Linearized Bradford Assay with β-Lactogloblin as Standard for Use with Snake Venoms

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Abstract

Analyzing the protein content of snake venom, a mixture of proteins, in solution is problematic due to the protein composition of venom being specific to each species and individual. A common method of determining the concentration of a protein in solution is to use the absorbance at 280 nm. The absorbance is due mostly to the presence of the amino acid tryptophan but is also influenced by the presence of tyrosine and cysteine. The problem with using this method is the interference from other chromophores such as DNA or other impurities that absorb near 280 nm. When analyzing snake venom samples purity can be an issue due to venom milking methods. Also the variability of the number and nature of proteins in the venom causes variability in the absorbance at 280 nm. In the linearized Bradford Assay, protein concentration is determined from the absorbance changes that occur when the protein binds a dye (Coomassie brilliant blue). For the linearized assay, the absorbance ratio, 590 nm/450 nm is used to calculate the amount of protein in the assay. The linearized Bradford Assay uses a standard protein and the concentration of the unknown protein is determined by comparison. Bovine Serum Albumin, BSA, is used commonly as a standard due to its ready availability, low cost, and known extinction coefficient. We show here that BSA poorly predicts the amount of snake venom in solution. Our results show that β-Lactogloblin is a better standard for snake venoms while also having a known extinction coefficient.

Introduction

Using protein absorbance from a specific wavelength of light is a common technique to ascertain the concentration of protein in solution. When using the spectrophotometric method the absorbance is taken at 280 nm where the "rule of thumb" is 1 A280 unit = 1 mg/ml of protein concentration in solution. This is based off of 1 tryptophan per protein though this can be affected by other amino acids such as cysteine and tyrosine. Though this is not entirely accurate due to variance in primary protein structure, number of absorbing amino acids, and light scattering. A correction for the light scattering can be done by subtracting out the absorbance at a further wavelength, 400 nm, from the absorbance at 280 nm (Aitken and Learmonth, 2002).

When using the Linearized Bradford Assay the main chromophore is a dye, Coomassie Brilliant Blue, in which the protein's absorbance is shifted due to binding with the dye (Bradford, 1976). Multiple concentrations of known protein are made along with multiple concentrations of an unknown protein in order to compare absorbance. The absorbance is now taken at 590 nm and 450 nm with a ratio of 590 nm/ 450 nm used to make a linearized standard curve. This increases the sensitivity of the Bradford assay (Zor and Selinger, 1996). Once the amount of protein is solved for the known protein and a linear equation is attained the amount of protein in the unknown sample can be calculated. Though how accurate this calculation is depends on the similarity between the known and unknown protein.

Results

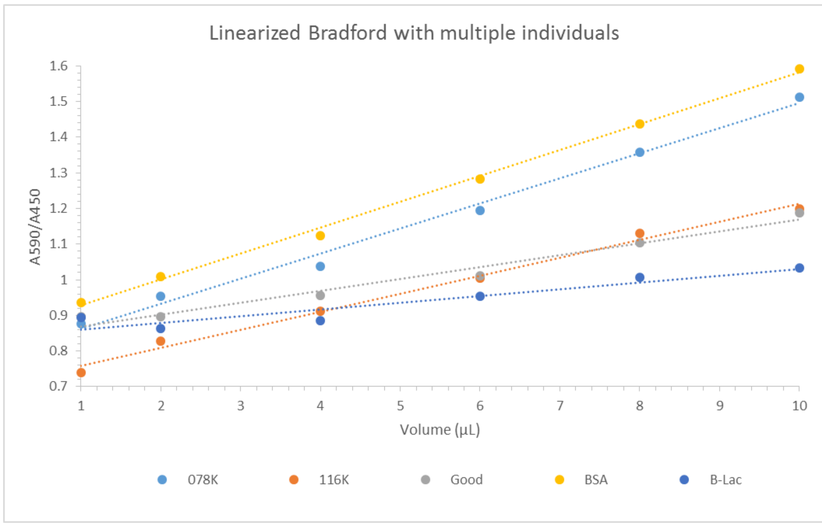

Figure 1: Standard curve from three individuals of the species *Crotalus Atrox* compared to β-Lactoglobulin and BSA.

Figure 2: Standard curve from three different species of venomous snakes compared to β-Lactoglobulin and BSA.

Methods and Materials

The venoms and standard proteins were acquired from Sigma with the "good" *C. Atrox* venom coming from the WTAM vivarium. All solutions were prepared in volumetric flasks to 1 mg/ml concentrations and phosphoric buffer was used to keep the venoms folded. In order to accurately know the concentration of the standard proteins the A280 was taken and using Beer's Law the concentration was calculated. All A280 absorbances were corrected by subtracting out the A400 absorbance. All linearized Bradford Assays were carried out with the different concentrations used being 1, 2, 4, 6, 8, and 10 µg/µL.

Disscusion

Comparing the wide range of snake venoms to the two standards, BSA and β-Lactoglobulin, showed that β-Lactoglobulin is the better standard of the two though it is not the ideal standard. When used with multiple individuals of the same species BSA, with a slope of 0.0727, was well above the average slope of the three individuals, 0.0514, though the data also showed the wide variance amongst even the same species. The standard deviation for the three individuals was 0.0151. β-Lactoglobulin had a slope of 0.0189 which was closer to the slopes of the individuals though in the higher volumes the standard curves quickly started to diverge.

When comparing the two standards to three different species' venoms the result was similar to the previous experiment. BSA had a higher slope, 0.1206, than the venoms, average 0.0825 with standard deviation of 0.0527, while β-Lactoglobulin had an average of 0.0194. The large standard deviation is due to the large slope differences between *C. Scutalatus* and the other two snake species.

While our experiments showed that β-Lactoglobulin is not the ideal standard for snake venoms it did show that the absorbance was closer to that of snake venoms compared to BSA. In future experiments other standards that have a similar structure to β-Lactoglobulin in hopes of finding a more ideal standard.

References

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Introduction