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Biological control of skeleton properties in echinoderms

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ABSTRACT: Echinoderms have a high-magnesium calcite skeleton whose cristallographic, chemical, and morphological properties make it different from abiotic calcites. The available data show that the single crystal behaviour, the conchoidal fracture properties, the high-magnesium content, and the stereom structure could be partly explained by the properties of the organic macromolecules associated with the mineral phase. Data suggest that these compounds could produce the stereom properties through stereochemical interactions with calcite.

1. INTRODUCTION

Echinoderms have a calcitic skeleton of mesodermal origin. This skeleton is developed by most postmetamorphic individuals as well as by larvae of echinoids and ophiuroids. The growing evidence indicates that the postmetamorphic and larval skeletons are homologous structures (see Emlet 1985, Parks et al. 1987, Drager et al. 1989, Dubois & Chen 1989, Richardson et al. 1989). The echinoderm skeleton may be considered as a paradigm of biologically controlled mineralized structures (Mann et al. 1989), differing by most of its properties from abiotically synthetized calcites.

Each skeleton element (the so-called ossicle) consists of a rounded tridimensional network of trabeculae, the stereom, devoid of any apparent crystalline feature (larval spicules correspond to isolated trabeculae) (Nichols & Currey 1968). Furthermore, the stereom surface has the characteristics of a so-called "periodic minimal surface" (viz. a surface that divides space into two interpenetrating regions, each of them being a single multiply connected domain with no connection with the other) (Donnay & Pawson 1969).

The mineral phase is a solid solution of $CaCO_3$ and $MgCO_3$ in a calcite lattice, i.e. a high-magnesium calcite (Chave 1952). The $MgCO_3$ content ranges from 3.0 to 43.5 mole% (Schroeder et al. 1969) and averages 13.5-16.2 mole% according to the class considered (Weber 1969). Such a concentration would make echinoderm skeleton metastable in standard inorganic temperature/ pressure conditions (Lerman 1965).

Most single ossicles show properties of light polarization and X-ray diffraction as though they were carved out of a single crystal of calcite even when they are several centimetres in length (Raup 1959, Nissen 1969, Donnay & Pawson 1969). Furthermore, contrary to what could have been supposed from these properties of the ossicles, trabeculae do not fracture along the usual rhombohedral cleavage of calcite but show conchoidal fractures (Nichols & Currey 1968, Nissen 1969).

This paper reviews the present knowledge of the biological mechanisms which control and generate the stereom properties.

2. CRISTALLOGRAPHY

Although a few ossicles were demonstrated to behave optically as polycrystals (Towe 1967, Donnay & Pawson 1969, Märkel et al. 1971, Märkel 1979), most of the echinoderm skeleton is made of phenotypically monocrystalline

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ossicles. Data are only available on the mechanisms which control the crystallography of the latter.

The properties characteristic of single crystals can result from either an actual monocrystal (i.e., a crystalline network devoid of discontinuities) or a highly ordered polycrystalline aggregate. In a biomineralization context, the presence of coherent organic material within the skeleton is a stepping stone in the demonstration of the polycrystalline nature of the mineral phase (Towe 1972).

Contrary to previous statements in the literature, the echinoderm stereom does contain organic macromolecules (see Wilt & Benson 1988 and Dubois & Chen 1989 for review). These are classically separated into EDTA-soluble and EDTA-insoluble constituents, viz. the soluble and insoluble matrix; SM and IM, respectively (the functional validity of this dichotomy is, however, debated; see Wheeler & Sikes 1989). The soluble components are principally proteins, several of them being acidic N-glycosylated glycoproteins (Weiner 1985, Benson et al. 1986, Di Virgilio & Dubois unpubl.). Addadi & Weiner (1989) proposed that these proteins are intracrystalline and not structured in a coherent framework. They consequently suggested that the intrastereomic organic macromolecules (IOM) only induce local discontinuities in the crystalline network, dividing the stereom element into mosaic blocks but not into discrete microcrystals (that would compose a true polycrystalline aggregate). However, Addadi & Weiner (1989) only considered the soluble constituents and overlooked the intrastereomic insoluble constituents. Since morphological investigations showed that a coherent organic material occurs in both larval spicules and postmetamorphic ossicles (Benson et al. 1983, Dubois 1988), it is most likely that this material introduces extensive discontinuities in the crystalline network of calcite, making thus echinoderm ossicles true polycrystalline aggregates composed of highly ordered microcrystals. This suggests that a biological control should be exerted over the microcrystal alignment. A first clue is given by Mizoguchi et al. (1981) who reported that spicules of echinoid embryos reared in presence of tunicamycin have no extinction position in polarized light. This indicates that (1) discrete microcrystals actually occurs in echinoid larval spicules, and (2) that it is possible to cause their misalignment by inhibition of the N-glycosylation of proteins, suggesting a direct involvement of the intraspicular glycoproteins in crystal alignment.

Conchoidal fractures of stereom trabeculae are probably also due to the presence of IOM. Berman et al. (1988) showed that calcite crystals which were abiotically grown in presence of SM contained specifically adsorbed SM on their $\{1\bar{1}00\}$* planes and cleaved "with difficulty" along a curved surface of glassy appearance reminiscent of the conchoidal fracture of echinoderm stereom. These authors concluded that, during cleavage, the SM adsorbed on the $\{1\bar{1}00\}$ planes creates a continuous interference with the $\{10\bar{1}4\}$ cleavage planes of calcite resulting in no well-defined cleavage direction, i.e. in a conchoidal fracture. Furthermore, the unusually high strength of the echinoderm stereom (Weber et al. 1969, Emlet 1982) is suggested to result from both the general presence of IOM (making the stereom a composite material) and from the specific inclusion of SM in cleavage-inhibiting planes.

Different treatments of the conchoidal fractures result in a differential etching pattern made of whiskered structures parallel to the general c-axis of the ossicle (Pearse & Pearse 1975, Okazaki & Inoué 1976, O'Neill 1981, Dubois & Jangoux 1985). Differential etching patterns of biomineralized structures may result from selective inclusion of organic material during mineralization (Addadi & Weiner 1989). Dubois (1988, see also Dubois & Chen 1989) suggested effective involvement of IOM in the generation of the whiskered structures. He showed that calcite crystals, which were epitaxially grown on fractured trabeculae in presence of SM in an abiotic system, take a similar whiskered appearance (whereas epitaxial cleavage rhombohedrons were formed in control experiments). Furthermore, Addadi & Weiner (1989) suggested that the whiskered structures observed within the trabeculae are mosaic blocks with IOM adsorbed on their $\{hki0\}$ faces. Indeed, the abiotically generated whiskered structures show morphological

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*. Miller-Bravais indices of the $(1\bar{1}00)$ and symetry related faces of calcite, according to the hexagonal notation.

similarities with some calcite truncations (Dubois 1988). Whether the whiskered structures correspond to mosaic blocks or to discrete microcrystals is still unclear. However, their dimensions correspond to the "sub-micrometre sized" mosaic blocks detected in a stereom element by Blake et al. (1984). This suggests that the echinoderm stereom could be composed of ordered microcrystals (separated by coherent layers of organic material and revealed by tunicamycin treatment) which are subdivided in mosaic blocks delimited by intracrystalline SM.

3.CHEMISTRY

Inorganic high-magnesium calcites are metastable in standard temperature/ pressure conditions (Lerman 1965). Moreover, in the same environmental conditions, the presence of magnesium ions in a supersaturated solution of calcium carbonate results in the final precipitation of aragonite (Kitano 1964, Glover & Sippel 1967, Kitano et al. 1979). On the contrary, the presence of some organic compounds (such as glycoproteins) in a precipitating medium containing calcium, magnesium, and bicarbonate ions induces the precipitation of magnesium calcite (Kitano 1964, Kitano & Hood 1965). The intrastereomic organic material which is partly composed of glycoproteins could thus account for the high magnesium concentration of echinoderm calcite. Concordantly, the temperature effect on skeletal magnesium concentration in echinoderms, which was emphasized for a long time, is now considered to be limited and the intrastereomic organic material is suggested to account for the observed variability (see Dubois & Chen 1989 for discussion).

4.STEREOM MORPHOLOGY

Echinoderm stereom is a quite unique three-dimensional structure which shows smooth rounded curves without any relationship with its crystalline properties. Three factors may act as potential regulators of stereom morphology: (1) the membrane of the calcifying site, (2) selective inhibitors of the crystalline growth, and (3) the incorporation of magnesium ions.

The membrane which limits the calcifying site is either the plasma membrane or a vacuolar membrane from the skeleton-forming cells (see Dubois & Chen 1989). This membrane frames the shape of the calcifying site before mineralization starts, prefiguring the morphology of the future trabecula (see Wilt 1987 and Dubois & Chen 1989, for review). Furthermore, experimental modifications of the shape of the calcifying vacuole in echinoid larvae induce corresponding modifications in the shape of the resulting spicule (Okazaki 1962). The mechanism by which the membrane controls stereom morphogenesis is not known. Two processes may be proposed: a spatial confinement of trabecular growth and/or an unspecific inhibition of trabecular growth by organic molecules occurring on or in the vicinity of the membrane. Märkel et al. (1986, 1989) suggested that the latter takes place in echinoid tooth calcification.

Growth directions of echinoid larval spicules (which are homologous to single trabeculae) follow well-defined crystallographic orientations (Okazaki 1975). Now, echinoid SM selectively adsorbs on $\{1\bar{1}00\}$ faces of abiotically growing calcite crystals and, as a consequence, inhibits crystal growth in these directions, i.e. in directions perpendicular to the c-axis (Berman et al. 1988). These data suggest that stereom morphogenesis could also be directed by secretion of SM into the calcifying space and that the SM would act as an inhibitor in specifically adsorbing on definite crystalline faces. Addadi & Weiner (1989) proposed that this specific adsorption depends on stereochemical factors.

Finally, the presence of magnesium in the calcite lattice (a presence which could depend on the IOM) may also act upon the trabecular morphology. Okazaki & Inoué (1976) showed that echinoid larvae reared in magnesium-free sea water formed abnormal spicules whose ends correspond to the cleavage faces of a calcite rhomb. On the other hand, larvae reared in sea water containing one-tenth of the normal calcium concentration (with the normal magnesium concentration) formed spicules whose ends had a globular shape. These experiments show that magnesium could be involved in concealing the crystalline faces of echinoderm high-magnesium calcite. This suggestion is supported by SEM observations of synthesized high-magnesium calcite crystals. These are deprived of crystalline edges and some of them show a rounded to spherical morphology (Devery & Ehlmann 1981, Bischoff et al. 1987, Koch 1989). Some of these crystals actually resemble trabecular fragments (see Bischoff et al. 1987: fig.2B).

5.CONCLUSION

The available data emphasize the paramount importance of the organic macromolecules associated with the mineral phase in controlling the properties of the echinoderm skeleton. The evidence suggests that these compounds act at a stereochemical level, as in other calcium carbonate biomineralizing systems.

Although the importance of the skeleton-forming cell (in confining and framing the calcification site) and of the ion transport systems (in qualitatively and quantitatively regulating the ion delivery to the calcification site) should not be underrated, advances in our understanding of the properties of the echinoderm skeleton will probably result mostly from a better knowledge of the stereom associated organic macromolecules. Thus, future investigations should focus on: (1) the assessment of the diversity of IOM and of their exact role in the calcification process, (2) the in situ ultrastructure of the IOM (this should definitely resolve the polycrystal vs mosaic crystal debate), (3) the molecular identification of the IOM, (4) the in vivo evaluation of abiotic models, especially the stereochemical control of crystallization.

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