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STR Markers: Pioneering Advances in Forensic Science and Genetic Research

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Abstract:

Forensic proteomics is a cutting-edge science that uses extensive protein analysis to handle various forensic difficulties, complementing traditional DNA-based approaches. Proteins' stability and abundance in biological samples make them important when DNA evidence is deteriorated, tainted, or inadequate. Advanced mass spectrometry methods make it easier to identify and characterize proteins and their post-translational changes, revealing important information about the biological condition and identity of persons engaged in criminal investigations.Proteomic analysis has several forensic uses, including identifying human remains, estimating post-mortem periods, and determining the reason and manner of death. Proteins taken from bones, teeth, and hair can provide valuable information about a person's age, gender, and perhaps lineage. Furthermore, examining protein breakdown patterns helps to estimate the period after death, which is an important component in forensic investigations. In violent crime cases, proteomic techniques may detect blood, sperm, saliva, and other body fluids, even in tiny amounts, using unique protein markers. Forensic proteomics also covers the examination of non-human proteins, which is essential in wildlife forensics and identifying animal species involved in illegal trade and poaching incidents.The resilience of proteins under varied environmental circumstances enables the examination of material exposed to hostile environments, increasing the scope of forensic investigations. Despite its intriguing premise, forensic proteomics confronts several hurdles, including the need for standardized techniques, large protein databases, and powerful bioinformatics tools for data analysis. Continued advances in mass spectrometry, sample preparation, and computer analysis are critical for overcoming these barriers and incorporating proteomics into standard forensic practice.

Keywords: *forensic proteomics, mass spectrometry, computational analysis, post-translational modifications*

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Introduction

DNA analysis has long been regarded as the gold standard in forensic science for identifying individuals and resolving crimes. However, sample deterioration, contamination, or insufficient quantity may limit the use of DNA alone in some circumstances. To address these issues and improve the robustness of forensic investigations, forensic proteomics has emerged as a valuable additional technique **(Ballou *et al.*, 2013).** Proteins, which are essential for organisms' biological processes, are usually more stable and plentiful in biological samples than DNA **(Kontopoulos, *et al.*, 2020).** Proteins are extremely useful in forensic investigations because of their stability, especially when DNA integrity is impaired. Forensic proteomics uses modern mass spectrometry methods to provide a thorough profile and identification of proteins and their post-translational changes. These alterations can provide important information about a person's biological condition, the period since death, and the presence of certain physiological fluids at crime scenes. Furthermore, forensic proteomics is not restricted to human materials. It includes wildlife forensics, which helps identify animal species engaged in illegal trade and poaching. The capacity to examine proteins in various situations broadens the toolset accessible to forensic investigators, allowing them to identify human remains, estimate post-mortem periods, and determine causes and ways of death. As forensic science advances, the incorporation of proteomics has the potential to dramatically improve the accuracy and reliability of forensic investigations. By supplementing DNA evidence with specific protein profiles and alterations, forensic proteomics provides a more complete and nuanced approach to criminal investigation, eventually improving the pursuit of justice.

History:

In the early twentieth century, forensic investigators began to investigate the use of protein-based approaches for identifying biological material. One of the first strategies involves using immunological tests to identify blood and other biological fluids at crime scenes. The precipitin test was very effective in distinguishing between human and animal blood, providing critical evidence in criminal trials.

Blood Group Typing

Karl Landsteiner's discovery of the ABO blood type system in 1900 completely changed the field of forensic serology. Forensic investigators now depend heavily on blood group typing to categorize blood samples according to specific antigens present on the surface of red blood **cells (Watkins and Morgan, 1959).** This technique was commonly utilized during the twentieth century to connect suspects to murder scenes, despite its limitations owing to the small number of blood groups and the likelihood of accidental matches **(Sensabaugh, 2016).**

Isoenzyme screening and electrophoresis

The development of electrophoresis techniques in the 1960s and 1970s made it possible to separate and analyse proteins according to their size and charge. Around this same period, isoenzyme analysis emerged, a technique that used variations in enzyme forms to identify individual differences. For example, phosphoglucomutase (PGM) typing enabled forensic investigators to examine genetic differences in this enzyme, hence increasing the selective power of protein-based evidence **(Butler, 2011).**

Application and future prospects

Today, forensic proteomics is evolving, including modern technology and procedures to improve the accuracy and reliability of forensic investigations. Modern uses of protein analysis include identifying human remains, estimating post-mortem periods, and detecting particular body fluids at crime scenes. Proteomic methods are also being studied for their potential to provide details on the age, gender, and health status of a deceased person. The continued advances in mass spectrometry, bioinformatics, and sample preparation techniques are beneficial to the field of forensic proteomics. These developments could lead to more thorough and insightful investigations by increasing the sensitivity and specificity of protein-based forensic research **(Divall, 1985).**

Fundamentals of proteomic mass spectrometry

Proteomic mass spectrometry advancements are transforming cell biology and biochemistry. Significant advancements have addressed four essential difficulties in proteomics. The first trend is the advancement of high-pressure liquid chromatography (HPLC) for protein and peptide separation and fractionation. Modern chromatography equipment can achieve greater, constant pressures and ultra-low flow rates **(Wilson *et al.*, 2015)**

Shotgun proteomic: Shotgun proteomic mass spectrometry utilizes trypsin for protein digestion, reducing and alkylating complex peptides, allowing for resolution through chromatography. Mass

spectrometry is used to acquire the mass of peptides and their fragments after they elute, enabling accurate analysis of their properties. The MS2 fragment spectra are compared to projected spectra from a protein database, which includes predicted peptides from the human genome projectThis method is analogous to large-scale shotgun DNA sequencing, which involves utilizing unbiased DNA libraries without targeting. There are limits to this method. Trypsin digests millions of peptides from over 10,000 genes expressed in all tissues, with abundances ranging by seven orders of magnitude. Variations between runs are unavoidable. In shotgun proteomics, peptide fragmentation is very stochastic and may not identify all peptides in each run. Mass detection is concentration-dependent, whereas scanner acquisition is rate-limited. This has important consequences for forensic proteomics since a lack of detection cannot be used as proof of absence. This analytical difficulty resembles the genetic analysis of low-read, low-coverage genomes. Mass detection is concentration-dependent, whereas scanner acquisition is rate-limited. This has important consequences for forensic proteomics since a lack of detection cannot be used as proof of absence. This analytical difficulty resembles the genetic analysis of low-read, low-coverage genomes. Stochasticity may be addressed through various inventions and data collection methodologies **(Michalski *et al.*, 2011).**

Targeted proteomics: Trypsin is utilized in mass spectrometry using a triple quadrupole spectrometer, allowing for targeted analysis of complex peptide combinations through reduction and alkylation processes. ESI is used to volatilize peptides, which are then filtered using a precise mass window. The target peptide is fragmented via collision-induced dissociation in a second quadrupole, and two or three targeted fragments are measured in the final quadrupole for peptide validation. Shotgun and targeted proteome mass spectrometry eliminated the need for several tests and assays for protein-based forensic investigation, which used up limited forensic material. Workflows have streamlined and converged on preparing a sample and putting it to mass spectrometry. Similar to PCR, a single study can provide genotypes from several loci. Current proteomic approaches are far more methodical, simple to execute, and powerful in terms of information acquisition. Forensic proteomics aims to identify the source tissue, analyze genetic information, and determine species **(Kulak *et al.*, 2014).**

Proteomics in forensic samples

Proteins are naturally more stable than DNA. The DNA backbone is high in mildly reactive electrophilic oxygen and nucleophilic amines. However, the amines and oxygen in the peptide backbone are nearby across the amide peptide link, making them less reactive. Protein persistence in forensic materials makes proteome mass spectrometry an effective method for analyzing contaminated samples. This is illustrated by the use of paleo-proteomics to identify prehistoric organisms. The oldest DNA on record comes from frozen mammoth samples that are over a million years old. Beyond that point, multiple paleo-proteomic dates have been discovered, the most recent being the detection of struthiocalcin protein in a 3.8-million-year-old ostrich egg. While contentious, collagen has been found in Tyrannosaurus rex and Brachylophosaurus canadensis bones dating back much further in antiquity. The capacity of paleo proteomics to distinguish between species is contingent on obtaining genetically meaningful information from severely contaminated protein samples. Protein is also an appropriate substrate for forensic sample analysis due to its similar features. Before proteomics may be widely employed in forensic contexts, practitioners must first acknowledge and account for the distinct biological, chemical, and analytical circumstances, as well as legal constraints, of processing evidence from a crime scene. The kind of sample studied is a key distinction in forensic proteomics: forensic settings produce degraded samples that are varied and frequently quite restricted. As with DNA-based approaches, forensic proteomic processes must account for considerable variations in the amount of material available for analysis. In forensic research, proteomic samples are often solid substrates like hair or bone, or dry, like bodily fluids or touch samples from skin or clothes. Forensic proteomic approaches should be compatible with other procedures, such as DNA typing, given their destructive nature. Furthermore, newly discovered peptide markers must be rigorously confirmed to survive legal and scientific examination. **(Aebersold, R *et al.*, 2018)**

Forensic environment and protein structure

Biological samples found at a crime scene will either have been taken from the body, come from a deceased person, or be in highly changeable, uncontrolled conditions. These might include greater temperatures, water availability, oxidative chemistry, pH extremes, and endogenous and ambient small molecules and metabolites. The repair mechanisms found in living cells and animals will not be able to reverse the build-up of denaturation and chemical alteration of proteins that occurs over time before collection. The number of intact, appropriately formed proteins have decreased. As previously noted, this reduces the sensitivity of bodily fluid detection and several, now-obsolete,

protein-based tests for genetic diversity. Many chemistries are inherent and influenced by protein mobility and environmental temperature. The well-studied and often seen of these is deamidation, in which the side chains of glutamine and asparagine react with the peptide bond to form glutamic or aspartic acid or its gamma counterparts. Another process is the racemization of amino acids from L- to D-enantiomers. Common environmental reactants, like oxygen, can alter sulphur-containing amino acid side chains, especially methionine and cysteine. Over time, these two processes enhance the variety of peptide masses, or peptidoforms, while decreasing the number of unmodified peptides accessible for investigation**.**

Preparation of a forensic sample

The majority of proteomics sample preparation follows a conventional methodology, which includes protein extraction, proteolytic digestion, and sample cleaning. This corresponds to the procedures used for DNA sample preparation. Rehydration, tissue homogenization, cell lysis, sample solubilisation, and centrifugation are all possible sample preparation methods. The protein matrix must be denatured so that proteases may enter and digest the exposed peptide backbones within. This is accomplished by the use of chaotropic substances like urea or guanidine, as well as powerful detergents. The disulphide linkages in the matrix are opened up by reluctant and then sealed by alkylation, preventing cysteines from reforming randomly and reforming the matrix. The most prevalent protease is trypsin, which cleaves the peptide backbone on the C-terminal side of positively charged arginine and lysine. Following digestion, materials can be cleaned and desalted before mass spectrum analysis by solid-phase extraction. Targeted protein or peptide capture using immobilized antibodies can also be employed to enrich a sample before analysis, focusing biomarker targets and reducing the impact of complex matrices. **(Duong *et al.*, 2021).**

Forensic proteomic tissues and bodily fluids

Forensic proteomics differs from other proteome approaches in terms of the types of samples studied. Body fluids are frequently freely available; therefore, proteomic specialists in the biological sciences have utilized them to show breakthroughs in mass spectrometry apparatus, technique, and analysis. The proteome composition of semen and vaginal fluid is important for both therapeutic and forensic purposes. The same goes for perspiration and vomiting. Forensically significant solid tissues, such as bone, teeth, hair, nail plates, and skin cells, have also been carefully processed and examined for both forensic and therapeutic purposes. Mineralized and keratinized tissues have also been studied from a paleontological and paleo-proteomic standpoint, which has forensic applications for the examination of extensively deteriorated human remains.

Tissue and proteomic body fluid identification

Forensic serology is the classification of biological fluids of forensic importance (such as blood, sperm, saliva, vaginal/menstrual fluid, and so on) discovered in the course of a criminal investigation. Detecting and identifying biological fluids gives critical contextual information for forensic investigations. While genetic testing can help determine where DNA came from, only serological testing can identify the bodily fluid or tissue from which a DNA profile originated. To get the maximum probative value out of a biological stain in a criminal investigation, you must first create an interpretable DNA profile and then identify the biological material from which the profile came. **(Legg *et al.*, 2016)** The proteins found in biological samples provide forensic contextual information. Transcriptional programs are tissue- or bodily fluid-specific. Proteomes, therefore, reflect tissue structure and physiological activity. The gene products present in a sample, as well as their relative levels, can be utilized to determine its tissue origin. However, modern forensic instruments for identifying bodily fluids are based on the same core procedures used throughout the history of forensic research. These include chemical interactions with bodily fluid components, detection of enzyme activity typical of a body fluid, and, in the case of sperm, direct observation of spermatozoa using microscopy. While these approaches are useful in forensic investigations, they have a number of significant test-specific limitations, the most noteworthy of which are related to specificity**. (Yang *et al.*, 2013).**

Proteomic genotyping

Proteins include genetic information in the form of single amino acid polymorphisms (SAPs) caused by non-synonymous SNPs. Proteomic genotyping is the process of detecting genetically variable peptides (GVPs) containing SAPs and then determining the existence of the associated SNP alleles in the genome of the individual who produced the protein sample. In aggregate, the profile of inferred SNP alleles, like any profile of nucleotide variation, may be used to compute the statistical connection between a person and a protein sample**. (Franklin *et al.*, 2020).**

Proteomes containing genetic information

Proteomic samples contain significant genetic information, with individual genomes containing between 3.5 and 4.3 million single nucleotide polymorphisms (SNPs), depending on genetic background. Non-synonymous SNPs alter codon assignment, resulting in single amino acid polymorphisms in genetically variable peptides.

Application of forensic proteomics

Finger-marks, often exposed to environmental damage, can be used for DNA analysis using low-copy-number DNA processing techniques. MALDI mass spectrometry can be performed in two dimensions, revealing significant molecules and information about an individual's biology, metabolism, and chemical exposure.

Protein markers for species identification

Forensic scenarios often involve identifying species of biological evidence, such as in criminal investigations, fish and wildlife regulations, customs, and food security. Proteins contain genetic variation and phylogenetic information, and as animal communities diversify, new alleles may become dominant. **(Welker, 2018).**

Proteomic sex estimate

Forensic proteomics is a crucial method for determining an individual's sex, particularly in the case of amelogenins, a sex-specific gene family found in enamel, the most durable human tissue. The amelogenin protein is essential for enamel production and is destroyed via endogenous proteases, resulting in peptides rich in biomarkers for the X and Y chromosomes. These markers can be used to predict the sex-chromosome karyotype of the enamel donor. Proteomic sex estimation is a reliable method for estimating sex in ancient DNA samples, outperforming genomic and osteology methods. This method has been successful in estimating fetal, baby, and partially cremated individuals. However, genomic and proteomic estimations occasionally disagree, especially in lower-quality DNA samples. Proteomic sex estimation is a confirmed technology with archaeological applications, but its value in forensic contexts is yet to be proven. Further validation testing is needed to evaluate its effectiveness and potential drawbacks.

Estimation of the post-mortem interval

Proteome degradation can be used to estimate post-mortem intervals, as environmental chemistry alters proteins over time. This data can be used to create algorithms for predicting post-mortem intervals. Housekeeping globular proteins, such as glycolytic enzymes, are more adaptable and susceptible to degradative chemical reactions. Proteomics does not rely on anatomical integrity, and protein changes may play a function in this context. Deamidation of hair proteins can be used to estimate post-mortem intervals, date archaeological material, and distinguish ancient proteins from current contaminated keratins. However, the dependent nature of chemical alterations, the large number of possible modifications, and the inability to accurately identify all proteoforms present challenges in estimating post-mortem time. **(Oonk *et al.*, 2018).**

Conclusion

Forensic proteomics, a rapidly growing subject in forensic science, uses extensive protein analysis to answer challenging forensic issues. Unlike DNA, proteins are more durable and plentiful in biological samples, making them useful when DNA is destroyed, contaminated, or inadequate. This discipline uses modern mass spectrometry techniques to detect and describe proteins and their post-translational changes, giving crucial information about a person's biological condition and identity in criminal investigations. Proteomic analysis has several forensic uses. It can help identify human remains, estimate post-mortem periods, and ascertain reasons and modes of death. For example, proteins isolated from bones, teeth, and hair can indicate a person's age, gender, and even lineage.

Forensic proteomics goes beyond human biology. It includes wildlife forensics, which is critical for identifying animal species implicated in illegal trade and poaching. Proteins' resistance to varied environmental conditions enables the study of materials exposed to hostile environments, increasing the scope of forensic investigations. Despite its high promise, forensic proteomics confronts a number of obstacles.

Forensic proteomics has its roots in the early twentieth century, when protein-based procedures for detecting biological materials were first used. Early procedures included immunological testing, such as the precipitin test, which discriminated between human and animal blood. The ABO blood group system, discovered by Karl Landsteiner in 1900, revolutionized forensic serology by allowing blood samples to be classified based on particular antigens. In the 1960s and 1970s,

electrophoresis and isoenzyme analysis were introduced, increasing the discriminating power of protein-based evidence. Protein sequencing and mass spectrometry, which were introduced in the late twentieth and early twenty-first centuries, marked important advances in forensic proteomics. These technologies enabled the accurate identification and characterization of proteins and their changes. The 2001 anthrax attack investigation demonstrated the potential of proteomic approaches in forensic science, which aided in determining the source of the bioterrorism event.

Today, forensic proteomics is evolving by merging cutting-edge technology and approaches to improve the accuracy and reliability of forensic investigations. Modern uses include identifying human remains, calculating postmortem periods, and detecting specific bodily fluids at crime scenes. Proteomic methods are also being investigated to reveal information on an individual's age, gender, and health state at the time of death. The discipline of forensic proteomics will benefit from current developments in mass spectrometry, bioinformatics, and sample preparation procedures. These enhancements promise to increase the sensitivity and specificity of protein-based forensic studies, allowing for more thorough and insightful investigations. By supplementing DNA evidence with specific protein profiles and alterations, forensic proteomics provides a more complete and nuanced approach to criminal investigations, eventually furthering the pursuit of justice.

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