Targeted Profiling of Common Metabolites in Saliva

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Saliva is an excellent biofluid for analysis by targeted profiling. Individuals with minimal training can easily collect saliva samples using noninvasive techniques. Targeted profiling of saliva samples can identify and quantify many small molecule metabolites commonly found in saliva, and may allow developing simple screening procedures for various diseases [1] [2]. In this note we present techniques for targeted profiling of saliva spectra with Chenomx NMR Suite.

Introduction

Saliva is easy to collect via noninvasive techniques used by individuals with minimal training, and can provide useful metabolic information [3]. You may collect samples multiple times per day, at times designed to maximize the amount of useful information gathered. Short-term and medium-term storage requirements are not onerous, and in many cases allow storing samples at room temperature. Concentrations of certain small molecule metabolites in saliva, including some hormones and many pharmaceuticals and drugs of abuse, are known to correlate quantifiably with concentrations in serum. Targeted profiling of saliva can directly identify and quantify many of these metabolites.

Preparing Samples and Acquiring Spectra

Analyzing saliva using targeted profiling is similar to analyzing blood serum or plasma, due to its protein content. In preparing saliva samples for analysis by targeted profiling, you must account for the effects of proteins in the sample. This can involve various methods, alone or in combination, as described previously for serum [4].

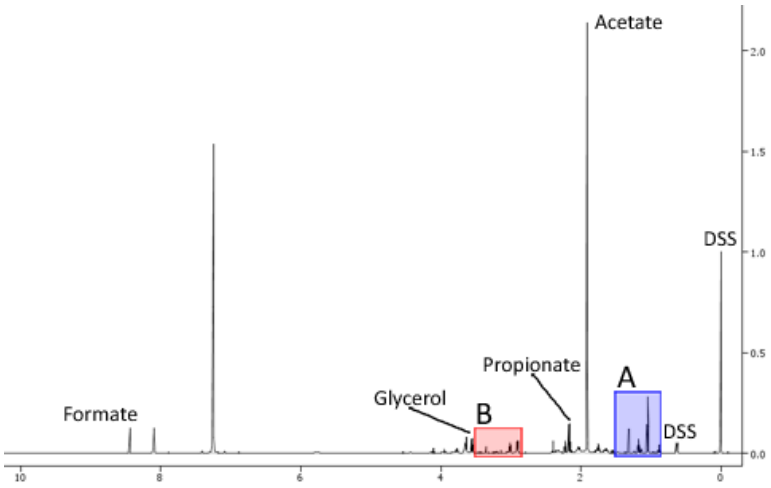

Figure 1. Spectrum of a filtered saliva sample at 600 MHz

At the sample preparation stage, ultrafiltration using microcentrifuge filtration tubes provides an effective method of removing both proteins and particulate matter from saliva samples. If you have filtered your saliva samples, then you can acquire NMR spectra with a NOESY presaturation pulse sequence. On the other hand, if you choose not to filter your samples, several relaxation-edited pulse sequences exist that can remove the broad protein resonances from your spectrum during acquisition, allowing for simpler identification of the small molecule metabolites in the sample.

In general, relaxation-edited pulse sequences are a simpler method of removing the effect of protein resonances from a sample, but analysis of a sample after acquisition is easier when the protein has been removed from the sample [4].

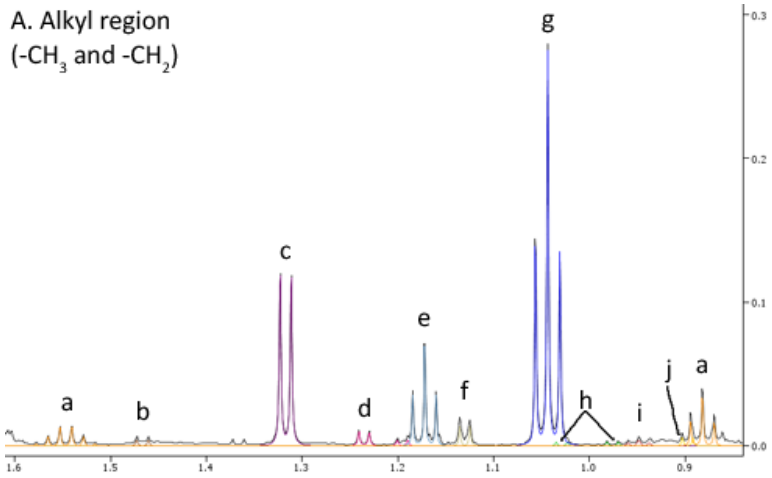

Figure 2. Region A of the sample spectrum, including signals from (a) butyrate, (b) alanine, (c) lactate, (d) fucose, (e) ethanol, (f) propylene glycol, (g) propionate, (h) valine, (i) leucine and (j) isovalerate.

Common Metabolites in Human Saliva

Small molecule metabolites found in human saliva can come from several sources. Depending on the method of collection, food components may be directly observed during targeted profiling of saliva. These can be solutes already dissolved in the food, like caffeine in coffee, solid components that rapidly dissolve into the saliva, like sugars from a cookie, or metabolites resulting from the action of enzymes in the saliva, like maltose from the action of α-amylase on starches.

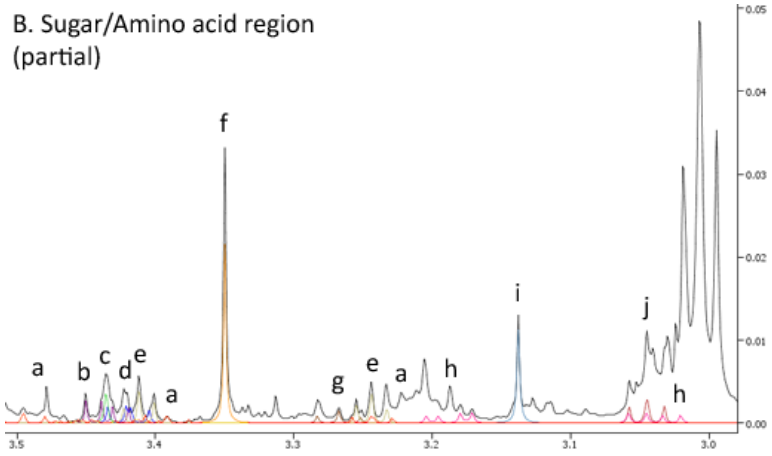

Figure 3. Region B of the sample spectrum, including signals from (a) glucose, (b) propylene glycol, (c) 4-hydroxyphenylacetate, (d) fucose, (e) taurine, (f) caffeine, (g) myo-inositol, (h) tyrosine, (i) malonate and (j) lysine.

Cellular debris from both human and bacterial sources may contribute various metabolites, and products of bacterial metabolism may also be present. Steroids, some other hormones, and many drugs and antibodies can diffuse from serum into saliva, as a result of both passive diffusion and active transport, including ultrafiltration through tight cell junctions [5]. In some cases, metabolites measured in saliva can provide information about metabolite concentrations that cannot be readily obtained from serum analysis [3].

Conclusion

You can readily use targeted profiling to analyze saliva samples, following ultrafiltration to remove interfering proteins and particulate matter. The remaining small molecule metabolites can provide insight into serum composition that is otherwise difficult to obtain.

References

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Metabolite List

Table 1. Common Metabolites in Human Saliva

Amines and Derivatives	
Caffeine	Methylguanidine
Choline	O-Phosphoethanolamine
Creatinine	Trigonelline
Dimethylamine	Trimethylamine
Methylamine	Trimethylamine N-oxide
Amino Acids and Derivatives	
4-Aminobutyrate	Methionine
Alanine	Ornithine
Aspartate	Phenylalanine
Glutamate	Proline
Glutamine	Sarcosine
Glycine	Threonine
Histidine	Tryptophan
Isoleucine	Tyrosine
Leucine	Valine
Lysine	
Carbohydrates and Derivatives	
Acetone	Glucose
Ethanol	Methanol
Fucose	myo-Inositol
Galactose	Propylene glycol
Glucitol	

Organic Acids	
3-Hydroxybutyrate	Isobutyrate
4-Hydroxyphenylacetate	Isocaproate
Acetate	Lactate
Acetoacetate	Phthalate
Butyrate	Propionate
Citrate	Pyruvate
Formate	Succinate
Glycolate	Valerate