Applicability of Electrophoresis in Wildlife Forensics

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Abstract

This research is important because it will help determine the applicability of electrophoresis to wildlife forensic. This project primarily focused on using sodium dodecyl sulfate polyacrylamide electrophoresis, SDS-PAGE. Most laboratories have all of the equipment needed, so this methodology will be easily accessible and inexpensive. The samples used in this project were proteins from the fibrous cartilage of short-fin mako sharks (*Isurus oxyrinchus*), blue sharks (*Prionace glauca*), and common thresher sharks (*Alopias vulpinus*). These samples were collected at various shark fishing tournaments throughout the summer of 2015. Shark finning has become illegal in certain areas so this research is trying to establish an inexpensive and quick way to determine what type of shark a fin has come from based on the proteins present. Fibrous cartilage from shark fins was chosen for this project because the fibrous cartilage has been shown to contain high levels of proteins. Two buffers were used in the procedure: a 10x Tris/Glycine/SDS solution as the running buffer and a 2x SDS-PAGE Laemmli as the sample buffer. Several Bradford Assays were conducted to determine the amount of proteins in solution. The Bradford Assays showed that there were not a lot of proteins present in solution.

Introduction

Electrophoresis is the movement of charged particles or ions through a medium under the influence of an electric field. There are many mediums that can be used for the different types of electrophoresis including: polyacrylamide gel, paper, cellulose acetate, and starch gel. Electrophoresis is often used in separating proteins and nucleic acids for analysis and purification. In traditional electrophoresis, the ions or particles of the sample bearing a net positive charge migrate toward the negative pole of the field, while the negative particles migrate toward the positive pole (Cammack, 2006).

There are several types of polyacrylamide gel electrophoresis, or PAGE: native PAGE, Blue native PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), Zymogram PAGE, 2-D electrophoresis, and Isoelectric Focusing. The type used in this project was SDS-PAGE. SDS-PAGE is the most popular form of protein electrophoresis and differs from native PAGE because the proteins are denatured. SDS denatures the proteins so they can be separated by size rather than charge. A protein's mobility in the presence of SDS is determined by the molecules' individual hydrodynamic "sizes" and molecular weights. Instead of a complex tertiary structure, the proteins form a long, rod-like conformation in the presence of SDS [Figure 1]. The SDS gives and overall negative charge on the proteins because it is negatively charged and masks the intrinsic charge of the proteins it binds to. SDS binds to proteins at a rate of 1.4 grams SDS per one gram protein, so it gives all of the proteins in the mixture a similar charge-to-mass ratio. When SDS was first being tested it was included in both the gels and the buffers, however it has been determined that SDS in the sample buffer is sufficient in denaturing the proteins.

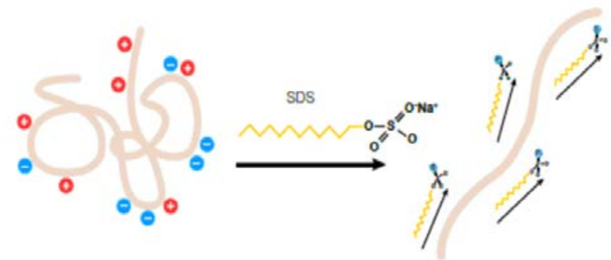

Figure One: Effect of SDS on protein

For this project sodium dodecyl sulfate polyacrylamide gel electrophoresis, better known as SDS-PAGE, was used. Some advantages to using SDS-PAGE are that it is efficient, fast, and economic because no sophisticated equipment is needed. This project will focus on how SDS-PAGE can be applied to wildlife forensic science. Specifically, this project will research speciation by SDS-PAGE of oligomeric proteins.

There are two types of cartilage present in shark fins: platelet and fibrous. For this project we used fibrous cartilage from the pectoral fins of short-fin mako sharks (*Isurus oxyrinchus*), blue sharks (*Prionace glauca*), and common thresher sharks (*Alopias vulpinus*) for samples. We chose shark fins from these three types of sharks because in recent years laws have been passed that have made shark finning illegal and these three types of sharks are common at several shark fishing tournaments we attended over the summer. Shark finning is when a shark's fins are cut off and the body is thrown back into the ocean (Verlecar, 2007). This project will be

used to determine an easy way to identify what species of shark a sample of cartilage came from without running a DNA test. This research is specifically focusing on whether or not SDS-PAGE is an efficient way to identify the species of shark from a sample of fibrous cartilage based on the proteins present. This project was conducted using a Mini-PROTEAN Tetra Cell to run the SDS-PAGE.

Materials and Methods

Throughout the summer shark fin samples were collected shark from shark fishing tournaments in New York and Rhode Island. Before any samples could be prepared for analysis or run in the Mini-PROTEAN Tetra Cell, the two buffers and gels must be made. The running buffer is made by mixing 30.3 grams Tris base, 144.1 grams Glycine, 10 grams SDS, and then adding deionized water until the volume is one liter. This sample buffer should have a pH of approximately 8.3. The sample buffer is made by mixing 3.75 mL of 0.5 M Tris-HCl with a pH of 6.8, 15 mL of 50% Glycerol, 0.3 mL 1.0% Bromophenol blue, 6.0 mL 10% SDS, and then adding deionized water until the volume is 30 mL. After the buffers and the gels have been made, we had to extract the proteins from the cartilage and prepare them for analysis. Fibrous cartilage from dried pectoral fins that had been previously skinned and separated was used in this experiment [Figure 2].

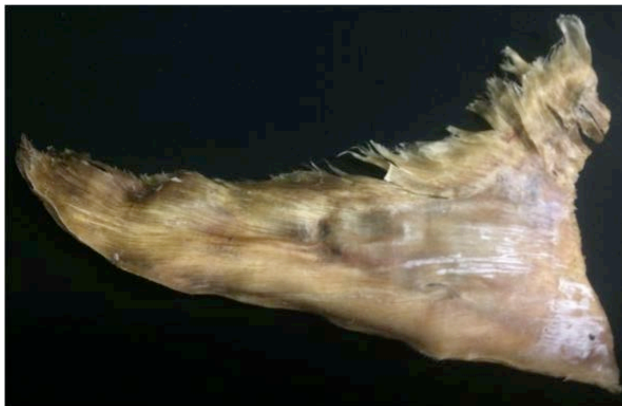

Figure Two: Fibrous cartilage from a previously skinned and separated shark fin

An approximately 3.0 gram piece of fibrous cartilage is cut from a dried pectoral fin, and then put in a 15 mL falcon tube with deionized water until rehydrated [Figure 3].

Figure Three: Sample of fibrous cartilage in a 15 mL falcon tube before rehydration

After the sample is rehydrated, it is pulverized using a Magic Bullet Blender™, then sonicated and centrifuged until it is separated. A Bradford Assay is used to determine the amount of proteins in solution. The sample is prepared for electrophoresis by mixing 5 µl of sample with 4.75 µl Laemmli sample buffer and 0.25 µl β-mercaptoethanol. After the sample solution is made, it is heated for ten minutes at 70°C. The gels were secured with glass plates in the frame and then were put into the electrophoresis tank. The inner chamber was then filled with running buffer and the samples were loaded into the wells of the gels with a pipette. Once the samples are loaded, 550 mL of running buffer was poured into the outer chamber and the inner chamber was topped off with running buffer. The Mini-PROTEAN Tetra Cell [Figure 4] was connected to the power supply and the electrophoresis was run at 200V for 30-40 minutes.

Figure Four: Mini-PROTEAN Tetra Cell, combs, glass plates, and casting frame for the gels

Once the electrophoresis was run, the cell was disconnected from the power supply and the running buffer was disposed of. After the running buffer was disposed of, the gels were removed from the system. Coomassie Blue was used to stain the gels once they were removed from the apparatus. The gels were left for sit in the Coomassie stain for 20-60 minutes and then were placed in a de-staining solution made of methanol and acetic acid. The gels were left in this solution until the bands were clearly visible. After this process, the gels are ready for analysis.

Results

The results from one of the Bradford Assays we have conducted is shown below [Table 1]. In the chart below, it can be seen that we only getting about the

Table One: Results of a Bradford Assay

Sample	Absorbance	K*Absorbance
0.05 Standard	1.319	1.3188
#1 unfiltered	1.181	1.1813
#1 centrifuged	1.035	1.0349
#2 centrifuged	1.195	1.1951
#2 filtered	1.314	1.3145
#1 below 50 K	0.67	0.6702
#1 above 50 K	1.308	1.3079
#2 below 50 K	0.761	0.7606
#2 above 50 K	1.735	1.735

same number of proteins as the 0.05 protein standard. All of the samples in the chart are from the same sample of fibrous cartilage besides the 0.05 standard. It can be seen that only three of the samples were almost the same as or higher than the standard. The sample #2 filtered, the sample #1 above 50 K, and the standard had an absorbance and K*absorbance around 1.3. The only sample that was higher than the standard was the sample #2 above 50K, with an absorbance and K*absorbance of 1.735.We have determined that there are proteins present in the solution, however, because the proteins will be further diluted with the sample buffer when running the SDS-PAGE we are trying to increase the amount of protein in solution before running any gels. We are trying to increase the amount of protein in solution because if there are not enough proteins present, the SDS-PAGE will not produce any visible protein bands. Which, in turn, would make analysis of the proteins present impossible.

Discussion

Extracting the cartilage proved to be more difficult than originally expected. Only very low levels of proteins were present according to the Bradford assays performed. Instead of wasting resources and trying to run tests with low levels of proteins, other methods were researched to see if there is a method that would produce more proteins in solution. Low amounts of proteins may not be able to produce any visible bands, therefore making analysis impossible. More research needs to be conducted to determine if other extraction methods will work better than the original one. Currently, only water, pulverization, and a centrifuge are being used to extract the proteins. The fibrous cartilage is made mostly of collagen, so there should be more proteins present than what is being obtained at the moment. This means either the extraction procedure is flawed or something else is causing a problem with the proteins. One possible problem is if how the shark fins are processed is causing the proteins to denature or be destroyed before they can be extracted. Processing the fins requires a number of steps. When the fins are obtained they are frozen until they can be dried. In order to separate the fibrous cartilage from the platelet cartilage, the skin must be removed. The skin is removed by rehydrating the fin for at least 24 hours, then soaking it in 53.5°C water for ten minutes. A scalpel is used to remove the skin and separate the two types of cartilage. Once the platelet cartilage is separated from the fibrous cartilage, it is left to dry. When the fins are completely dry, the samples are prepared for SDS-PAGE. The repeated drying and rehydrating, and the added heat could be causing the proteins to denature before they can be extracted Heat has been proven in past experiments to denature proteins. Even though the water is not

boiling, the heat could still be affecting the proteins in the sample. After higher levels of proteins can be detected, the samples of cartilage will be run in SDS-PAGE to determine if this method is applicable to wildlife forensics.

Conclusion

This study looked at the applicability of electrophoresis, specifically SDS-PAGE, to wildlife forensics. At this moment the research has not been concluded and there is no definitive conclusion about the applicability of electrophoresis. In addition to being shared with the forensic science community, this research is a proof of concept for a graduate student at the University of New Haven. She is using capillary electrophoresis, or CE to study the differences or similarities in the proteins of fibrous shark cartilage; however, for that project the samples will not be denatured with SDS.

Acknowledgements

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References

Cammack, R., T. K. Attwood, P. N. Campbell, J. H. Parish, A. D. Smith, J. L. Stirling, and F. Vella, eds. "Electrophoresis." Def. 1. *Oxford Dictionary of Biochemistry and Molecular Biology*. 2nd ed. New York City: Oxford UP, 2006. 10. *Knovel*. Web. 10 Aug. 2015.

Carwardine M, Watterson K (2002). *The Shark Watcher's Handbook; A guide to sharks and where to see them.* New Jersey: Princeton University Press.

Hardin, Charles C. Knopp, James A. . (2013). Biochemistry - Essential Concepts. Oxford University Press.

Laemmli UK (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680–685.

O'Farrell PH (1975). High resolution two-dimensional electrophoresis of proteins. J Biol Chem 250, 4007–4021.

Ornstein L and Davis BJ (1964). Disc electrophoresis-I: Background and theory. Ann NY Acad Sci 121, 321–349.

"Protein Electrophoresis Methods." *Protein Electrophoresis Methods*. N.p., n.d. Web. 10 Aug. 2015.

Verlecar, X. N., Snigdha, Desai, S. R., & Dhargalkar, V. K. (2007). Shark hunting -- an indiscriminate trade endangering elasmobranchs to extinction. *Current Science (00113891)*, *92*(8), 1078-1082

Biography

Briana Smith

Briana Smith is currently a sophomore at the University of New Haven. Briana is a Forensic Science and Biotechnology double major. Following graduation, she plans to continue her studies and work her way up to a PhD. Briana plans to have a career in either a forensic or biology laboratory.