

MECHANISMS OF PROTEIN DEGRADATION BY MICROBIAL CONSORTIA AND ROUTES FOR THE PRESERVATION OF PROTEIN IDENTITY

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Protein comprises the largest compartment of nitrogen in phytoplankton (e.g. Lourenço et al., 1998) and thus provides a major source of carbon, but more importantly nitrogen, in marine systems. Despite the rapid recycling of protein in the environment, proteinaceous remnants have been recognised in sediments and changes in molecular weight, acidity and amino acid distribution have been related to diagenesis (e.g. Nguyen and Harvey, 2003; Nguyen et al., 2003). Little is known, however, about the mechanisms by which intact proteins are transformed and the conditions (if, indeed they exist) in which sufficient sequence is preserved to identify the original protein sources and their routes to survival.

Incubations were performed in which an individual protein was followed through the initial stages of microbial degradation and the structures of the resulting products characterised. Cytochrome c, a protein with substantial sequence coverage achievable by liquid chromatography-mass spectrometry (LC-MS) analysis, was selected as a model protein. Estuarine waters amended with exudates of growing algae provided the microbial community and a mixed carbon substrate. As chemical modification has been proposed as one route for protein preservation, Cytochrome c was also glycosylated (with D-Glucose) at multiple positions and subjected to the same conditions. Total protein concentrations of innate and glycosylated Cytochrome c were monitored over time. After an initial lag both Cytochrome c and its glycosylated counterpart decreased in concentration (Figure 1), with the glycosylated Cytochrome c removed significantly slower than the unmodified protein.

Protein samples were extracted by adsorption onto Nylon filters and subsequently recovered. LC-MS analysis of extracts revealed the generation of peptides ranging from 11 to 20 amino acid residues in length during degradation and giving an overall sequence coverage of 65%. Seven peptides were seen in both native and glycosylated protein incubations with two additional peptides found in the glycosylated protein decay series. In both treatments all released peptides persisted at detectable levels after total protein concentrations had reached baseline levels.

The cleavage sites and retention of specific regions of the protein did not correlate with features in either the amino acid sequence or secondary structure (e.g. α -helices and β -sheets). Notably, portions of the protein that were retained were all in close proximity to, and in one case covalently bound to the heme around which the protein is folded. It is suggested that, following interruption of the protein chain through selected cleavages, heme provides a focus for aggregation of nearby peptides, affording the aggregate increased protection against enzymatic attack. The generation and persistence (albeit at very low levels) of these peptides indicates that protein hydrolysis by natural microbial consortia is not an arbitrary process and that sequence information sufficient to provide source and/or functional information about a protein can survive the initial stages of diagenesis. Furthermore, the proposed role played by the porphyrin in the survival of Cytochrome c-specific peptides has an interesting parallel in the geochemical record in the potential survival of identifiable photosystem proteins *via* interaction with their chlorin ligands.

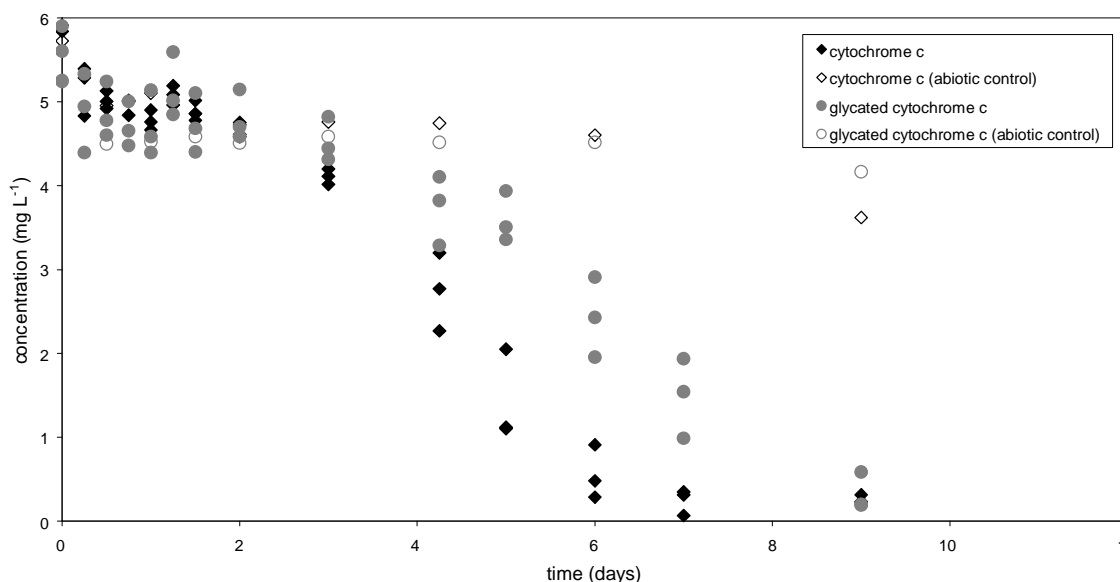


Figure 1. Changes in total protein concentrations during bacterised and abiotic incubations of Cytochrome c and its glycated derivative.

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