G88 Polymorphic Salivary Glycoproteins Recognized by the Carbohydrate-Binding Protein Peanut Agglutinin

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The goal of this presentation is to characterize inter-individual variation (polymorphisms) in a subset of salivary glycoproteins that bind to the galactose binding protein peanut agglutinin. This research was undertaken because there is: (a) lack of knowledge on buccal cell glycoproteins; and, (b) characterization of both buccal cell and salivary fluid glycoproteins will facilitate testing these proteins as biomarkers for pathological conditions and forensic applications such as indicators of exposure to toxins and identity testing through protein polymorphisms.

This presentation will impact the forensic science community by showing how lectins such as peanut agglutinin may be used to detect and characterize salivary fluid and buccal cell glycoproteins. The identified glycoproteins may be further tested for applications as forensic and biomedical markers.

Methods used in this study included: (1) SDS gel electrophoresis and tests of Peanut agglutinin (PNA) binding to proteins electrophoretically transferred to nitrocellulose sheets (blotting); (2) treatment of samples with the enzyme neuraminidase which removes terminal sialic acid from oligosaccharides; (3) microtiter plate binding assays; and, (4) fluorescence microscopy of buccal cells by the use of fluorescent PNA.

An initial analysis was performed to determine the nature of salivary glycoproteins that might be recognized by the galactose-binding protein peanut agglutinin (PNA). Different samples of cell free salivary fluid were analyzed by electrophoresis and blotting with PNA. A protein of molecular mass around 150,000 Daltons bound to PNA in six out of eight salivary fluid samples. This approximately 150,000 Dalton protein had slightly different electrophoretic mobilities in the various samples, being close to 150,000 Daltons in two samples, greater than 150,000 Daltons in three samples and less than 150,000 Daltons in one sample. In the one sample, this approximately 150,000 Dalton protein consisted of two closely spaced bands. The staining intensity of this protein also varied among samples suggesting that different amounts of this protein bound to PNA in different saliva samples. No proteins in human serum bound to PNA. In additional analyses, 45 salivary fluid samples were analyzed for PNA reactivity and 39 of the 45 samples contained the 150,000 Dalton PNA-reactive protein, but six samples were negative. Twelve of the positive samples reacted very strongly with PNA compared to the others. Thus in a total of 53 salivary fluid samples, 84.9% (45) contained a 150,000 Dalton PNA-reactive protein and 15.1% (eight) did not under native conditions.

In another analysis, three matched pairs of buccal cell preparations and corresponding salivary fluid samples from the same donors were compared for PNA-binding proteins. Buccal cells and their corresponding salivary fluid fractions contained proteins of similar electrophoretic mobility that migrated at positions of molecular mass between 200,000 to 150,000. The buccal cell PNA-binding proteins were slower in mobility and of slightly larger molecular mass than the corresponding salivary fluid proteins.

PNA binds to terminal galactose residues on oligosaccharides that are attached to glycoproteins by O-glycosidic linkages. Galactose is sometimes terminated by sialic acid (neuraminic acid sugar residues) which prevent binding of PNA. To further examine the nature of the 150,000 Dalton salivary protein, the binding of PNA was tested with and without treatment of electrophoretically transferred (blotted) salivary proteins with a Clostridium perfringens enzyme neuraminidase which removes sialic acid from oligosaccharides. For untreated samples of salivary fluid, only three out of eight samples had 150,000 Dalton proteins that bound PNA and one of these bound very weakly. After treatment with neuraminidase, seven out of the same eight samples had 150,000 Dalton proteins that bound PNA and the staining of the three samples that were previously reactive with PNA was increased. For a serum sample that was also tested in this experiment, no binding of PNA was observed without neuraminidase treatment, but after neuraminidase treatment very high molecular bands became reactive with PNA.

These results indicated that a salivary fluid protein of approximately 150,000 Dalton varies in reactivity with PNA because individuals vary in how much terminal sialic acid is present on oligosaccharides that are attached to this protein.

An experiment was performed to determine if inter-polypeptide disulfide bonds were involved in the structure of the 150,000 Dalton PNA-binding salivary fluid protein. Four different salivary fluid samples were subjected to SDS gel electrophoresis under non-reducing and reducing conditions and electrophoretic transfers of the gels were analyzed for PNA binding. No shift in electrophoretic mobility was observed for the 150,000 Dalton protein in reducing conditions compared to non-reducing conditions.

The results indicated that this protein did not form inter-chain disulfide bonds with any polypeptide that was large enough to cause a significant shift in electrophoretic mobility. The results also suggested that the 150,000 Dalton PNA-reactive salivary fluid protein did not contain extensive intra-polypeptide bonds of a sort that would cause changes in electrophoretic mobility.

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Two different samples of buccal cells were also analyzed by PNA binding after gel electrophoresis under both reducing and non-reducing conditions and electrophoretic transfer to nitrocellulose sheets. The PNA-reactive protein migrated as a very diffuse band whose mobility was the similar under both conditions. This result indicated that the PNA-reactive buccal cell protein did not form inter-chain disulfide bonds with any polypeptide sufficiently large to alter its electrophoretic mobility.

The presence and relative amounts of PNA-binding proteins in salivary fluid and buccal cell was also confirmed by microtiter plate binding assays which showed that PNA bound to both buccal cells and salivary fluid coated onto microtiter plate wells. PNA binding to salivary proteins produced higher absorbance readings than certain other lectins such as soy bean agglutinin and galanthus nivalis agglutinin which recognize different oligosaccharides than PNA. However other lectins such as artocarpus integrifolia bound in greater amounts than PNA to salivary proteins and produced higher absorbance readings. Fluorescence microscopy by the use of fluorescent PNA or streptavidin and biotinylated PNA confirmed that all buccal cells tested bound PNA to their surfaces.

From the results of this study, it was concluded that buccal cells and salivary fluid contain glycoproteins of molecular mass between 200,000 to 150,000 that vary in amount of terminal galactose and molecular mass among individuals. These proteins may be further examined for use as forensic and biomedical biomarkers.

Saliva, Glycoproteins, Biomarkers

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