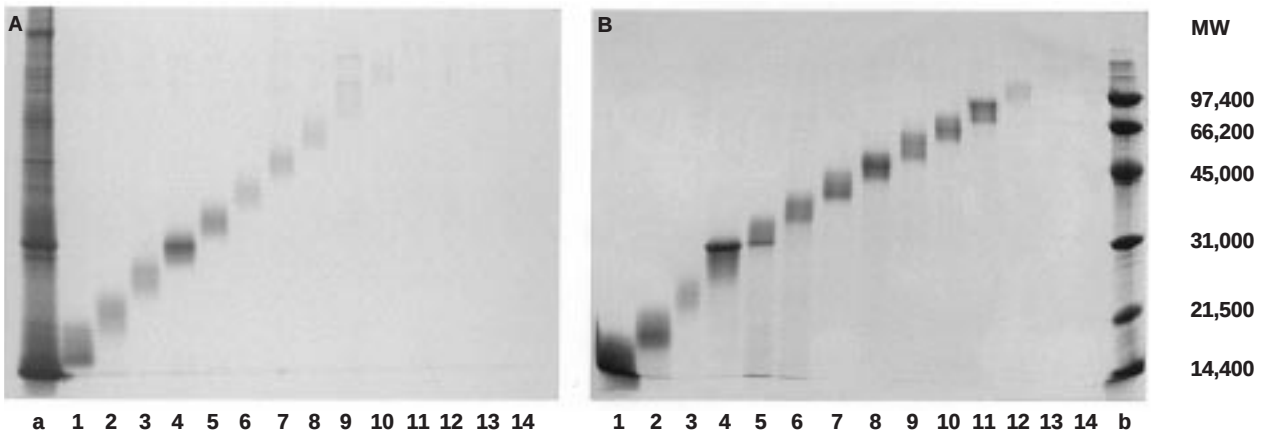

Fig. 1. SDS-PAGE profiles of protein fractions (components of sheep louse components) electro-eluted from mini SDS-PAGE gels using Mini Whole Gel Eluter. Gel A – electro-elution with buffer B: 10 mM phosphate buffer, 0.005% SDS, pH 7.8. Gel B–electro-elution with buffer C: 25 mM Tris, 19.2 mM glycine, 0.01% SDS, pH 8.3. Louse homogenate–gel A, lane a; Low molecular weight standards–gel B, lane b.

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It was obvious that the buffer (C) containing Tris and a higher SDS concentration (0.01%) was a much better choice, as it resulted in the elution of virtually all proteins present in the louse homogenate ranging from <14,000 to >100,000 daltons. Increased SDS concentration also yielded approximately 2–3 fold greater amount of protein eluted in each of 14 fractions (Figure 1B).

Protein fractions electro-eluted on the Mini Whole Gel Eluter with buffer C, containing 0.01% SDS, pH 8.3, were transferred on Prosorb, desalted, and subjected to automated N-terminal amino acid sequencing. Of three major proteins analyzed, sequence information was obtained for two. One of the proteins contained a sequence that was similar to that of a reported allergen.

References

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