

OPTIMIZATION OF HUMAN HAIR PROTEOMIC PROCESSING TO MAXIMIZE TOTAL AND GENETICALLY-VARIANT PEPTIDES

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Human identification from forensic hair evidence, primarily using microscopic comparisons, has been criticized for its subjectivity and minimal reproducibility. Hair mitochondrial DNA analysis is scientifically and statistically rigorous, but the DNA can degrade under environmental conditions. The peptide bonds in protein are more resilient to breakage than phosphodiester bonds in DNA. Using high resolution mass spectrometry, single amino acid polymorphisms can be detected in the amino acid sequences of keratins, keratin-associated proteins, and other hair shaft proteins. As a result, Genetically-Variant Peptides (GVPs) can be used to help identify an individual and to provide information on ancestral origin. To increase peptide yield from hair protein, and to maximize GVP and ancestral information, we examined the conditions for reduction and protein digestion. Typical hair processing is accomplished using a detergent, reducing agent, alkylating agent, and proteolytic enzyme. Harsher treatments use long incubation periods of up to several days and higher temperatures during reduction, while gentler methods use a cleavable detergent, room temperature, and shorter digestion periods. In this research, temperature, time, agitation method, and concentrations of the reagents have been varied. Results indicate that for unique peptide number, lower temperatures were best, yielding 2441 ± 18 unique peptides versus 2020 ± 108 at higher temperatures. Agitation by stirring has given higher solubilization than swirling, reflected in an average solubilization of $76\% \pm 7\%$ by mass via stirring and $43\% \pm 5\%$ by mass via swirling. A time course quantifying the residual insoluble fraction has shown that trypsinization for 6 hours solubilizes $73\% \pm 2\%$ of the hair by mass and produces the largest number of unique and total peptides in the soluble fraction, yielding 2778 ± 37 unique peptides versus 2068 ± 15 for the 3 day digestion. The optimized hair processing procedure with shorter times and lower temperatures for both reduction and digestion has yielded more detectable GVPs than the previous method, with 37 versus 16 GVPs in European hair and 22 versus 12 GVPs in African hair. Another gentle processing method using urea and a cleavable detergent was tested on the European hair, yielding 25 GVPs versus the 37 by the optimized method. Thus optimizing sample processing is anticipated to improve hair analysis for individual identification. Overall, this research will enhance the use of novel GVP analysis in forensic science by helping to optimize GVP yields and discovery, as well as assuring uniformity in results across different hair types.