

B122 Lectin Blot-Based Profiling of Salivary Fluid Glycoproteins Distinguishes Different Patterns Among Individuals

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Learning Overview: The goal of this presentation is to show that salivary fluid glycoprotein patterns differ among individuals and how they can be detected by Sodium Dodecyl Sulfate (SDS) gel electrophoresis and Western blotting with lectin staining.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing new knowledge of the differences (polymorphisms) in salivary glycoproteins. This study may facilitate the identification of the origin of saliva samples.

It is hypothesized that individuals have unique profiles or patterns of carbohydrates on their salivary glycoproteins. It is further hypothesized that differences in salivary glycoprotein profiles may be used in forensics to identify the source of saliva. There are precedents in the available literature of inter-individual differences in protein carbohydrates. For example, ABO blood group antigens are carbohydrate structures present on salivary mucins. Furthermore, whether individuals can express ABO blood group structures in saliva is dependent on a second genetic system—the Secretor gene. It is proposed that there are other inter-individual differences in salivary protein carbohydrate structures to be discovered per previous work.^{1,2}

The specific goal of this research was to define the patterns of carbohydrate structures on salivary glycoproteins from different individuals that were detected by selected lectins (carbohydrate binding proteins) and determine if individuals have distinct profiles that differ from others and that can serve as a means of identification.

In regard to methods, glycoproteins in salivary fluid samples were separated by Polyacrylamide Gel Electrophoresis in the presence of Sodium Dodecyl Sulfate (SDS PAGE) on the basis of size. Staining of the pattern of proteins after transfer to nitrocellulose with carbohydrate binding proteins (lectin blots) was used to characterize the pattern of the glycoproteins in the different proteins. This technique has been previously validated by researchers in other institutions and works well on salivary glycoproteins.¹⁻⁵

Biotinylated lectins derived from plants were used on salivary fluid from eight individuals and were: UEA-I (*Ulex europaeus* I), PNA (peanut agglutinin, *Arachis hypogaea*), ECA (*Erythina cristagalli*), TL (tomato lectin, *Lycopersicon esculentum*), and STA (potato lectin, *Solanum tuberosum*).

The results obtained showed that generally each lectin reacted with a different pattern of protein bands so that the patterns were mostly distinct for each lectin for the different samples . When the staining patterns for the five lectins were compared side by side for all samples, the patterns were different. It was found specifically that UEA-I did not stain proteins in two samples, weakly stained one sample, and stained proteins in five samples. PNA stained one major band in four samples and multiple bands in four other samples. ECA only faintly stained six samples and reacted more strongly with six others. TL stained all samples, but one had a high molecular mass component absent from the others. STA stained high molecular mass bands of different size in three samples but not in three other samples.

The conclusion was that a panel of five lectins UEA-I, PNA, ECA, TL, and STA had different patterns of reactivity that distinguished saliva samples from different sources. This finding may be further investigated for forensic applications.

Reference(s):

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- ^{2.} Austin K.S., Goolsby D., and Vanderpuye O.A. ABO Blood Group Antigens and Mucin-Like Proteins in Human Buccal Cells. *Proceedings of the American Academy of Forensic Sciences*, 63rd Annual Scientific Meeting, Chicago, IL. 2011, vol. 17, A24, p34.
- ^{3.} Carpenter, G.H., Pankhurst, C.L., and Proctor, G. 1999. Lectin binding studies of parotid salivary glycoproteins in Sjögren's syndrome. *Electrophoresis* 1999, 20, 2124±2132.B.
- ^{4.} Rohringer R. and Holden, D.W. 1985. Protein Blotting: Detection of Proteins with Colloidal Gold, and of Glycoproteins and Lectins with Biotin-Conjugated and Enzyme Probes. *Analytical Biochemistry*, 144, 118-127.
- ^{5.} Carpenter, G.H. and Proctor, G. 2000. Double electrophoretic separation and lectin analyses of the component chains of secretory immunoglobulin A from human saliva. *Electrophoresis*, 21, 1440-1453.

Lectins, Saliva, Glycoproteins