CHAPTER SEVENTEEN
Introduction to the Analysis of Protein Residues in Archaeological Ceramics

H. Barnard, L. Shoemaker, O.E. Craig, M. Rider, R.E. Parr, M.Q. Sutton and R.M. Yohe II

Hans Barnard, Research Associate; Cotsen Institute of Archaeology; P.O.-box 951510; Los Angeles, CA 90095; USA; <wendrich@barnard.nl>; Lori Shoemaker; Pasarow Mass Spectrometry Laboratory, UCLA; Los Angeles, CA 90095; Micala Rider; Department of Anthropology, University of Arizona; Department of Anthropology; 1009 E. South Campus Drive; Tucson, AZ 85721-0030; Oliver Craig, Dipartimento di Biologica, Università di Roma 'Tor Vergata'; Via della Ricerca Scientifica; 100133 Rome; Italy; Robert Parr, Mark Sutton and Robert Yohe; Laboratory of Archaeological Sciences, California State University, Bakersfield; 9001 Stockdale Highway; Bakersfield, CA 93311; USA. We would like to thank Alek Dooley, Kym Faull, William Longacre and Willeke Wendrich for their help in preparing this chapter.

As shown from the increasing volume of publicatons on this subject (Chapter 1), including the chapters in this volume, organic residues in archaeological ceramics have the potential to yield important information that is not easily recovered by other, more traditional reseach techniques. The analysis of lipid residues and their association with food products is now relatively well established (Charters et al. 1997; Copley et al. 2005; Eerkens 2005; Evershed et al. 1997; Malainey et al. 1999a; b), although the interpretation of the results can still be problematic (Barnard et al. 2007). Several approaches have been suggested to expand the understanding of archaeological lipid residues, including the determination of fatty acid ratios (Eerkens 2005; Malainey et al. 1999a; b), stable isotope analysis of individual lipid compounds (Copley et al. 2005), or of the complete organic residue (Ambrose 1993; 2000; Morton and Schwarcz 1988; 2004). Organic compounds other than lipids that may survive in an archaeological context include polysaccharides (such as starches and cellulose), DNA and proteins.

Preserved ancient polysaccharides have been isolated from archaeological pottery (Oudemans et al. 2007), historical paints (Bersani et al. 2004; Stevanato et al. 1997; Vallance et al. 1998) and from the surfaces of stone tools (Del Pilar Babot and Apella 2003; Lombard and Wadley 2007). They could potentially serve as diagnostic markers for specific foodstuffs, but are generally soluble in water, causing them to leach out of context, and are rapidly digested by micro-organisms in the environment. Furthermore, the problems with the archaeological interpretation of the biochemical analysis will prove very similar to those concerning lipids. The amount of residual DNA, theoretically the most informative biomolecule, is very low in most food products, with the possible exception of meat products, and even lower in archaeological food residues, due to the loss of molecules by leaching and the activity of micro-organisms. Proteins, on the other hand, are generally much more abundant in natural substances than DNA. They could provide more specific information concerning their origin than lipids or polysaccharides because they are the direct products of the DNA of the plant or animal species in which they were synthesized.

In this chapter we present a short overview of the various methods available for the identification of protein residues from food in archaeological ceramics. This is preceded by some remarks on the fate of proteins in geological and archaeological contexts, and followed by five case studies, three of which represent our own research. This chapter is not a review of all the work done in the past nor a step-by-step manual, but rather an introduction for those planning to enter this promising field of study as well as those who seek a better understanding of the publications on this subject. For more details on protein biochemistry, we refer to Appendix II of this volume, which also has a small glossary, or to any textbook or website on this subject.

The Preservation of Proteins

After deposition in an archaeological or geological context, proteins and peptides are subject to rapid attack by micro-organisms and relatively slow chemical decomposition (such as hydrolysis of the peptide bonds and racemization, decarboxylation and deamination of the amino acids, as discussed below). These will usually destroy the proteins, which will only survive under special circumstances. One of the best studied examples is collagen in buried bone (Collins et al. 2002). Collagen is a water-insoluble protein that appears to be protected from the extracellular enzymes produced by the micro-organisms in the soil (Child 1995; Hedges 2002). In favorable conditions, bone and collagen can be preserved for millennia due to physical and chemical changes resulting from a complex of interrelated processes collectively referred to as diagenesis (Collins et al. 2002; Hedges 2002). Other biomolecules, including amino acids (Jun 1974), heme (Schweitzer et al. 1997), and osteocalcin (Collins et al. 2000; Nielsen-March et al. 2002), have also been described in fossilized bones, as

well as in ancient ostrich eggshell (Brooks et al. 1990), teeth enamel (Bada 1991), and geological contexts (Bada 1991; Belluomini et al. 1986; Cheng et al. 1975). Although this serves to show that the search for proteins in archaeological contexts is certainly warranted, the time scale and the nature of the above processes place them mostly in the fields of geology and paleontology.

The most important process concerning archaeological proteins from food sources, next to microbiological digestion, is denaturation as a result of food preparation. Denaturation alone does not alter the primary structure (the amino acid sequence) of a protein, but it will typically render proteins irreversibly non-functional and often insoluble. It may help to impede microbiological attacks, but at the same time promotes hydrolysis of the peptide bonds (Child 1995; Collins et al. 2002). In studies using immunological techniques, denaturated proteins may no longer be recognized by antibodies raised against their native form (Barnard et al. 2007; Downs and Lowenstein 1996; Fiedel 1996, but see Craig and Collins 2000; De Jong et al. 1974; Lowenstein 1981; Newman et al. 1997; Schweiter et al. 2002). Another process directly related to food preparation is the non-enzymatic, heat-induced condensation reaction between peptides and oligosaccharides (sugars). This process is known the Maillard reaction, commonly refered to as 'browning'. This reaction usually results in ketosamines, after Amadori rearrangement, and melanoidins, after polmerization. Browning obviously modifies the involved peptides, but also contributes to their preservation by inhibiting microbiological attacks and the hydrolysis of peptide bonds (Bada 1991).

Denaturation and browning are the basis of various food preparation techniques, but likewise occur as the result of many other events and certainly do not indicate an anthropogenetic origin of the organic residue. The conditions favorable for the preservation of proteins, primarily by reducing the extent of the digestion by micro-organisms, are generally the same as those during food preparation and conservation (Collins et al. 2002). Proteins from prepared food seem thus more likely to survive than those in other archaeological or geological contexts.

Proteins as Archaeological Biomarkers

As proteins are present in substantial quantities in most organic materials (including food), are the direct products of DNA and appear to survive in the right environment for significant periods of time, they are potentially ideal biomarkers for the origin of an archaeological organic residue (Fankhauser 1994; 1997; Evershed and Tuross 1996). However, several factors frustrate the realization of this potential. First, as discussed above, archaeological proteins are likely to be denatured and fragmented by microbiological enzymes and hydrolysis. Also, some of the amino acids will have been chemically altered by decarboxylation, deamination and racemization; processes that advance over time and increase at higher temperatures (cooking). An anaerobe post-depositional environment will inhibit microbiological attacks and decarboxylation of the amino acids, while an arid environment will inhibit hydrolysis of the peptide bonds and racemization. Archaeological finds from cold and either very dry or water-logged (anaerobe) deposits can therefore be expected to yield better preserved peptides. Important to the study of archaeological proteins is that residual ancient proteins may not be intact proteins, but rather a mix of peptides with various modifications, as a result of changes over time as well as the complex depositional milieu.

Second, as a result of genetic conservation, molecules that serve similar functions in different species are often almost identical. Myoglobine, for instance, is thought to have evolved several hundred million years ago and parts of its amino acid sequence have never changed and are the same in virtually all animals. On the other hand, different proteins that have evolved to perform a similar task may have converged towards the same, most effective composition and shape. Convergent evolution explains why whales and dolphins (marine mammals) look like fish, and why echidnas (monotremes), porcupines (rodents) and hedgehogs (insectivores) are so similar in appearance and behavior. For the analysis of archaeological proteins, 'conservation' and 'convergent evolution' mean that diverse sources may leave residues of very similar proteins that may be impossible to distinguish, even after most of the amino acid sequence has been determined. Antibodies raised against a protein produced by one species may react with a similar protein from another species when both have epitopes, chemical groups that trigger an immune-response, in common. Such 'cross-reactivity' can make the interpretation of research using immunological techniques a hazardous affair (Fiedel 1996, but see Newman et al. 1997). Where the amino acid sequence is sufficiently specific, however, mass spectrometry techniques may allow differentiation for species assignment.

Third, oligopeptides and amino acids are a common constituent of the soil (humus). Here, peptides are preserved in anaerobic conditions or by browning, as melanoidins (Bada 1991; Child 1995; Evershed and Tuross 1996). Some of these molecules may leach into the buried ceramic vessel and contaminate the anthropogenetic residue that was present in the vessel before it was discarded.