PRELIMINARY IDENTIFICATION OF AN ALANINE RACEMASE GENE IN *MYA ARENARIA*

Robert L. Preston[1], Benjamin D. Crisp[1], Heather D. Lewis[1] and Kyomi Gregory[2]
[1]Department of Biological Sciences
Illinois State University, Normal, IL. 61790-4120
[2]Queens Gateway to the Health Sciences High School, Queens, NY 11433

D-amino acids occur in the free amino acid pools in tissues of marine invertebrates in diverse phyla (Preston, R.L. Comp. Bioch. Physiol. 87B: 55-62, 1987). We have shown that D amino acids are transported and metabolized by invertebrate tissues (Preston, R.L. Comp. Bioch. Physiol. 87B: 63-71, 1987; Preston, R. L. In *Comparative Aspects of Sodium, Cotransport Systems*. Comp. Physiol., RKH Kinne (*ed*). Basel, Karger, pp1-129). The principal route of metabolism utilizes racemases, enzymes that interconvert D- and L-amino acids (Preston, R.L., et al., Bull. MDIBL 36: 86, 1997). We have partially purified and characterized the alanine racemase from gill tissue from the soft-shelled clam, *Mya arenaria*. Our kinetic studies have shown that this enzyme is specific for alanine and has a Km of 9 mM (measured in the L-ala → D-ala direction). Polyacrylamide SDS gel electrophoresis of purified gill extracts have led us to the tentative conclusion that the alanine racemase has a molecular weight of ~60 kD (Preston and Middaugh, unpub. data).

In an extensive series of studies, we attempted to identify the racemase gene by probing *Mya* gill cDNA using degenerate primers based on well characterized bacterial racemase sequences, without success. Recently, two eukaryotic racemase genes have been sequenced, serine racemase from mouse brain (Wolosker et al., PNAS 96: 13409-13414, 1999) and alanine racemase from a pathogenic plant fungus, *Cochliobolus carbonum* (Cheng and Walton, J. Biol. Chem. 275: 4906-11, 2000). We report here the tentative identification of a gene fragment that is amplified using PCR primers from *C. carbonum*.

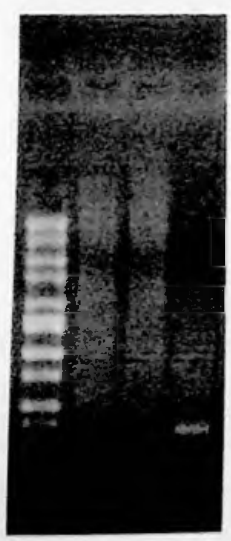

Fig 1. PCR products from Mya cDNA using alanine racemase primers.

Mya gill mRNA was isolated and cDNA synthesized using standard RT-PCR methods (Qiagen, Inc.). The primers based on *C. carbonum* alanine racemase sequences were:
Forward: 5'-TGTTTCGACTAACCGGTAGCAGGG-3'
Reverse: 5'-CGATTCATTTTAGGGTGTGCCAGAT-3'.
The primary band detected was ~330 bp (Ala; Fig 1). Primers (Ser A, Ser B) derived from serine racemase sequences from mouse brain (Wolosker et al., *ibid*) were also used but gave very weak bands under low stringency conditions. PCR conditions: 95°C, 3min; 95°C, 45 sec denaturation; 40°C, 30 sec annealing; 72°C, 90 sec elongation (35 cycles); 72°C termination. The ready detection of an alanine racemase gene but not the serine racemase gene is consistent with our physiological studies that show that serine racemase activity is absent from *Mya* gill tissue. To our knowledge these data are the first to report racemase gene identification in marine invertebrates. Sequencing is in progress on the amplified alanine racemase cDNA from *Mya*. (Kyomi Gregory was a Hancock County Scholar funded in part by the SETH Program. Benjamin Crisp was supported by a NSF-REU grant NSF DBI-9820400).