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In-silico study on interaction of active binding sites of proteins with calcium oxalate monohydrate

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ABSTRACT

Bijarnia RK, Kaur T, Naik PK, Singla SK, Tandon C, In-silico study on interaction of active binding sites of proteins with calcium oxalate monohydrate, Online Journal of Bioinformatics, 9 (2):92-107, 2008. Calcium oxalate monohydrate (COM), the thermodynamically most stable form, is observed frequently in clinical stones. So far, many urinary proteins are believed to inhibit its growth. Further understanding of the molecular mechanism behind this modulation of COM growth is crucial for controlling urolithiasis. We have examined the interaction of COM with the various active binding sites of proteins (viz. bikunin, osteonectin, urinary prothrombin, CD59 and MAp19) known to inhibit COM crystallization. The active binding sites in these proteins were predicted and their binding affinity with COM was estimated using MOE (Molecular Operation Environment) software. On further analysis of the active binding sites which showed strong binding affinity, it was observed that amino acids having carboxyl group interact strongly through hydrogen bonding with calcium ion of COM. In addition, amino acids having hydroxyl group also showed considerable attraction for calcium

ion by forming hydrogen bond with it. This study shows that active binding sites of these proteins effectively interact with COM. For stronger affinity, various amino acids together interact either through hydrogen bonds or hydrophobic interactions. It could be interpreted that although acidic amino acids are of paramount importance in establishing strong binding with COM, nevertheless, the involvement of other amino acids is also significant as it strengthen this interaction.

Keywords: calcium oxalate monohydrate (COM), crystallization, hydrogen bonding, hydrophophic interactions, inhibitory protein, kidney stone**.**

Abbreviation: CGU: gamma carboxy glutamic acid; MAp19: Mannan-binding lectin (MBL)-associated plasma protein 19; CA: calcium ion; COM: calcium oxalate monohydrate.

INTRODUCTION

Kidney stone is a common chronic disorder affecting 10-15% of the general population world wide. Calcium oxalate monohydrate (COM) is the primary constituent of the majority of stones formed in the urinary system of patients with urolithiasis. Calcium oxalate monohydrate, the thermodynamically most stable form, is observed more frequently in clinical stones than calcium oxalate dihydrate (COD) and it has greater affinity for renal tubular cells, thus responsible for the formation of stones in kidney [1]. The saturation state of body fluids with respect to stone-forming constituents and the presence of various biomolecules (inhibitors/stimulators) in the body fluids as well as organic matrix are known to influence mineralization [2,3,4,5]. The matrix displays a variable and complex composition and a few proteins of matrix are common in various stones. It may act as a catalyst to induce mineral phase formation from metastable solutions or the biomolecules associated with the matrix may control mineralization by acting as inhibitors/stimulators. It is observed that certain macromolecules isolated from normal urine (*i.e.*, healthy individuals) inhibit COM crystal growth *in vitro*. In normal individuals, kidney stone formation is suppressed by these urinary inhibitors [6] and some of such inhibitors are proteins. These kidney stone inhibitory proteins may keep the crystals from aggregating or accumulating as additional layers and thus prevent their growth.

More recently, the growth of selected faces of calcium oxalate monohydrate have been studied at atomic level by atomic force spectroscopy [7] and osteopontin mainly affected (010) surface of COM. Electrostatic forces between COM and proteins surfaces showed that acidic amino acids effectively interact with all COM surfaces equally [8]. Despite the studies on calcium oxalate monohydrate with the surface of these proteins, the interaction of active binding sites of proteins with COM crystals have not been previously studied at molecular level.

Although improved non-surgical techniques, such as extracorporeal shock wave lithotripsy, percutaneous nephrolithotomy and ureteroscopy have been used in the management of urolithiasis successfully, its high recurrence still poses a serious problem. Therefore, understanding the etiology of renal calculosis at the molecular level could lead to its better management. The present work is thus aimed to study the interaction of COM with kidney stone inhibitory protein viz. bikunin, osteonectin, urinary prothrombin (prothrombin fragment 1), CD59, and MAp19. The main focus of the present study is to investigate how well various active binding sites of these proteins interact with COM crystals (most prominent in kidney stones).

MATERIALS AND METHODS

Molecular modeling was performed using Molecular Operation Environment (MOE) software package (Chemical Computing Group, Montreal, Canada). The unit cell structure of COM was prepared using crystallographic data derived from Tazzoli and Domeneghetti [9]. The low temperature monoclinic structure with space group $P2_1/c$ was selected. Because hydrogen atoms were not included so they were computationally added to COM structure. Energy minimization was preformed to COM by using force field MM2 with dielectric constant equal to 1.2. This minimized structure of COM is treated as ligand for further docking simulations.

Prediction and modeling of binding sites: The coordinates of inhibitory proteins viz. bikunin (PDB ID:1BIK), osteonectin (PDB ID:1SRA), urinary prothrombin (PDB ID:2PF2), CD59 (PDB ID:2J8B), MAp19 (PDB ID:1SZB) was taken from Protein Data Bank (www.rcsb.org) (Table 1).

	Protein	PDB ID	Resolution (Å)	Source organism
1	Osteonectin	1SRA	2.00	Homo sapiens
2	CD59	2J8B	1.15	*Homo sapiens*
3	Bikunin	1BIK	2.50	*Homo sapiens*
4	Urinary Prothrombin	2PF2	2.20	*Bos Taurus*
5	MAp19	1SZB	2.50	*Homo sapiens*

Table 1: Information of proteins procured from Protein data Bank (www.rcsb.org)

After addition of hydrogen atoms, Amber99 force field was used to assign partial charge on protein and all structure minimizations. Different binding sites of each protein were predicted with MOE-Site Finder which predicts hydrophilic and hydrophobic interaction sites and cavities. After adding the hydrogen atoms and prior to the docking calculations, an energy minimization using MMFF94 forcefield was performed on all the binding sites using the minimization protocol of Steepest Descent (SD), Conjugate Gradient (CG) and Truncated Newton (TN) methods. During the minimization, non-hydrogen atoms were held fixed and the RMSD gradient used in SD was 1,000, in CG was 100, and in the TN step the RMSD was 0.1. The iteration limits in SD and CG are 100 in TN 200.

Docking Simulations: Docking simulations were done using MOE-Dock which utilizes a Monte Carlo simulated annealing process for the docking calculation. A docking energy is calculated from a set of energy grids centered in the binding site of the protein. The protein coordinates were fixed during calculation, while the ligand is flexible and moves on the grid and searches the grid to locate the best binding orientation and conformation based on the docking energy. In all docking calculations, a docking box with a grid consisting of 60 X 60 X 60 points was employed. The spacing of the grid was 0.375 Å. The iteration limit was set to 20,000, the numbers of cycles were set to 20 and numbers of runs were set to 25, producing a molecular database with 25 docked configurations for each calculation. After a preferable binding structure was obtained from docking simulation, the complex was

used to determine atoms involved in hydrogen bonds and distance between atoms were determined using LIGPLOT [29].

Molecular mechanics and binding free energies: For the calculations of free energy of binding (FEB) of the ligand within the corresponding binding sites, only the docking result and the best scoring pose of the ligand was taken into consideration. eMBrAcE developed by Schrodinger was used for the physics-based rescoring procedure [10] For each binding site, the energy of protein–ligand complex (*E*lig–prot), the free protein (*E*prot) and the free ligand (*E*lig) were all subjected to energy minimization in implicit solvent (water) using the OPLS_2001force field with a constant dielectric electrostatic treatment of 1.0 [11,12]. It uses traditional MM methods to calculate ligand–receptor interaction energies (*Gele, GvdW, Gsolv*) by a GB/SA method [13] for the electrostatic part of solvation energy and solvent-accessible surface for the non-polar part of solvation energy. A conjugate gradient minimization protocol with default values was used in all minimization. eMBrAcE minimization calculations were performed using an energy difference mode, in which the calculation is performed first on the receptor, then on the ligand and finally on the complex, taking complexes obtained after docking analysis (MOE-dock outputs) as input. The energy difference is then calculated using the equation:
$\Delta G_{binding} = Ecomplex - Eligand - Eprotein$

RESULTS

On using MOE Site finder to depict active binding sites, each protein showed more than four such active binding sites and they were named arbitrarily. After their energy minimization, these active binding sites were then docked with COM unit cell. Table 2 represents docking score and the free energy of binding of those active binding sites which showed negative free energy of binding on interaction with COM. We observed that there is one or more than one binding site in each inhibitory protein that efficiently binds to COM (Table 2).

Table 2: The docking score and estimated free energy of binding ($\Delta G_{binding}$) of functional motifs (from MOE site finder) with the unit cell of COM.

	Protein	Active binding site	Sequence of active binding sites	Docking score (kcal/mol)	$\Delta G_{binding}$ (kcal/mol)
1	Bikunin	Bikunin-1	YACETEYGCQG	-45.863	-27.496
		Bikunin-2	FGKCERCGV	-31.230	-27.123
2	CD59	CD59-1	YADCKNNFNE	-115.110	-26.898
		CD59-2	NFKCW	467.665	-1.531
		CD59-3	KAGQENELT	581.227	-2.6
3	MAp19	Map-1	YFTHFDQAPGKHHCHRA	-147.063	-25.193
		Map-2	YFTHFDQESPGKDHCHR	-212.368	-26.46
		Map-3	LELGQTEKF	1053.544	-14.28
4	Osteonectin	Osteonectin-1	FDIDGLKVI	818.686	-17.573
		Osteonectin-2	RDWNAGDHLP	48.608	-24.937
5	Urinary prothrombin	Uripro-1	ANKGFLXXVRKGNLXRX CLXXPCSRXX	47.726	-27.22

*X = CGU (gamma carboxy glutamic acid)

Table 3 shows those active binding sites of the inhibitory proteins which showed minimum free binding energy. It is observed that active binding site of bikunin-1 showed minimum free energy of binding, thus pertaining to be most effective in interacting with COM unit cell.

Table 3: Geometry of hydrogen bonds and hydrophobic interaction of functional motifs (from MOE site finder) showing negative free energy of binding ($\Delta G_{binding}$).

Hydrogen bonding			Hydrophobic interaction		
Hydrogen donar group (HD)	Hydrogen acceptor (A)	Distance (Å)	COM	Protein receptor	Distance (Å)
Bikunin (bikunin-1)					
50 Ca	GLU A 69 OE1	2.89	54 C1	GLU A 52 CB	3.30
			54 C1	GLU A 52 CA	3.65
Bikunin (bikunin-2)					
48 Ca	GLY A 133 O	2.03	53 C1	VAL A 134 C	3.79
48 Ca	ARG A 129 O	2.47	53 C1	ARG A 129 CD	3.28
			53 C1	ARG A 129 CG	2.88
			16 C2	ARG A 129 CG	3.53
			53 C1	ARG A 129 CB	2.85
			53 C1	ARG A 129 CB	3.68
			53 C1	PHE A 100 CE2	3.24
CD59 (CD59-1)					
			53 C1	GLU A 73 CD	2.61
			16 C2	GLU A 73 CD	3.34
			53 C1	GLU A 73 CG	2.79
			16 C2	GLU A 73 CG	3.06
			53 C1	TYR A 4 CE1	3.44
			16 C2	TYR A 4 CE1	3.58
			53 C1	TYR A 4 CD1	2.75
			16 C2	TYR A 4 CD1	3.40
			53 C1	TYR A 4 CG	3.07
			16 C2	TYR A 4 CG	3.88
			53 C1	TYR A 4 CB	3.41
MAp19 (Map-1)					
44 Ca	HIS A 140 ND1	2.98	63 C2	HIS A 140 CE1	2.93
			3 C1	HIS A 140 CE1	3.45
			54 C1	GLY B 87 C	3.82
			64 C2	ALA B 85 CB	3.63
			24 C1	GLN B 77 CG	3.84
			64 C2	GLN B 77 CB	3.66
			24 C1	GLN B 77 CB	3.82
			64 C2	GLN B 77 C	3.72
MAp19 (Map-2)					
49 Ca	GLU A 78 OE1	2.58	5 C4	HIS B 140 CG	3.65
49 Ca	GLU A 78 0	2.30	5 C4	HIS B 140 CB	3.68
48 Ca	GLN A 77 OE1	3.33	63 C2	LYS A 88 CE	3.26
			3 C1	LYS A 88 CE	3.04
			63 C2	LYS A 88 CD	3.66
			4 C3	LYS A 88 CD	3.41
			3 C1	LYS A 88 CD	3.01
			4 C3	LYS A 88 CG	3.79
			53 C1	GLU A 78 C	3.32
			53 C1	HIS A 48 CE1	3.89

Table 3 Continued….

Hydrogen bonding			Hydrophobic interaction		
Hydrogen donar group (HD)	Hydrogen acceptor (A)	Distance (Å)	COM	Protein receptor	Distance (Å)
MAp19 (Map-3)					
			26 C4	PHE A 112 CZ	3.46
			25 C3	PHE A 112 CZ	3.55
			26 C4	PHE A 112 CE2	3.41
			63 C2	GLU 109 CD	3.86
			3 C1	GLU 109 CD	3.89
Osteonectin (osteonectin-1)					
48 Ca	PHE A 218 O	3.32	53 C1	ILE A 286 CD1	2.28
			16 C2	ILE A 286 CD1	2.77
			53 C1	ILE A 286 CG1	2.47
			16 C2	ILE A 286 CG1	2.68
			53 C1	ASP A 222 CG	3.34
			53 C1	ASP A 222 CB	3.76
Osteonectin (osteonectin-2)					
48 Ca	PHE A 218 O	3.32	53 C1	ILE A 286 CD1	2.28
			16 C2	ILE A 286 CD1	2.77
			53 C1	ILE A 286 CG1	2.47
			16 C2	ILE A 286 CG1	2.68
			53 C1	ASP A 222 CG	3.34
			53 C1	ASP A 222 CB	3.76
Urinary prothrombin (uripro-1)					
47 Ca	CGU A 7 OE1	3.31	53 C1	CGU A 15 CD1	2.79
52 Ca	VAL A 9 O	2.81	53 C1	CGU A 15 CG	3.74
50 Ca	SER A 24 OG	2.46	53 C1	CGU A 15 CB	3.77
			53 C1	CGU A 15 CA	3.12
			17 C2	CGU A 15 CD1	3.30
			54 C1	ASN A 2 CG	3.72
			37 C2	ASN A 2 CG	3.81
			63 C1	ASN A 2 CB	3.84
			63 C2	ASN A 2 C	3.86

Fig. 2 represents the interaction of amino acids of active binding site bikunin-1 with COM unit cell. It is clear that GLU at 69th position of this site is involved in hydrogen bonding with CA through atom O$^{\varepsilon}$ and GLU at position 52 is involved in hydrophobic interactions with carbon atom (C1) of oxalate group in COM (Table 3). Bikunin-2 is also possessing comparable strong interactions with COM unit cell. Its ARG (basic amino acid) at position 129 is involved in both hydrogen bonding and hydrophobic interactions. CD59-1 active binding site of CD59 has showed maximum attraction towards COM in comparison to its other active binding sites screened by MOE site finder. Although CD59-1 have GLU and ASP in its active binding site, yet only GLU showed hydrophobic interactions with COM and ASP is not at all involved (Table 3). Active binding sites of MAp19 identified by MOE site finder showed that out of all identified sites, Map-2 binding with COM unit cell is strongest followed by Map-1 and Map-3. Map-1 and Map-2 sites have quite similar sequences except for GLU and ASP present in Map-2 instead of ALA and HIS in Map-1. It is further observed that GLU of Map-2 is involved in hydrogen bonding with CA of COM. Although Map-3 active binding sites possesses GLU in its sequence, still its GLU is not involved in hydrogen bonding (Fig. 3)

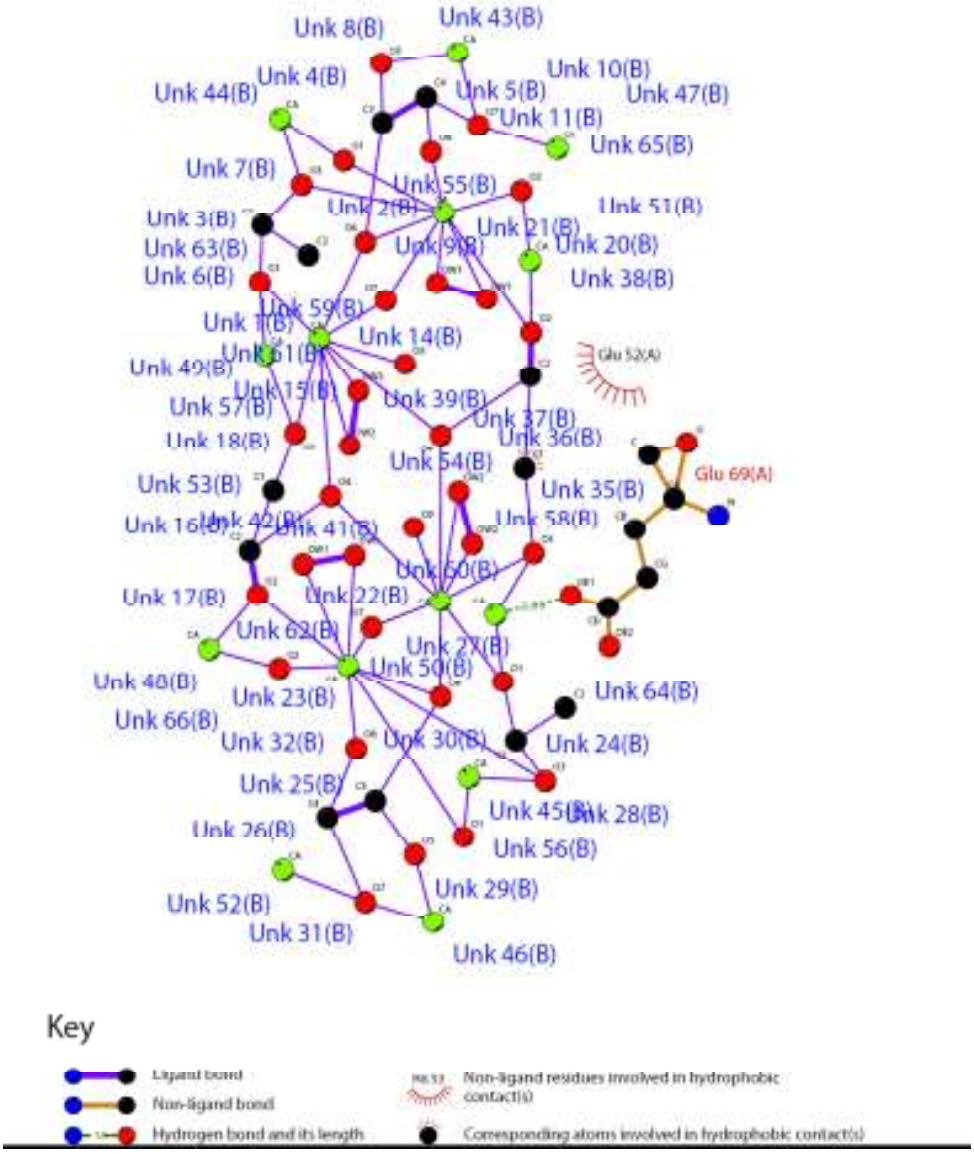

Figure 2: Two dimensional representations of the interactions observed between COM unit cell and active binding site bikunin-1 of protein bikunin. Dashed lines denote hydrogen bonds, and numbers indicate hydrogen bond lengths in Å. Hydrophobic interactions are shown as arcs with radial spokes. The figure was made using LIGPLOT [29]

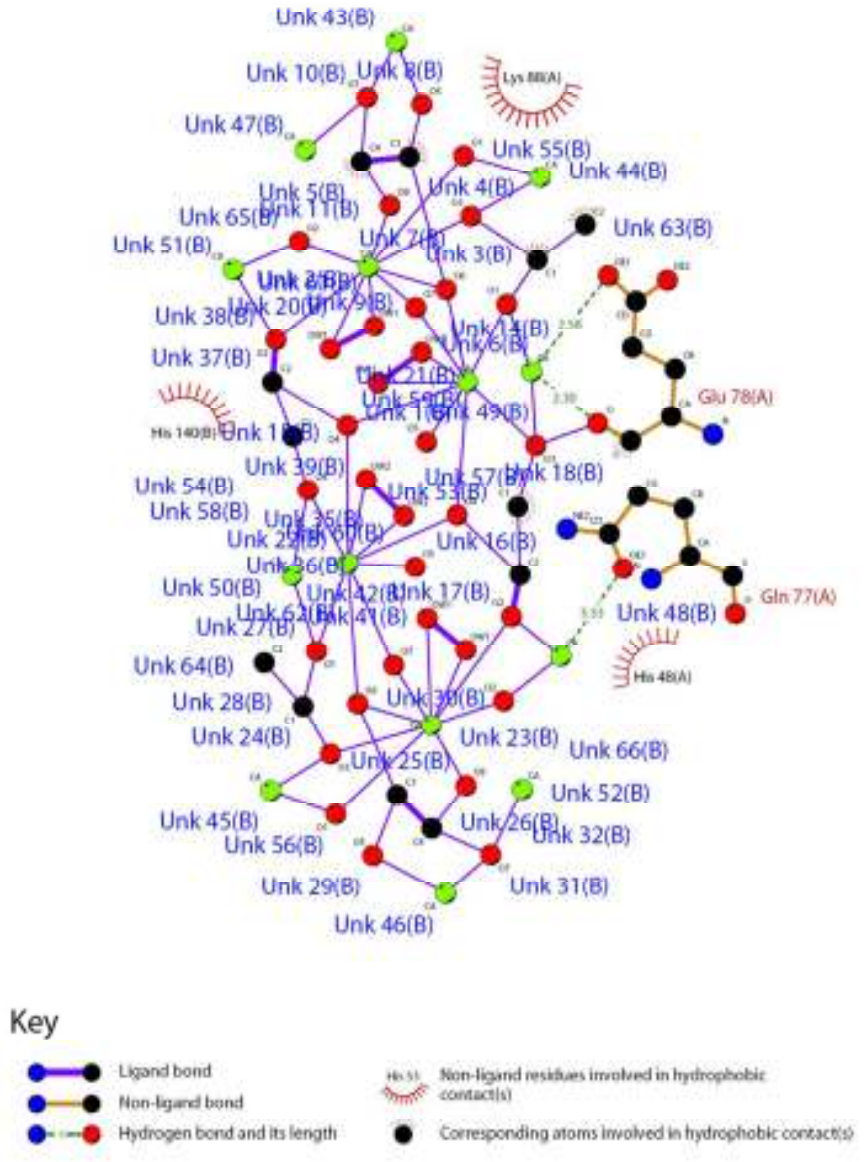

Figure 3: Two dimensional representations of the interactions observed between COM unit cell and active binding site Map-2 of MAp19. Dashed lines denote hydrogen bonds, and numbers indicate hydrogen bond lengths in Å. Hydrophobic interactions are shown as arcs with radial spokes. The figure was made using LIGPLOT [29]

On estimating the interaction between active binding sites of osteonectin and COM, it was observed that osteonectin-1 and osteonectin-2 showed comparable binding which is fairly strong. In addition to GLU and ASP, osteonectin-2 also showed involvement of basic amino acids like LYS and aromatic amino acid like TRY and TRP

(Table 3). Involvement of aromatic amino acids in COM interactions is further supported by osteonectin-1 (Fig. 4) active binding site where oxygen atom (O) of PHE is involved in hydrogen bonding with CA of COM unit cell. Only one active binding site uripro-1 of urinary prothrombin having gamma carboxy glutamic acid (CGU), showed strongest binding with COM in comparison to its other active binding sites.

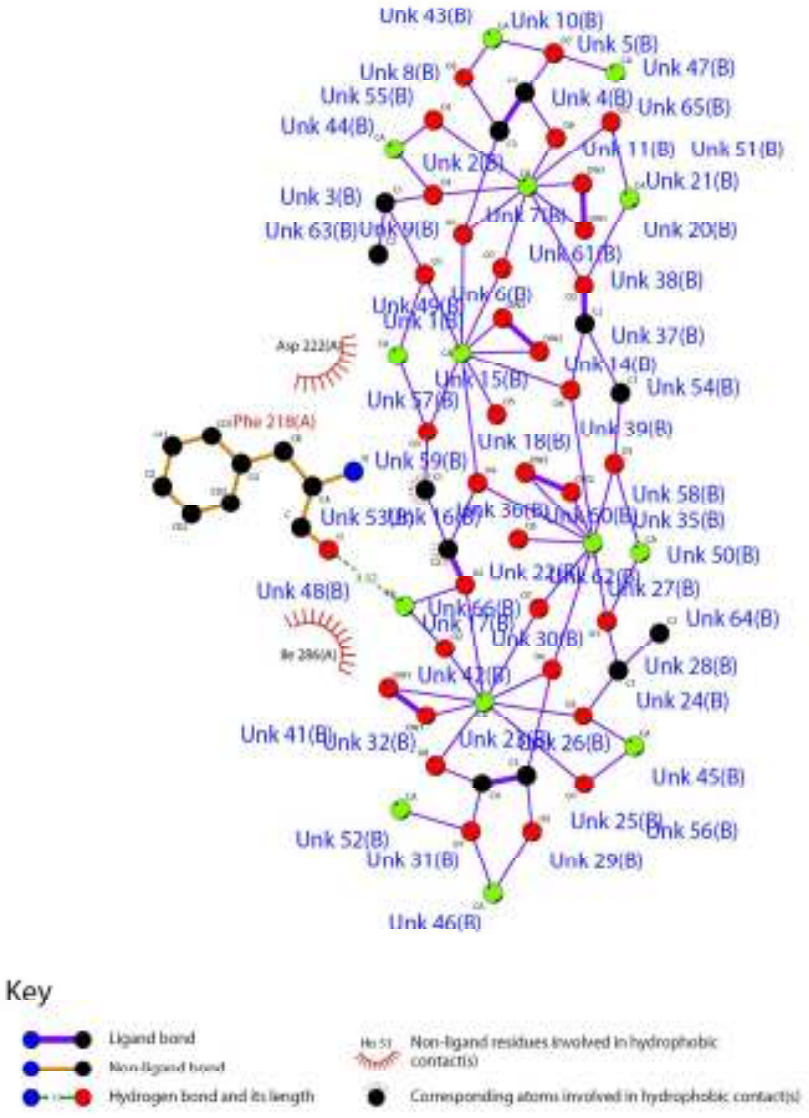

Figure 4: Two dimensional representations of the interactions observed between COM unit cell and active binding site osteonectin-1 of osteonectin protein. Dashed lines denote hydrogen bonds, and numbers indicate hydrogen bond lengths in Å. Hydrophobic interactions are shown as arcs with radial spokes. The figure was made using LIGPLOT [29]

Gamma carboxy glutamic acid is involved in both hydrogen bonding and hydrophobic interactions (Table 3 and Fig 5). Carboxylic group in the side chain of CGU is forming hydrogen bond with CA at 47th position (Fig. 5).

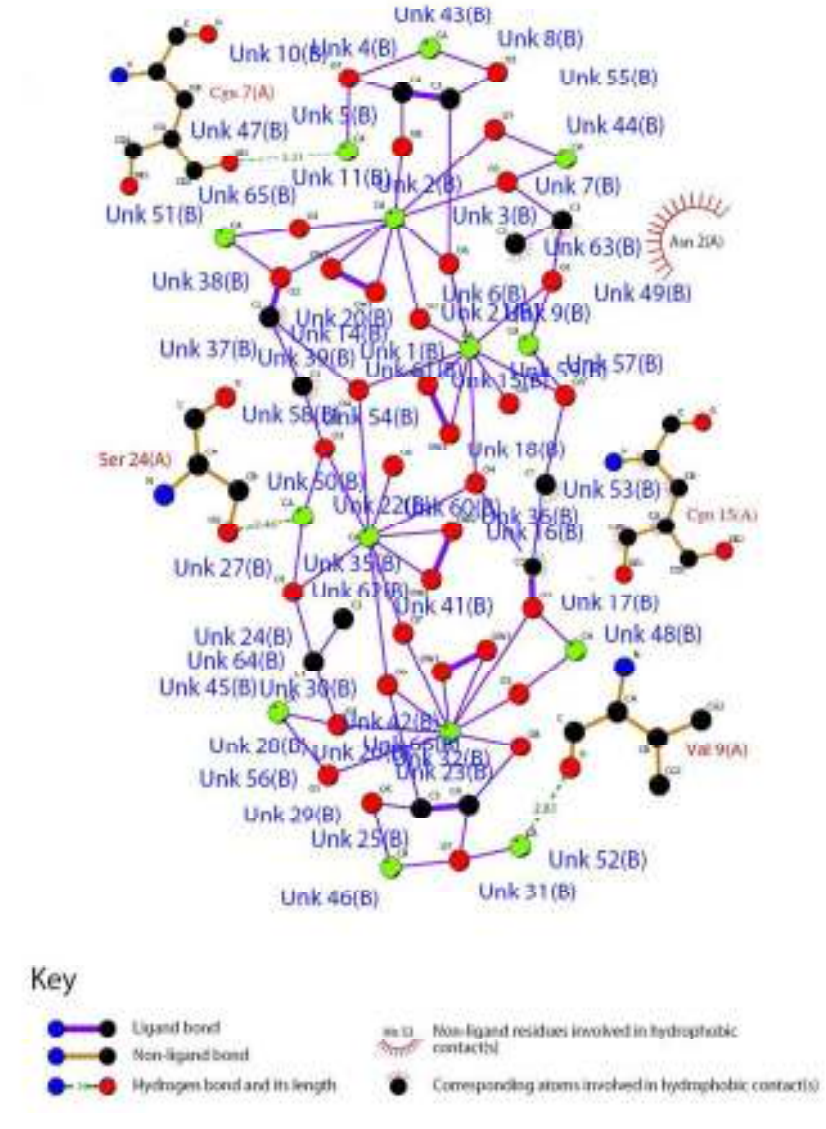

Figure 5: Two dimensional representations of the interactions observed between COM unit cell and active binding site uripro-1 of urinary prothrombin. Dashed lines denote hydrogen bonds, and numbers indicate hydrogen bond lengths in Å. Hydrophobic interactions are shown as arcs with radial spokes. The figure was made using LIGPLOT [29]

Carbon (C1) at position 53 and carbon (C2) at 17^{th} position of COM crystal are mainly forming hydrophobic interactions with C^{δ}, C^{γ}, C^{β} and C^{α} of CGU. The other two contributors of strong interactions with COM are SER and VAL. Here O^{γ} at 24^{th} position of SER forms hydrogen bonding with calcium ion at 50^{th} position and O of VAL at 9^{th} position form hydrogen bond with calcium ion at 50^{th} position.

DISCUSSION

The absence of a realistic concept of renal stone generation in important number of cases is, to a considerable extent, caused by the fact that this process cannot be observed directly *in vivo* and all hypotheses have to be based on results of *in vitro* experiments [14]. The use of molecular modeling and docking of compounds into the target sites of molecular models derived directly from resolved crystal structures has already proven valuable for discovering new ligands [15]. In the present study we have incorporated docking simulations to analyze the interactions between calcium oxalate monohydrate and proteins which are known to inhibit its growth. Docking energies are calculated as the sum of the intermolecular interactions (electrostatic + vander Waals) plus the conformational energy of the ligand-domain complex determined by molecular mechanics [16]. More negative the docking score, stronger is the binding between ligand (COM in our study) and protein's active binding site. These strong interactions between protein active binding site and COM crystal, specifically at the growing sites would predict inhibition of COM crystal.

The structure of calcium oxalate monohydrate is shown in figure 1. In this, the coordination polyhedra of atoms Ca(l) and Ca(2) are present as distorted square antiprisms (calcium polyhedra). Each calcium polyhedron shares three edges with three adjacent calcium polyhedra and these polyhedral layers are formed parallel to (100) plane. Within the layers, Ca atoms occur at the vertices of hexagons which have at their center one of the two independent oxalic groups. These oxalic groups also lie in the (100) plane with the C(l) and C(2) carbon atoms of oxalate group. The layers are connected to one another through the second series of oxalic groups and the water molecules. This oxalic groups which are alternating with the water molecules lie in (010) plane. From the figure it is shown that unit cell of COM possess atoms like calcium ion (CA), carbon (C) and oxygen (O) which are freely available to further enlarge COM crystal. Strong binding at these sites would definitely retard the growth of COM crystal.

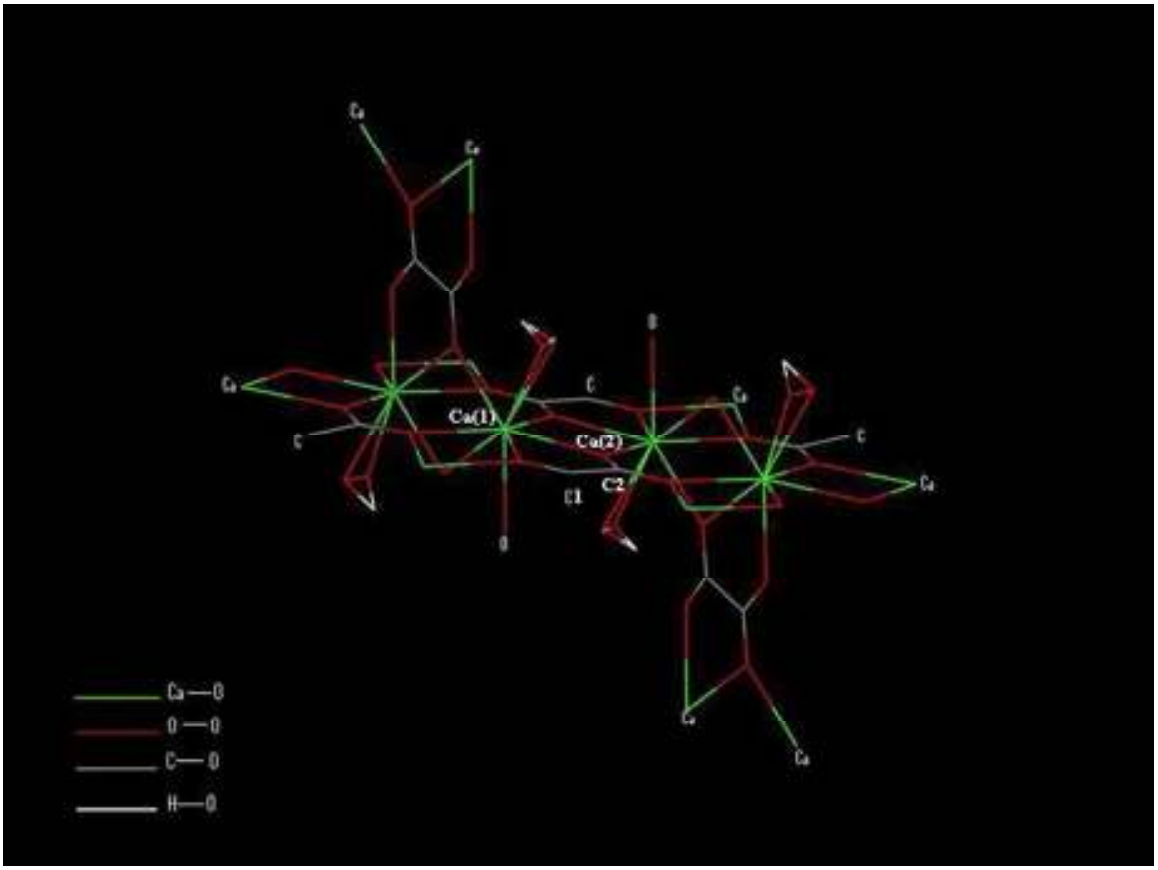

Figure 1: Structure of Calcium oxalate monohydrate (COM) unit cell showing coordination polyhedra of atoms Calcium 1 [Ca (l)] and Calcium 2 [Ca (2)].

There are many reports suggesting that proteins either partially or completely denature in the proximity of hydrophobic or charged surfaces [17, 18]. Inspite of such reports some authors documented that no loss of secondary structure was observed following absorption of fibrinogen on hydroxyapatite [19]. In addition to this, various proteins have been isolated from calcium oxalate kidney stones itself in their active form [4, 20]. This suggests that all proteins do not loose their complete structure in proximity to ionic solid surfaces. So, the following inferences are made assuming that even though certain conformational changes in proteins occurred due to COM, the active site sites remained intact.

There have been many reports showing interactions between surface of protein and COM crystal [7,8]. Still there has been no report about the interactions of COM with the amino acids incorporated in the vicinity of the proteins. It could be suggested that the active binding site, if could resist any conformational change, strongly interact with COM in its initial growing phase and prevent its further crystallization.
Each protein possessing the property of inhibiting COM crystallization taken in the present study showed diverse kind of interactions, supporting the fact that most of the kidney stone inhibitory proteins are multifunctional proteins. The property common to them is the ability to strongly interact with the free available growing sites (CA, C and O atom) (Fig.1) of COM. It is known that COM crystal growth is slow in some directions since certain macromolecules adsorb on it and prevent them from lattice ions. Face (-101) of COM crystals is more active as it presents more closely

packed calcium atoms and has significantly more adsorptive characteristics for many macromolecules (proteins) [21]. In the present investigation it was observed that calcium ions of COM form hydrogen bonding with varied amino acids of active binding sites and carbon of oxalate group gets involved in hydrophobic interactions. It is a fact that hydrogen-bonding are of primary significance in establishing strong complex between ligand and protein active binding site, but nevertheless hydrophobic interactions also act as a stabilizing factor and addition of a hydrophobic group not only allows hydrophobic bonding but also strengthens existing hydrogen bonds and the increased hydrogen bond strength can be an important factor in determining the overall binding energy [22].

The interaction of amino acids is also dependent on the conformation of the active sites, same amino acid is one instance is effectively involved in hydrogen bonding and in other instance same amino acid is involved in week hydrophobic interactions as is shown in the case of active binding site bikunin-1. This dependence is purely steric hindrance, thus suggesting that not all amino acids which could strongly bind with calcium ions, although repeatedly present in the active binding site, interact with COM. Thus there is no advantage of repeated GLU residues until they are structurally available to interact. Strong hydrogen bonding of GLU and ASP with calcium ion of COM crystal supports the hypothesis that acidic amino acids which are negatively charged are attracted to positively charged calcium ions [23]. The results presented by CD59-1 binding site supports the findings of Wesson et al. [24] that the charge of the side group was not the sole determinant to cause this effect since GLU and ASP present in this binding site do not interact with COM. It is further perceived that proteins rich in gamma carboxy glutamic acid (CGU) possess two negative carboxylate groups have better binding to calcium sites of COM [25]. Here again it was observed from the results that although active binding site uripro-1 of protein urinary prothrombin have repeated CGU monomers, still all the CGU are not involved in COM interactions. This evidence further suggests that conformational and interface chemistries interact in a complex manner to inhibit aggregation of COM and an understanding of such interactions may help to determine and control the factors affecting kidney stone formation.

In addition to inhibition of COM by acidic amino acids, certain other amino acids also showed optimal binding with COM unit cell. Osteonectin-2 and uripro-1 active binding site, showed formation of hydrogen bonding by TYR and SER, both are form hydrogen bond through the hydroxyl group of their side chain with calcium ion. A recent study by Wang et al [26] presented that addition of serine spacer in poly aspartate peptide increased their ability to inhibit COM crystallization. They suggested that the hydroxyl groups (-OH) of serine may have contributed in the interaction by directly binding to calcium ions and form hydrogen bonds. Our results confirmed their hypothesis as oxygen of the hydroxyl group in the side chain of both SER and TYR are involved in hydrogen bond formation with calcium ion.

Additionally, aromatic amino acids like HIS and PHE and basic amino acids like LYS and ARG are also showing optimum interactions with COM by forming both hydrogen bonding and hydrophobic interactions or either of them. Tandon et al [5] isolated a protein causing inhibition of calcium phosphate mineralization from rabbit aorta and it was found to possess higher content of aromatic amino acids followed by basic amino acids. As depicted from our results these amino acids form hydrogen bonds with calcium ion. It could be suggested that the calcium phosphate inhibitory protein from rabbit aorta possess this property as they could interact with calcium ions and prevent its further mineralization. The study done by Gul et al [8] also suggested the

involvement of basic amino acids like ARG and LYS with the oxalate ions of COM crystal. Our results further supported this possibility as from the ligplot analysis it is clear that ARG and LYS are forming strong hydrophobic interactions with carbon of oxalate group

Various findings till date have documented on the inhibition of calcium oxalate crystals by these proteins. It has been confirmed by scanning electron microscopy that the reduction in particle size of calcium oxalate stones by urinary prothrombin, resulted from a decrease in the degree of crystal aggregation [27]. Recently the Gla domain of urinary prothrombin has shown to possess an important role in calcium oxalate inhibition [28]. It was documented that on modifying gamma carboxy glutamic acid (CGU) in the Gla domain of urinary prothrombin, to gamma methyleneglutamic acid, a decrease of about 65%, in inhibitory activity of urinary prothrombin towards calcium oxalate stones was observed. Thus it suggests inhibitory role of active binding sites of such proteins towards calcium oxalate crystallization.

This study put forth the possibility of COM and active binding site interactions. Additionally, this study documents selective binding of amino acids with COM crystal. Furthermore the selectivity is imposed by stereochemical characteristics of active binding site. From these results we can also conclude that amino acids interact with calcium ion by forming hydrogen bonds and it binds with carbon of oxalate group in COM crystal, through hydrophobic interactions. Although acidic amino acids are primarily involved in establishing strong binding with COM but involvement of other amino acids is also important as it strengthen this interaction.

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