

## Resistance of Peptide Degradation Products in Seawater

Much of marine dissolved organic matter (DOM) produced by photosynthetic microorganisms is remineralized in the water column, but a small fraction is preserved. Of particular importance to global C and N budgets is the inherent resistance of organic matter to decay and its transformation into refractory substances, which remove it from active biotic and abiotic marine cycles. Little is known about the chemical composition of refractory DOM, the mechanisms contributing to its resistance, and the processes that affect the character of much of the DOM pool. In living cells, proteins account for a major fraction of the C and most of the N. Not surprisingly, amide N dominates in the high molecular weight (HMW) DOM pool, and dissolved proteins have been found in seawater. Although smaller peptides are believed to be important intermediates in the degradation of protein, their presence has not yet been documented. <sup>15</sup>N-NMR spectra suggest that much of the HMW DON pool is derived from compounds with peptide linkages. However, the relatively low contributions of hydrolyzable amino acids to the total N pool suggest that: a) protein N is not the dominant form of HMW DON, b) protein and peptide N is rapidly degraded and consumed in the environment, and/or c) that analytical methods currently being employed underestimate the contribution of proteins to this fraction of the DOM pool. Historically, peptides and proteins have been considered very labile, but evidence is increasing that some is preserved in the aquatic environment. In systems with high concentrations of particulate matter, chemisorptive attachment to mineral surfaces and sequestration within small pores are important processes for the preservation of labile organic matter in sediments. However, proteinaceous material can survive in systems where minerals are absent or in low amounts.

In this proposal, we will compare the relative importance of three peptide reaction mechanisms in seawater: 1) chemical transformation of peptides by glycosylation, oxidative coupling, AGE formation, and Michael reactions, to the lability of peptides in seawater; 2) physical transformation of peptides by sorption to colloidal organic matter; and 3) loss of peptides due to extracellular hydrolysis by bacteria and/or algae. We will synthesize labeled and unlabeled peptides of biological interest and monitor rates of production and removal independently and in competition experiments. This would lead eventually to comparisons of different environments to see what the relative importance of the various chemical and biological loss mechanisms are with changes in light, pH, nutrients, temperature, etc.

Our approach is made possible by the ability at ODU to synthesize labeled and unlabeled peptides of biological interest using a newly acquired automated peptide synthesizer system, and to spectroscopically examine the peptides interacting with DOM or other organic fractions. The ability to monitor reaction rates, physical transformations, and biologically-mediated processes independently and in competition will ultimately enable us to elucidate the relative importance of these competing processes with respect to environmental variables such as light, pH, nutrients, temperature, community structure, etc. We will use synthesized (but real) peptides to determine products of diagenetic reactions. In addition, we will also test the susceptibility of these products to attack by hydrolytic enzymes present in the environment and associated with algae and bacteria in seawater. This will enable us to determine whether we might expect to see these products accumulate in seawater and under what environmental conditions, and will advance our ability to apply state-of-the-art chemical techniques to natural samples.