



B25 An Investigation Into the Use of Amino Acid Ratios to Distinguish Between the Hairs of Similar Individuals

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Learning Overview: After attending this presentation, attendees will gain an understanding of how amino acid concentration ratios of hair proteins can be used to distinguish between the hairs of demographically similar individuals.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing an alternate method of hair analysis for when DNA is not present in a hair sample and for when different hair samples appear similar under a microscope.

Hair is a very important piece of trace evidence to forensic scientists because it is commonly found at crime scenes and can be a vital piece of evidence in a criminal case. Currently, forensic hair analysis is performed by both Microscopic Hair Comparison (MHC) and DNA analysis, but they are not always reliable. For example, MHC is very subjective and is not supported with statistical analysis. On the other hand, DNA analysis can be problematic if there is not enough undamaged nuclear DNA to sample from or if the nucleated cells within a hair follicular tag are not intact or are not present. Therefore, a new technique of analyzing hair, such as proteomic analysis, must be developed to replace MHC and DNA analysis for instances in which no beneficial results can be obtained from those methods. In this study, amino acid analysis was investigated as a new method to analyze hair proteins.^{1,2} Human hair is made up of several proteins, such as keratin, and these proteins can have slight genetic variations in the form of Genetically Variant Peptides (GVPs). These GVPs contribute to an individual's genetic variation because they can alter amino acid sequences.³ This means that the amount of amino acids can vary in people's hair based on their genetics.

First, hair samples were washed with deionized water and methanol. Then, the hair proteins were broken down into their corresponding amino acids via acid hydrolysis with hydrochloric acid. After digestion of the hair proteins, norvaline, an amino acid not synthesized by humans, was added to the hair samples as an internal standard. The amino acids were then derivatized with N,O-Bis(trimethylsilyl)trifluoroacetamide and identified using gas chromatography/mass spectrometry. The amino acids present in hair were quantified relative to norvaline, and the values were used to construct amino acid ratios. The amino acid ratios of various hair samples from individuals of common demographics and from monozygotic twins are compared to distinguish one hair sample from many others.

Hair samples were obtained on various days and from various areas of the head. The aim was to show that people's amino acid ratios do not change based on when the hair samples were taken or where the hair samples were taken from the head. Initial results suggest that amino acid ratios of hair proteins do not change over a short period of time and do not change among different areas of the head. Next, hair samples were taken from two people who have similar demographics. The goal was to use amino acid ratios to distinguish between the persons' hairs. Preliminary data supports the hypothesis that hair amino acid analysis can be used to distinguish between demographically similar individuals. For example, one person's proline/valine ratio was three times higher (5.46 ± 1.68) than the same ratio in the other person's hair (1.84 ± 0.27). Differences were also observed for the phenylalanine/leucine ratio, with one person's ratio being seven times higher (0.51 ± 0.07) than the other person's ratio (0.07 ± 0.02). Other ratios that were different between the hairs include aspartic acid/threonine, serine/leucine, tyrosine/valine, and several others. However, a larger population must be investigated further, including multiple individuals with similar demographics as these two people, as well as comparing the hair amino acid profiles of monozygotic twins.

In conclusion, hair amino acid analysis has the potential to enhance the current identification methods of individuals. Although it is not an individualizing technique, it could help provide law enforcement with a person's demographics based on hair found at a crime scene. This could help to narrow a suspect pool or rule out specific individuals.

Reference(s):

1. Sirena Lam. *Investigating the Use of Amino Acid Ratios in Human Hair to Assist in the Differentiation of Individuals Using Gas Chromatography/Mass Spectrometry (GC/MS)*. (Thesis, University of New Haven, 2018).
2. Ayat H.B. Rashaid, Peter B. Harrington, and Glen P. Jackson. Amino Acid Composition of Human Scalp Hair as a Biometric Classifier and Investigative Lead. *Analytical Methods* 7 (2015): 1707-1718.
3. Glendon J. Parker et al. Demonstration of Protein-Based Human Identification Using the Hair Shaft Proteome. *PLoS ONE* 11, no. 9 (2016): 1-26.

Amino Acids, Hair Proteins, GC/MS