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Proteomic Approaches *vis-a-vis* Forensic Evidence Analysis: Forensic Proteomics a Valuable Tool

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Abstract

Proteomics is a valuable tool for identifying biological systems because it allows researchers to study all the proteins in an organism or system on a large scale. Advanced proteomic techniques can be used in the forensic field as a confirmatory and complementary approach to well-designed genomic analysis. When nucleic acids are missing or destroyed at the crime scene, it is particularly beneficial for efficient and reproducible peptide and protein analysis, identification, and characterization in a variety of biological and non-biological contexts. In forensic sciences, this could help identify new biomarkers. Unlike DNA markers, protein markers prevent sample contamination while giving quantifiable and highly reliable data that can be evaluated employing databases. They are used in forensics to identify body fluids, ethnic groups, genders, and individuals, and to estimate post-mortem intervals from decomposition fluid, bone and muscle samples. Our current review focuses on a wide spectrum of human samples used in forensic proteomics, as well as recent research achievements. This review is for forensic investigators who want to learn and explore more about proteomics and how to get more information from biological evidence.

Keywords: Proteomics, Forensic, serology, body fluid, evidence.

Introduction

Forensic proteomics is still in its early phases of development. It has been utilized in forensics for tissue and body fluid identification, protein toxin identification and quantification, human individualization, detection of protein pharmaceuticals and hormones in sports, and post-mortem interval estimate. Forensic proteomics is still in its early phases of development. It has been utilized in forensics for tissue and body fluid identification, protein toxin identification and quantification, human individualization, detection of protein pharmaceuticals and hormones in sports, and post-mortem interval estimate. A crime scene is a

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location where the crime is believed to have occurred. At the scientific level, the investigator must learn to recognize, identify, secure, and collect physical evidence.[1]

Protein can thus be used to identify the source of a sample's tissue or bodily fluid, a fundamental forensic context that is still used today. Protein molecules also have genetic information in the form of polymorphisms in amino acids. However, the protein molecules can explore vast information regarding forensic samples, even though it is extremely complex.[4] Some of this information may be important to investigators because it provides context regarding the origin of the material from the donor tissue. Proteomic advances, for the most part, mass spectrometry, have had a considerable impact on the life sciences, and they comprise the potential for forensic investigations as well. The impact of these proteomic opportunities on forensic practice is an open topic that will be explored in the future. Our current review focuses on a wide spectrum of human samples used in forensic proteomics, as well as recent research achievements[2].

Fig. 1: Various type of samples for Forensic Proteomics analysis.

Advancements in proteomics

Genes encode proteins, which are complex molecules that catalyze activities, transmit information, and build three-dimensional cellular support structures that are structured spatially and temporally. The goal of several of the early proteomic research was to link individual proteins to biological processes. Even though this effort was successful, it is essential to mention that proteins do not always have a specific task or role in physiology and one of the major challenges of proteomics is identifying the various cellular functions and roles of proteins in cells. Proteomics has advanced to the point that it can now identify post-translational changes in proteins, protein-protein interactions, and protein locations inside cells. Recent proteomic investigations aim to discover protein structures or folding on a large scale and in vivo. Advances in cryogenic electron microscopy (cryo-EM) have resulted in a massive rise in the number of challenging protein structures identified. However, because most of these studies have been conducted in vitro, it is critical to evaluate how these structures relate to those seen in cells. Mass spectrometry (MS) has been used successfully to analyze native proteins and native protein complexes, and it is currently being utilized to research whole cells in an attempt to determine the state of folding of proteins within whole proteomes.[3] New instruments and approaches are required to enhance the acquisition of proteomic information.

Proteomics of Forensic Samples

Proteomics is an effective method to know more information about biological systems. Recent advances in LC-MS /MS have made it possible to analyze peptides and proteins in samples in a time frame similar to next-generation sequencing (NGS). Proteomics can save time and money when compared to immunological procedures that need antibodies and polymerase chain reaction (PCR) with particular primers. In a single experiment, proteomics methods allow the exact identification of a particular protein.[4]

The first technique is to analyze the effectiveness of the peptide matching procedure in shotgun proteomics datasets internally and statistically. The second way of validation is to compare the reported peptide masses and mass spectra to approved standards using an assigned peptide sequence. Finally, standard peptides can be used to compare spectra of known peptides with spectra of peptides found in a sample.[5]

Proteomic genotyping

Proteomic genotyping explains the finding of genetically variant peptides (GVPs) containing Single amino acid polymorphisms (SAPs) along with corresponding single nucleotide polymorphisms (SNP) alleles in the genome of the sample donor. It has been confirmed in the literature that GVPs can accurately identify nearly 500 non-synonymous SNP alleles. Proteomic genotyping is useful for forensic investigators in contexts where obtaining DNA-based information is complicated.[6]

Instrumental analysis of proteins

Proteomics is an effective method for exploring biological systems. Recent advancements in LC-MS/MS have made it possible to analyze peptides and proteins in samples quickly, in a manner comparable to next-generation sequencing (NGS). The expansion of proteomics including liquid chromatography-tandem mass spectrometry (LC-MS/MS) to analyze peptides and proteins in samples in a time frame comparable to next-generation sequencing (NGS).[7]

Forensic serology

Forensic serology classifies biological fluids of forensic interest (such as semen, urine, breast milk, blood, saliva, vomit, and vaginal/menstrual fluid) recovered in connection with a crime scene (Table 1).

Sample types	Proteomic methods	Biomarkers	Applications	References
Blood	Liquid chromatography-tandem mass spectrometry (LC-MS/MS)	Phospholipase A2	Identified snake venom	8
Bone	Liquid chromatography-tandem mass spectrometry (LC-MS/MS)	Collagen alpha-1(III) chain, decorin, olfactomedin-like protein 3 (OLFL3)	Accurate post mortem interval (PMI) and age-at-death (AAD)	9
Skin	Liquid chromatography-tandem mass spectrometry (LC-MS/MS)	Genetically variable peptides (GVPs)	Human identification.	10
Blood	Capillary reversed-phase liquid chromatography MS/MS	Apolipoprotein A1 (ApoA1) and α-1 antitrypsin	Diagnosis of drowning	11
Hairs	Liquid Chromatography-Tandem Mass Spectrometry	Genetically variant peptides (GVPs)	Protein-based human identification	12
	High-Resolution Tandem Mass Spectrometry	Genetically variant peptides (GVPs).	Protein-based human identification	13

Sample types	Proteomic methods	Biomarkers	Applications	References
Tissue	Western Blotting	Fosl1 protein	Wound age estimation	14
Saliva	Phadebas Paper	α-amylase	Identification of saliva	15
Urine	Liquid chromatography-tandem mass spectrometry (LC-MS/MS)	Osteopontin and Tamm-Horsfall protein (THP)	Identification of body fluids	16
Sweat	Liquid chromatography-tandem mass spectrometry (LC-MS/MS)	Dermcidin	Identification of body fluids	16

Table 1. Proteomic techniques used to identify biological markers in forensic human samples.

Proteomic analysis of blood/body fluids

Blood plays an important role in the analysis of available components in the blood, it is the most important body fluid among others because it is one of the most important indicators of a violent incident and is difficult to remove from the scene of a crime. Every biological body fluid serves a unique purpose, the composition and concentration of proteins present in each fluid are also unique. Swanson et al. devised a thorough screening approach for forensic toxicological analysis utilizing mass spectrometry and enzyme-linked immunosorbent assay (ELISA) in a recent study. In this comparative investigation, a method for recognizing drugs was devised using postmortem blood samples with minimal sample preparation consisting only of protein precipitation. For forensic specimen analysis, the LC/MS-MS approach performed as well as or better than the other methods.[17]

In contrast, studies using mass spectrometry to identify proteins in menstrual blood have identified several different markers, including orexin-A (OREX) and the semaphorin receptor (PLXA1, D1), involved in cord vascular tissue formation and extracellular matrix adhesion, respectively. Kennedy et al. developed a proteomics method based on MALDI MS for reliable detection of blood, differentiation of human and animal blood, determination of animal blood species, and identification of sperm.[18] Based on the success of forensic proteomics, peptidomics can be used to identify potential. peptide biomarkers in these fluids and also has a wide range of applications in forensic science. Peptide mapping software such as SWPepNovo and Peptigram has been developed. Peptide biomarkers in seminal fluid corresponding to known seminal protein biomarkers have also been identified, including prostate-specific antigen, semenogelin I and II, and prostate acid phosphatase.[19]

Hair Proteome

Hair shafts are common at almost all crime scenes but are underutilized forensically. Keratinization of epidermal keratinocytes leads to their formation. The strong hair tissue is due to extensive isopeptide and disulfide cross-linking, which effectively transforms the hair shaft into a single protein molecule. In a recent study, it was found that keratin peptides in human hair can be used to determine the gender (using keratin peptides K81, K83, and K86 of type II peptides) and ethnicity (using type I and type II keratin K33b, K81, K83, and K86 peptides).[20]

Bone Proteome

The extracellular matrix contains bone proteins, most of which are collagen proteins (90 percent). Due to their interconnected structure and the protection provided by the bone matrix, bone collagens are extremely stable. Prieto-Bonete et al. employed proteome analysis to identify 32 proteins that might distinguish between postmortem intervals of different time intervals in 40 femur bones from 40 different cadavers.[21] Notably, the muscle protein fructose bisphosphate aldolase A (ALDOA) decreased in abundance as PMI increased, whereas the proteins peptidylprolyl cis-trans isomerase (PPIA) and coagulation factor VII (F7A) might be used as biomarkers to differentiate samples from terrestrial and aquatic environments.[22]

Muscles

The degradation of skeletal muscle proteins can be used to estimate Mid-PMI (approximately 24–120 h PMI) by using gel electrophoresis, Western blotting, and casein zymography. Choi et al. recently used proteomic profiling to examine global alterations in rat and mouse skeletal muscle proteomes at 0, 24, 48, 72, and 96 hours after PMI, and found two proteins, elongation factor 1alpha 2 (eEF1A2) and glyceraldehyde3phosphate dehydrogenase (GAPDH). These proteins were found to be viable for PMI estimation in human autopsy cases, as confirmed by traditional Western blot experiments.[23]

Fingernail

A study has reported the detection of cornulin (a protein biomarker for vaginal fluid) up to 5 h and hemoglobin (a protein biomarker for blood) up to 18 h post-deposition in fingernails.[24]

Protein markers for species identification

In forensic scenarios, identifying the originating species of biological evidence is required as part of the standard criminal investigation. Proteins contain phylogenetic information as well as information about genetic variation in human populations. Baniasad et al. recently studied how forensic evidence could be used in protein-based human genetically variable peptides (GVP) analysis in the future to help in-person identification. A reliable protein extraction (RapiGest) and digestion (trypsin enzymatic digestion) approach for skin proteins are critical for downstream GVP analysis and the advancement of this technology may improve the analysis of forensic queries.[25]

Challenges and Future of Forensic Proteomics

In the future of Forensic Science, Proteomics will play an important role. Various studies have reported potential applications of proteomics in forensic sciences. However, forensic proteomics may face several difficulties. Less amount of samples is generally encountered by forensic scientists. So less abundant proteins are difficult to analyze in these types of cases. A standard operating protocol (SOP) should be regulated by investigation agencies. So, the collection, and preservation of evidence will be performed appropriately. In the scenario of Proteomics many standard protocols, regulations, etc have already been applied. This is the need of the hour that these regulations, SOPs should also be applied in the field of Forensic Science. Proteomics attracted forensic scientists for the answers to several questions which remained unsolved. Forensic Proteomics is highly beneficial for those problems which were unsolved by the DNA profiling technique.

Conclusion

This article has discussed the role of proteomics in the context of forensic evidence analysis. Proteomics techniques have been used to discriminate between different body fluids and tissues in the context of unidentified forensic biological samples, as well as to identify unfamiliar proteins and species. The protein population in a biological sample is large and complicated, with chemical, biological, and genetic features that are relevant to the investigator. Advances in cryogenic electron microscopy (cryo-EM), LC-MS/MS, and Next-generation sequencing (NGS) play important roles in protein identification. One of the most problematic aspects of forensic proteomics is that trace evidence samples can be quite small, making sample preparation difficult. Low-abundance proteins cannot be recognized when the sample amount is very little. Bottom-up proteomics, on the other hand, has been developed to allow the analysis of modest sample amounts. The ability to analyze the protein composition of evidence more efficiently and effectively gives the potential for those looking to extract as much information as possible from a crime scene.

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