

Xue, Q.-G., M. E. Hellberg, K. L. Schey, N. Itoh, R. I. Eytan, R. K. Cooper and J. F. La Peyre. 2010. A new lysozyme from the eastern oyster, *Crassostrea virginica*, and a possible evolutionary pathway for i-type lysozymes in bivalves from host defense to digestion. BMC Evolutionary Biology, in press.

Lysozymes are enzymes that lyse bacterial cell walls, an activity widely used for host defense but also modified in some instances for digestion. The biochemical and evolutionary changes between these different functional forms has been well-studied in the c-type lysozymes of vertebrates, but less so in the i-type lysozymes prevalent in most invertebrate animals. Some bivalve molluscs possess both defensive and digestive lysozymes. We report a third lysozyme from the oyster *Crassostrea virginica*, cv-lysozyme 3. The chemical properties of cv-lysozyme 3 (including molecular weight, isoelectric point, basic amino acid residue number, and predicted protease cutting sites) suggest it represents a transitional form between lysozymes used for digestion and immunity. The cv-lysozyme 3 protein inhibited the growth of bacteria (consistent with a defensive function), but semi-quantitative RT-PCR suggested the gene was expressed mainly in digestive glands. Purified cv-lysozyme 3 expressed maximum muramidase activity within a range of pH (7.0 and 8.0) and ionic strength (I=0.005-0.01) unfavorable for either cv-lysozyme 1 or cv-lysozyme 2 activities. The topology of a phylogenetic analysis of cv-lysozyme 3 cDNA (full length 663 bp, encoding an open reading frame of 187 amino acids) is also consistent with a transitional condition, as cv-lysozyme 3 falls at the base of a monophyletic clade of bivalve lysozymes identified from digestive glands. Rates of nonsynonymous substitution are significantly high at the base of this clade, consistent with an episode of positive selection associated with the functional transition from defense to digestion. The pattern of molecular evolution accompanying the shift from defensive to digestive function in the i-type lysozymes of bivalves parallels those seen for c-type lysozymes in mammals and suggests that the lysozyme paralogs that enhance the range of physiological conditions for lysozyme activity may provide stepping stones between defensive and digestive forms.