Pancreatic juice is supersaturated with calcium carbonate. Calcite crystals therefore may occur, obstruct pancreatic ducts, and finally cause a lithiasis. Human lithostathine, a protein synthesized by the pancreas, inhibits the growth of calcite crystals by inducing a habit modification: the rhombohedral (10 14) usual habit is transformed into a needle-like habit through the (11 20) crystal form. A similar observation was made with the N-terminal undecapeptide (pE1R11) of lithostathine. We therefore aimed at discovering how peptides inhibit calcite salt crystal growth. We solved the complete x-ray structure of lithostathine, including the flexible N-terminal domain, at 1.3 Å. Docking studies of pE1R11 with the (10 14) and (11 20) faces through molecular dynamics simulation resulted in three successive steps. First, the undecapeptide progressively unfolded as it approached the calcite surface. Second, mobile lateral chains of amino acids made hydrogen bonds with the calcite surface. Last, electrostatic bonds between calcium ions and peptide bonds stabilized and anchored pE1R11 on the crystal surface. pE1R11-calcite interaction was stronger with the (11 20) face than with the (10 14) face, confirming earlier experimental observations. Energy contributions showed that the peptide backbone governed the binding more than did the lateral chains. The ability of peptides to inhibit crystal growth is therefore essentially based on backbone flexibility.

Biominalization has occurred for millions of years (1). Half of these biogenic minerals contain calcium (2); for example, the teeth of the sea urchin contain calcium carbonate (CaCO₃) in the form of calcite (the most common polymorph existing in nature), and primitive mollusks have aragonite spicules. In humans, biominalization is observed not only during skeletal formation, but also in biological fluids generally supersaturated with a calcium salt, such as oxalate in urine, phosphate in saliva, or carbonate in pancreatic juice. As a result, calcium salt crystals form spontaneously. While beneficial for tooth or bone mineralization, precipitation of calcium salts can be extremely harmful in fluids because it leads to the formation of stones and to the development of a lithiasis.

Various structural motifs emerge at calcite surfaces. Adsorption of additives, i.e. foreign substances, takes place either on growth sites or along the steps that spread over the faces or even onto the flat area of the faces. Since growth proceeds mostly through kinks, i.e. defects, blocking these sites is sufficient to hinder crystal growth. In some cases, adsorption is irreversible. In other cases, however, adsorption of additives is temporary and reversible: the oncoming growing units continuously repulse the additive molecules, which try to incorporate into the crystal lattice, in front of faces in growth. Mineralization must therefore be controlled at the molecular level (3). This control is ensured mainly by macromolecules, essentially proteins. Since the discovery of dentin (4), an acidic protein in vertebrate teeth, many other proteins involved in crystal growth control in humans have been described (for a review, see Ref. 5).

Among these proteins, lithostathine has been well documented. It is a protein of 144 amino acids that is produced by acinar cells of the pancreas and secreted into pancreatic juice. The structure of human lithostathine has been reported at 1.5 Å resolution (6); no structural information was obtained on the N-terminal domain (residues 1–13), whereas the C-terminal part, which belongs to the C-type lectin superfamily, was fully characterized. Yet, lithostathine is very susceptible to proteolysis. It produces a soluble N-terminal undecapeptide (pE1R11) and a C-terminal form of 133 amino acids that precipitate and form fibrils (7). The specific association of lithostathine with CaCO₃ suggested that it could be involved in the control of crystal growth (8). This suggestion was confirmed by Bernard et al. (9). However, several other groups published conflicting results depending on how crystal growth measurements in solution had been made (10, 11). Looking at the calcite crystal morphology in the presence of lithostathine, we previously observed that lithostathine changes the growth of calcite crystal by inducing a habit modification in vitro: the rhombohedral (10 14)¹ usual habit is first transformed into a subcubic.

¹ Crystal planes are denoted by the Miller indices h, k, i, and l, where i = −(h + i), which define their orientation relative to the crystal axes.
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EXPERIMENTAL PROCEDURES

X-ray Structure Determination

Crystallization and Heavy Atom Search—The purification and crystallization of human lithostatine have been previously described (14). Crystals belong to the hexagonal P6₃ space group with a = b = 48 Å and c = 111 Å and contain one molecule of lithostatine/asymmetric unit. Soaking crystals for 24 h in a solution containing 0.5 mM only 17.4 and 18.6%, respectively.

Model Building and 1.3-Å Refinement—As no interpretable density data for N-terminal residues 1–13 were obtained, a 1.3-Å resolution data set was subsequently collected on a MarResearch imaging plate system at the Hamburg synchrotron radiation facility (Germany). Data were processed with DENZO and SCALEPACK (17). The atomic coordinates resulting from the 1.55-Å resolution refinement were used as the starting model. Energy was first minimized with REFMAC (18) using individual isotropic B-factors. The (2mFo – DFc) and (mFo – DFc) electron density maps, calculated with SIGMAA (18), clearly showed an extended density around residue 14, into which it was possible to build the N-terminal part of the protein (residues 1–13). Residues 9–11 were built as alanine residues since the density was not interpretable for building the side chains. Finally, a new refinement procedure, with an electron density calculation data set overlapped with the model including the complete protein chain. After energy minimization using REFMAC, the R-factor and the free R-factor were only 17.4 and 18.6%, respectively.

Peptide Synthesis, Purification, and Characterization

The N-terminal undecapeptide pyro-EEAQTELPGQ (pE₅R₁₁) was assembled according to the method of Barany and Merrifield (19) on 4-hydroxymethylphenoxymethylpolystyrene and 1% divinylbenzene-preloaded resin (0.10-0.65 mmol; Perkin-Elmer) on an automated synthesizer (ABI 433A, Perkin-Elmer). To avoid derivatives with deletion, 4-hydroxymethylphenoxymethylpolystyrene and 1% divinylbenzene-assembled according to the method of Barany and Merrifield (19) on 0.1% trifluoroacetic acid, and buffer B was acetonitrile with 0.1% trifluoroacetic acid. The elution gradient consisted of increasing the buffer B ratio from 20 to 40% in 40 min with a 2-ml/min flow rate. HPLC analysis was done with a C8 reverse phase column (4.75×250 mm; Merck). Buffer A was water with 0.1% trifluoroacetic acid, and buffer B was acetonitrile with 0.1% trifluoroacetic acid. The elution gradient consisted of increasing the buffer B ratio from 20 to 40% in 40 min with a 2-ml/min flow rate. Electrospray mass spectrometry was carried out with a single quad PE-SCIEX API 150ex (Perkin-Elmer).

Amino acid composition was analyzed on a Beckman Model 6300 amino acid analyzer.

Circular Dichroism Measurements

Initial Conditions—Calcite unit cell parameters were taken from the literature (20): space group R3c (rhombohedral system), Z = 6, a = 4.990 Å, and c = 17.092 Å. For partial charges on calcite atoms, the values of Catti et al. (21) obtained from ab initio calculations were used: +1.865 e⁻ on calcium, +1.075 e⁻ on carbon, and −0.980 e⁻ on oxygen. As the overall surface charge of calcite is neutral at physiological pH (22), no specific surface charge or surface ionic species like -CO₂⁻ or -CO₂H groups were taken into account. Periodic boundary conditions were applied on a simulation box to get rid of boundary effects. The simulation box size was chosen to allow the undecapeptide to move freely on the calcite surface. To avoid pE₅R₁₁ adsorption under the top of the box where periodic boundary conditions replicate the calcite interface, a 100-Å box height was used. This height gave enough space for this undecapeptide to move over the calcite surface. Under these conditions, the (10 14) and (11 20) surface areas were 1411.52 Å² (35 unit cells) and 2057.20 Å² (42 unit cells), respectively. Finally, a slice depth of three unit cells was necessary to properly evaluate the interactions of the undecapeptide with calcite faces.

Docking Studies—Molecular dynamics simulations were carried out in the NVT ensemble (molecule number, volume, and temperature are kept constant during the simulation). They were performed with Ceres2 running on a Silicon Graphics R5000PC workstation. The simulation box contained a fixed calcite interface, representing either the (10 14) or the (11 20) face, and the pE₅R₁₁ peptide, whose coordinates were taken from x-ray data, without the carbohydrate moieties. A 0.5-fs integration step was used. Temperature was kept constant at 310 K using the Nose-Hoover equation of motion with a 0.3 relaxation factor value that guaranteed a proper convergence of the dynamics simulation. No solvent molecules were added in our model. The undecapeptide was then introduced into the box and set at a 20-A distance from the calcite interface. A typical dynamics simulation included a 20-ps relaxation period of the system during which the undecapeptide moved toward the calcite surface and reached rather stable location and conformation. It was followed by a simulation period of several tens of ps. Snapshots taken from the dynamics simulation trajectory files were then analyzed. The interaction energy between pE₅R₁₁ and the calcite surface was computed as the difference between the energy of the whole system and the energy of pE₅R₁₁ alone plus the energy of the calcite crystal. Ewald parameters were kept constant for all the calculations. As physical equivalence could vary from one simulation snapshot to another, we expressed the undecapeptide-calcite interaction energy E_i (J/mol of unit cell) in terms of microscopic adhesion work W_i (J/mol) as proposed by Lin et al. (24): W_i = -E_i/2N_A S_ad, where S_ad is the interface area, N_A is the Avogadro number, and n_i is the number of molecules each covering a contact area S_i (n_iS_i = n_A S_ad).

RESULTS

Quality of the X-ray Model and Overall Protein Structure—The final refinement process was carried out with SHELX (25). Anisotropic temperature factor refinement led to subsequent...
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Table 1

|                     | Native 1 | Native 2 | Derivative |
|---------------------|----------|----------|------------|
| X-ray data collection parameters and refinement statistics |          |          |            |
| R-factor = \( \frac{\sum_{\alpha} F_{\alpha} - F_{\alpha}}{\sum_{\alpha} F_{\alpha}} \) |          |          |            |
| Rmsd \( F_{\alpha}/\text{residual} \) | 1.5      | 1.6      | 1.3        |
| Rvalues           | 0.042    | 0.097    | 0.084      |
| Refinement        |          |          |            |
| Program           | REFMAC   | SHELX    |            |
| Type of B refinement | Isotropic | Anisotropic |            |
| Protein atoms     | 1132     | 1132     |            |
| Sugar atoms       | 45       | 45       |            |
| Water molecules   | 135      | 135      |            |
| R-factor          | 17.4     | 13.2     |            |
| Free R-factor     | 18.6     | 15.9     |            |

\( ^a \) Root mean square.

R-factor and free R-factor values of 13.2 and 15.9, respectively, for all 32,058 reflections in the resolution range of 8.0 to 1.3 Å. X-ray data are provided in Table 1. A Ramachandran plot (26) showed that 88.1% of the residues were in the most favored regions, and none of the non-glycine residues were in the disallowed regions (data not shown). The final model included 135 water molecules and the entire protein (144 residues) (Fig. 1A). Surprisingly for such a small protein, the complete high resolution structure of human lithostathine showed two domains: a well organized globular C-terminal domain (residues 14–144) and a flexible N-terminal region (residues 1–13) that do not interact. The C-terminal domain belongs to the C-type lectin superfamily, although it does not bind carbohydrate (6). It is separated from the N-terminal domain by the C-14–C-25 disulfide bridge. The N-terminal domain is a 13-residue peptide chain that stretches out of the heart shape of the C-type lectin domain. Three residues (positions 9–11) are involved in a helix turn motif that represented the only secondary element of this domain. As a result, the N-terminal domain was much more agitated in the crystal (averaged B-factor of 36 Å²) than the rest of the protein (averaged B-factor of 22 Å²). This is consistent with the fact that we needed x-ray data at 1.3 Å resolution to build this domain.

Earlier studies have shown that lithostathine is O-glycosylated on Thr⁵ (27). During structure refinement, we observed a large electron density around Thr⁵, clearly indicating the presence of O-linked sugars to the Thr⁵ side chain. This enhanced density of synthetic pE₁R₁₁ was confirmed by semi-empirical quantum chemical method calculations using the program MOPAC from the Cerius² package (data not shown). This carbohydrate moiety was divided into two carbohydrate chains perpendicular to the protein backbone (Fig. 1A). Furthermore, because of the high quality of our electron density maps (Fig. 1B), it was possible to assign our isoform to isoform K (27) and to build three sugar residues (NeuAc(1→3)GalNAc(2→6)GalNAc(91→3)Gal) out of the four forming the carbohydrate chain. In addition, mass spectroscopy results for the crystallized protein (17,199 Da) agreed very well with the theoretical weight of this isoform (17,194 Da) (data not shown).

CD Experiments—That the N-terminal domain remained disordered in the crystal prompted us to test the behavior of the synthetic counterpart, pE₁R₁₁, which is also an inhibitor of calcite crystal growth, under physiologic conditions (pH 7, 37 °C). The CD spectrum (Fig. 2) was typical of a random coil structure (28) and strengthened our x-ray data. The negative band near 200 nm had an intensity in the range of those observed with model peptides with no steric constraints or internal hydrogen bonds. Random coil CD spectra were characterized by an intense negative band at 200 nm due to \( \pi-\pi^* \) transition and low intensity bands near 210 nm due to \( n-\pi^* \) transitions (28). No typical secondary structures could be deduced from this spectrum. Similar results were obtained in trifluoroethanol or SDS (data not shown). Both x-ray and CD experiments therefore suggest that pE₁R₁₁ is a highly flexible molecule that can display many configurations depending on the environment.

(10 14 and 11 20) Structures of Calcite Faces—Calcite lattice energy computed with DREIDING 2.21 (2663 kJ·mol⁻¹) was within 5% of that calculated from the Born-Fajans-Haber thermodynamic cycle (2804 kJ·mol⁻¹, 29) and justified the use of this force field for our docking studies. (10 14) and (11 20) faces used in dynamics simulation are displayed in Fig. 3 (A and B, respectively). They both exhibit evenly spaced rows of calcium and carbonate ions. However, on the (10 14) face, the planar carbonate ions are parallel to the surface and always the same \( \text{O}_2\text{CO} \) pattern. Conversely, on the (11 20) face, Y-shaped carbonate ions display alternately one and two protrud-
Docking Studies—A qualitative analysis of the dynamics simulation trajectories showed that, as pE1R11 approached the calcite face (10 1\(^{-}\)4) or (11 2\(^{-}\)0), it gradually unfolded (data not shown). Simultaneously, it attached to the calcite surface through electrostatic bonds between its oxygen atoms from C\(_5\)O groups and calcium ions regularly emerging from the surface and by means of hydrogen bonds between its hydrogen atoms from N–H or O–H groups and oxygen atoms from the carbonate ions. This dual bonding strengthened the pE1R11-calcite surface interaction. Both types of bonds participated in the global interaction. Interestingly, we noticed that electrostatic bonds do not break during the course of simulation studies, which indicates very strong binding. Indeed, once the Ca\(_{\text{surface}}\)-O\(_{\text{undecapeptide}}\) bonds were established, the undecapeptide stayed rather still, only making or breaking hydrogen bonds. With such an inertia, the undecapeptide is a good crystal growth inhibitor candidate: it sticks to the calcite surface long enough to impede the attachment of crystal growth units and thus reduces the face growth rate. Furthermore, dynamics simulation assays with pE1R11 surrounded by water molecules showed a similar tendency, but convergence time was beyond our computer time capacity (data not shown).

Interaction of the pE1R11 Peptide with the (10 1\(^{-}\)4) Face—Fig. 4A shows a top view of the pE1R11 peptide adsorbed on the (10 1\(^{-}\)4) calcite interface. The most interesting feature was that pE1R11 was stabilized by 15 bonds: 5 hydrogen bonds (H\(_{\text{undecapeptide}}\)-O\(_{\text{calcite surface}}\)) and 10 electrostatic bonds (Ca\(_{\text{surface}}\)-O\(_{\text{undecapeptide}}\)). These hydrogen bonds included four O\(_{\text{calcite surface}}\)-H-N\(_{\text{undecapeptide}}\) bonds (two from backbone, Leu\(^{7}\) and pyro-Glu\(^{5}\), and two from lateral chains, Gln\(^{4}\) and Arg\(^{11}\)) and one O\(_{\text{calcite surface}}\)-H-O bond of the hydroxyl group of Thr\(^{5}\). Among the 10 electrostatic bonds, 9 showed the oxygen atom located just above the calcium ions, whereas one oxygen atom settled between two calcium ions (Ca\(_{\text{surface}}\)-O\(_{\text{undecapeptide}}\)-O\(_{\text{undecapeptide}}\)). Under these conditions, the Ca\(_{\text{calcite surface}}\)-O\(_{\text{undecapeptide}}\) distance was 2.4 Å, but reached 2.5 Å when two calcium ions were involved in the bonding. Experimental measurements of Ca–O distances agreed on a 2.4-Å value regardless of the environment of the oxygen atom (30) and therefore validated our simulation method. During the course of the dynamic simulations, the average number of Ca–O–C\(_{\text{undecapeptide}}\) and -CO\(_{2}\)C\(_{\text{cal}

![Diagram](image_url)

Fig. 2. Circular dichroism (\(\Delta \varepsilon\)) and absorption (A) spectra of the lithostatine N-terminal undecapeptide. The two spectra were measured simultaneously from 260 to 178 nm. The absorption spectrum shows a positive band at 190 nm due to the amide chromophore. The intensity of this band was similar to that of any peptide at 1 mg/ml. The CD spectrum is typical of a random coil structure.
hydrogen nature (−37.5 and −11.4 kcal mol⁻¹, respectively). Last, for all those bonds, the van der Waals contribution to the interaction energy with the (10 1¯4) calcite interface was positive because the repulsive term of the van der Waals potential dominates its attractive term at the short distances involved.

Interaction of the pE₁R₁₁ Peptide with the (11 2¯0) Face—Fig. 4B shows a top view of the pE₁R₁₁ peptide adsorbed on the (11 2¯0) calcite interface. Anchoring of the peptide was ensured by 18 bonds that represent 70% of the total interaction energy: 9 hydrogen bonds (Hundecapeptide–Ocalcite surface) and 9 electrostatic bonds (Casurface–Oundecapeptide). Hydrogen bonds were divided as follows: three with the peptide bond (H–N), Glu⁶, Ala¹⁰, and Arg¹¹; six with lateral chains, pyro-Glu¹, Gln⁴, and Gln⁹; and three with Arg¹¹. For the electrostatic bonds, four came from the peptide bond (OₓC), Gln⁴, Glu⁶, Pro⁸, and Arg¹¹; and five came from lateral chains, pyro-Glu¹, Glu², Gln⁴, Thr⁵, and Gln⁹. In addition, five oxygen atoms settled between two calcium ions (Casurface–Oundecapeptide–Casurface bonds). As Ca–O distances were similar (2.4 or 2.5 Å) whether one or two calcium atoms were involved, the electrostatic attraction energy of the Ca–O–Ca bond was roughly double the energy of the Ca–O bond. Bond distances were similar to those observed with the (10 1¯4) face. Table III summarizes the energy values obtained.

The same observations as for the interaction of pE₁R₁₁ with (10 1¯4) applied (see Table II). However, the total energy measured was greater with the (11 2¯0) face than with the (10 1¯4) face (−302.1 versus −214.2 kcal mol⁻¹) for two reasons. First, the calcium row spacing is larger on the (11 2¯0) face than on the (10 1¯4) face, Ca surface–Oundecapeptide–Casurface bonds are more numerous on the (11 2¯0) face. Second, as this type of bond has a larger electrostatic interaction energy than do Ca surface–Oundecapeptide bonds and as the total interaction energy is mostly of electrostatic nature, the pE₁R₁₁ peptide binds more strongly on the (11 2¯0) face than on the (10 1¯4) face.

Coulombic Interactions and Microscopic Adhesion Work—Qualitatively, the interactions of the undecapeptide with the (10 1¯4) and (11 2¯0) faces were almost equivalent. Quantitatively, however, they differed. Indeed, because of row spacing, calcium ions are more accessible on the (11 2¯0) face than on the (10 1¯4) face. Consequently, coulombic interactions between a negatively charged atom of pE₁R₁₁ and a calcium ion were greater on the (11 2¯0) face (−213.4 versus −105.6 kcal mol⁻¹). By performing many simulations and analyzing several snapshots (data not shown), we calculated the mean microscopic adhesion work. It amounted to 350 mJ/m² for the interaction of pE₁R₁₁ with the (10 1¯4) face and 550 mJ/m² with the (11 2¯0) face, i.e. 1.5 times greater. This result clearly shows a stronger attachment of pE₁R₁₁ to the (11 2¯0) face than to the (10 1¯4) face. According to theories of crystal growth with additives (35, 36), strongly attachment would lead to the appearance of the (11 2¯0) face when pE₁R₁₁ is present, thus confirming our previous experimental observations (12).
tying down such layers should adopt a regular β-sheet conformation. For instance, this is the case with acidic glycoproteins from mollusk shells (37, 38) or poly-Asp (39), which adsorb strongly onto calcite surfaces. Other studies have suggested there might be bonds between calcium atoms and carboxylate groups belonging to small synthetic α,ω-dicarboxylic acids (40). CBP1, an artificial regular α-helical peptide, has been shown to bind to calcite surfaces, inducing morphological changes (13). It was therefore concluded that regular secondary structures, either of the β-sheet or α-helix type, are a prerequisite for the peptide to bind to crystal surfaces and to disturb their growth.

All proteins display dicarboxylic acids and regular secondary structures such as β-sheet or α-helix; but only a few are good inhibitors of crystal growth, thus ruling out the lattice matching model. It has become obvious that other factors such as stereochemical, charge-dependent, or electrical polarization ones are also required (41, 42). The growth of crystals proceeds by a two-step mechanism: growth unit diffusion toward the surface and subsequent integration into the crystal lattice. We believe that inhibition of calcite growth by pE1R11, and more generally by any protein inhibitor, proceeds by a similar mechanism. First, the undecapeptide pE1R11 unfolds as it approaches and then diffuses over and binds to the calcite surface. This diffusion means that the peptide and water–calcite hydrogen bonds are progressively broken and replaced by peptide–calcite interactions. Then, through electrostatic bonds, pE1R11 progressively integrates the crystal lattice, so that it cannot totally desorb. Flexibility is therefore the key point for permanent trapping into the crystal. This trapping hinders the smooth flow of growing steps, which are somehow forced to flow between additives, e.g., the pE1R11 peptide. Similar conclusions have been drawn by Sicheri and Yang (43) with the antifreeze protein from winter flounder. They showed that the current ice-binding models (44, 45), also based on ice-lattice matching, were no longer adequate.

Interestingly, human serum albumin, an excellent binder of calcium oxalate crystals, does not inhibit their growth. Conversely, it is a strong nucleation enhancer of calcium oxalate dihydrate crystals by ionotropic effect (46). The structure of albumin shows a repetitive configuration of three helical homologous domains, each formed by two smaller subdomains. This is a highly organized structure (47, 48) conferring a notable rigidity that prevents albumin from disrupting some of its own hydrogen, van der Waals, or electrostatic bonds. Consequently, albumin is easily rejected from the crystal surface by oncoming growing units. This rejection explains why albumin is not an inhibitor of crystal growth and confirms why flexibility is the key point in the inhibition of calcium salt crystal growth.

This “unfolding-binding” mechanism mirrors our crystallographic data and the deduced resulting structural organization of lithostathine. Indeed, in previous experiments, no structural information was obtained for the first 13 amino acids, including pE1R11. Consequently, as this domain was disordered in our current crystallographic data and the deduced resulting structural organization of lithostathine.

**DISCUSSION**

Our results show that the ability of peptides to inhibit crystal growth is essentially due to their backbone flexibility. Yet, until now, the theoretical model explaining the inhibition of crystal growth by proteins was based on lattice matching. Indeed, a neutral but ionic crystal surface like calcite shows a periodic array of localized binding sites for ions of opposite charges. This periodicity prompted several authors to speculate that proteins...


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The 15 bonds identified, as well as the splitting of the total interaction energy in terms of interaction type (6–12 Lennard-Jones-like van der Waals type, coulombic type, and 10–12 Lennard-Jones-like hydrogen bond type), are shown. In this particular snapshot, the undecapeptide surface coverage represents 27% of the (10 1 4) interface area.

| Crystal face–undecapeptide bond type | Interaction type | van der Waals | Coulombic$^a$ | Hydrogen bond | Total kcal/mol$^-1$ |
|-------------------------------------|-----------------|--------------|-------------|--------------|-------------------|
| $\text{pE}_{1\text{R}11}$         |                 | -26.0        | -175.4      | -12.8        | -214.2            |
| 5 $\text{O}_\text{surface}$–$\text{N}$-undecapeptide bonds |                 | +8.0         | -37.5       | -11.4        | -40.9             |
| 10 $\text{Ca}_\text{surface}$–$\text{O}$-undecapeptide bonds |                 | +15.2        | -105.6      | -90.4        |                   |

$^a$ Computed using a dielectric constant ($\varepsilon$) value equal to 1.

The 18 bonds identified, as well as the splitting of the total interaction energy in terms of interaction type (6–12 Lennard-Jones-like van der Waals type, coulombic type, and 10–12 Lennard-Jones-like hydrogen bond type), are shown. In this particular snapshot, the undecapeptide surface coverage represents 20.6% of the (11 2 0) interface area.

| Crystal face–undecapeptide bond type | Interaction type | van der Waals | Coulombic$^a$ | Hydrogen bond | Total kcal/mol$^-1$ |
|-------------------------------------|-----------------|--------------|-------------|--------------|-------------------|
| $\text{pE}_{1\text{R}11}$         |                 | +6.5         | -292.1      | -16.6        | -302.1            |
| 9 $\text{O}_\text{surface}$–$\text{N}$-undecapeptide bonds |                 | +10.1        | -33.3       | -15.5        | -38.7             |
| 9 $\text{Ca}_\text{surface}$–$\text{O}$-undecapeptide bonds |                 | +32.5        | -213.4      | -170.9       |                   |

$^a$ Computed using a dielectric constant ($\varepsilon$) value equal to 1.

Hydrate moiety is involved in specific localization of the protein.

In conclusion, a prototypic protein inhibits calcium salt crystal growth through three basic rules. First, it must be flexible enough to spread onto the crystal surface. Second, it must adopt a conformation that allows it, through its backbone, to develop strong coulombic interactions with the crystal surface. Last, it must further stabilize these interactions through interactions of the crystal surface and lateral chains. Since calcium oxalate and calcium phosphate crystals are related to calcite, mechanisms of growth inhibition in other biological fluids should proceed in the same way.

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