B73 A Comparison of D-Amino Acid Levels in Historical Parchment and Leather

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Learning Overview: After attending this presentation, attendees will understand the differences in racemization rates of certain amino acids among historical parchments and leathers as old as 5,000 years old. While parchment shows exceptionally high levels of D-tyrosine and relatively high levels of D-phenylalanine, the levels in leather are virtually nonexistent for both. This is attributed to differences in processing and preservation methods.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by enhancing the knowledge of amino acid racemization behavior in historical parchment and leather. Association of the proteinaceous artifacts with carbon dated human remains establishes a point of comparison and allows the amino acid levels to be investigated for external contributors to their conditions. Ultimately, the cause of unusually high levels of D-tyrosine in historical parchments will be assessed.

Recently, Moini et al. introduced a new racemization dating technique which implements capillary electrophoresis mass spectrometry (CE-MS) analysis of HCl hydrolyzed proteinaceous specimens to determine D to L ratios of various amino acids. Moreover, the technique uses proteomics to identify and quantify the specimens' impurities, if any, using only ~50 µg of protein. Previously studied were the effects of environmental factors, such as extreme pH and temperature, on racemization rates of silk amino acids. Compared to their naturally aged counterparts, the D/L ratios of aspartic acid, phenylalanine and tyrosine increased significantly.[1] More recently, a combined 18 samples of parchment and leather received from various institutions in the world were dated to range from modern to 5000 years old. Some leather samples were dated by association with carbon dated human remains. To confirm the age of the leather, the amino acid racemization (AAR) dating technique was used. Notably, the analysis of parchments revealed exceptionally high levels of D-tyrosine and relatively high levels of D-phenylalanine, while that of leather revealed miniscule levels of both.

Both parchment and leather are made from animal skins which are soaked in lime and scraped to remove the hair, washed, then pumiced to smooth for writing. Another method uses salt and flour rather than lime. Leather is then tanned—a process which alters the protein structure of a skin and reduces its susceptibility to bacteria and deterioration. Parchment especially is prone to degradation if not preserved almost immediately after the skin is removed from the animal. Therefore, this investigation of unusually high levels of D-tyrosine in parchment focuses on chemical and biological phenomena that are known to enhance the formation of D-tyrosine.

First, the effects that historical parchment preservation techniques have on the racemization of tyrosine and phenylalanine were studied. Research demonstrates that the presence of acetic anhydride significantly increases the racemization rate of certain amino acids, including tyrosine, in NaOH and acetic acid solutions.[2] It is possible that acetic anhydride is used in the production or preservation processes or may be a byproduct of the chemicals used. This theory may be tested by applying to parchment the chemicals known to increase racemization of tyrosine and those used in the preservation process. A comparison can determine if the combination of chemicals will produce the same effect as acetic anhydride. The results of this investigation will be presented.

Secondly, biofilm formation was investigated in relation to parchment. Certain bacteria or microbial communities can lead to the formation of biofilms; the bacteria of biofilms protect themselves from being chewed up by other bacteria by incorporating D-amino acids which are prone to enzymatic proteolysis. A concentration of 5nM D-tyrosine proves to be effective in inhibiting biofilm formation with respect to the bacteria Pseudomonas aeruginosa.[3] These proteolytic bacteria are the most common bacteria found in parchment and leather.[4] While biofilms create an infinitesimal layer compared to the parchment itself, the bacteria could run throughout the artifact. The samples will be examined for the presence of these bacteria using proteomics data. While the synthesis for most D-amino acids is due to the enzymatic effect of racemase, the same study suggests D-tyrosine in parchment to be produced primarily through the effects of the liming process and lengthy exposure to alkalinity.

Links between these studies and ours of parchment are still under investigation. With a better understanding of this scientific anomaly, the dating of these historical artifacts could be improved.

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Amino Acid Racemization Dating, Parchment and Leather, CE/MS and Proteomics

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