ISOTOPIC AND MOLECULAR CHARACTERIZATION OF MODERN AND FOSSIL PROTEINS IN OSTRICH EGGSHELL

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Stable carbon and nitrogen isotopes in fossil ostrich eggshell (OES) proteins have the potential to provide paleodietary information on ostriches through the Late Pleistocene in arid and semiarid regions of the Old World. Carbon isotopes may be used as a monitor for floral composition (C_3 vs. C_4 plant abundance), for example. The stable isotope composition of the organic matter in modern OES and Middle Holocene fossil OES samples from Bir Tarfawi (an archaeological site in southern Egypt) are presented, as well as the nature of modern proteins in OES and the degree to which they are preserved in the fossil samples.

The organic fraction was extracted from OES samples and subsequently desalted by (1) decalcification in 0.5 N EDTA, followed by dialysis against water in >6-8,000 molecular weight cut off (MWCO) tubing, or (2) hydrolysis in 6N HCl for 20 minutes at 153°C, followed by the addition of concentrated HF to facilitate the precipitation of CaF_2. There is good agreement between the stable isotope results of the two organic fractions from modern OES samples ($\delta^{13}C$ = -23.51‰±0.76 and -23.53‰±0.39, and $\delta^{15}N$ = +6.23‰±0.15 and +6.42‰±0.33 for the >6-8,000 MWCO and HF preparations, respectively). A 2‰ and 4‰ enrichment between ostrich diets and the organic fraction was found for carbon and nitrogen, respectively. These relationships support a vegetarian diet comprised almost exclusively of C_3 plants. Result from the HF preparation show higher variability in both the $\delta^{13}C$ and $\delta^{15}N$ values in the fossil samples than in the modern. This increased variability may be due to isotopic fractionation accompanying protein degradation or to inadequate methodologies for isolating the bulk organic matter. These effects could be minimized by analyzing individual molecules throughout the study.

The >6-8,000 MWCO fraction of the modern and fossil OES samples were electrophoresed on SDS polyacrylamide gels. The modern sample shows predominant bands corresponding to apparent molecular weights of 14 kilodaltons (kD), 28 kD, and 35 kD, and a light smear at the top of the gel. Preliminary results of the fossil sample show a pronounced band corresponding to an apparent molecular weight of 80 kD, and a light smear over the whole gel. Results of an enzyme linked immunosorbent assay (ELISA) on the modern material demonstrate a positive antibody-antigen reaction between the >6-8,000 MWCO fraction and osteocalcin, osteonectin, and albumin.

Due to the "closed-system" nature of the ostrich eggshell matrix and the ease with which it can be accurately dated through the Late Pleistocene, it provides an ideal medium for studying protein diagenesis. Subsequent isotopic analyses, using the newly developed technologies of GC/IRMS, offer an alternative method for using the paleodiets of ostriches as a tool for paleoenvironmental reconstructions in Africa and Asia.