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Research paper

Amino acid racemization in four species of ostracodes: Taxonomic, environmental, and microstructural controls

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ABSTRACT

Here we quantified the aspartic acid and glutamic acid racemization rates of the four main ostracode species (*Herpetocypris reptans*, *Candona neglecta*, *Ilyocypris gibba* and *Cyprideis torosa*) present in several Iberian Peninsula localities covering a wide chronological range (ca. 1 Ma to present). At low *D*/*L* values (at Asp *D*/*L* < 0.40; and Glu *D*/*L* = 0.09–0.18), *H. reptans* racemized at higher rates than *C. neglecta*, *C. torosa* and *I. gibba*. In contrast, for Asp *D*/*L* > 0.4 and Glu *D*/*L* > 0.18, *H. reptans*, *C. neglecta* and *C. torosa* showed similar racemization rates. *I. gibba* exhibited the lowest *D*/*L* values in old samples (Middle and Lower Pleistocene). We attribute these differences in amino acid racemization rates mainly to variations in valve protein composition. We found that the microstructure of the valves of each species (size, morphology, and arrangement of crystals) differed, but did not appear to change over time (at least for the last ca. 1 Ma). Such differences may also be linked to the type of proteins involved in the respective calcification processes of these organisms. On the basis of our results, and given that other studies have demonstrated that the majority of inter-crystalline proteins are leached early after death (a few centuries or millennia), we propose that the degradation rates of the most resistant inter- and intra-crystalline proteins in each species differ depending on the protein composition of the valves. Although further research is required, we suggest that amino acid racemization in each ostracode species might be related to valve microstructure.

1. Introduction

Ostracode valves are made of low-magnesium calcite (Kesling, 1951; Sohn, 1958), which is a more stable mineral than aragonite. The characteristics of these valves make them particularly useful for amino acid racemization (AAR) dating purposes (McCoy, 1988; Oviatt et al., 1999; Kaufman, 2000, 2003; Ortiz et al., 2004, 2009; Torres et al., 2005; Colman et al., 2006; Owen et al., 2007; Jayko et al., 2008; Bright et al., 2010; De Santis et al., 2010; Bright and Kaufman, 2011a). They show excellent preservation of amino acids (Kaufman and Manley, 1998; Kaufman, 2000; Ortiz et al., 2002), and the amino acid abundance allows the analysis of a small sample size (even a single ostracode valve ≈ 0.01 mg). The sample size required for ostracodes (in many cases just one valve) is much lower than for other organisms, such as mollusks (5 mg; Lajoie et al., 1980; Goodfriend and Mitterer, 1988; Wehmiller, 1990, 2000; Goodfriend, 1991; Torres et al., 1997; Ortiz et al., 2002; Penkman et al., 2008). Thus, many ostracode samples from a single bed can be analyzed, thereby enabling easier identification of anomalous results, and estimation of the time-averaging of the dated event. Recent studies by Bright and Kaufman (2011a,b) and Reichert et al. (2011) shed light on the processes that affect AAR in ostracode valves and conclude that *D*/*L* values are influenced by taxonomy, temperature and environmental pH.

Given that AAR is a genus-dependent process (Lajoie et al., 1980) (as well as being time and temperature-dependent), monogeneric samples are required to reduce taxonomically controlled differences in *D*/*L* values. However, in some previous studies, various ostracode genera have been analyzed together from the same horizons/localities in order to establish age, with the assumption that there are only small differences between *D*/*L* values of distinct genera (cf. Oviatt et al., 1999; Kaufman et al., 2001; Kaufman, 2003; Ortiz et al., 2004, 2009; Torres et al., 2005; De Santis et al., 2010). It has been demonstrated that for *Candona* and *Limnocythere*, which belong to separate phylogenetic ostracode groups, the *D*/*L* values of valves from the same horizons (Table 1) showed only slight differences (McCoy, 1988; Oviatt et al., 1999; Kaufman, 2000, 2003; Kaufman et al., 2001; Reichert et al., 2011). Nevertheless, Bright and Kaufman (2011a) reported that the abundance and relative

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Table 1
Classification of modern ostracodes used in this study. Compiled using Meisch (2000) and the Integrated Taxonomic Information System. On-line database. www.itis.gov/index.html. Accessed: July, 15, 2011.

Class CRUSTACEA Pennant, 1777
Subclass OSTRACODA Latreille, 1806
Order PODOCOPIDA Muller, 1894
Suborder PODOCOPINA Sars, 1866
Superfamily CYPRIDOIDEA Baird, 1845
Family CYPRIDIDAE Baird, 1845
Subfamily HERPETOCYPRIDINAE Kaufmann, 1900
Genus HERPETOCYPRIS Bradyi and Norman, 1889
Family CANDONIDAE Kaufmann, 1900
Genus CANDONA Baird, 1846
Family ILYOCYPRIDIDAE Kaufmann, 1900
Subfamily ILYOCYPRIDINAE Kaufmann, 1900
Genus ILYOCYPRIS Brady and Norman,
Superfamily CYTHEROIDEA Baird, 1850
Family CYTHERIDEIDAE Sars, 1925
Subfamily CYTHERIDEINAE Sars, 1925
Genus CYPRIDEIS Jones, 1857

proportions of amino acids vary among ostracode genera, even when these belong to the same family. The most striking compositional difference was observed in *Heterocypris incongruens* (Bright and Kaufman, 2011a), which contained twice the relative proportion of aspartic acid (Asp) compared to the other taxa. Bright and Kaufman (2011b) observed that the racemization rate of Asp is higher in *Heterocypris* valves than in *Candona* and *Ilyocypris* ones, thereby suggesting a taxonomic effect. Likewise, the same authors reported that *D*/*L* values differ considerably across family boundaries in the Family Cypridoidea, thus indicating that these differences are linked to taxonomic variability in protein composition. In this regard, Kaufman (2003) noted that differences in *D*/*L* values are likely to result from taxon-dependent arrangement of amino acids, or from morphological and structural differences that influence the differential loss of free and peptide-bound amino acids.

Ostracode valves consist of small crystallites of calcium carbonate (80–90%, Sohn, 1958) embedded in a chitinous and protein matrix (Bate and East, 1972, 1975; Langer, 1973; Keyser, 1982; Rosenfeld, 1979) accounting for 2–15% of the valve weight (Sohn, 1958). Depending on the taxonomy, the microstructure of the adult valve is expressed in a variety of forms. As in arthropods, the main structure of ostracodes consists of a continuous cuticular integument that forms the exoskeleton, which is partly calcified (Fig. 1) (Bate and East, 1972, 1975). In ostracodes the integument is formed by epidermal cells, which periodically secrete crystalline layers and a cuticular membrane over the external surface (Bate and East, 1972, 1975) which comprises mainly chitin and other proteic material (e.g. arthropodine, resiline, sclerotine and cuticuline), as well as calcite crystals. These materials confer hardness and high resistance to the valve. A layer of granules secreted by the epidermis was found to consist of calcite and apatitic calcium orthophosphate (Rosenfeld, 1979, 1982; Bate and Sheppard, 1982; Keyser and Walter, 2004), the main function of which is considered to be the construction of a new calcareous valve during molting. This granular layer is present only in living ostracodes, and it probably recrystallizes to form the calcite layers of the valves (Rosenfeld, 1982). The secretion of a new valve occurs within a few hours of molting (Kesling, 1951; Turpen and Angell, 1971). In all arthropods the cuticle consists of two layers (Richards, 1951), namely the epicuticle (the outer part) and the procuticle (the innermost area).

The epicuticle is a thin layer that accounts for only 5% of the whole cuticle (Fig. 1), and its structure is simpler in ostracodes than in other arthropods. This layer does not contain chitin, but rather lipids and polyphenols (Jeuniaux et al., 1986). The procuticle comprises chitin and is segregated by the epidermic cells before molting takes place (Bate and East, 1972).

From a biochemical, structural, and functional point of view, the procuticle shows two distinct layers, namely the endocuticle and exocuticle layers (Fig. 1). In most ostracodes, the latter is thin, calcified, and pigmented. This layer shows a cross-lattice of chitin fibers positioned perpendicular to the surface in the calcareous layer (Bate and East, 1972, 1975; Dépêche, 1982), and also secondary chitin fibrils without any preferential orientation. The mineralized part of the valve is made up of prisms of calcite disposed in a mosaic of irregular outlines (Langer, 1971, 1973; Dépêche, 1982); however, Jørgesen (1970) reported that these prisms are perpendicular to the valve surface. According to Dépêche (1982), calcite prisms and fibrils are of the same scale, and fibrils occur both outside and inside calcite crystals (Jørgesen, 1970). Chitin layers envelop calcitic layers and are highly relevant for the preservation of fossil ostracode valves (Oertli, 1975).

The endocuticle is the internal zone of the procuticle and is present in all ostracodes. This layer is calcified and can show pigment granules that provide some coloration (Bate and East, 1972, 1975). The endocuticle is made of chitin fibers arranged in a reticular matrix (feathered-like), although with a certain degree of layering (Bate and Sheppard, 1982).

The size, morphology and arrangement of crystals in the microstructure of biomineralized tissues is regulated by proteins,