JOP. J Pancreas (Online) 2003; 4(2):92-103.

An Insight into the Role of Human Pancreatic Lithostathine

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Summary

Human lithostathine was initially isolated from pancreatic stones in patients with alcoholic calcifying chronic pancreatitis. It is secreted into the pancreatic juice where it was believed to inhibit stone formation. The N-terminal undecapeptide was assumed to play an important role in the mechanism, by adsorption to the crystal surface. Later, the role of lithostathine in calcite formation and growth was questioned, together with the associated mechanism of action. In particular, although lithostathine adsorbs on calcite crystal, this property does not now seem to be specific. Moreover, the N-terminal undecapeptide is not likely to have, by itself, the function of the entire protein. The different aspects of this controversy are reviewed and discussed, particularly in the light of recent structural biology. Comparative biological data now available allow us to draw an interesting parallel between lithostathine and other related proteins. Finally, lithostathine might affect stone formation and may also have another function which could be investigated in the other proteins belonging to the same structural family.

Introduction

Human pancreatic lithostathine is encoded by *reg* gene (regenerating gene) [1], is located on the short arm of chromosome 2 in 2p12 [2], spans about 3,000 base pairs and is composed of six exons [3]. It is encoded as a 166-residue preprotein, with a 22 residue N-terminal signal sequence [4]. Lithostathine is a soluble 144 amino acid glycoprotein with three disulfide bridges, existing under eleven isoforms (17-22 kDa) and accounts for 5 to 10% of the secreted proteins [5]. Lithostathine S2-5 corresponds to the four isoforms distinguishable by SDS-PAGE [6] and was believed to inhibit calcite crystal growth [7] and, therefore, stone formation, a property from which it takes its present name [8]. A non-glycosylated and cleaved form (trypsin-like cleavage between Arg 11 and Ile 12), lithostathine S1, is also observable in the pancreatic juice collected in the absence of proteolytic enzyme inhibitors. Lithostathine S1 was independently discovered by Gross *et al*. in human [9] and bovine [10] pancreatic secretions and called pancreatic thread protein because of its ability to form fibrilla at a neutral pH. Historically, lithostathine C was initially isolated as the major proteic component of the pancreatic stone in patients with alcoholic calcifying chronic pancreatitis and was consequently called pancreatic stone protein [11]. It shares the same polypeptidic chain with lithostathine S1. *In vitro* trypsin hydrolysis products of lithostathine S2-5 are called lithostathine H1 (133 amino acids peptide), and H2 (N-terminal 1-11 undecapeptide), (Figure 1) [12]. Therefore, it appears that lithostathine C, S1, and H1 are different names found in the literature for the same protein. Finally, the Reg protein is also

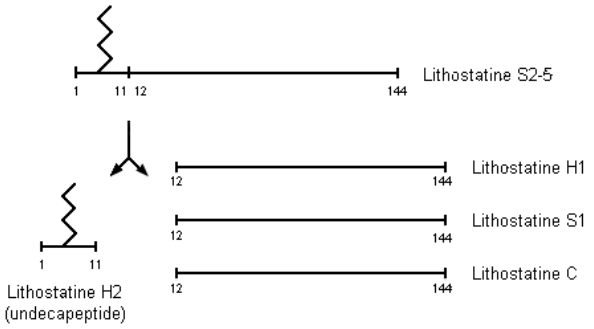

Figure 1. The different forms of human lithostathine. Lithostathine S2-5 is the generic name of the eleven glycosylated isoforms with 144 amino acids of lithostathine secreted by pancreatic acinar cells in the pancreatic juice. The *in vivo* trypsic-like hydrolysis of the Arg11 – Ile12 bond of the protein generates lithostathine S1 with 133 amino acids, the *in vitro* one lithostathine H1 and an N-terminal glycosylated undecapeptide (lithostathine H2). Lithostathine C is the 133 residue-isoform isolated from the pancreatic stones. (the zig-zag line represents the glycosidic chain on the Thr 5) (adapted from [13]).

another name found for lithostathine, as the gene product of the *reg* gene. This gene was discovered to be expressed in regenerating liver or regenerating islets in the pancreas, but not in the equivalent normal tissues [4]. The function of the Reg protein is not fully understood. It could stimulate the regeneration and/or growth of pancreatic beta-cells [13].

The historical circumstances of the discovery of proteins in pancreatic stones have influenced researches on the properties of proteins. Early studies have focused on a role in the pathological modifications observed in the pancreas during alcoholic calcifying chronic pancreatitis. Sarles *et al*. [14] suggested that lithostathine would prevent pancreatic stone formation by inhibiting calcite crystal nucleation and growth in the pancreatic juice.

However, this property is now very controversial and the specificity of lithostathine with respect to this function is now being questioned [15, 16]. Lithostathine could even promote the nucleation of calcite crystals, generating many small crystals that can be easily washed out by the juice flow [17]. The different aspects of this controversy are reviewed. In particular, the presumed functions (inhibition of calcium carbonate precipitation and inhibition of calcite nucleation and growth) and mechanisms of action (calcium binding or adsorption) are discussed in the light of recent data, including structural and comparative biology.

Lithostathine Function

Inhibition of Calcite Growth

Bernard *et al*. [7] proposed that the inhibitory activity was carried out by the N-terminal undecapeptide. However, the concentration at which the N-terminal peptide or its synthetic analogue are active is not clear. These investigators [7] observed an inhibitory effect for natural and synthetic peptide concentrations (1.2-5.9 and 3.0-9.0 μM, respectively), similar to that at which lithostathine S2-5 is also active (0.6-5.9 μM). Bimmler *et al*. [15] did not observe any effect of the synthetic peptide even at a concentration of 243 μM (nucleation test) or 81 μM (crystal growth test). Consistent with this result, Geider *et al*. [17] showed that peptide concentration must reach 500 μM in order to observe modifications of the crystal habits (see below). In our study [16], the peptide was active only at a concentration above 80 μM.

Various interpretations are found for these divergent results. Bimmler *et al*. [15] explained that the difference between their results and those of Bernard *et al*. [7] was due to peptide preparation and purification protocols or to a contamination by acids in the preparation used by Bernard *et al*. Therefore, they question the physiological meaning of this inhibitory property. For Geider *et al*. [17], the size and bulk of the undecapeptide is much smaller than that of lithostathine S2-5. Consequently, the inhibitory effect requires more molecules of undecapeptide, leading to an active concentration about 100 times higher than for lithostathine S2-5. Consistent with this idea, comparison of the dissociation equilibrium constants of lithostathine S2-5/crystal (0.9

μmol/L), N-terminal glycopeptide/crystal (3.0 μmol/L) or synthetic peptide/crystal (4.1 μmol/L) complexes indicates that the C-terminal part of the protein is likely to stabilise the interaction of the undecapeptide with the crystal. Indeed, similar inhibitory properties of calcite nucleation and growth are found for lithostathine C and S2-5. This does not seem compatible with an activity site exclusively carried by the N-terminal undecapeptide (present only in lithostathine S2-5). In agreement with this conclusion, only the C-terminal peptide of rat recombinant lithostathine would present a significant inhibition of calcite nucleation and growth [15].

However, we proposed that the inhibitory property reported for lithostathine was, in fact, due to the presence of a high concentration of Tris buffer (500 mM Tris) [16]. Indeed, in our study, Tris was found to totally inhibit calcite crystal formation from Ca^{2+} ions at a concentration of 1 mM. Therefore, the calcite crystal growth inhibition by lithostathine could be a "side effect" resulting from sample preparation.

This specificity was also further challenged by Bimmler *et al*. [15]. This author found that recombinant rat lithostathine exhibited calcite crystal inhibitor activity. After limited proteolysis, only the C-terminal peptide still displayed this activity. However, under the same conditions, other pancreatic (bovine trypsinogen) and extrapancreatic (human serum albumin, soybean trypsin inhibitor) proteins presented inhibitory activities of calcite crystal growth comparable to that of lithostathine. Furthermore, we have shown that NaCl, phosphate and, to a certain extent, trypsinogen and chymotrypsinogen inhibit calcite crystal growth [16]. Addadi and Weiner [18] also reported an unspecific inhibition of calcite crystal growth by various proteins at concentrations higher than 0.5 μg/mL, as found in lithostathine.

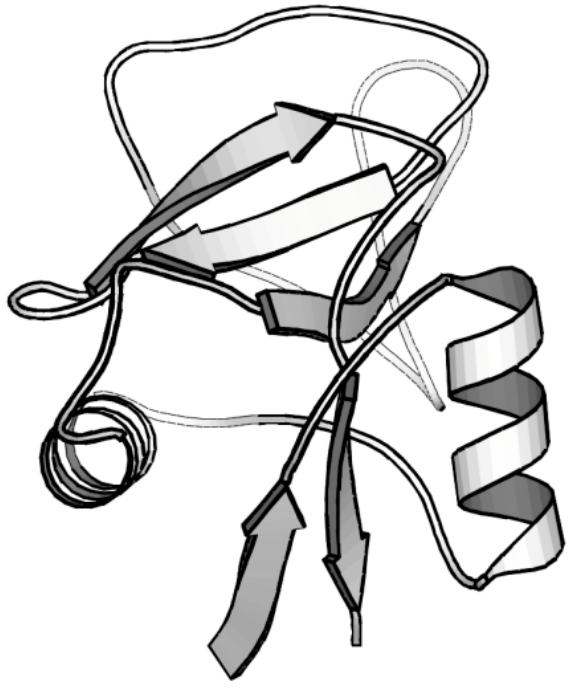

Figure 2: Crystallographic structure of lithostathine at 1.55 Å resolution.

Inhibition of Calcite Precipitation and Nucleation

Lithostathine C extracted from human pancreatic stones [19] and lithostathine S2-5 purified from pancreatic juice [7] would both increase the induction period and decrease the precipitation rate of calcium carbonate.

However, only a decrease in the precipitation rate was observed for the protein extracted with disodium EDTA, from the human pancreatic stones of patients with alcoholic chronic pancreatitis and purified by HPLC [20]. An increase in the induction period was not found, although this could be due to a different purification protocol. Furthermore, we reported that lithostathine does not present any inhibitory effect in calcite nucleation [16]. The activity found by Bernard *et al*. [7] could be due to the presence of a Tris/glycine buffer. Low Tris/glycine concentrations indeed inhibit calcite nucleation (and crystal growth), in accordance with the inhibition curves initially attributed to lithostathine. Even more strikingly, Geider *et al*. [17] observed that, on the contrary, lithostathine S2-5 promotes calcite crystals nucleation. The protein would induce the nucleation of many seeds, leading to many non-pathological crystals of small sizes. This could account for the presence of microcrystals that can be

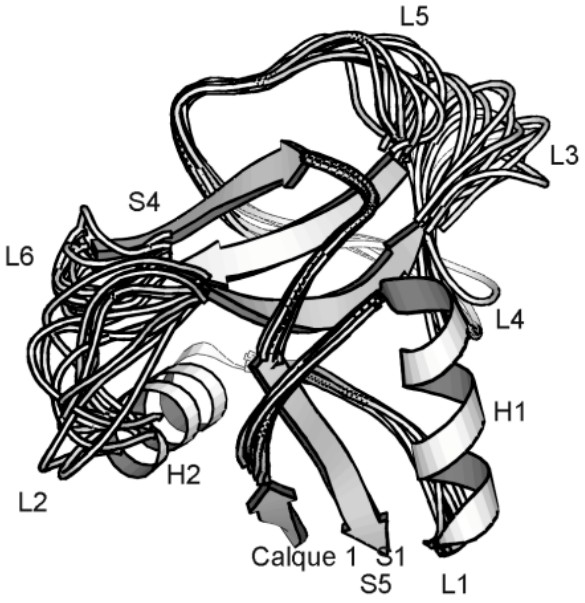

Figure 3: Modelled lithostathine structure. Backbone superposition with ribbon representation of 10 calculated structures of lithostathine.

easily washed out in the pancreatic juice of healthy subjects.
Therefore, the biological role initially attributed to lithostathine in the inhibition of calcite crystal growth is much debated. We will discuss the mechanisms of action that were proposed to rationalize this function. The structure of lithostathine will be presented, since it will be of interest for the discussion.

Structure of Lithostathine

The crystallographic structure of lithostathine was first determined at 1.55 Å resolution (Figure 2) [21]. Subsequently, we built a three dimensional structural model of lithostathine, using an original reconstruction method developed by our group. This model was validated by the analysis of the secondary structure elements deduced from the NMR spectra (Figure 3), and is very similar to the crystal structure [22].
The structure of lithostathine consists of two non-interacting domains: a globular C-terminal domain (residues 14-144) and a flexible N-terminal one (residues 1-13). The C-terminal domain contains two major alpha helices, one helice turn, six beta strands arranged in two triple-stranded antiparallel beta sheets and many loops. This domain adopts the overall fold described for C-type lectins. It is separated from the N-terminal domain by a C14-C25 disulfide bridge. The N-terminal domain is a 13-residue peptide chain that stretches out of the C-terminal domain. Three residues (9-11) are involved in a helix turn motif.
This structure allows us to discuss the function and mechanism of action of lithostathine. Indeed, a number of proteins have a similar C-type lectin-like domain, although they share a sequence identity with lithostathine which can be less than 10%, and can present very diverse established or assumed functions. These proteins are of particular interest for the discussion of lithostathine function as discussed later on.

Lithostathine: Mechanism of Action

The Calcium Binding Hypothesis

Equilibrium dialysis experiments [23], performed in the presence of radioactive ^{45}Ca, indicated that lithostathine C has four equivalent and independent calcium binding sites (Kd in the mM range). Lohse and Kraemer [23] suggested that calcium binding is likely to modify the physico-chemical characteristics of the protein, leading to the formation of protein plugs preceding calcification, which could explain the presence of the protein in all the layers of pancreatic stones. However, a Kd in the mM range does not correspond to a specific binding. Similarly, the absence of calcium observed by Multigner *et al*. [24] and by Pitchumoni *et al*. [25] in the proteic core of some stones is not in agreement with the calcium binding assumption. Moreover, Multigner *et al*. [19] have indicated that $CaCO_3$ crystal growth inhibition by lithostathine C could not be explained solely by the calcium binding to the protein. Indeed, at a lithostathine C concentration that totally inhibits nucleation (5.6 µg/mL), more than 98% of calcium ions would be free [19, 26].

Mariani *et al*. [27] also found that the polypeptides of the core of radiolucent stones have no affinity for calcium.

However, a renewed interest in the calcium binding to lithostathine arose from the sequence homology found with animal C-type lectins (lectins whose activity depend on calcium) [28, 29] and from the ability of lithostathine H1 and S2-5 to induce a calcium dependent bacterial aggregation [30]. Using quasi-elastic light scattering techniques, Cerini *et al*. [31] suggested a possible lithostathine dimer formation in the presence of $CaCl_2$, as revealed by the modifications of the mean diffusion coefficient and of the mean hydrodynamic radius. In this context, it is interesting to compare the 3D structure of lithostathine with that of various calcium binding or non-binding proteins belonging to the same structural family.

Besides lithostathine, nine structures are available today in the C-type lectin family. Seven of these proteins have a calcium-dependent activity: rat serum mannose-binding protein A (MBPA) [32], carbohydrate recognition domain (CRD) and epidermal growth factor-like (EGF) domain of human E-selectin [33], human tetranectin [34], CRD domain of the H1 subunit of the asialoglycoprotein [35], rat lung surfactant protein A [36], human lung surfactant protein D [37] and TC14, a tunicate C-type lectin from *Polyandrocarpa misakiensis* [38]. Two proteins (Factor IX and Factor IX/X binding proteins from *Trimeresurus flavoridis*) have no lectin activity but present a calcium-dependent binding function to blood coagulation factor IX or X [39, 40, 41]. Finally, the type II antifreeze protein of the sea raven has no lectin activity. Furthermore, its ability to protect the fish of polar oceans from freezing in icy water does not depend on calcium [42]. These structures present similar overall topologies and three types of calcium binding sites have been identified in calcium-binding CRDs, involving Glu, Gln, Asn, or Asp residues. Site 1 is composed of 4 amino acids. It is found in MBPA, tetranectin, lung surfactant proteins and the CRD domain of the H1 subunit of the asialoglycoprotein, although the local geometry of this site differs within these protein structures. Site 2 is composed of 5 amino acids and is involved both in calcium and sugar binding. All CRDs with a demonstrated lectin function have this site in common. It is present for example in MBPA, E-selectin, tetranectin and the CRD domain of the H1 subunit of the asialoglycoprotein. It is absent in both subunits of factor IX and factor IX/X binding proteins and in the sea raven antifreeze protein which do not present a lectin function. Similarly to site 1, site 3 is involved only in calcium binding. It is composed of two residues (Ser and Glu) and also involves water molecules. It is found in both subunits of factor IX and factor IX/X binding proteins, and in the CRD domain of the H1 subunit of the asialoglycoprotein.

Figure 4 shows that none of these sites are present in lithostathine: only two residues of site 1 are conserved, one of site 2 and none of site 3. Consequently, lithostathine is not likely to present a lectin function (otherwise, site 2 would be conserved) or a calcium-dependent function. These conclusions are

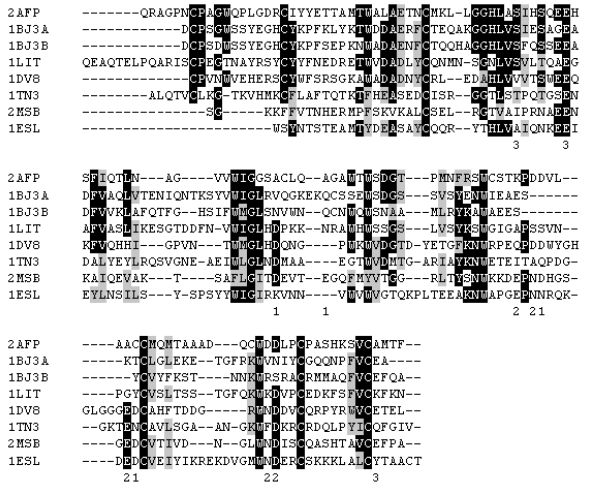

Figure 4: Structural alignment of antifreeze protein from sea raven - 2AFP, Protein Data Bank (PDB) database: http://www.rcsb.org/pdb/cgi/explore.cgi?pid=143951044353134&pdbId=2AFP [56] -, subunits A and B of factor IX/X binding protein (1BJ3A and B), human lithostathine (1LIT), CRD domain of H1 subunit of asialoglycoprotein (1DV8), tetranectin (1TN3), mannose-binding protein A (2MSB), human E-selectin (1ESL). Numbers indicate position of residues involved in each type of Ca^{2+} binding.

consistent with the absence of sugar binding found for lithostathine [30], and with its low and probably unspecific affinity for Ca^{2+} ions, in the mM range [23]. They are also consistent with the observation that all three sites are absent in the antifreeze protein from sea raven, which is not calcium dependent.
Furthermore, the reported ability of the 133-amino acid isoform of lithostathine to induce a calcium dependent bacterial aggregation appears intriguing [30] and this function has, by the way, never been confirmed. In particular, bacterial aggregation was observed between pH 5.5 and 8.5, although this isoform is, in fact, insoluble above pH 5 [43].
In conclusion, analysis of the structure of lithostathine does not allow us to identify calcium binding sites which could account for its inhibition of calcite nucleation, precipitation and crystal growth.

The Adsorption Hypothesis

The adsorption of lithostathine on calcite has been proposed as a mechanism for the inhibition of stone formation. Multigner *et al*. [19] proposed a mechanism in which the protein could have a greater affinity for the crystal than for free calcium ions. Similarly, De Caro *et al*. [11] concluded that there was a greater affinity of lithostathine C for the crystals than for free calcium ions. The adsorption of lithostathine S2-5 on a pre-formed calcite crystal surface (absence of growth) was also demonstrated by an ELISA-type technique [7].
Sarles *et al*. proposed a mechanism based on the adsorption of the N-terminal undecapeptide to the crystal surface. The decrease of the level in the pancreatic juice of patients, and the protein precipitation after cleavage of the N-terminal glycopeptide, would be responsible for the appearance of calcified stones characteristic of calcifying chronic pancreatitis.
Therefore, the adsorption of lithostathine and of the N-terminal peptide has been the subject of many studies.
Using immunofluorescent techniques, Geider *et al*. [17] found that lithostathine does indeed adsorb specifically on the crystal in relation to the c axis edges of the growing $\{10\bar{1}4\}$ faces. They studied the modifications of the habit of seeds of calcite rhombohedra, in the presence of lithostathine. The adsorption of the protein generates six rough and striated $\{11\bar{2}0\}$ faces. Their growth, at the expense of the $\{10\bar{1}4\}$ faces, explains the successive transitions observed from the rhombohedral to the cubic habit and then to the olive-shaped habit of the crystals when lithostathine concentration increases. The N-terminal undecapeptide causes similar habit modifications, but at a higher concentration (500 μM). However, it is important to stress that these experiments were performed under calcite growth conditions. Therefore, this study does not demonstrate that adsorption of lithostathine is a mechanism for the inhibition of crystal growth. It can only conclude that there is a modification of the crystal shape in the presence of lithostathine.
Furthermore we found that the lithostathine affinity for calcite, expressed as the half-life of bound iodinated protein in the presence of an unlabelled competitor, is lower than that of albumin. Moreover, the quantities of adsorbed lithostathine and albumin per unit of surface are in the same range [16]. Finally, the adsorption of lithostathine on calcite is not much higher than for an amorphous phase (glass). These observations are not in favour of the adsorption of lithostathine on calcite as a specific interaction.

Modelisation of Lithostathine/Crystal Interaction

Gerbaud *et al*. [21] used molecular dynamics simulations to study the interaction between calcite and the N-terminal peptide. Their studies lead to an "unfolding-binding" model of the mechanism for adsorption on crystal. In addition to the interaction of the N-terminal peptide, the overall distribution of charged residues in the structure of the C-terminal domain confers a dipolar moment [44], which could initially play a role in protein orientation with respect to the crystal surface. Another mechanism could, however, be

invoked in the protein approach; the crystallographic structure indeed revealed that, in the C-terminal domain, acidic residues are arranged on the same side of the molecule, in two stretches separated by approximately 6 Å. This periodicity, compatible with that of the calcium ions on several calcite crystal planes, could enable electrostatic interactions between the C-terminal domain and the crystal, through a "lattice matching" model.
This model could explain the adsorption of lithostathine on calcite, but does not prove, by itself, either the inhibition function of calcite growth or the specificity of lithostathine adsorption. Lithostathine adsorption on calcite appears to be an unspecific phenomenon not necessarily linked to its biological function.
None of the above studies were able to clearly identify a function or a mechanism of action for lithostathine. It is interesting to use comparative biology data in order to get other insights into the function of lithostathine.

Elements of Comparative Biology

We will first present proteins known or suspected to be involved in crystallisation processes. Then we will compare them with structurally related proteins which do not fit the theory and appear to be even more relevant today.

Baculovirus Expressed Recombinant Rat Lithostathine [45, 46]

The baculovirus expressed rat lithostathine also presents an inhibitory effect on $CaCO_3$ spontaneous precipitation and crystal growth, at concentrations in the µM range. The studies were carried out on each of the two fragments (N-terminal undecapeptide and C-terminal polypeptide). They showed that, contrary to human lithostathine, only the C-terminal polypeptide presented an inhibitory activity on calcite nucleation and crystal growth. This activity was nevertheless reduced compared to the full size protein. Both the N-terminal undecapeptide and its synthetic homologue were inactive, even at higher concentrations. However, other proteins, such as bovine trypsinogen, human serum albumin and soybean trypsin inhibitor, presented a comparable effect. Therefore, as in the human form, the authors question the specificity and the physiological meaning of this inhibitory property.

Antifreeze Proteins: Similar Properties with Respect to Other Crystals

Many marine teleost fish from polar oceans and north temperate seas can be protected from freezing in icy sea water by serum antifreeze proteins or glycoproteins. Four distinct types of antifreeze proteins have been identified. These macromolecular antifreezes are all believed to function in the same non-colligative manner, by binding to the surface of ice crystals and preventing their growth. The type II antifreeze proteins (AFPs) from the sea raven, smelt and herring share a high protein sequence identity (25% to 29%) with human lithostathine and are homologous to C-type lectins [47]. The solution structure of a recombinant sea raven Type II AFP has been determined by NMR spectroscopy [42]. A model of the herring type II AFP was also constructed from the crystallographic structures of MBP and E-selectin [48]. Site-directed mutagenesis studies have shown that the ice-binding site of the herring AFP (whose function is calcium dependent) corresponds to the carbohydrate-binding site in C-type lectins [49]. However, mutagenesis studies on sea raven AFP (whose function does not depend on calcium) showed that the epicenter of the ice binding surface of sea raven type II AFP lies near Ser 120 [50]. This site is different from that of the herring, but it could correspond to a calcite-binding site of low specificity, in the C-terminal domain of lithostathine [50].

Proteins Potentially Controlling Mineral Growth

The mechanisms by which proteins control mineral nucleation and growth (bones, teeth,

mollusc shells) may be quite different. They use spatial (localisation, size, shape, orientation, habit) and temporal control of crystal growth. These proteins sometimes also have a structural role inside the edifice of which they control the mineralization.

Among these proteins, ovocleidin, a major protein of the avian eggshell calcified layer recently isolated and sequenced [51], presents 30% of sequence identity with human lithostathine and consists of a single C-type lectin domain. None of the 3 calcium binding sites are conserved in this protein. The function of ovocleidin has not yet been established but it is suspected to play a role in the eggshell matrix formation.

Perlucin, a 17 kDa protein, was isolated from the shell of the mollusc *Haliotis laevigata* [52]. The analysis of its sequence reveals that this protein also belongs to the group of proteins consisting of a single C-type lectin domain. Calcium binding site 2 is fully conserved, together with several residues of sites 1 and 3. Perlucin promotes the precipitation of $CaCO_3$, although the authors emphasize that it may be only one functional aspect of this protein. Interestingly, this observation recalls that of Geider *et al*. [17] for lithostathine (lithostathine would promote formation of small non-pathological $CaCO_3$ crystals), and could also be correlated to the suspected role of ovocleidin. Taken together, these observations do not fit the idea of a role for lithostathine in the inhibition of calcium carbonate precipitation and crystal growth.

Finally, human tetranectin (which contains sites 1 and 2) is a plasminogen-binding protein belonging to the C-type lectin family. It has a potential role as a bone matrix protein expressed during mineralization [53]. It has recently been thought to play a role in tissue growth and remodelling, due to its expression in developing bone and muscle [54].

Pancreatitis-Associated Protein

The pancreatitis-associated protein (PAP), also called hepatocarcinoma intestine pancreas (HIP) protein, synthesised and secreted by the pancreas, shares high sequence similarity with lithostathine (43% identity, 54% similarity). PAP shares the basic features of a lectin-binding domain and it is a formidable candidate for function elucidation. There is no publication demonstrating the effect of PAP on crystal nucleation, growth or habit modification. The function of PAP remains unknown, although both lithostathine and PAP synthesis and secretion are increased in response to pancreatic stress. In the C-type lectin family, only these two proteins (and their homologues in other species) present a trypsin cleavage site (between residues Arg and Ile) leading to proteins which are essentially insoluble, with a tendency to form thread structures. This probably constitutes an important common feature, indicating that these two proteins probably share common functions. It has recently been proposed that the active form of these proteins could be the cleaved insoluble forms, displaying outstanding resistance to protease cleavage. The authors suggested that the dense extracellular fibrillar complexes formed under stress conditions provide a luminal matrix which would help regeneration of the ductal structures.

The observation that lithostathine and PAP levels are elevated in the early stage of Alzheimer's disease, and remain elevated during the course of the disease is again consistent with a functional overlap between the two proteins [55].

Conclusion

This article has reviewed the controversy about one of the functions and mechanisms of action of lithostathine. The results of the functional studies are conflicting, but the most recent studies do not support a specific role for lithostathine in the inhibition of calcite crystal growth [15, 18]. The N-terminal undecapeptide does not seem to bear, on its own, the function of the whole protein. Lithostathine is able to adsorb on calcite crystal, although this adsorption is not specific and is shared by other pancreatic proteins. The modifications of calcite crystal

habit upon lithostathine adsorption were performed under crystal growth conditions, which inherently do not demonstrate that it plays a role in crystal growth inhibition. Taken together, these results do not argue in favour of such a function. The fact that lithostathine may adsorb on calcite and present an inhibition effect on its growth does not exclude another biological function.

This review emphasizes comparative biology studies, including proteins only recently identified. In fact, it is noteworthy that several proteins related to lithostathine are even suspected of playing a role in the control of crystallisation processes. In this context, elucidation of the functions of ovocleidin 17 (30% sequence identity with lithostathine), perlucin and tetranectin would certainly give an insight into that of lithostathine. The recent comparative studies between PAP and lithostathine also appear very promising. The vast number of names (lithostathine, pancreatic stone protein, pancreatic thread protein, Reg protein) reflects the various circumstances in which lithostathine has been identified, and the different functions proposed. These very diverse suggestions show that, despite many studies, the debate on lithostathine function remains wide open. Moreover, the fact that the *reg* gene is expressed in a variety of human tissues, where calcium carbonate is not present, suggests a pleiotrophic function for the gene product [3].

Received October 17th, 2002 - Accepted January 27th, 2003

Keywords Antifreeze Proteins; Asialoglycoproteins; Lectins, C-Type; Pancreatitis; Pancreatitis, Alcoholic; Selectins

Abbreviations AFP: antifreeze protein; CRD: carbohydrate recognition domain; MBP: mannose-binding protein; NMR: nuclear magnetic resonance; PAP: pancreatitis-associated protein; PDB: Protein Data Bank

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