

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.

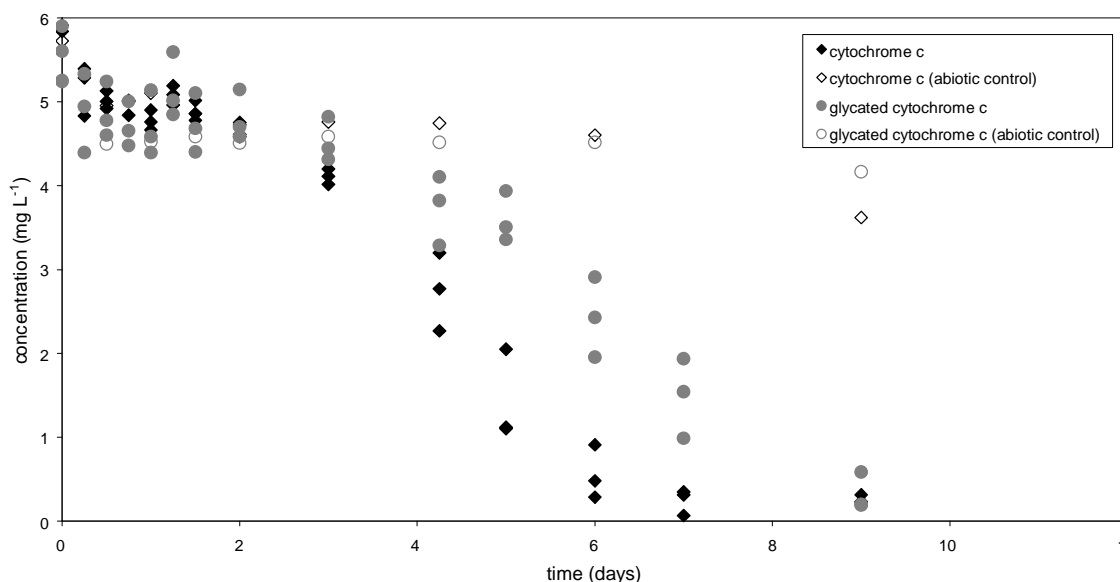


Figure 1. Changes in total protein concentrations during bacterised and abiotic incubations of Cytochrome c and its glycated derivative.

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SORPTION OF ORGANIC MATTER ON CLAY MINERALS IN AQUATIC SYSTEM: INFLUENCE ON SEDIMENTARY ORGANIC PRESERVATION. AN EXAMPLE OF A LACUSTRINE ENVIRONMENT (LAC PAVIN, FRANCE)

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Sorption of organic molecules on clay surfaces in aquatic environment influence organic sedimentation fluxes to bottom water and to sediment. It is well known that physical and chemical interactions produce the flocculation of organo-mineral complexes. Flocculation decrease the transfer time across the water column and thus the risks of oxidation and/or bacterial recycling in this environment. Other studies, based on analyses of organic matter in sediments, revealed also that direct contacts between clay surfaces and organic molecules influence molecule availability to benthic fauna and bacteria. Many studies conclude that mineral sorption can be considered as one of the preservation mechanisms, leading to incorporation of metabolisable organic matter in sediments. The most part of these studies based their conclusions on analyses of sediments but never fully considered sorption processes resulting to organic matter preservation.

Many questions in this topic remain unresolved. Does sorption of organic molecules on clay minerals can occur in the water column, i.e. before sedimentation? Which organic molecules can be fixed on clays? What is the reactivity of these organo-mineral complexes in deep water? Does interaction can be considered as an efficient process for organic molecules protection, leading an efficient transport of organic matter from the dynamic water column to sediments?

The aims of this work are first to test the possible *in situ* sorption of organic molecules on clay minerals, in a lacustrine environment, and secondly to test the stability of the organo-clay complexes formed under these conditions. In this respect, the behaviour of natural organic matter in presence of synthetic saponites (high- and low-charge Na-smectites) has been studied in oxic and anoxic levels of an oligotrophic meromictic crater lake (Lac Pavin, Massif Central, France). Clay samples were positioned in traps, closed by two membranes, allowing only circulation of dissolved organic matter. Interactions were interrupted on the spot, after 3, 10 and 21 days of immersion.

Bulk chemical analyses showed the presence of organic carbon, associated with clays after interaction with the water lake. Low-charge saponites present higher organic carbon concentrations than high-charges, suggesting the non-prominence of cation bridge linkages between organic molecules and clay surfaces. Molecular pyro-GC/MS investigations, performed on clays, revealed the sorption of n- and iso- fatty acids, alkanes and alkenes, in significant amounts (Figure 1). The molecular pyro-GC/MS analyses of organic extracts carried out on waters emphasized the existence of selectivity during fixation, since the same molecular fractions are present in waters and associated to clays, but in contrasted relative proportions.

X-ray diffraction analyses, performed on the two saponites, proved the lack of molecular intercalation in clay mineral structure. ESCA analyses showed a loss of sodium in the interlayer space of the two saponites, and its probable substitution by divalent iron in anoxic deep water. Failure of extractions by saponification and organic solvents, tested on sorbed molecules, indicates the strong stability of the assemblages between clays and organic molecules. This strong relationship could explain the organic matter preservation under lacustrine water conditions.

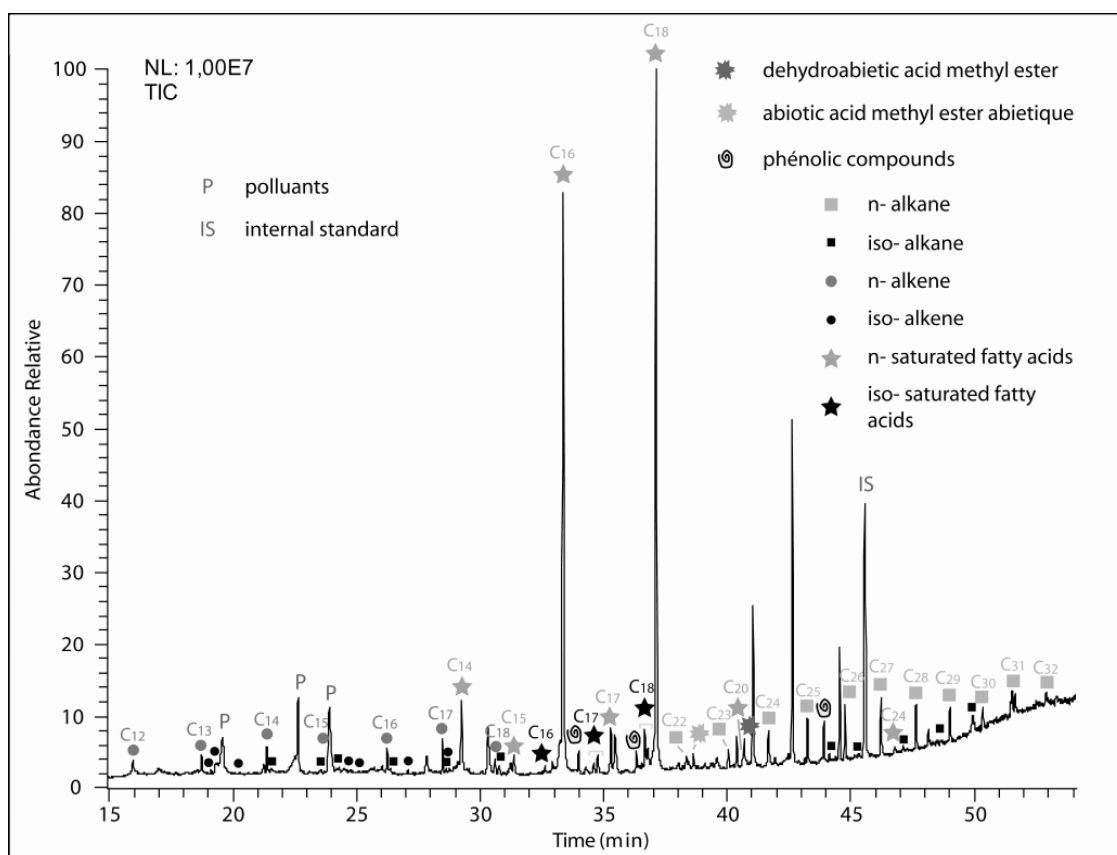


Figure 1. TIC chromatogram of py-GC/MS analyses with TMAH performed on low charge saponite before 10 days of interactions in the anoxic level of the Pavin lake.

CHARACTERISTIC OF ORGANIC MATTER IN SURFACE SEDIMENTS FROM THE PERU MARGIN – IS THE PRESERVATION OF ORGANIC CARBON RELATED TO THE GENERAL DEGRADATION STATUS?

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The Peru margin offers a unique opportunity to study the composition of pre-burial organic matter that accumulates at the sediment – water interface. Such studies provide the baseline characteristics of organic matter that will be modified during diagenesis and ultimate burial (Arthur et al. 1998). The upwelling of Peru is perennial, wind-driven, and presently concentrated in the zones 7°-8°S, 11°-12°S and 14°-16°S. The upwelling system is dominated by two currents: the equator-ward O₂-rich Peru-Chile-current in the upper 200 m of the water column, and the pole-ward flowing Peru Undercurrent underneath (Hill et al. 1998). The latter transports oxygen-poor, nutrient rich water, which is brought to the euphotic zone by upwelling. This results in high primary production, which leads to high sedimentation rates. Remineralization of the large amount of sinking organic material results in an oxygen minimum zone (OMZ) which is located at ~50-650 m water depth (Emeis et al 1991; Lückge and Reinhardt 2000). High primary production, high sedimentation rates, shallow water depth, and oxygen limitation in the water column and sediments favor the accumulation of organic rich sediments (e.g. Thiede and Suess 1983). The sedimentary OM is predominantly of marine origin, as input from the dry coastal area is limited (Niggemann 2005).

Although the characteristics of organic matter on the Peru margin have been studied previously (see intensive study by Arthur et al. 1998 and refs in there), there has been no previous intensive characterization of surface sediments by use of multiple amino acid based freshness and preservation indicators along several onshore – offshore transects across the water column redox gradient. The investigated region off Peru reached from 9.5°S to 13.5°S latitude and included both shelf and slope and sediments within and outside the OMZ. The diagenetic indicators applied in the present study were the percentage of total organic carbon present as amino acid carbon (%T_{AA}C) and the percentage of total nitrogen present as amino acid nitrogen (%T_{AA}N), which are particularly useful as diagenetic indicators; they are sensitive to different stages of alteration and appear to be uncompromised by source variations (Cowie and Hedges 1994). We gained further information on the degradation state of organic matter from the relative abundance of non-protein amino acids, which generally increases during diagenesis. The result of this is a decrease in the ratio between the protein

precursor (e.g. aspartate) and the non-protein degradation product (e.g. β -alanine; Cowie and Hedges 1994). Applied together, %T_{AA}C, %T_{AA}N and the ratio between source protein amino acid and its non-protein degradation product offer congruent information on the relative diagenetic stage and reaction potential of natural organic material (Cowie and Hedges 1994, Keil et al. 2000, Lomstein et al. 2006). In addition, the diagenetic status of the sediment was evaluated by use of the amino acid composition based degradation index (Dauwe and Middelburg 1998). Finally the reactivity of THAA was evaluated from estimated THAA-N mineralization rates. Preservation of organic carbon, in the form of bacterial cell walls (live+dead + remains) was inferred from the concentrations of D-aspartate, D-glutamic acid, D-serine and D-alanine. This method has been successfully applied to give indications on the importance of bacteria in the preservation of organic carbon in coastal Chilean sediments (Lomstein et al. 2006). Sampling was carried out during RV Sonne cruise 147 in June 2000. Sediment cores from 20 stations were retrieved by multicorer from water depths between 50 and 1369 m.

All the applied diagenetic indicators showed consistent trends with water depth, indicating that organic matter was increasingly decomposed when it reached the sediment surface at greater water depth (i.e. residence time of organic matter in the water column had been longer). At present we are still working on the D-amino acid data, which at the moment do not allow us to conclude on the contribution of bacterially derived amino acids to bulk THAA and thus the importance of bacteria in the preservation of organic carbon. Results from this work will be available in due time before the meeting and be incorporated in the presentation.

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