Purification and Functional Analysis of a 40 kD Protein Extracted from the *Strombus decorus persicus* Mollusk Shells

Boaz Pokroy,[†] Emil Zolotoyabko,[†] and Noam Adir*[,‡]

Department of Materials Engineering and Department of Chemistry, Technion - Israel Institute of Technology, Haifa 32000, Israel

Received July 19, 2005; Revised Manuscript Received November 15, 2005

A 40 kD protein has been extracted from the biomineral matrix of the calcium carbonate gastropod shell of *Strombus decorus persicus*. The protein was isolated by decalcification and ion exchange HPLC. We have named this protein ACLS40, i.e., aragonite crossed-lamellar structure protein. A partial sequence of the isolated ACLS40 and amino acid analysis both indicate that it does not belong to the family of very acidic proteins, i.e., rich in aspartic and glutamic residues. The shell-extracted protein shows the ability to stabilize calcium carbonate in vitro, in the form of thermodynamically unstable vaterite polymorph, and to inhibit the growth of calcite.

Introduction

Numerous organisms require shields built of inorganic materials in order to survive. An example is mollusk shells which provide protection for their soft-bodied inhabitants against predators.[1] The shells of mollusks are in fact composite materials built of a calcium carbonate (ceramic phase) and organic molecules.[2] The ceramic phase is usually composed of one of the two main polymorphs of $CaCO_3$, aragonite or calcite. If both polymorphs are present in a particular shell, they are typically separated in different layers.[3] The organic molecules secreted by mollusks are present in small concentrations (0.1−5 wt %),[4] but have a decisive effect on the internal architecture of the shells and their superior mechanical properties.[5] The organic phase is responsible for stabilizing the metastable aragonite polymorph[6,7] and for controlling the morphology[8−10] and orientation of the crystals.[2] The organic molecules can be divided into two groups: macromolecules that are soluble in the aqueous phase when the shell is demineralized and those that remain insoluble.[11] This topic has been recently reviewed by Marin and Luquet.[1]

The majority of the organic phase in the seashells is located between crystallites. In the nacre microstructure, the intercrystalline organic phase has a layered structure composed of highly ordered β-chitin, which is responsible for the overall interlamellar sheet structure.[12] The chitin fibrils are arranged in parallel arrays within each interlamellar sheet. The second major component of the organic matrix is silk, which is intimately associated with the β-chitin and is in the form of an amorphous gel phase.[12] It was also shown that the organic molecules can penetrate into the crystals of biogenic calcite[13] and biogenic aragonite.[14]

Soluble macromolecules have been extracted from a large variety of mineralized tissues and have been found to be rather anionic (i.e., rich in aspartic and glutamic acids). In addition, the presence of phosphorylated amino acids and/or acidic sulfated polysaccharides[15] has also been identified. It is important to note that all the aspartic-acid-rich shell proteins isolated to date are associated with calcitic microstructures,[1] while a majority of proteins extracted from aragonitic nacre have not been found to be acidic.[1] A number of extremely acidic proteins associated with calcitic prisms have recently been characterized, and their sequences show that most probably they play an important role in the shell formation.[16] In contrast to other aragonitic proteins previously described, an acidic 8 kDa protein was extracted recently from the abalone nacre (aragonite) and shown to influence calcite morphology in vitro.[17] Some of the proteins described above, and in some cases their associated sulfated polysaccharides, have been shown to interact with calcium in vitro.[18] Indeed, calcium appears to facilitate the formation of β-sheet structures in some of these macromolecules.[19,20]

Only a very few soluble shell proteins have been isolated to homogeneity and characterized. Most of these proteins are from the nacreous microstructure. Mucoperlin has been extracted from the nacreous layer of the fan mussel *Pinna nobilis* and was found to be a mucin-like protein.[1] The sequence of Lustrin A from the abalone (*Haliotis rufescens*) nacre has similarities with collagen (the presence of glycine-rich repeats) and has a modular structure where cysteine and serine−proline-rich domains alternate.[21] Three proteins from the nacre of the Japanese oyster (*Pinctada fucata*) have been genetically characterized. Two of them are very similar to silk protein (MSI31 and MSI60),[22] while the third one, called Nacrein, has been shown to possess carbonic anhydrase activity and most likely has the ability to chelate calcium ions.[23] Several other shell-associated proteins have been reported, including three proteins from the mollusc *Haliotis laevigata* (with molecular weights of 13, 15, and 17 kDa)[24] and a 5 kDa peptide called Pelovaterin from *Pelodiscus sinensis* turtle eggshells. This last peptide was shown to stabilize the calcium carbonate polymorph vaterite.[25] In most of these studies, the amino acid composition has been determined, but protein functionality has been difficult to address. A number of studies have indicated the limited usefulness of sequences in understanding the mechanism of action performed by these proteins.[26−28]

In the current study, we have extracted the organic macromolecules that are soluble in aqueous solution from *Strombus decorus persicus* gastropod seashells. These aragonitic shells comprise almost exclusively the crossed-lamellar microstructure

* To whom correspondence should be addressed. E-mail: nadir@tx.technion.ac.il.

[†] Department of Materials Engineering.

[‡] Department of Chemistry.

10.1021/bm050506f CCC: $33.50
Published on Web 01/26/2006

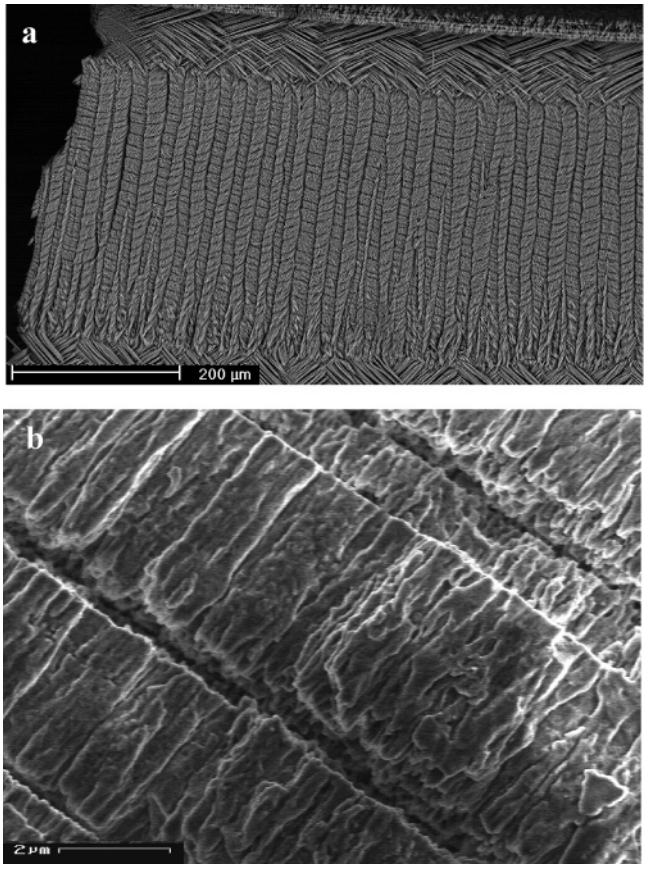

Figure 1. (a) SEM image of the crossed-lamellar microstructure. (b) SEM image of the inner prismatic layer.

and a very thin (a few microns) inner prismatic layer, but no nacre. An example of the microstructure is given in Figure 1. Detailed characterization of this gastropod shell microstructure was performed by Pokroy and Zolotoyabko.[29] Here, we show that a 40 kD protein isolated from this aragonitic seashell is deeply involved in calcium carbonate polymorphism in situ.

Materials and Methods

Samples. Fresh, mollusk-contained *Strombus decorus persicus* gastropod seashells were harvested at the coast of Haifa bay of the Mediterranean Sea. The *Strombus decorus persicus* was first described by Swainson in 1821. This mollusk has emigrated from the Arabian Sea through the Suez Canal.

Protein Purification and Characterization. After the mollusks were removed, the shells were washed extensively in 5% NaOH, followed by washing successively in a detergent, double distilled water (DDW), and acetone. The shells were allowed to air-dry for an hour and then crushed by a mortar and pestle to a fine powder, which was decalcified by the addition of 50 mL of EDTA 1 M pH 8 to every 5 g of the shell's powder. The slurry was stirred at 4 °C overnight and then was centrifuged at 21 000 g for 15 min with a Sorvall Super 21 centrifuge, which separated the supernatant (soluble) and insoluble fractions. The soluble fraction was filtered through a 0.45 μm filter (Minisart) and then desalted through exchange with DDW by dialysis. Complete removal of salts and EDTA was required in order to perform the purification protocol. The protein-containing medium was concentrated by ultrafiltration on an Amicon centricon, and products having molecular weights above 30 000 were collected to a total volume of approximately 50 mL.

The protein mixture was further fractionated by anion exchange HPLC (PL-SAX 1000 Å, Polymer Laboratories). Bound proteins were eluted using a linear gradient of 0−1.4 M NaCl over 80 min. The eluted

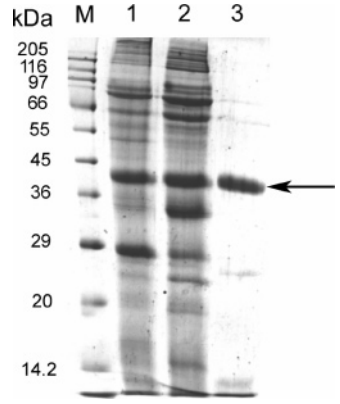

Figure 2. SDS−PAGE of the soluble proteins extracted from the shell with 1 M EDTA pH 8. Lane M shows molecular weight markers. Lanes 1 and 2 are soluble proteins, extracted as is described in the text from two different batches of shells. Lane 3 is the purified 40 kD protein (arrowed) obtained by HPLC using an anion exchange column.

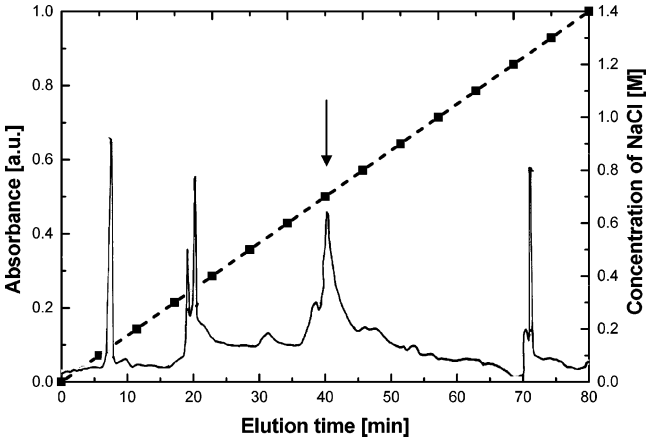

Figure 3. Anion exchange HPLC separation of soluble shell proteins. The elution peaks correspond to different protein fractions presented in Figure 2, lanes 1 and 2. The arrow indicates the position where the ACLS40 protein elutes.

fractions and the purity of the final protein preparation were verified by SDS−PAGE.

Both Edman degradation and de novo MS/MS protein sequencing methods were performed at the Smoler Protein Center of the Department of Biology, Technion. Total amino acid analysis was performed by the Chemical Research Support Unit of the Faculty of Chemistry, Weizmann Institute of Sciences.

$CaCO_3$ Crystallization. $CaCO_3$ crystallization at room temperature (22 ± 2 °C) and normal pressure was accomplished in a sealed desiccator by slow diffusion of $NH_4(CO_2)_2$ vapor into 1 mL of 10 mM $CaCl_2$ placed on amorphous silica disks within the wells of a 24-well tissue culture box, tightly sealed by transparent polyethylene film (similar to Falini et al.[7]). The purified protein was mixed prior to crystallization at different concentrations with the 10 mM $CaCl_2$. After preparation of the crystallization systems, identical diffusion outlets were produced in all wells by a 21G needle. This procedure ensured equivalent crystallization parameters for all samples involved and allowed us to obtain very reproducible results of the crystallization experiments. When crystallization was complete, the wells were allowed to air-dry so that all crystals produced remained on the silica disk.

Physical Measurements. Phase analysis was carried out by means of conventional powder X-ray diffraction (XRD). We used a Philips X-Pert diffractometer in Bragg−Brentano focusing geometry, equipped with a Cu sealed tube ($\lambda = 1.5406$ Å) and a curved graphite monochromator to reduce the white radiation background. Experimental data were collected at 40 kV and 40 mA.

The structural types of crystallites grown in the presence of the extracted protein were determined by use of a single-crystal X-ray

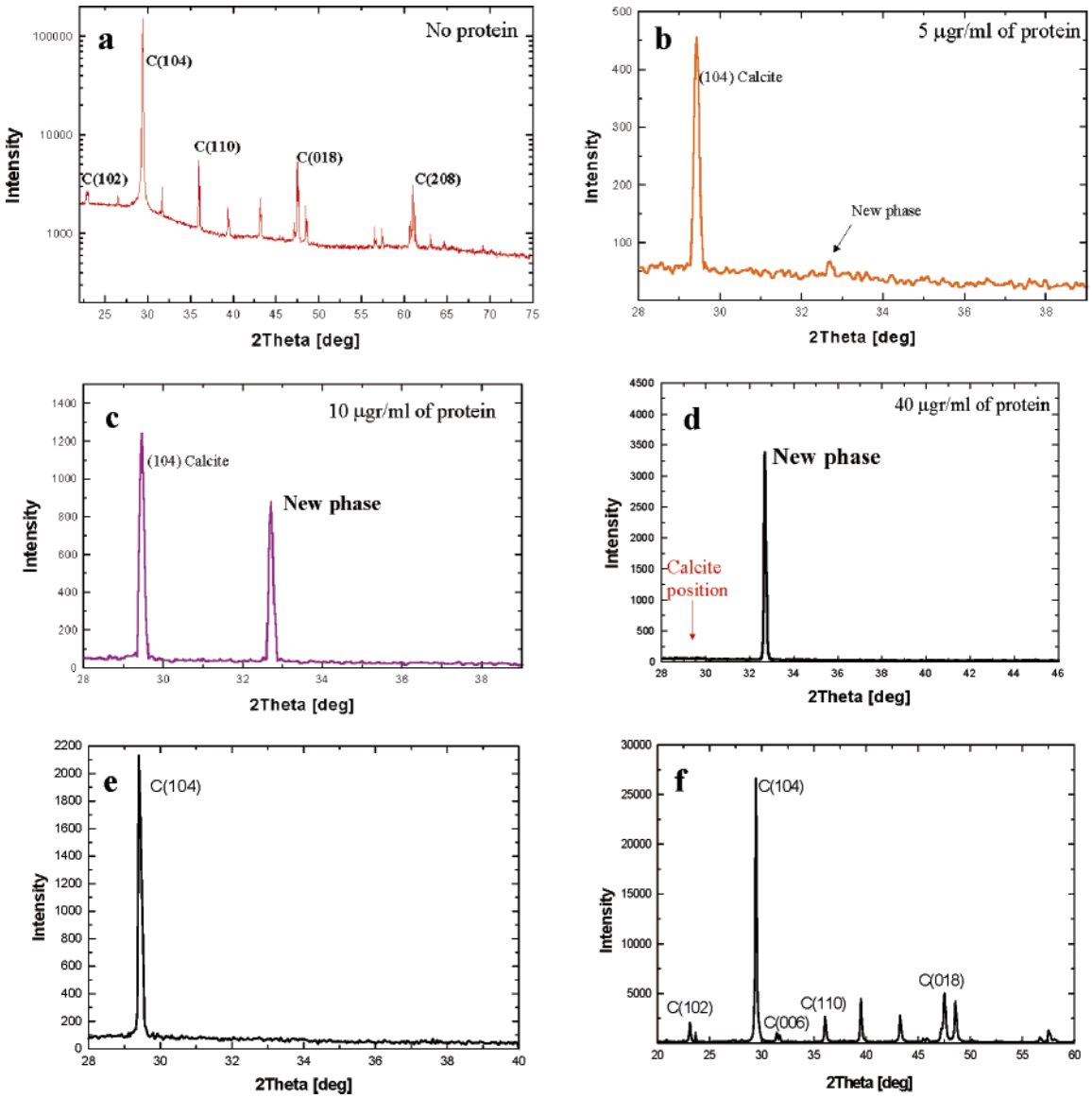

Figure 4. XRD patterns taken from the crystals grown in the absence or presence of ACLS40. (a) In the absence of ACLS40, the only phase detected is calcite, the thermodynamically stable polymorph of $CaCO_3$. Several high-intensity reflections of calcite are marked. (b−d). XRD patterns produced by crystals grown in the presence of 5, 10, and 40 μg/mL of the extracted protein, respectively. The dominant (104)-calcite peak decreases in intensity with a concomitant increase in a peak from new phase, which appears at $2\theta = 32.7°$. (e) XRD pattern produced by crystals grown in the presence of 40 μg/mL serum albumin. The only peak observed is that of (104)-calcite. (f) XRD pattern produced by crystals grown in the presence of the filtrate obtained during the protein concentration process. The only phase present is calcite (selected calcite reflections are marked).

diffractometer (Nonius KappaCCD), equipped with a Mo sealed tube ($\lambda = 0.7071$ Å) and a CCD camera.

Scanning electron microscopy (SEM) micrographs of the shell microstructure (cross-sections) and grown crystals (plane views) were taken by means of a Leo Gemini 982 microscope.

Micro-Raman measurements were carried out in a wet environment by a Dilor XY system with $\lambda = 632.8$ nm, 2 mW unpolarized laser light focused to a spot of 1 μm in diameter.

Light microscopy images of grown crystals were taken by a Zeiss Axiophot microscope equipped with a polarizing stage.

Results and Discussion

Isolation of a 40 kDa Protein from the Shell Matrix. We extracted the soluble proteins from fresh (mollusk-contained) *Strombus decorus persicus* gastropod seashells as described in the Materials and Methods section. The protein mixture (see Figure 2, lanes 1 and 2) was further fractionated by anion exchange HPLC, resulting in the separation of a number of protein-containing fractions (see their elution signals in Figure 3). In this research, we focus on the prominent 40 kDa protein (see Figure 2, lane 3) that eluted at ~0.7 M NaCl. This protein's purity was verified by SDS−PAGE (Figure 2, lane 3). We named this protein ACLS40, since it was extracted from the aragonite crossed-lamelar structure.

ACLS40 Stabilizes the Vaterite Polymorph in Vitro. To ascertain the possible effect of the isolated 40 kDa protein on the formation of the shell, the $CaCO_3$ crystallization experiments were performed. $CaCO_3$ was brought to a state of supersaturation within a crystallization growth well with or without the addition of the isolated protein. The crystals obtained were then analyzed by XRD. When no protein was added during crystallization, the XRD pattern clearly showed the presence of only calcite crystallites, which have different orientations (Figure 4a). When the purified shell extracted protein was added at increasing

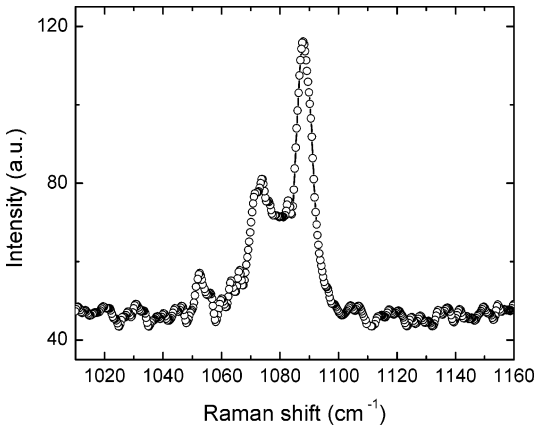

Figure 5. Micro-Raman spectrum in the vicinity of the carbonate peak, showing doublet with components at 1088 and 1075 cm^{-1}.

concentrations to the crystallization solution, remarkable changes in the XRD patterns of $CaCO_3$ were detected.

Figure 4b presents the XRD pattern taken from $CaCO_3$ crystals grown in the presence of 5 μg protein/mL in the crystallization solution. It is clear that the majority of crystallites are still in the form of calcite, since the (104)-calcite peak is dominant in the XRD pattern; however, all crystallites are similarly oriented, since the calcite peaks other than (104) are absent. However, in addition, a small peak is clearly visible at $2\theta = 32.7°$, which does not belong to calcite and indicates the formation of a new phase. When crystallization was performed in the presence of 10 μg protein/mL, the diffraction peak of this phase became higher with a concomitant decrease in the (104)-calcite peak (Figure 4c). Following addition of 40 μg protein/mL to the crystallization solution, the (104)-calcite peak completely disappeared, and only the peak of the new phase was observed (Figure 4d).

Control experiments were also performed to ensure that the effect identified here is indeed caused by the addition of the 40 kDa protein: (i) Crystal growth was performed in the presence of bovine serum albumin at concentration of 40 μg/mL (see Figure 4e). (ii) Crystal growth was performed in the presence of the filtrate obtained during the protein concentration process (and thus contained all components of a molecular weight smaller than approximately 20 kDa) (see Figure 4f). These control growths revealed only peaks of calcite in the XRD spectrum. On this basis, we concluded that the formation of a new $CaCO_3$ phase together with complete suppression of the calcite growth was due to the presence of the isolated protein.

To shed additional light on the polymorph growth in the presence of proteins, we performed a number of measurements by means of different experimental techniques. For example, Figure 5 shows a micro-Raman spectrum that exhibits the splitting of the peak from carbonate group (doublet at 1088 and 1075 cm^{-1}), which is characteristic for the vaterite polymorph.[30]

Examination of grown crystallites by light microscopy revealed spherical objects encapsulated in a noncrystalline phase (Figure 6). The central section of the grown objects is certainly crystalline, since it produces birefringence in polarized light. The rim regions are most probably amorphous and correspondingly do not reveal any birefringence effect. Since it is known that vaterite precipitates have spherical shapes,[31,32] the crystals obtained here are most probably composed of the vaterite polymorph of $CaCO_3$. The noncrystalline phase might be the remains of the amorphous calcium carbonate.

SEM images taken from individual crystallites show flower-like microstructure (see Figure 7a) composed of the radial fragments issuing from the center of the crystallite. Under higher magnification, the hexagonal arrangement of facets is clearly seen (Figure 7b).

Unequivocal determination of the polymorph type was accomplished by measuring X-ray diffraction patterns from an individual crystallite with the aid of single crystal diffractometer. To collect the maximum intensity and to imitate to some extent powder diffraction conditions, the investigated crystal was rotated around the sample rotation axis. The obtained Debye rings (see Figure 8) totally coincided with those expected in the case of vaterite, the third polymorph of $CaCO_3$ in decreasing thermodynamic stability sequence. Complete analysis of X-ray diffraction data shows that the peak from new phase visible in XRD patterns in Figure 4 b,c,d is the (114) vaterite reflection. The presence of the single XRD peak implies that this phase is grown in the form of oriented crystals, which is also supported by SEM images (see Figure 7).

We stress that in the presence of a high concentration of ACLS40 there is complete suppression of calcite growth. It has been reported that soluble proteins from mollusk shells when in solution inhibit the crystal growth initiation; however, when adsorbed onto a suitable template, they nucleate a specific polymorph of $CaCO_3$ (aragonite or calcite, depending on the shell layer from which they were extracted).[33] However, the question still remains: Why does vaterite nucleate but aragonite does not in our experiments, despite the fact that aragonite is thermodynamically more stable than vaterite?

Studies of crystallization of $CaCO_3$ in the presence of macromolecules extracted from the aragonite layers of some mollusks have shown aragonite formation in vitro only if macromolecules were preadsorbed on β-chitin and/or silk fibroin.[7] In the absence of silk fibroin, the macromolecules extracted from the aragonitic layers were able to induce the growth of either a vaterite/calcite mixture or calcite only.[7] Falini

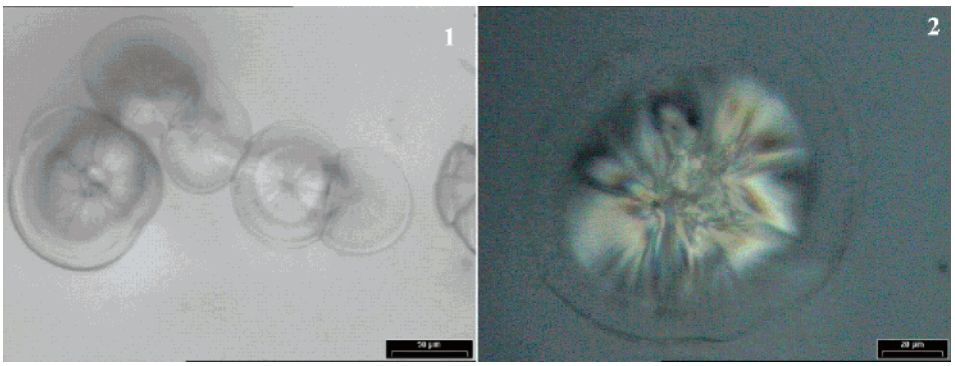

Figure 6. Transmission light microscopy images of vaterite crystallites grown in the presence of ACLS40: (1) nonpolarized light, scale bar − 50 μm; (2) polarized light, scale bar − 20 μm.

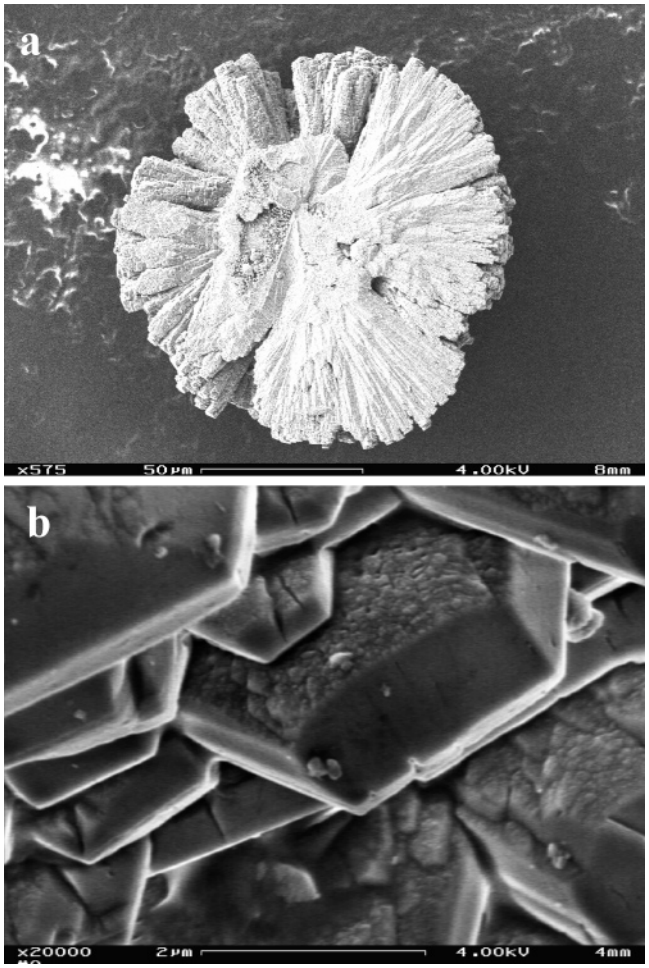

Figure 7. Typical SEM images (plane views) taken from crystallites grown in the presence of 40 μg/mL of ACLS40 protein in crystallization solution: (a) low magnification × 575; (b) higher magnification × 20 000.

et al.[7] proposed that the formation of vaterite occurs because of adherence of the system to the Ostwald−Lussac law of stages, which predicts that the initial phase formed from a solution supersaturated with respect to more than one mineral phase is the one with the highest solubility, which in the case of $CaCO_3$ is vaterite (following from either amorphous $CaCO_3$ or its hydrate).

One might expect that if the appearance of the vaterite polymorph was determined solely by the state of supersaturation, then one could obtain vaterite in the absence of macromolecules or when calcite-inducing macromolecules are present but in the absence of the silk fibroin.[7] However, since in our experiments vaterite is obtained only in the presence of the ACL40, it is feasible that the precipitation of vaterite in the aragonite-inducing protein solution does not occur as a result of supersaturation, but rather is due to the direct interaction between the supersaturated $CaCO_3$ and the protein. This proposal is further supported by the fact that there is a strong correlation between the protein concentration and an increasing ratio between the amounts of precipitated vaterite and calcite.

It is quite likely that in the calcium carbonate crystallization systems the first precipitated phase is amorphous $CaCO_3$, which is transformed to calcium carbonate monohydrate and then subsequently to vaterite, according to the Ostwald−Lussac law of stages. It has been previously shown that calcium carbonate hydrate transforms to vaterite.[34] In early development larval stages of mollusks, the first phase precipitated is the amorphous $CaCO_3$.[24] Additionally, it has been shown that some biological systems have the ability to stop the process of phase sequencing at a particular phase that would otherwise transform into a more stable crystalline polymorph.[33] The mechanism of phase sequence inhibition may be due to proteins such as ACLS40.

It is interesting to note that, in the work of Falini et al.,[7] the proteins extracted from the aragonitic shell of the gastropod *Strombus tricornis* (belonging to the same family as *Strombus*

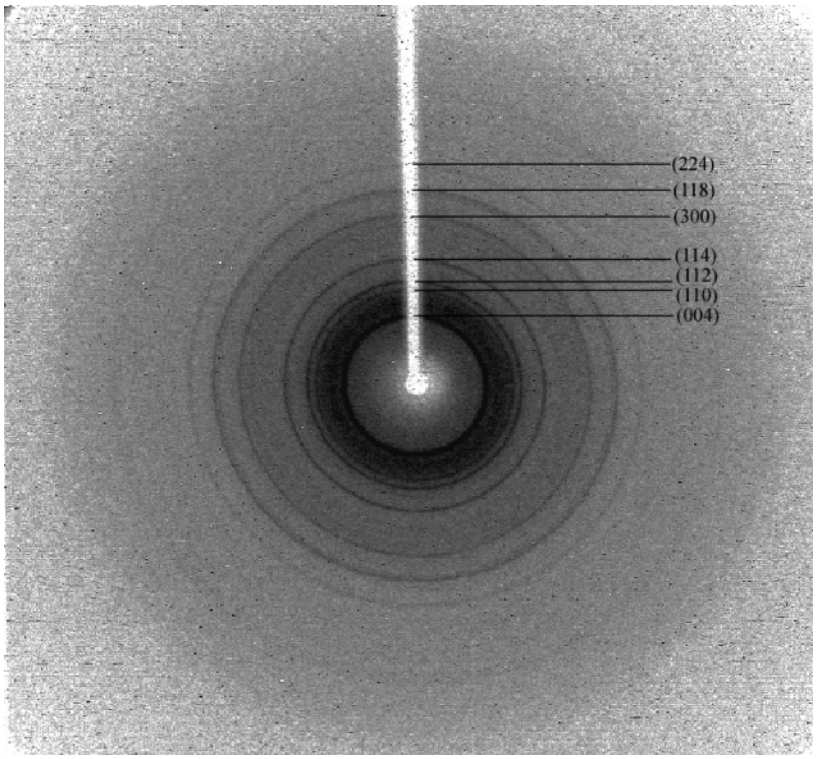

Figure 8. Debye rings taken from one of the spherical-shaped crystallites visible in Figure 6. The marked reflections, according to the JCPDS file #33-0268, are from the vaterite polymorph.

Table 1. Amino Acid Analysis of the ACLS40 Protein

amino acid	weight fraction (%)
AspX	12.25
Ser	5.94
GlX	13.46
Gly	0
His	2.1
Arg	5.85
Thr	6.51
Ala	11.97
Pro	7.78
Thy	1.80
Val	7.2
Met	0.18
Lys	5.7
Ile	4.76
Leu	8.39
Phe	3.18

decorus persicus) induced vaterite growth even when both β-chitin and silk fibroin were present.

Recently, it was found that amorphous calcium carbonate is a precursor phase for aragonite formation in two other larval mollusk shells.[24] It was observed that the first phase precipitated was amorphous calcium carbonate, and after a period of three days, this phase transformed to aragonite. It was also shown that some proteins extracted from the nacre can first induce the in vitro appearance of an amorphous phase prior to the appearance of aragonite.[28]

N-Terminal Sequencing of ACLS40 and Amino Acid Analysis. Very few gene sequences from mollusk species have been determined. To obtain further information on the possible role of ACLS40 in shell growth, we performed N-terminal sequencing. Unfortunately, the N-terminal appeared to be blocked for Edman degradation. Nevertheless, two internal sequences were obtained by partial tryptic cleavage of the ACLS40. Application of the Edman degradation method to one of the internal fragments resulted in the sequence T(D)-VVTDQEGLTSVDVFH. We also derived a second internal sequence, WGVGGAEEG, by de novo MS/MS sequencing. Both of these sequences were used in an extensive search of all known protein sequences in the various gene banks, but no match could be identified. This indicates that the ACLS40 is a novel protein that may only exist in certain mollusk families. Many of the shell proteins already characterized have been found to possess a large number of negatively charged residues,[28] especially aspartic acid residues. The sequences presented here did not reveal this structural signature; however, it is possible that other parts of the protein do include larger amounts of aspartic acid and glutamic acid residues. We thus performed total amino acid analysis to ascertain the apparent acidic content of the protein. The results of this analysis are presented in Table 1. We see that about 25% of the residues are Asx or Glx. Since the average content of a large subset of proteins contains about 20% of these four residues,[35] we contend that the protein is far less acidic than proteins isolated from calcitic shells.

Summary

In this work, we have extracted, purified, and characterized a novel 40 kD protein, ACLS40, from the soluble matrix of the calcium carbonate gastropod shell of *Strombus decorus persicus*. An amino acid analysis of the isolated ACLS40 indicates that it does not belong to the family of very acidic proteins (i.e., rich in aspartic residues), which agrees with the previous findings concerning the aragonite-associated proteins.

From the in situ crystallization experiments, we found that this protein has the ability to stabilize calcium carbonate in the form of the thermodynamically unstable vaterite polymorph, and thus may play an important role in the feasible process by which amorphous calcium carbonate finally transforms (via vaterite) into the aragonite crystalline blocks found in the mature shell.

Acknowledgment. The authors thank Dr. Ingrid M. Weiss for helpful discussions, Dr. Meira Frank for her technical assistance, and Dr. Moshe Kapon for the single-crystal X-ray diffraction. We thank the Chemistry Research Support unit of the Faculty of Chemistry, Weizmann Institute of Science for the amino acid analysis. This work was supported by the Technion V.P.R. Fund (grant #2004116) and also partially supported by the Israel Science Foundation founded by the Israel Academy of Science and Humanities (grant #15/03-12.6)

References and Notes

(1) Marin, F.; Luquet, G. *C. R. Palevol* **2004**, *3*, 469−492.
(2) Weiner, S.; Addadi, L. *J. Mater. Chem.* **1997**, *7*, 689−702.
(3) Lowenstam, H. A.; Weiner, S. In *On biomineralization*; Oxford University Press: New York, 1989; p 324.
(4) Hare, P. E.; Abelson, P. H. *Yearb. Carnegie Inst. Wash.* **1965**, *65*, 223−234.
(5) Smith, B. L.; Schaffer, T. E.; Viani, M.; Thompson, J. B.; Frederick, N. A.; Kind, J.; Belcher, A.; Stucky, G. D.; Mors, D. E.; Hansma, P. K. *Nature (London)* **1999**, *399*, 761−763.
(6) Belcher, A. M.; Wu, X. H.; Christensen, R. J.; Hansma, P. K.; Stucky, G. D.; Morse, D. E. *Nature (London)* **1996**, *381*, 56−58.
(7) Falini, G.; Albeck, S.; Weiner, S.; Addadi, L. *Science* **1996**, *271*, 67−69.
(8) Aizenberg, J.; Hanson, J.; Ilan, M.; Leiserowitz, L.; Koetzle, T. F.; Addadi, L.; Weiner, S. *FASEB J.* **1995**, *9*, 262−268.
(9) Albeck, S.; Aizenberg, J.; Addadi, L.; Weiner, S. *J. Am. Chem. Soc.* **1993**, *115*, 11691−11697.
(10) Michenfelder, M.; Fu, G.; Lawrence, C.; Weaver, J. C.; Wustman, B. A.; Taranto, L.; Evans, J. S.; Morse, D. E. *Biopolymers* **2004**, *73*, 291.
(11) Wilbur, K. M.; Simkiss, K. Calcified shells. In *Comprehensive Biochemistry*; Elmer, H. S., Florkin, M., Eds.; Elsevier Publishing Co.: Amsterdam, 1962; p 80.
(12) Levi-Kalisman, Y.; Falini, G.; Addadi, L.; Weiner, S. *J. Struct. Biol.* **2001**, *135*, 8−17.
(13) Berman, A.; Addadi, L.; Weiner, S. *Nature (London)* **1988**, *331*, 546−548.
(14) Pokroy, B.; Quintana, J. P.; Caspi, E. N.; Berner, A.; Zolotoyabko, E. *Nat. Mater.* **2004**, *3*, 900−902.
(15) Weiner, S. *Calcif. Tissue Int.* **1979**, *29*, 163−167.
(16) (a) Gotliv, B. A.; Kessler, N.; Sumerel, J. L.; Morse, D. E.; Tuross, N.; Addadi, L.; Weiner, S. *ChemBioChem* **2005**, *6*, 304−314. (b) Marin, F.; Amons, R.; Guichard, N.; Stigter, M.; Hecker, A.; Luquet, G.; Layrolle, P.; Alcaraz, G.; Riondet, C.; Westbroek, P. *J. Biol. Chem.* **2005**, *280*, 33895−33908. (c) Tsukamoto, D.; Sarashina, I.; Endo, K. *Biochem. Biophys. Res. Commun.* **2004**, *320*, 1175−1180.
(17) Fu, G.; Valiyaveettil, S.; Wopenka, B.; Morse, D. E. *Biomacromolecules* **2005**, *6*, 1289−1298.
(18) Crenshaw, M. A. *Biomineralisation* **1972**, *6*, 6−11.
(19) Worms, D.; Weiner, S. *J. Exp. Zool.* **1986**, *237*, 11−20.
(20) Jodaikin, A.; Traub, W.; Weiner, S. *Arch. Oral Biol.* **1986**, *31*, 685−689.
(21) Shen, X. Y.; Belcher, A. M.; Hansma, P. K.; Stucky, G. D.; Morse, D. E. *J. Biol. Chem.* **1997**, *272*, 32472−32481.
(22) Sudo, S.; Fujikawa, T.; Nagakura, T.; Ohkubo, T.; Sakaguchi, K.; Tanaka, M.; Nakashima, K.; Takahashi, T. *Nature (London)* **1997**, *387*, 563−564.
(23) Miyamoto, H.; Miyashita, T.; Okushima, M.; Nakano, S.; Morita, T.; Matsushiro, A. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 9657−9660.
(24) Weiss, I. M.; Tuross, N.; Addadi, L.; Weiner, S. *J. Exp. Zool.* **2002**, *293*, 478−491.
(25) Lakshminarayanan, R.; Chi-Jin, E. O.; Loh, X. J.; Kini, R. M.; Valiyaveettil, S. *Biomacromolecules* **2005**, *6*, 1429−1437.

(26) Bowen, C. E.; Tang, H. *Comp. Biochem. Physiol. A* **1996**, *115* (4), 269−275.
(27) Samata, T. *Venus* (*Jpn. J. Malacol.)* **1998**, *42* (2), 127−140.
(28) Gotliv, B. A.; Addadi, L.; Weiner, S. *ChemBioChem* **2003**, *4*, 522−529.
(29) Pokroy, B.; Zolotoyabko, E. *J. Mater. Chem.* **2003**, *13*, 682−688.
(30) Gauldie, R. W.; Sharma, S. K.; Volk, E. *Comp. Biochem. Physiol. A* **1997**, *118*, 753−757.
(31) Meldrum, F. C. *Int. Mater. Rev.* **2003**, *48*, 187−224.
(32) Walsh, D.; Lebeau, B.; Mann, S. *Adv. Mater.* **1999**, *11*, 324−328.
(33) Levi, Y.; Albeck, S.; Brack, A.; Weiner, S.; Addadi, L. *Chem.—Eur. J.* **1998**, *4*, 389−396.
(34) Coleyshaw, E. E.; Crump, G.; Griffith, W. P. *Spectrochim. Acta, Part A* **2003**, *59A*, 2231−2239.
(35) McCaldon, P.; Argos, P. *Proteins: Struct., Funct., Genet.* **1988**, *4*, 99−122.

BM050506F