Goldschmidt 2000
September 3rd–8th, 2000
Oxford, UK.

Journal of Conference Abstracts
Volume 5(2), 883

New Insights into Ancient Proteins from Traditional and Novel Mass Spectrometric Approaches

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Sequence analysis of proteins from fossils affords the intriguing possibility of phylogenetic reconstruction. However, traditional Edman sequencing is frequently confounded by the small quantities and diagenetic properties of ancient proteins. In addition we have a poor understanding of how long proteins can survive in the geological record. We have developed a method to extract, concentrate, purify and confirm the sequence of minute quantities (picomolar) of osteocalcin (OC) from small amounts (mg) of fossil bone. We are also able to detect possible substitutions in the sequence caused by diagenetic changes. The approach involves the application of matrix-assisted laser desorption ionisation mass spectrometry (MALDI-MS) for molecular weight determinations, peptide mass mapping, and sequencing of tryptic fragments using post source decay (PSD) analyses. MALDI-MS data shows that in-tact OC resides in fossils that are more than 53,000 years old. Furthermore, data from an artificial diagenesis experiment shows that OC undergoes degradation but can be detected in bones heated to 100 degrees Celsius for 200 hrs. In addition to the MALDI-MS data, we present new perspectives on the resiliency of stable isotopic and amino acid characteristics of collagenous and non-collagenous protein fractions of fossils. Although the majority of our efforts have focused on OC, MALDI-MS is applicable to other bone proteins. Our data opens new possibilities to obtain phylogenetic information from organisms with obscured taxonomic affinities.