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Acidic peptides acting as growth modifiers of calcite crystals†

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Small acidic peptides comprising a repeating Phe–Asp sequence motif exert control, *in vitro*, on the morphology of calcite crystals similar to natural proteins from calcified tissues.

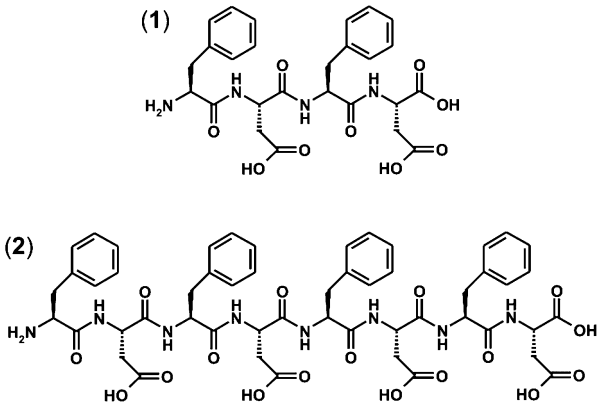
Crystallization of inorganic solids in the presence of organic molecules is an important step in biomineralization[1] and crystal engineering.[2] Investigations into the amino acid composition of natural proteins which are associated with $CaCO_3$ biomineralized tissues reveal characteristic sequence motifs which are particularly rich in aspartic and glutamic acid residues.[3] It has been suggested that mollusc shell acidic proteins may act as $CaCO_3$ crystal nucleation promoters. These proteins, upon adsorption onto a framework of water-insoluble macromolecules, might adopt a β-pleated sheet structure, where the carboxylate groups are placed exclusively on one side of the β-sheet.[4] The structure model implies that the ordered arrangement of carboxylate residues could act as a two-dimensional template for the formation of a $CaCO_3$ crystal nucleus. Other acidic proteins extracted from different calcified tissues were shown to exert vast control on polymorph selection, texture and morphology of $CaCO_3$ crystals.[5] However, up to now only a few artificial oligopeptides have been designed and tested for specific interactions with $CaCO_3$ single crystals *in vitro*.[6]

Amphiphilic peptides comprising alternating hydrophilic (Asp) and hydrophobic (Phe) amino acid residues, for instance, in fact tend to adopt β-sheet arrangements if spread as a monolayer at the air–water interface.[7] In this communication we describe the properties of two such acidic peptides (Scheme 1) designed to imitate the epitopes of acidic proteins from calcified tissues.

The amphiphilic peptides H–(Phe–Asp)$_2$–OH (**1**) and H–(Phe–Asp)$_4$–OH (**2**) specifically interact with distinct crystal faces of calcite. The morphological features of calcite crystals grown in the presence of **1** or **2** are very similar to calcite crystals grown from solutions containing natural acidic proteins. The peptides were synthesized according to the Fmoc/*t*-butyl strategy on a 2-chlorotrityl resin (see Electronic Supplementary Information).† Crystallization of $CaCO_3$ was performed within a sealed desiccator by slow vapor diffusion of CO_2 and ammonia into a solution containing calcium chloride and peptide.[8] The resulting crystals were harvested at different stages and examined by light and scanning electron microscopy. IR spectroscopy and powder X-ray diffraction of the resulting crystals show that in all cases calcite has formed exclusively (see ESI).† In addition to the normal {10.4} faces, the calcite crystals grown in the presence of **1**, at a concentration of 1 mM, or in the presence of **2**, at a concentration of 50 μM, respectively, develop a set of stepped diamond-shaped {01.2} crystal faces (Fig. 1). These are clearly distinguishable from the set of prismatic {11.0} faces which are also expressed. In contrast, $CaCO_3$ crystals grown in the presence of monomeric aspartic acid (3 mM) were indistinguishable from control experiments lacking additives.[9]

The newly formed crystal faces were identified by stereological analysis of scanning electron micrographs taken from crystal specimens at different viewing directions. The assignment of {11.0} and {01.2} faces was derived from measuring the characteristic interfacial angles of the appropriately oriented crystal specimen and comparing the results to computer models of truncated calcite rhombohedra (Fig. 1).[10]

The appearance of polar faces on biogenic calcite crystals belonging to index class {01.*l*} with l = 1–2 has been reported frequently. Calcite crystals grown in the presence of proteins isolated from sea urchin spicules, sponge spicules, and mollusc shells have been shown to induce the formation of {01.*l*} faces with l = 1–1.5.[11] Macromolecules extracted from the calcitic layer of



Scheme 1 H–(Phe–Asp)$_2$–OH (**1**) and H–(Phe–Asp)$_4$–OH (**2**).

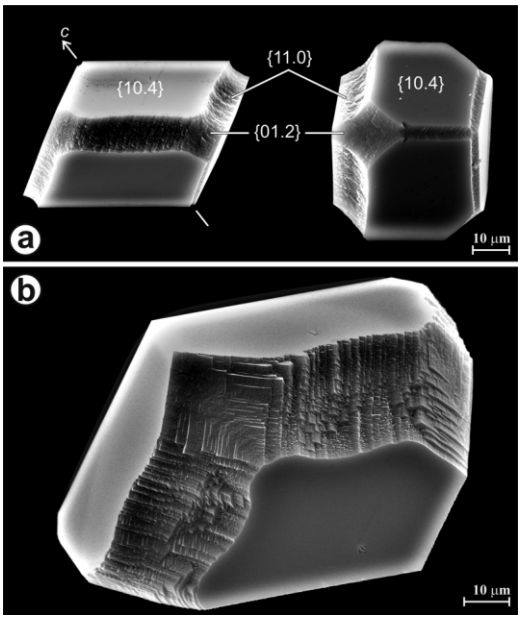


Fig. 1 Scanning electron micrographs of calcite crystals grown in the presence of **2** (ratio of **2** : Ca = 1 : 180). (a) Crystals isolated after 5 h, (b) crystal isolated after 18 h. (Photomontage showing selected crystal specimen).

† Electronic Supplementary Information (ESI) available: full analytical characterization of **1** and **2** as well as experimental details on $CaCO_3$ crystal growth and crystallographic analysis of the calcite crystal morphology. See http://www.rsc.org/suppdata/cc/b4/b405613b/

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tunic spicules of ascidians induce exactly the same calcite crystal habit morphology with well-developed stepped faces, indexed as {01.*l*} (*l* = 1.5).[12] Inhibition of {01.2} crystal faces seems to play an important role in controlling the texture and crystal shape of calcitic sponge spicules.[13]

The {11.0} crystal faces of calcite are less abundant in biological samples but it has been suggested that nucleation of the R-units of *Emiliania huxleyi* coccoliths originates from this particular crystal face.[14,15]

It should be noted that the {01.2} and {11.0} calcite crystal cleavage planes bear few common features with regard to their symmetry and electrostatic properties (Fig. 2). Surface models show that carbonate ions emerge almost perpendicular to the crystal faces, and it has been argued that this orientation should be favourable for interaction with acidic macromolecules containing Asp- or Glu-rich domains by virtue of stereochemical and geometrical recognition.[16] However, it is still an open question if epitaxial or stereochemical correlations which are implicit in many literature schemes showing an adsorbate layer of organic molecules sticking to a particular crystal cleavage plane represent a physically realistic model of the interface.[17] $CaCO_3$ crystals grown underneath monolayers of amphiphilic oligoacids such as calixarenes,[18] or resorcarenes,[19] for instance, often expose a single {01.2} crystal plane facing the monolayer. There is sound experimental evidence that this particular crystal orientation is caused by non-directional forces such as average charge density or mean dipole moment of the monolayer (the crystal growth plane, respectively). Since the peptide molecules we are using are very flexible, a template or epitaxy mechanism is currently ruled out although it would certainly be possible to construct foldamers of **1** and **2** which possess a spacing of the carboxylic acid residues that is commensurate with the positions of Ca ions in the inhibited crystal planes.

The expression of {01.2} and {11.0} faces of calcite induced by synthetic peptides containing a repeating Phe–Asp sequence motif bears novel insights into the crystallization of $CaCO_3$ under biological control. Thus, amphiphilic peptides such as **1** or **2** and similar peptides containing glutamic acid residues are ideal candidates for imitating epitopes of acidic proteins which are abundant in calcified tissues. To the best of our knowledge this is the first example where artificial peptides exert the same influence on the growth habit of calcite crystals as has been reported for natural acidic proteins isolated from skeletal elements of various organisms. Moreover, this is the first example where a single peptide selectively interacts with two different faces of calcite which might indicate that the peptide possesses distinct, energetically favourable conformations. In order to elucidate the predominant structure of the peptides in solution 2D-NMR investigations are currently under way.

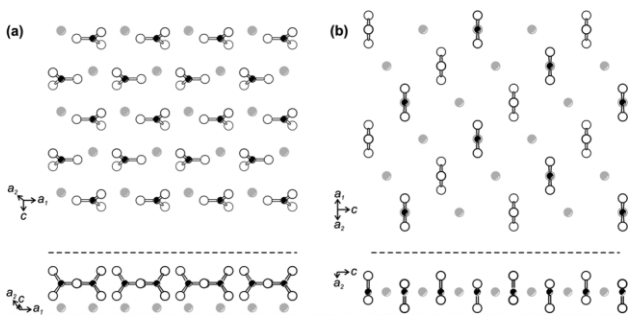

Fig. 2 Calcite surface layers of (a) the {01.2} cleavage plane and (b) the {11.0} cleavage plane.

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