Urol Res (2007) 35:63–71
DOI 10.1007/s00240-007-0087-3

ORIGINAL PAPER

Models for protein binding to calcium oxalate surfaces

Asiya Gul · Peter Rez

Received: 5 September 2006 / Accepted: 27 February 2007 / Published online: 20 March 2007

Abstract It is widely believed that proteins rich in Asp, Glu or Gla (γ carboxyglutamic acid) interact strongly with calcium oxalate surfaces and inhibit calcium oxalate crystal growth. An alternative hypothesis would be that the interaction of Asp, Glu and Gla residues with surfaces could facilitate nucleation and crystal aggregation. Prothrombin fragment 1 and bikunin have been studied extensively as inhibitors, β-microglobulin, transferrin and antitrypsin have been found in stone matrix and tubulin has been observed in the attachment of crystals to cell surfaces. The aim of this study is to examine how well carboxylate groups in proteins found either in stone matrix, or proposed as inhibitors, could fit with the calcium ion sub-lattice of both calcium oxalate monohydrate and dihydrate surfaces. The carboxylate groups in the acidic Asp, Glu and Gla residues were marked in the Protein Data Bank structures and matched to calcium oxalate surfaces using the Cerius 3D molecular modeling program. A contact was defined if a carboxylate oxygen atom approached a surface calcium atom in such a way that the separation was less than 6 Å but greater than 2.4 Å, the sum of the ionic radii. If the proteins maintain their 3D structure, the number of contacts was no more than 3 or 4 for all the proteins studied, irrespective of the calcium oxalate surface.

Keywords Calcium oxalate · Whewellite · Wedellite · Protein · Phospholipid · Modeling

A. Gul · P. Rez (✉)
Department of Physics and Astronomy,
Arizona State University,
Tempe, AZ 85287-1504, USA
e-mail: atppr@exchange.asu.edu

Introduction

Calcium oxalate is the most significant component of urinary stones, usually in its monohydrate form [1]. The mechanisms for the formation of calcium oxalate urinary stones are still not understood, though it is thought that organic macromolecules, in particular proteins, play a significant role. It is widely believed that proteins act as inhibitors of crystal growth and aggregation. Prothrombin fragment 1 (PFT1) [2], nephrocalcin [3], bikunin [4, 5] and osteopontin [6] have all been shown to inhibit growth or aggregation or both in crystallization experiments. More recently the growth of selected faces of calcium oxalate monohydrate has been studied at the atomic level by atomic force microscopy (AFM) and it was shown that osteopontin mainly affected the (010) surfaces [7]. The specific mechanism by which these proteins might interact with calcium oxalate surfaces has never been discussed in detail, but following work in other areas of biomineralization [8] it has been suggested that acidic amino acid residues such as Asp and Glu, that are expected to be deprotonated and negatively charged at urinary pH, are attracted to the positively charged Ca ions. Recent functionalized AFM measurements give experimental support to this hypothesis [9]. It would therefore be expected that proteins rich in γ carboxyglutamic acid, Gla, such as PFT1, with two deprotonated carboxylate groups would be even better at electrostatically binding to calcium sites [10, 11].

It could also be argued that organic macromolecules might instead facilitate heterogeneous nucleation of crystals by providing a suitable template, as appears to be the case in some instances of calcium carbonate biomineralization [8]. They could also help stick small crystallites together, thus facilitating aggregation. Good

candidates for such promoter molecules would be those proteins found in stone matrix, if one believes that matrix components play an active role in stone formation and their presence is not merely the adventitious result of growth in urine rich in proteins. Dussol et al. [12] found human serum albumin, α1 acid glycoprotein, α1-microglobulin, agolipoprotein, transferrin, α1-antitrypsin, retinol-binding protein, renal lithostatine and β2-microglobulin in stone matrix. Urinary PFT1, also known as crystal matrix protein [13], and nephrocalcin [14] have been found in calcium oxalate crystals precipitated from urine, and bikunin [15] has been reported in both calcium oxalate and calcium phosphate crystals. Warpehoski et al. [16]and Lian et al. [10], found from amino acid analysis that stone matrix contained up to 50% Asp, Glu and Gla residues. This seems implausibly large given that the average protein has about 10–15% of these residues, though it fits well with the calcium-binding hypothesis proposed as a way of explaining inhibitor behavior.

Whether a protein or other macromolecule acts as an inhibitor of growth and aggregation or a promoter of nucleation and aggregation implies that there must be some mechanism to explain the interaction with the mineral oxalate surfaces. The attraction between calcium and Asp, Glu and Gla is certainly plausible, but it is equally conceivable that basic residues that are normally protonated at urinary pH and positively charged might experience an attraction toward negatively charged oxalate groups. In either case steric constraints from the 3D conformation of the molecule might limit the number of these simple interactions.

Prothrombin fragment 1 and bikunin, which have been postulated as inhibitors, β-microglobulin, antitrypsin and transferrin which have been found in stone matrix, all have structures available from the Protein Data Bank (PDB) [17]. Tubulin was examined because there is some evidence that it facilitated crystallization on cell surfaces [18], and osteocalcin since it had been found in human urine [19]. Tubulin and transferrin might be products of the stone disease from cellular injury but they may still play a role in facilitating further aggregation. All the structures had been determined by X-ray diffraction of the protein crystallized in water.

To investigate how well these proteins might interact with the most prominent calcium oxalate monohydrate and dihydrate surfaces we used the Cerius 3D molecular visualization software to match proteins with surfaces by maximizing the number of contacts between surface calcium atoms and carboxylate oxygen atoms. This is very similar to the approach Hoang et al. [20] used to demonstrate the affinity of porcine osteocalcin to calcium hydroxyapatite.

Materials and methods

Calcium oxalate dihydrate (COD) usually appears as bipyramids with (101) being the dominant face. Calcium oxalate monohydrate (COM) can show many different morphologies but in single crystal growth experiments the most prominent facets are (100), (001), (10I), (021). The Cerius molecular visualization and modeling program was used to prepare (100), (001), (021), (10I), (010) surfaces for COM using Tazzoli and Domeneghetti's [21] low temperature monoclinic structure, space group $P2_1/c$. For COD (001), (010), (011), (110) faces were prepared from Tazzoli and Domeneghetti's tetragonal structure [21], space group I 4/m. Figures 1 and 2 show representative surfaces for COM and COD respectively. The fundamental repeating unit on a crystal surface, analogous to the unit cell in the 3D crystal, is the unit mesh. It is defined by the two vectors, **a** and **b**, and the angle between them. Unit mesh dimensions, areas and the number of Ca or oxalate ions per unit area are summarized in Table 1.

Even though the exact termination of the surfaces is not known, electrostatic energy considerations would suggest that surfaces are non-polar with equal numbers of positive and negative ions. The exception might be the COM (100) surface which would appear to be positively charged as it seems that there are twice as many Ca ions as oxalate ions within 4 Å of the surface. The unit cell as a whole is neutral so there will be a net surface dipole. In fact there are two distinct groups of Ca atoms near the surface, and it is possible that the small separation between these groups might well increase due to surface relaxation. There is also the possibility of surface reconstruction, though there has been very little work on ionic surfaces in vacuo let alone solution. As a starting point we shall assume that the surfaces are as shown in Figs. 1 and 2, and that they are both non-polar and unreconstructed.

Structures of osteocalcin, PFT1, bikunin, β-microglobulin, antitrypsin, tubulin and transferrin were downloaded from PDB [17]. The carboxylate oxygen atoms in the Asp, Glu and Gla residues were marked as fluorine so that they would show up as a distinct color. The protein molecules were then manually aligned with respect to the calcium oxalate surfaces in such as way as to bring about the maximum number of close contacts between surface calcium atoms and the carboxylate oxygen atoms, defined as approaching within 6 Å, while ensuring that that the calcium oxygen distance was never less than 2.45 Å. This distance, which corresponds to the sum of the ionic radii, is also, not surprisingly, the spacing between oxygen and calcium atoms in both the COM and COD structures.

Ideally the electrostatic forces between the protein and the calcium oxalate surfaces would be calculated from an exact charge distribution, including any possible charge

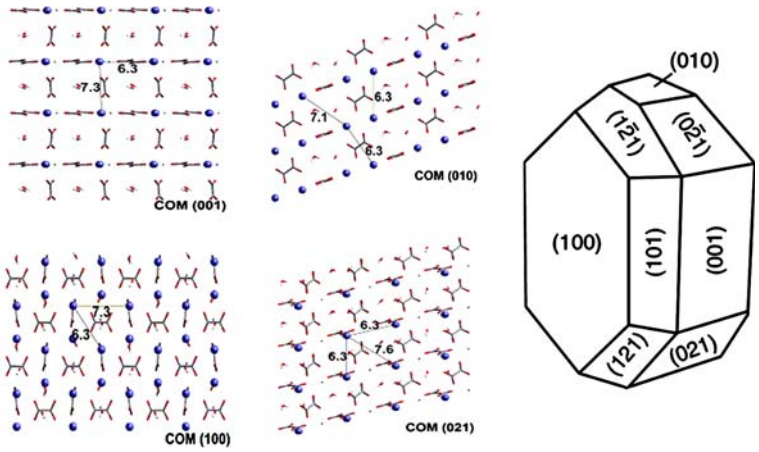

Fig. 1 Calcium oxalate monohydrate surfaces. Oxygen represented by *red sticks*, hydrogen represented by *white sticks*, calcium represented by *blue spheres*

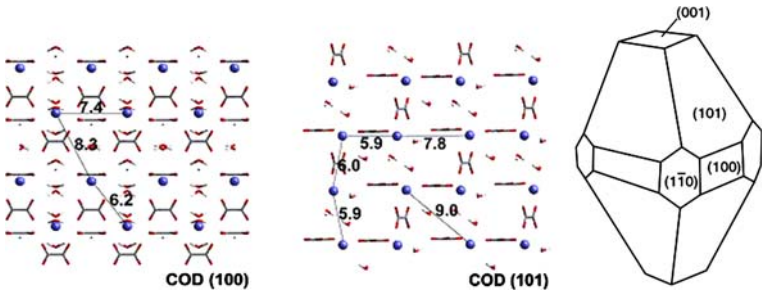

Fig. 2 Calcium oxalate dihydrate surfaces. Color scheme same as in Fig. 1

Table 1 Summary of unit mesh dimensions defined by the repeat lengths *a* and *b* and the angle between them, unit mesh areas and area per Ca or oxalate ion on calcium oxalate surfaces

	a (Å)	*b* (Å)	Angle (deg)	Area (sq Å)	No. of CaOx	Area per Ca or Ox (sq Å)
COM (001)	6.29	7.28	91.5	45.9	1	45.9
COM (010)	6.29	10.30	70	60.9	2	30.5
COM (100)	6.30	7.30	55	37.7	1	37.7
COM (021)	6.26	6.29	105	37.9	1	37.9
COM (101)	9.98	7.29	90	72.8	2	36.4
COD (011)	12.37	14.39	90	178	4	44.5
COD (001)	12.37	12.37	90	153	2	38.3
COD (100)	12.37	7.36	90	91	4	45.5

The unit mesh is the fundamental 2D repeating unit on the surface of a crystal

transfers. This would require a full quantum mechanical treatment of both the crystal and the protein, which is not a practical proposition for systems of this size. Our results are not really dependent on the exact value of the charge, only that electrostatic interactions are the dominant attractive force between the protein and the calcium oxalate surfaces.

The VMD program [22] was also used to visualize the combination of protein and calcium oxalate surface and to prepare the figures.

Results

For all the proteins we considered except transferrin the same residues gave the best contact irrespective of which of the four COM surfaces or four COD surfaces we examined. To illustrate how the proteins make contact we have, for convenience, selected only COM (001), the atomic arrangements being shown as Figs. 3, 4, 5, 6, 7, 8, and 9. A summary of the residues that make the best contact, as defined in the materials and methods section, as well as the distances between the carboxylate oxygen atoms, O1 and O2, and the closest surface Ca atom in the (001) surface are listed in Table 2 for antitrypsin, bikunin, β-microglobulin and tubulin.

For PFT1 the same Gla residues, Gla 33, Gla 26 and Gla 30 made the best contact with the COM and COD surfaces. Since it is conceivable that oxygen atoms from both carboxylate groups could be in close contact with surface Ca ions the distances between all four oxygen atoms on the

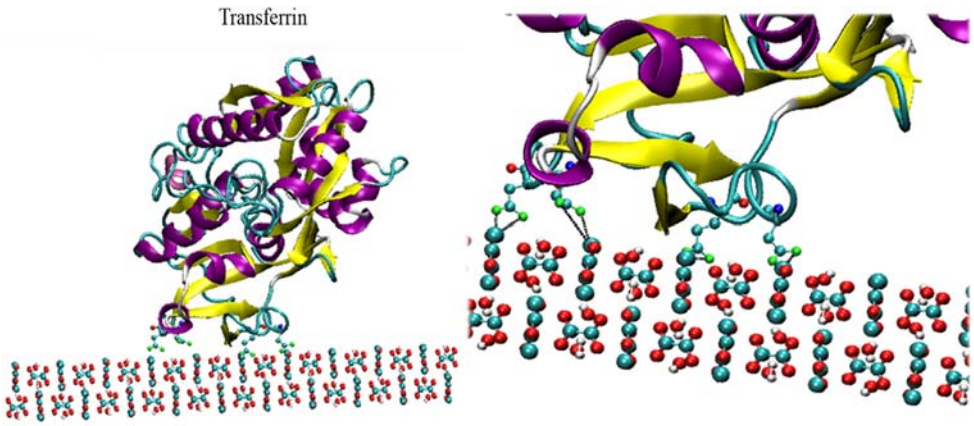

Fig. 3 Transferrin on COM (001). COM oxygen represented by *red spheres*, carboxylate oxygen represented by *green spheres*, COM carbon represented by *small blue spheres*, COM calcium represented by *large blue spheres*, protein nitrogen represented by *purple spheres*. The ''bonds'' between COM calcium (shown as *larger opaque spheres*) and carboxylate oxygen are shown as *dark lines*

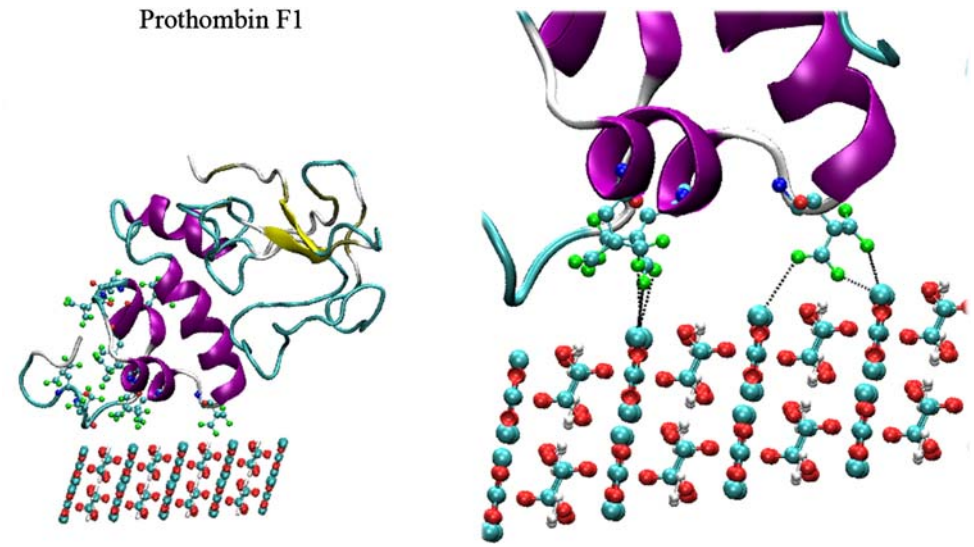

Fig. 4 Prothrombin fragment 1 on COM (001). Color scheme same as in Fig. 3

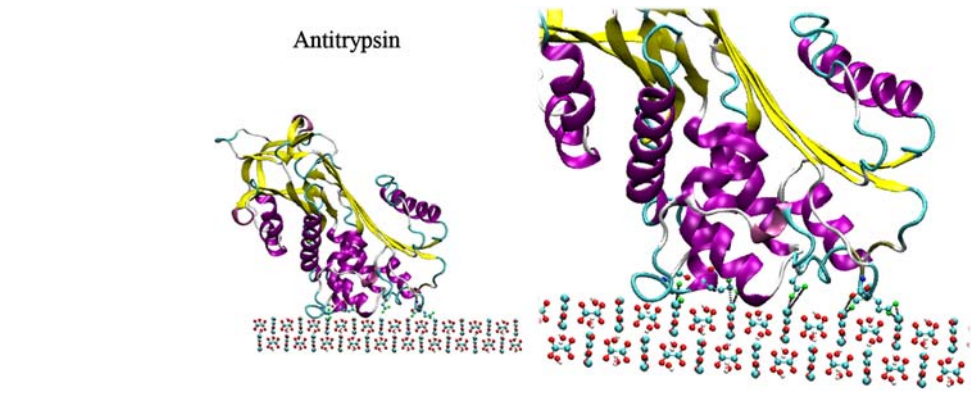

Fig. 5 Antitrypsin on COM (001). Color scheme same as in Fig. 3

carboxylate groups and the nearest Ca ion are listed in Table 3 for the COM (001) surface.

It is interesting to note that in all cases only three or four amino acid residues made good contact, since steric considerations hindered close approach of other Asp, Glu or Gla residues. The only exceptions were antitrypsin, where the Asp 19 residue did not come close to some of the COM surfaces, and transferrin. In all cases for transferrin Asp 240 and Glu 237 were involved in contact with both COM and COD surfaces, while Asp 221 was more important for the COD surfaces except (011) and Asp 229 for the COM surfaces except (001). Glu 224 and Asp 236 also were

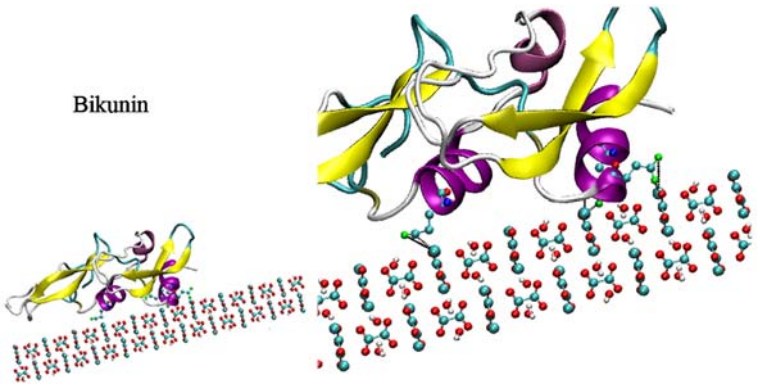

Fig. 6 Bikunin on COM (001). Color scheme same as in Fig. 3

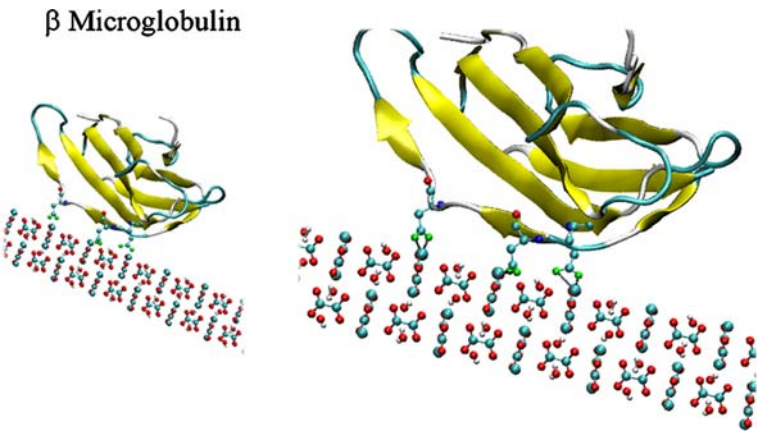

Fig. 7 β-Microglobulin on COM (001). Color scheme same as in Fig. 3

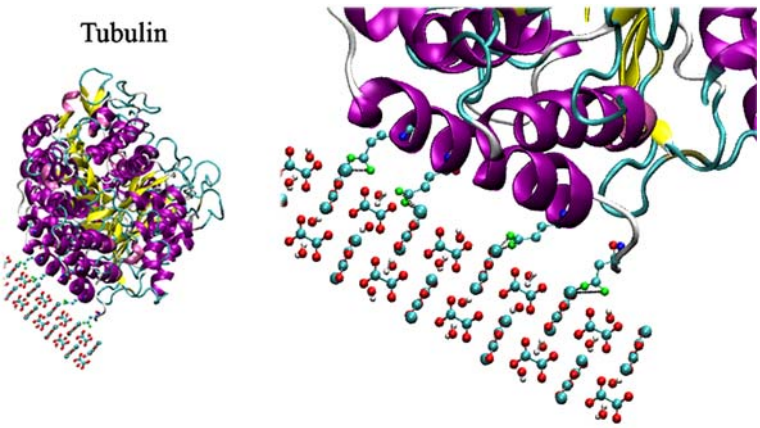

Fig. 8 Tubulin on COM (001). Color scheme same as in Fig. 3

significant for COD (010). The separation of the carboxylate oxygens from the nearest surface Ca ions for transferrin are summarized as Table 4.

We also considered the possible contact between the Lys and Arg amide groups and oxalate ions in the surface in addition to the contacts between Asp and Glu and surface calcium atoms. The results are summarized as Table 5.

A summary comparing the numbers of contacts for all the proteins is given as Table 6.

Discussion

If protein molecules retain their 3D shape then the number of points of contact with calcium oxalate mineral surfaces is limited. Small molecules like bikunin (Fig. 6) and β-microglobulin (Fig. 7) appear to be better than larger molecules like transferrin (Fig. 3) or tubulin (Fig. 8). The shape of the molecule is significant, the carboxylate oxygen atoms in the relevant residues for bikunin (Fig. 6) can get

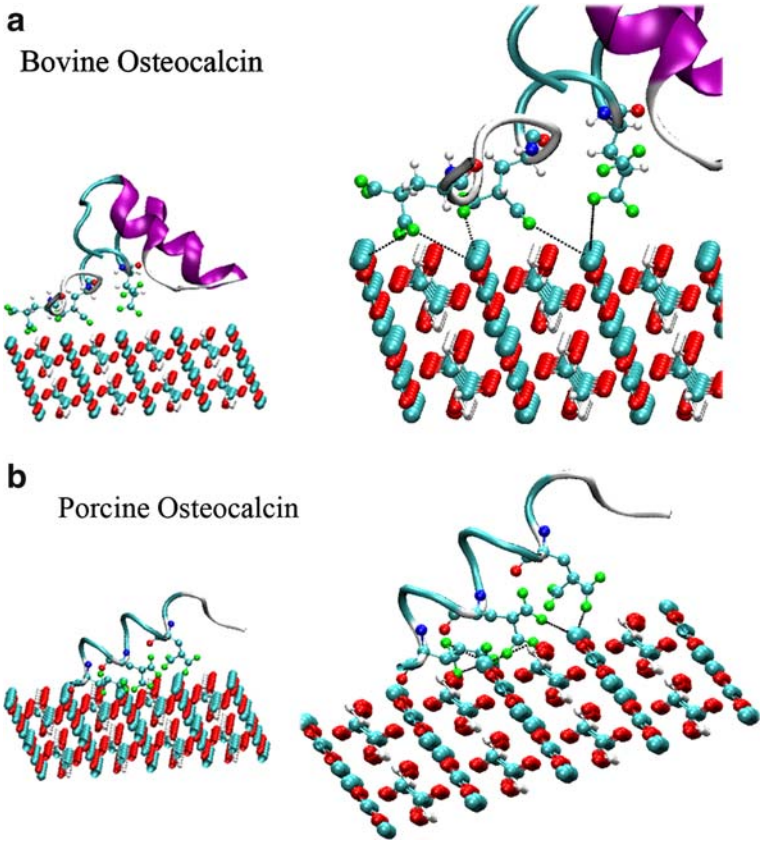

Fig. 9 **a** Bovine osteocalcin and **b** Porcine osteocalcin on COM (001). Color scheme same as in Fig. 3

Table 2 Summary of separation in Å between the two carboxylate oxygen atoms, labeled O1 and O2, and the closest surface Ca ion for residues making the best contact on COM (001)

Protein	Residue	Distance Ca–O1 (Å)	Distance Ca–O2 (Å)
Antitrypsin	Glu 317	5.1	5.1
	Glu 89	5.2	5.6
	Glu 323	4.5	5.3
	Glu 324	4.1	2.5
	Asp 19	3.3	4.3
Bikunin	Glu 71	2.9	4.4
	Glu 130	2.4	3.3
	Glu 127	6.6	6.8
	Glu 125	4.2	2.7
β-Microglobulin	Glu 68	3.1	2.4
	Glu 49	2.5	2.4
	Glu 52	2.4	2.5
Tubulin	Glu 420	3.7	2.4
	Glu 423	3.8	4
	Glu 434	3.1	4
	Glu 438	2.7	4.6

closer to the surface calcium atoms because they are on a relatively flat side of the molecule. On the other hand not all the Gla carboxylate oxygen atoms in the PFT1 Gla domain can come close to surface Ca since the Gla domain is wrapped around a cone on one side of the molecule (see Fig. 4) which allows only a few of the groups to come close to the oxalate surface. Simple steric considerations alone would suggest that there is no particular advantage to Gla over Glu, since it would not be possible to bond between both carboxylate groups and surface Ca spaced at approximately 6.3 or 7.3 Å.

What might be more significant is that with the exception of transferrin, and possibly antitrypsin, the number of contacts is the same irrespective of the surface. Recent work on the association of different urinary proteins with both COM and COD crystals grown in artificial urine [23] found that there was no specific interaction with any particular face as would be expected from our results, though they did report a slight preference for association with COD rather than COM. Ryall et al. [24] showed that PTF1 associated with COM at low Ca concentrations while osteopontin associated with COD at high Ca concentrations. They hypothesized that higher Ca concentrations blocked

Table 3 Summary of the separation in Å between the four γ carboxyglutamate carboxylate oxygen atoms labeled O1–O4 and the closest surface Ca ion for PFT1 residues making the best contact on COM (001)

Residue	Distance Ca–O1 (Å)	Distance Ca–O2 (Å)	Distance Ca–O3 (Å)	Distance Ca–O4 (Å)
GLA 33	4.6	2.4	3	5.2
GLA 26	5	5.4	5.2	4.5
GLA 30	3.8	3.6	6.6	4

Table 4 Summary of residues and contact lengths in Å for transferrin on COM and COD surfaces

Surface	Asp 240	Glu 237	Asp 104	Asp 221	Asp 229	Glu 224	Asp 236
COD (001)	3.5	3.4	3.6	3.8			
	5.5	2.4	3.2	3.4			
COD (010)	2.9	2.4	3.3	4.6		3.6	5.8
	3.4	3.3	5.6	5.8		5.8	7.3
COD (011)	4.2	4.1	2.9		3.8		
	3.5	4.1	2.4		4.2		
COD (110)	3.9	3.5	2.4	4.2			
	5.4	4.2	3.4	5.5			
COM (001)	3.4	4.3	4.9	3.8			
	3	2.5	3.8	3.9			
COM (010)	4.1	3.1	2.9		4.7		
	4.2	3.9	3.1		6.2		
COM (021)	2.9	2.4			2.5		
	2.9	3.2			4.6		
COM (101)	3.6	3.2	3.3		3.2		
	5.5	2.8	2.4		3.5		

For each surface the shortest distances in Å between the two carboxylate oxygens (one line for each) and the nearest calcium atom in the calcium oxalate surface is given

Table 5 Contact lengths in Å between transferrin and COM (001) including Lys oxalate interactions

Residue	Distance amide oxalate	
Lys 136	3.4	
Lys 125	3	
Lys 129	3.3	
	Distance Ca–O1	Distance Ca–O2
Glu 132	3.2	3.9
Glu 323	2.5	2.5
Glu 324	3.2	4.2
Asp 128	3.4	3.1

The shortest distance between a lysine amide group and a surface oxalate group is given in Å. For the Glu and Asp residues the distance between the two carboxylate oxygens, labeled O1 and O2, and the closest surface Ca is given in Å

the carboxylate groups in the Gla domain. They did not investigate whether either of these proteins associated with a specific face of either COM or COD.

The real issue is whether these 3D structures, mainly determined from X-ray diffraction, represent protein molecular structure in close proximity to oxalate surfaces. It is conceivable that proteins either partially denature by losing their tertiary structure or completely denature in proximity to ionic surfaces. The evidence from the literature is ambiguous as summarized in the recent review by Gray [25] since there are no techniques that can give atomic scale surface structure of proteins at a mineral surface. It could be argued that hydrophilic surfaces would provide an environment like water while hydrophobic surfaces would promote denaturation as suggested by studies on lysozyme [26]. Molecular dynamics (MD) simulations of crambin and rubredoxin on clay mineral surfaces showed considerable changes in secondary and tertiary structure [27], though limitations in the size of systems that can be studied by MD make it difficult to generalize these results to other systems. Work on fibronectin adsorption on different mineral surfaces suggested that the number of water molecules of crystallization controlled whether the protein adsorbed or not, and surfaces with the fewest water molecules adsorbed the most protein molecule [28]. Circular dichroism (CD) can be used to probe changes in α helical structure while fluorescence resonance energy transfer (FRET) gives information on large scale structural displacements in a molecule. CD measurements for fibrinogen absorption on TiO_2 and hydroxyapatite showed little change in the fraction of α helices [29] which implied that secondary structures remained intact, though the authors did suggest that electrostatic interactions and conformational changes were important. However a recent FRET study came to the conclusion that more denaturation took place on hydrophilic surfaces contrary to expectation [30]. Clearly further studies need to be done on how proteins implicated in urolithiasis interact with oxalate surfaces.

Even if the proteins were fully denatured and could be considered as polypeptide chains the presence of numerous Asp, Glu or Gla residues is not a sufficient condition for a strong interaction with the mineral surface. Neighboring acidic residues are too close to match either the 6.3 or 7.4 Å spacing in the dominant COM surfaces (see Fig. 1). Furthermore electrostatic repulsion would favor a coil structure for a domain of repeated acidic residues. For matching to the COM (100) surface the optimum sequence would appear to be an acidic residue such as Asp alternating with a small spacer such as Gly. It is of interest to note that the proteins involved in calcium carbonate biomineralization [8] all have a β sheet configuration that

Table 6 Summary of proteins with number of contacts and most significant residues involved

Protein	PDB ID	Contacts	MW (kD)	Residue 1	Residue 2	Residue 3	Residue 4	Residue 5
Porcine osteocalcin	1Q8H	5	5	Gla 17	Gla 21	Gla 24	Asp 34	Asp 30
Bovine osteocalcin	1Q3M	3	5	Gla 17	Gla 21	Gla 24		
Prothrombin fragment 1	2PF2	3	16	Gla 33	Gla 26	Gla 30		
Bikunin	1BIK	4	16	Glu 71	Glu 130	Glu 127	Glu 125	
β-Microglobulin	1BMG	3	11	Glu 68	Glu 49	Glu 52		
Tubulin	1JFF	4	50	Glu 420	Glu 423	Glu 434	Glu 438	
Antitrypsin	1HP7	5	44	Glu 317	Glu 89	Glu 323	Glu 324	Asp 19
Transferrin	1B3E	4	36	Asp 240	Glu 237	Asp 104	Asp 221	

allowed maximal regular contact between the acidic residues and surface ions. If this is typical, then the conformation and the arrangement of acidic residues are just as significant as the number present.

Another factor that has to be considered is that proteins are flexible structures, not static arrangements of atoms, and that natural flexibility of parts of the 3D structure could bring about more contacts. Modeling using normal mode analysis [31] and constraint networks [32] would suggest that these motions make perturbative changes to the structure and are insufficient to bring about contact from a group that is more than about 10 Å from the surface. It could be argued that surface steps would change the picture, but as can be seen from Figs. 3, 4, 5, 6, 7, 8, and 9, steps of even one or two unit cells up to 15 Å in height might at most bring one more acidic residue into contact. Qiu et al. [7] have argued that steps of this size could explain why osteopontin changes growth morphology on COM (010), but not on COM (100) where steps propagated freely without interference from the osteopontin absorbate. One would expect that steps of this magnitude would be easy to detect in AFM imaging. From size considerations alone it would appear that steps are much less significant for large molecules, such as proteins, than small molecules such as citrate. The presence of water surrounding the protein molecule does nothing to change the conclusions. Water is a polar molecule with a dielectric constant of 81, which effectively shuts off all long range electrostatic interactions. The only way to get a strong interaction between a macromolecule and an ionic surface is a direct electrostatic interaction with no intervening water molecules.

It could be argued that many of the protein structures do not come from humans and there could be significant differences in conformation. In Fig. 9 we show how porcine osteocalcin can fit the surface much better than bovine osteocalcin. This is in agreement with Hoang et al. who showed a close affinity for the binding of porcine osteocalcin with calcium hydroxyapatite [20].

The fundamental structure of osteocalcin, two α helices linked by a disulfide bridge between cysteine 23 and cysteine 29 is well conserved. However the bovine osteocalcin does not fit as well since the aromatic ring in tryptophan 5 prevents the aspartate 34 and glutamate 31 from getting closer to the surface as can be seen in Fig. 9. Ignoring differences at the C and N termini human osteocalcin found in urine has some similarity to both porcine and bovine osteocalcin. The tryptophan 5 from bovine osteocalcin is still present but like porcine osteocalcin there is arginine 18 substituting for lysine 18. How both these changes would affect the structure is unknown.

Finally there is always the possibility that proteins from stone formers and non-stone formers are sufficiently different to bring about changes in their interaction with mineral surfaces. It would be very hard to prove whether this was indeed the case as it would involve not only isolation of the relevant protein but also its structural determination.

Unless the protein molecules studied here denature on contact with oxalate mineral surfaces there are no more than three or four contacts between acidic Asp, Glu and Gla residues and surface calcium atoms. Even this weak attachment might still be sufficient to slow down growth or inhibit aggregation. The residues that made best contact were the same for all COM and COD surfaces considered which reflects the constraints brought about by the protein structure.

Acknowledgments We are indebted to Dr J. Wesson and Dr G.W. Drach for many useful discussions and a critical reading of the manuscript. We also acknowledge use of facilities in the Center for Solid State Science, and the assistance of Mr Jon Mull and Mr Craig Jolley.

References

1. Drach GW (1992) Urinary lithiasis etiology, diagnosis, and medical management. Campbell's Textbook of Urology, p 2085
2. Grover PK, Ryall RL (1998) Inhibition of calcium oxalate crystal growth and aggregation by prothrombin and its fragments in vitro. Eur J Biochem 263:50
3. Nakagawa Y, Abram V, Kezdy FJ, Kaiser ET, Coe FL (1983) Purification and characterization of the principal inhibitor of

calcium oxalate monohydrate crystal growth in human urine. J Biol Chem 258:12594–12600
4. Atmani F, Khan SR (1995) Characterization of uronic acid rich inhibitor of calcium oxalate crystallization isolated from rat urine. Urol Res 23:95
5. Atmani F, Mizon J, Khan SR (1996) Identification of uronic acid rich protein as urinary bikunin, the light chain of inter-α-inhibitor. Eur J Biochem 236:984
6. Wesson JA, Johnson RJ, Mazzali MA, et al (2003) Osteopontin is a critical inhibitor of calcium oxalate crystal formation and retention in renal tubules. J Am Soc Nephrol 14:139–147
7. Qiu SR, Wierzbicki A, Orme CA, et al. (2004) Molecular modulation of calcium oxalate crystallization by osteopontin and citrate. Proc Natl Acad Sci USA 101:1811
8. Addadi L, Weiner S (1985) Interactions between acidic proteins and crystals: stereochemical requirements in biomineralization. Proc Natl Acad Sci USA 82:4110
9. Sheng XX, Jung TS, Wesson JA, Ward MD (2005) Adhesion at calcium oxalate crystal surfaces and the effect of urinary constituents. Proc Natl Acad Sci USA 102:267
10. Lian JB, Prien EL, Glimcher MJ, Gallop PM (1977) The presence of protein bound γ-carboxyglutamic acid in calcium-containing renal calculi. J Clin Invest 59:1151
11. Ryall RL, Fleming DE, Grover PK, et al (2000) The hole truth: intracrystalline proteins and calcium oxalate kidney stones. Mol Urol 4:391
12. Dussol B, Geider S, Lilova A, et al (1995) Analysis of the soluble organic matrix of five morphologically different kidney stones. Urol Res 23:45
13. Doyle IR, Ryall RL, Marshall VR (1993) Inclusion of proteins into calcium oxalate crystals precipitated from human urine: a highly selective phenomenon. Clin Chem 37:1589
14. Nakagawa Y, Ahmed MA, Hall SL, et al (1987) Isolation from human calcium oxalate renal stones of nephrocalcin, a glycoprotein inhibitor of calcium oxalate crystal growth. J Clin Invest 79:1782
15. Atmani F, Glenton PA, Khan SR (1998) Identification of proteins extracted from calcium oxalate and calcium phosphate crystals induced in the urine of healthy and stone forming subjects. Urol Res 26:201
16. Warpehoski M, Buscemi PJ, Osborn DC, et al (1981) Distribution of organic matrix in calcium oxalate renal calculi calcif. Tissue Int 33:211
17. Berman HM, Westbrook J, Feng Z, et al (2000) The protein data bank. Nucleic Acids Res 28:235–242
18. Khoul H (2003) Personal Communication
19. Ivaska KK, Hellman J, et al (2003) Identification of novel proteolytic forms of osteocalcin in human urine. Biochem Biophys Res Commun 306:973
20. Hoang QG, Sichert F, Howard AJ, Yang DSG (2003) Bone recognition mechanism of porcine osteocalcin from crystal structure. Nature 425:977
21. Tazzoli V, Domeneghetti C (1980) The crystal structures of whewellite and weddellite: re-examination and comparison. Am Mineral 65:327
22. Humphrey W, Dalke A, Schulten K (1996) VMD: visual molecular dynamics. J Mol Graph 14:33
23. Walton RC, Kavanagh JP, Heywood BR, Rao PN (2005) The association of different urinary proteins with calcium oxalate hydromorphs. Evidence for non-specific interactions. Biochim et Biophys Acta 1723:175
24. Ryall RL, Chauvet MC, Grover PK (2005) Intracrystalline proteins and urolithiasis: a comparison of the protein content and ultrastructure of urinary calcium oxalate monohydrate and dihydrate crystals. BJU Int 96:654
25. Gray JJ (2004) The interaction of proteins with solid surfaces. Curr Opin Struct Biol 14:110
26. Lu JR, Su TJ, Thirtle PN (1998) The denaturation of lysozyme layers adsorbed at the hydrophobic solid/liquid surface studied by neutron reflection. J Colloid Interface Sci 206:212
27. Yu CH, Norman MA, Newton SQ, et al (2000) Molecular dynamics simulations of the adsorption of proteins on clay mineral surfaces. J Mol Struct 556:95
28. Hanein D, Geiger B, Adaddi L (1993) Fibronection adsorption to surfaces of hydrated crystals. An analysis of the importance of bound water in protein-substrate interactions. Langmuir 9:1058
29. Yongli C, Xiufang Z Yandao G, et al (1999) Conformational changes of fibrinogen adsorption onto hyroxyapatite and titanium oxide nanoparticles. J Colloid Interface Sci 214:38
30. Baugh L, Vogel V (2004) Structural changes of fibronection adsorbed to model surfaces probed by flourescence resonance energy transfer. J Biomed Mater Res A 69:525
31. Tama F, Sanejouand YH (2001) Conformational change of proteins arising from normal mode calculations. Protein Eng 14:1–6
32. Rader AJ, Hespenheide BM, Kuhn LA, Thorpe MF (2002) Protein unfolding: rigidity lost. Proc Natl Acad Sci USA 99:3540