RACEMIZATION OF AMINO ACIDS BY MARINE INVERTEBRATES

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Previous work in our laboratory has demonstrated that D-amino acids occur in the free amino acid pools in tissues of a wide variety of marine invertebrates from at least 11 phyla (Preston, R.L. Comp. Bioch. Physiol. 87B: 55-62, 1987; Preston, R.L. Comp. Bioch. Physiol. 87B: 63-71, 1987). We also demonstrated that D-amino acids were both transported and metabolized by these invertebrates. One of the principal routes of metabolism utilizes a racemase, an enzyme which interconverts D- and L-amino acids. Ordinarily, racemases have equilibrium constants near unity and thus act to equilibrate D- and L-amino acid pools in tissues with this enzyme. Detection of D-amino acid therefore implies that the tissue also contains racemase (and vice versa). We report here analysis of selected species in which racemase occurs without detectable D-amino acids.

Animals were collected from Salsbury Cove, ME. The tissues selected were extracted in deionized water (10:1 v/w), homogenized and centrifuged at 15,000 x g for 5 min. An aliquot of the supernatant was reserved for D-amino acid analysis while the remainder was treated with 80% ethanol to precipitate proteins. This was centrifuged, the pellet washed 3 times in 80% ethanol and then extracted with deionized water to solubilize the racemase. We have shown that this procedure recovers racemase activity while removing most other proteins. D-Amino acid content and racemase activity was measured using a coupled enzyme microassay. Typical assay conditions to detect neutral D-amino acids were: tissue extract (50 µl), tetrasodium pyrophosphate (NaPP) buffer saturated with the chromophoric peroxidase substrate, *o*-dianisidine (50 mM NaPP, pH 8.5; 110 µl), D-amino acid oxidase (0.06 units; 20 µl) and horseradish peroxidase (0.08 mg; 20 µl) and water 50 µl. The reaction was run at room temperature in a 96 well microplate and the product formation (oxidized *o*-dianisidine) measured at 490 nm using an ELISA plate reader. Each condition was done in quadruplicate and multiple positive (containing D-alanine) and negative controls were always included. The racemase assay used the same procedure but 200 mM L-alanine was added to the extract and the formation of D-amino acid measured. This assay can reliably detect D-amino acids at concentrations from 0.05 mM or higher although some tissue extracts inhibit the coupled enzyme assay and thus the data reported here should be regarded as minimum concentrations.

The following species showed detectable (> 0.5 mM apparent tissue concentration) levels D-amino acids and racemase activity (phylum shown in parentheses): (Nemertea) *Micrura leidyi*; (Annelida) *Lepidonotus squamatus*; (Mollusca) *Mya arenaria*, *Spisula solidissima*; (Arthropoda); *Crangon septimspinosus*, *Carcinus maenas*; (Echinodermata) *Asterias vulgaris*. In (Annelida) *Glycera dibranchiata* coelomocytes D-amino acids were detected (est. concentration 20 mM) but no racemase activity. In the mollusks, *Lunatia heros* and *Buccinum undatum* racemase activity was found but not D-amino acids. In *G. dibranchiata* this suggests there may be other pathways (transaminases?) which can yield D-amino acids. In *B. undatum* and *L. heros*, the data suggest rapid metabolism of D-amino acids generated by the racemase and perhaps rather different equilibrium constants for this process. This assay may not be sensitive enough to detect the residual D-amino acid pool under these circumstances. These data are the first observations of racemase activity without concomitant detectable levels D-amino acids in the literature and suggests that amino acid racemization may be more common than previously assumed on the basis of D-amino acid occurrence in tissues.

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