Glycoconj J (2006) 23: 251–257
DOI 10.1007/s10719-006-7930-6

Search for fucose binding domains in recently sequenced hypothetical proteins using molecular modeling techniques and structural analysis

Sujata Majumder · Madhumita Patra · Chhabinath Mandal

Abstract The crystal structure of a fucose-binding lectin from the bacteria *Pseudomonas aeruginosa* in complex with α-L-fucose has been recently determined. It is a tetramer; each monomer displays a nine-stranded, antiparallel, β-sandwiched arrangement and contains two calcium ions that mediate the binding of fucose in a recognition mode unique among protein-carbohydrate interactions. In search of this type of unique interactions in other newly discovered protein sequences, we have used molecular modeling techniques to predict and analyze the 3-D structures of some proteins, which exhibited reasonable degree of homology with the amino acid sequence of the bacterial protein. A BLAST search with the sequence of *Pseudomonas aeruginosa* as query in the non-redundant sequence database identified four proteins from different species, three organisms from bacteria and one from archaea. We have modeled the structures of these proteins as well as those of the complexes with carbohydrates and studied the nature of physicochemical forces involved in the complex formation both in presence and absence of calcium. The calcium-binding loops have been found to be highly conserved both in terms of primary and tertiary structures in these proteins, although a less acidic character is observed in *Photorhabdus* lectin due to the absence of two aspartic acid residues on the calcium-binding loop which also resulted in lower binding affinity. All these structures exhibited highly negative electrostatic environment in the vicinity of the calcium-binding loops which was essential for neutralizing the positive charges of two closely situated Ca^{+2} ions. The comparison of the binding affinities of some monosaccharides other than fucose, e.g. mannose and fructose, showed higher binding energies confirming the fucose specificity of these proteins.

Keywords Fucose-binding proteins · Molecular modeling · Calcium-mediated fucose binding

Abbreviations

Chromo	Chromobacterium violaceum
Bcepa	Burkholderia cepacia
Photorb	Photorhabdus luminescens
Methkand	Methanopyrus kandleri
BLAST	Basic local alignment-search tool
MOLMOL	Molecule analysis and molecule display
CBL	Calcium binding loop

Introduction

Lectins represent a specific class of carbohydrate binding proteins different from enzymes or antibodies [2]. They are found in a wide range of organisms, including viruses, bacteria, plants, and animals, and belong to one of several different families, many of which have been characterized structurally [3]. Bacterial lectins are believed to play an important role in infection, such as specific recognition of or attachment to target cells. Structural data on bacterial lectins are limited. We are interested mainly in the bacterial fucolectins because cell surface glycoconjugates carry fucose as the terminal residue on complex oligosaccharide antigen of the Lewis a (Le^a) or Le^x series. The fucose-binding lectins on the pathogenic bacteria recognize the fucosylated terminals which can be prevented by docking fucose into the binding site leading to competitive inhibition of the binding of the pathogenic bacteria to a human blood group. Although limited information

S. Majumder · M. Patra · C. Mandal (✉)
Drug Design, Development and Molecular Modelling Division
Indian Institute of Chemical Biology Jadavpur, Kolkata 700 032, India
e-mail: cnmandal@iicb.res.in

is available on the structures of the bacterial fucolectins, fortunately the crystal structure of a bacterial fucolectin, PA-IIL from the pathogenic bacterium *Pseudomonas aeruginosa* has been determined with 1.3 Å resolution [1]. In the present study we are in search of newly discovered bacterial protein sequences which may have a structure similar to that of the fucolectin from *Pseudomonas aeruginosa*. *Pseudomonas aeruginosa*, an important opportunistic pathogen associated with chronic airway infections, synthesises two lectins, LecA and LecB (formerly named PA-IL and PA-IIL, respectively) [4]. PA-IL is galactophilic [5–7] and PA-IIL exhibits a high specificity for L-fucose and its derivatives [1,4,8]. PA-IIL contributes to the virulence of this pathogenic bacterium, a major cause of morbidity and mortality in cystic fibrosis patients. The crystal structure of PA-IIL reveals a tetrameric structure of four identical subunits, each of them containing two Ca^{2+} ions and each binding one α-D-fucose molecule. The overall-fold of PA-IIL is that of a nine stranded antiparallel β-sandwich [1]. The two close calcium ions mediate the binding of fucose in a recognition mode unique among carbohydrate-protein interactions.

Using BLAST search we have identified four sequences, three from the bacteria, *Chromobacterium violaceum* ATCC 12472 (ACCESSION: NP_901414), *Burkholderia cepacia* R18194 (ACCESSION: ZP_00218018), and *Photorhabdus luminescens* subsp. Laumondii TTO1 (ACCESSION: NP_931416) and one from *Methanopyrus kandleri* AV19 (ACCESSION: NP_614303), an archaea. *Methanopyrus kandleri* is a rod-shaped organism that grows optimally at temperatures near and above the boiling point of water. The microbe was isolated from the sea floor at the base of a 'black smoker' chimney in the Gulf of California [15]. The organism is a rod-shaped Grampositive methanogen that grows chemolithoautotrophically at 80 to 110°C in the H2–CO2 atmosphere [16]. *M. kandleri* proteins show an unusually high content of negatively charged amino acids, which might be an adaptation to the high intracellular salinity. The genome of *M. kandleri* is very GC-rich. These are the potential candidates for such unique lectin folds. The reason behind selecting these four sequences lies in the fact that the residues that are playing an important role in fucose binding as well as in calcium binding in PA-IIL are found to be almost conserved here. The 3D structures of these four hypothetical proteins have been predicted by using a homology modeling technique taking the crystal structure of PA-IIL as template. Fucose molecule has been docked to arrive at the structures of the complexes using computer-aided modeling techniques. Analysis of these structures allowed us to examine the feasibility of the formation of such folds and the nature of interactions of fucose with these proteins. We have also studied the binding affinities of the protein-sugar complexes in presence and absence of calcium in order to analyze the role of calcium in sugar binding. Another striking observation was the highly negatively charged environment of the fucose-binding pocket. In order to verify if this is a necessary condition of fucose binding, we calculated the electrostatic potentials of all the modeled proteins and examined their nature in the vicinity of the binding pocket. A comparative study of the sugar-binding affinities of two other sugars α-D-mannose and β-D-fructose were done in order to see the fucose-binding specificity of the protein. Information from these predicted structures of these molecules and their complexes will provide valuable data to develop anti-therapeutics in the future.

Methods

Initial structures of the fucose-binding proteins were modeled by knowledge based homology modeling using our in-house software package of ANALYN and MODELYN [10]. The starting scaffold for homology modeling was the x-ray crystallographically determined structure of PA-IIL (PDB ID: 1GZT). These structures were refined using the InsightII 2000.1 of Accelrys (San Diego, CA) equipped with DISCOVER as the energy minimization and molecular dynamics module. Structural optimization involved energy minimization (100 steps each of steepest descent and conjugate gradient methods) using cff91 force-field followed by dynamics simulations. A typical dynamics run consisted of 1000 steps of one femto-second after 100 steps of equilibration with a conformational sampling of 1 in 10 steps at 300°K. At the end of the dynamics simulation, the conformation with lowest potential energy was picked for the next cycle of refinement using the ANALYSIS module. This combination of minimization and dynamics were repeated until satisfactory conformational parameters were obtained. Special care was taken in the structural zones where major insertion or deletions were made. Structures of the complexes were obtained by the superposition of the modeled protein structures with the experimental structure of a fucose complexed with PA-IIL (1GZT) followed by optimization with repeated energy minimization and dynamics simulations. Position constraints were applied to the atoms, which were more than 10 Å away during energy minimization and molecular dynamics of the complexes. During the regularization of the structures of the complexes with calcium and fucose the distance between the two calcium ions and the atoms involved in coordination with the calcium ions were kept constant by applying 'generic distance constraints' from the DISCOVER module of InsightII.

ANALYN was used for the homology analysis of prealigned sequences of the target and scaffold proteins and was run on IBM-compatible PC. MODELYN was used for automated prediction of the target structure and its structural analysis after refinement; it was run on both on IBM-compatible PC in the windows environment and OCTANE

workstation of Silicon Graphics, Inc. in the UNIX environment. InsightII was run on OCTANE workstation of Silicon Graphics, Inc. in the UNIX environment. CLUSTALW [11] was run through the Internet for multiple alignment of the amino acid sequences. The electrostatic potential surfaces of the proteins were determined by MOLMOL [12]. PROCHECK [13] was used for checking the structural parameters. Both MOLMOL and PROCHECK were run on OCTANE in the UNIX operating system. Hydrogen bonding patterns of the modeled and x-ray structures were obtained by adding hydrogen (x-ray structures lacked hydrogen atoms, and DISCOVER needs these atoms for minimization and dynamics) followed by optimization of the complex by energy minimization and molecular dynamics. The binding affinities are of the complexes were studied using the DOCKING module of InsightII. Structural superposition was done using ABGEN on both PC and UNIX environments. Protein BLAST [14] was used through the Internet for finding homologous sequences.

Results and discussion

In this study we are in search of bacterial fucolectins, and we have found out that the structure of a bacterial fucolectin from the pathogenic bacteria *Pseudomonas aeruginosa* has been determined with 1.3 Å resolution. To find out the protein sequences homologous to that of *Pseudomonas aeruginosa* (PA-IIL), BLAST search was performed with its amino acid sequence as the query. Among the BLAST hits we have selected four sequences on the basis of their high homology in a particular region of the sequence with the query sequence; and that portion of the query sequence formed the calcium-binding loop in the structure which plays an important role in fucose binding. Three of these selected protein sequences belong to the bacteria and one to the archaea. The conserved hypothetical protein from *Chromobacterium violaceum* (Chromo) shows 60% sequence identity and about 89% sequence similarity with the query sequence. Bearing such a high sequence homology, it is expected that it will also bear a high degree of structural similarity. This was found to be true with the study of our modeled structure which also showed similarity with its interaction with calcium as well as fucose. The hypothetical protein from the bacteria *Burkholderia cepacia* (Bcepa) showed 53% identity and about 68% similarity with the query sequence and the hypothetical protein from *Photorhabdus luminescens* (Photorb), an enterobacterium pathogenic to a wide range of insects but lives in symbiosis with soil entomopathogenic nematodes showed 40% identity and 65% similarity with PA-IIL sequence. The life cycle of the bacterium is composed of a symbiotic stage in the nematode and a virulent stage in the insect larvae. The uncharacterized protein from the archaea *Methanopyrus kandleri* (Methkand) showed only 35% identity and about 46% similarity but surprisingly the residues involved in calcium binding in PA-IIL are found to be conserved here. This led to the assumption that this archaea may also have a fucose-binding lectin similar to that of the bacteria *Pseudomonas aeruginosa* having similar calcium-mediated fucose-binding. A sequences of these four proteins were used as target sequences for 3-D structure prediction and analysis for the calcium-mediated carbohydrate recognition-fold described recently [1]. After the initial structures were predicted by the combination of ANALYN and MODELYN, they were refined by regularization of segments with major deletion or insertion using DISCOVER module of InsightII. Final predicted structures were checked for main chain conformations using PROCHECK which showed that more than 80% of the ϕ–ψ plots were in the core regions and less than 3% were in the disallowed regions of Ramachandran's plots. Root mean square deviations of bond lengths and bond angles were within 0.03 Å and 3.5 degrees from the standard values indicating reasonably good structural parameters of the predicted structures. The ClustalW multiple sequence alignment of the query and the modeled proteins is shown in Fig. 1 which shows the highly conserved amino acid residues.

```
PA-IIL      ATQGVFTLPANTRFGVTAFANSSGTQTVNVLVNN--ETAATFSGQSTNNAVIGTQVLNSG
Chromo      AQQGVFTLPARINFGVTVLVNSAATQHVEIFVDN--EPRAAFSGVGTGDNNLGTKVINSG
Bcepa       ERDGLFNLPPNIAFGVTALVNSSAQQTIEVFIDDNPKPAATFQGAGTQDANLNTQIVNSG
Photorb     TGKHCFQLPQSIRFGLTAYNNTNIQQTVKVYIDD--LLVDTLTGKGTNN-PMATKTYTSG
MethKand    EPLIPGEVHPDALGAVVIPFKPGEWPGVALCSDP--TTAATFRR------LLDRPELRTI
                      :      .:.   :.     : :  :       ::          :      :
PA-IIL      SSGKVQVQVSVNGRPSDLVSAQVILTNELNFALVGSEDGTDNDYNDAVVVINWPLG
Chromo      S-GNVRVQITANGRQSDLVSSQLVLANKLNLAVVGSEDGTDMDYNDSIVILNWPLG
Bcepa       K-GKVRVVVTANGKPSKIGSRQVDIFKKTYFGLVGSEDGTDGDYNDGIAILNWPLG
Photorb     T-GKVCIEIEGDGKPSKLRYFDNTLDGKPGTAIIGAENGTNNNYNDCVVILNWPLV
MethKand    LNGPVIVTTEVQGRPCCIVRR-------TPDGFEFFEDGTDADYNDAVIEVTLEAV
              * * :    :*: . :              ..   *:**: :*** :  :.
```

Fig. 1 Clustal W multiple alignment of the template protein (PA-IIL) sequence and the target (modeled) sequences. The conserved amino acid residues are marked in bold

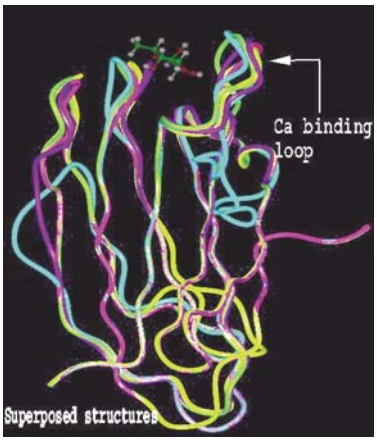

Fig. 2 Ribbon representation of the superposed structures of the modeled proteins with the crystal structure of PA-IIL. The ligand molecule is shown in balls-and-sticks representation and is colored by atom. The pink ribbon represents PA-IIL, light green represents Chromo, Bcepa is shown in yellow, Photorb in violet, and Methkand in light blue

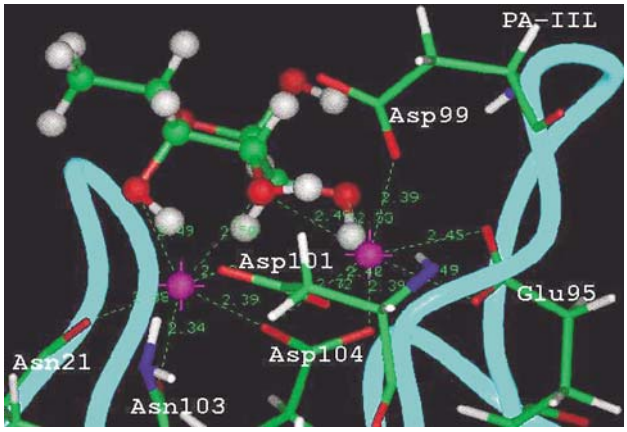

Fig. 3 Mode of calcium binding in PA-IIL. The two calcium ions are shown in pink. The residues of protein involved in binding are colored by atom shown in stick representation and the fucose is shown in balls-and-sticks representation. The distances of calcium ions from their coordinating atoms are also shown. Six residues of protein and O2, O3, O4 atoms of fucose molecule are involved in coordination with the two calcium ions

In PA-IIL the amino acid sequence from 95 to 104 (EDGTDNDYND) forms the calcium binding loop, and this sequence is found to be almost conserved in the target sequences. The modeled proteins Chromo, Bcepa, and Methkand have only one amino acid residue differing from that of PA-IIL. But Photorb showed less homology with only 6 residues out of 10 remained conserved. The three Asp residues found in other sequences are found to be replaced by Asn this protein. The absence of these Asp residues also affects fucose binding which will be discussed later. Figure 2 shows the superimposed form of the modeled structures with the template structure.

In PA-IIL it is seen that the calcium-binding pocket consists of two calcium-binding loops. The calcium-binding site contains six acidic groups, five of which are involved in calcium binding. Both calcium ions have a classical seven ligand coordination; loop CBL-1 only participates in calcium binding via Asn-21 main chain O, whereas the side chains of Glu95, Asp99, Asp101, Asn103, and Asp104 of loop CBL-2 are all involved in calcium-binding. Figure 3 shows the mode of calcium-binding in PA-IIL. In our modeled structures also we have found similar calcium-binding environment (Fig. 4). In Chromo the calcium-binding loop consists of the residues from 94 to 103, the residues involved in calcium binding are Asn-21 main chain O and the side chains of Glu94, Asp98, Asp100, Asn102, and Asp103. All the residues involved are identical to that of PA-IIL. The situation is identical in case of Bcepa and Methkand also. In Bcepa the calcium binding loop consists of the residues from 96 to 105 and the residues participating in calcium coordination are identical to that of PA-IIL (Asn21, Glu96, Asp100, Asp102, Asn104, and Asp105). Methkand although have less sequence similarity with PA-IIL but the residues forming the calcium binding loop are highly conserved. Here only the Asn residue is replaced by Lys21 and all the other residues are identical (Glu83, Asp87, Asp89, Asn91, and Asp92). But in Photorb due to the absence of three Asp residues, the calcium binding is somewhat affected. Two of the three Asp residues absent in Photorb are directly involved in calcium binding in PA-IIL. We have seen that the absence of these residues not only weakens the calcium-binding but also results in lower fucose-binding affinity.

α-L-fucose has been docked into the binding site of the modeled structures and the binding affinities are studied both in presence and absence of calcium. In all cases including the crystal structure of PA-IIL we have found that the binding affinity is decreasing in absence of calcium, suggesting an important role of calcium in carbohydrate binding. It is also seen beside their interaction with the protein molecule the O2, O3, and O4 atoms of fucose are also taking part in calcium coordination so the absence of calcium leads to lower binding affinity. Another reason is that in absence of calcium the calcium binding loop is deviated away from their position. Figure 5 shows the deviation of the calcium-binding loop in absence of calcium in one of the modeled structures. This deviation leads to less interaction of the protein molecule with fucose resulting in lowering of binding affinity. Table 1 shows the binding affinity of the crystal structure and the modeled structures in presence and absence of calcium. The electrical energy plays the major role in the binding affinity, and it is also interesting to note that in absence of calcium the van der Waals contribution remains more or less same where as the electrical energy is decreasing leading to the decrease of binding affinity. The binding affinity is found to be least in the case of Photorb, and the reason behind it is due

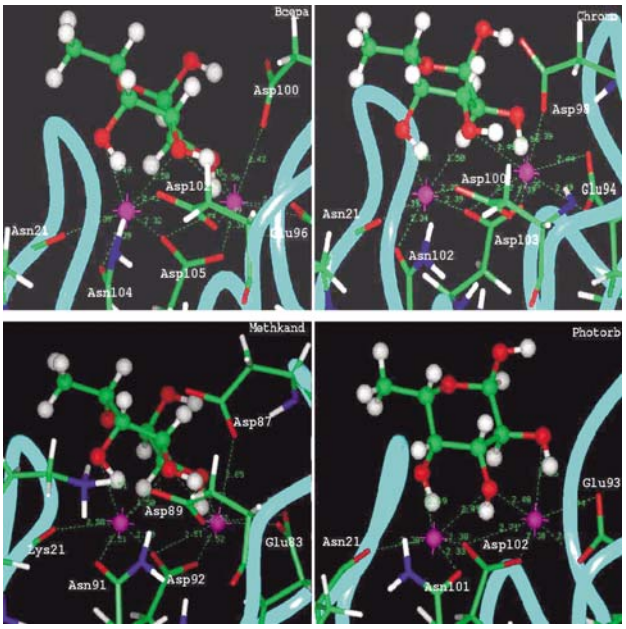

Fig. 4 Mode of interaction of Ca with protein and sugar molecule in the modeled structures. The residues of protein involved in coordination with the Ca ions are shown in stick representation. Six residues are involved in Ca binding in the case of Bcepa, Chromo and Methkand similar to that of PA-IIL whereas only four in the case of Photorb

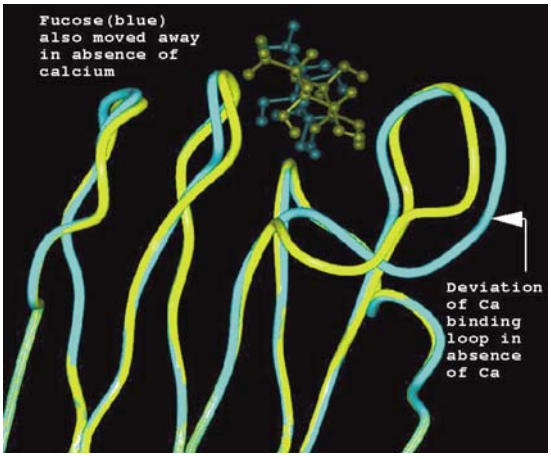

Fig. 5 Ribbon representation of the superposed form of a modeled protein (Chromo) in presence (yellow) and absence (light blue) of Calcium ions. The position of the ligand is also shown in balls-and-sticks representation in the presence (yellow) and absence (light blue) of calcium. The average deviation of the superposed structures is about 0.19 Å where as the deviation is 0.8 Å in the Ca-binding loop

to the absence of the three Asp residues which has mentioned earlier.

The fucose locks onto both calcium ions, unique among protein-carbohydrate interactions in PA-IIL, and this same mode of interaction is observed in the case of all the modeled structures. Three of the fucose hydroxyl groups participate in the coordination of calcium ions: O2 to calcium site 1, O4 to site 2, and O3 to both sites. Apart from their interaction with the calcium ions the fucose molecule is also interacting with the protein molecule via hydrogen bonding. The same three hydroxyl groups (O2, O3, O4) in addition to directly coordinating the calcium ions also hydrogen bond to acidic groups in the calcium-binding site. Important hydrogen bonding found to be highly conserved in all the modeled structures is the one formed with the main chain nitrogen of amino acid residue 23 with the fucose-ring oxygen. This residue is Ser in the crystal structure as well as in Bcepa where as it is Ala, Asn, and Gly in Chromo, Photorb and Methkand respectively. Other hydrogen bondings which are found to be conserved are HO2 and HO3 of fucose with the Asp residues present in the binding site. A hydrogen bond with the HO1 of fucose and the OG1 of Thr residue is found in all the modeled structures excepting Bcepa. In addition the fucose methyl group at position 6 creates hydrophobic

Table 1 Emperical binding energies of the complexes of proteins (crystal structure and the modeled structures) with fucose in presence and absence of calcium ions

	Binding Energies with the Ca^{2+} ions Kcals/mol			Binding Energies without involving the Ca^{2+} ions Kcals/mol		
Complexes	VdW*	Elec.*	Total	VdW*	Elec.*	Total
1. PA-III (1GZT in complex with α-L-fucose)	−16.14	−60.64	−76.78	−14.99	−41.49	−56.48
2. Chromo (Modeled protein from *Chromobacterium violaceum* in complex with α-L-fucose)	−14.86	−60.77	−75.63	−16.14	−38.40	−54.54
3. Bcepa (Modeled protein from *Burkholderia cepacia* in complex with α-L-fucose)	−13.45	−55.88	−69.33	−13.66	−43.89	−57.55
4. Photorb (Modeled protein from *Photorhabdus luminescens* in complex with α-L-fucose)	−15.74	−42.95	−58.69	−13.97	−33.62	−47.59
5. Methanopyrus (Modeled protein from *Methanopyrus kandleri* in complex with α-L-fucose)	−10.71	−58.63	−69.34	−13.66	−42.07	−55.73

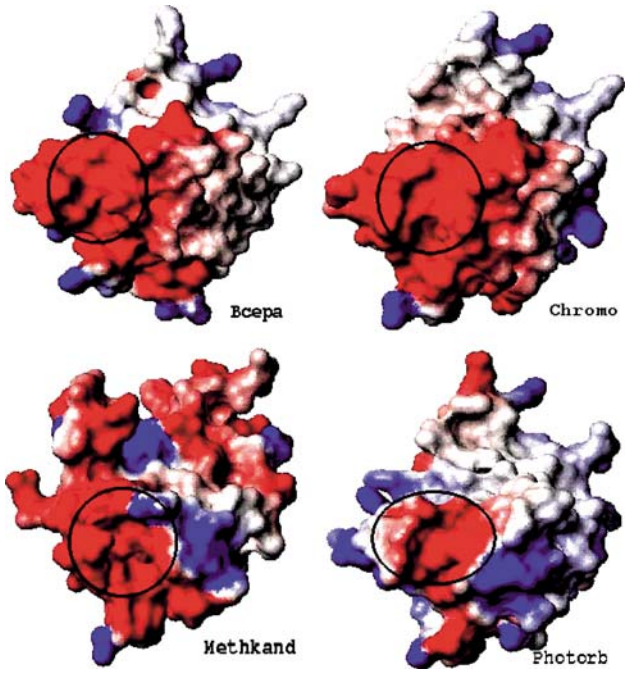

Fig. 6 Electrostatic potential surfaces of the modeled proteins. The blue region represents positively charged environment, red for negatively charged, and white for hydrophobic surroundings. The fucose binding site as well as the calcium binding site are shown by black circles

interactions with the methyl and hydroxymethyl group of Ser and Ala, respectively, and also with Thr in Bcepa, Chromo, and Methkand. This Thr residue is present in all the sequences excepting Photorb.

The study of electrostatic potential shows a highly negatively charged region around the binding site. This is obvious because the binding site is surrounded by acidic residues, mainly Asp. All the modeled structures show a similar environment although in case of Photorb the environment is slightly less acidic due to the absence of three Asp residues and in Methkand a small positively charged patch is observed due to the presence of Lys residue at 21 position. The electrostatic potential surfaces of the crystal structure of PA-IIL as well as the modeled structures are shown in Fig. 6. The highly acidic electrostatic potential surfaces suggest that, for this type of unique protein-sugar interaction mediated by two calcium ions, such an environment is necessary.

We have also made a comparative study of the binding affinities of α-L-fucose with α-D-mannose and β-D-fructose. The common factor in the structure of α-L-Fucose, α-D-mannose and β-D-fructose lies in their 2, 3, and 4 positions; 180° rotation of α-D-mannose gives the same conformation as α-L-fucose. Although the O2, O3, and O4 are atoms of the sugar are involved in coordination with calcium, still there is some difference in their mode of binding with PA-IIL. α-L-fucose forms the most stable complex with the protein (Table 2) due to its greater binding affinity. The cause of greater binding affinity of α-L-fucose may be due to the favourable hydrophobic interaction of the CH_3 group of fucose with the Thr (46) residue of protein leading to better stability of the protein-sugar complex.

Table 2 Comparative study of the binding of different sugars to the protein molecule

Protein	Sugar	Potential Energy of the complex in kcal.
1. PA-III (1GZT)	α-L-Fucose	−96.30
	α-D-Mannose	−88.97
	β-D-Fructose	−87.47
2. Chromo	α-L-Fucose	−91.05
(Modeled protein from	α-D-Mannose	−80.99
Chromobacterium violaceum)	β-D-Fructose	−75.25

Conclusions

We have modeled the structure of four fucose-binding lectins, taking PA-IIL (1GZT) as the template. Three of these hypothetical proteins were from bacteria eg. *Chromobacterium violaceum*, *Burkholderia cepacia*, and *Photorhabdus luminescens* subspecies Laumondii, and another protein from *Methanopyrus kandleri* which belonged to the archaea class. Another interesting finding is that the uncharacterized protein from *Methanopyrus kandleri* has less sequence similarity (46%) with PA-IIL but surprisingly, the acidic residues forming the calcium-binding loop are found to be conserved here. Our modeled structure of this hypothetical protein shows a high affinity for fucose also (−69.34 Kcal/mol). Thus it can be concluded that this hypothetical protein from the arachea fold in a manner similar to that of PA-IIL with a calcium mediated carbohydrate-recognition domain. The two calcium ions play a very important role in fucose binding in all the four modeled proteins similar to that of the PA-IIL. The O2, O3 and O4 of fucose are found to be involved in coordination with the two calcium ions. The acidic residues surrounding the binding site involving in coordination with the calcium ions forms a calcium-binding loop in PA-IIL. It is also found to be conserved in the modeled protein sequences, excepting *Photorhabdus* due to the absence of three Asp residues, and we have seen that this results in lower binding affinity (−58.69 Kcal/mol). The study of the binding energies without involving the calcium ions shows a decreased binding affinity in all cases. This is due to the absence of interaction of the three hydroxyl groups (O2, O3, O4) with calcium and also due to the deviation of the calcium-binding loops in absence of calcium leading to lesser protein-sugar interaction. The comparative study of the binding affinities involving the other two sugars α-D-mannose and β-D-fructose shows the

specificity of α-L-fucose toward binding PA-IIL as well as to the modeled proteins which may be due to the favorable hydrophobic interaction of the fucose-methyl group with the Thr residue. Finally, it can be emphasized that the knowledge of these modeled structures and particularly of their carbohydrate recognition domains may provide valuable information in developing potent therapeutics.

Acknowledgments This work was financially supported by the Mission Mode Project No CMM0017 of CSIR, India.

References

1. Mitchell, E., Houles, C., Sudakevitz, D., Wimmerova, M., Gautier, C., Perez, S., Wu, A.M., Gilboa-Garber, N., Imberty, A.: Structural basis for oligosaccharide-mediated adhesion of *Pseudomonas aeruginosa* in the lungs of cystic fibrosis patients. Nat. Struct. Biol. **9**, 918–21 (2002)
2. Barondes, S.H.: Bifunctional properties of lectins: lectins redefined. Trends. Biochem. Sci. **13**, 450–82 (1988)
3. Loris, R.: Principles of structures of animal and plant lectins Biochim. Biophys. Acta. **1572**, 198–208 (2002)
4. Gilboa-Garber, N.: Pseudomonas aeruginosa lectins. Methods Enzymol. **83**, 378–85 (1982)
5. Gilboa-Garber, N.: Inhibition of broad spectrum hemagglutinin from Pseudomonas aeruginosa by Dgalactose and its derivatives. FEBS Lett. **20**, 242–44 (1972)
6. Garber, N., Guempel, U., Belz, A., Gilboa-Garber, N., Doyle, R.J.: On the specificity of the D-galactosebinding lectin (PA-I) of Pseudomonas aeruginosa and its strong binding to hydrophobic derivatives of D-galactose and thiogalactose. Biochim. Biophys. Acta. **1116**, 331–333 (1992)
7. Chen, C.P., Song, S.C., Gilboa-Garber, N., Chang, K.S., Wu, A.M.: Studies on the binding site of the galactose-specific agglutinin PA-IL from Pseudomonas aeruginosa. Glycobiology **8**, 7–16 (1998)
8. Garber, N., Guempel, U., Gilboa-Garber, N., Doyle, R.J.: Specificity of the fucose-binding lectin of Pseudomonas aeruginosa. FEMS Microbiol. Lett. **48,** 331–34 (1987)
9. Mandal, C., Kingery, B.D., Anchin, J.M., Subramanium, S., Linthicum, D.S.: ABGEN: A knowledge based automated approach for antibody structure modeling. Nature Biotechnol. **14**, 323–28 (1996)
10. Mandal, C.: MODELYN – A molecular modelling program version PC-1.0 Indian Copyright No 9/98. (1998)
11. Thompson, J.D., Higgins, D.G., Gibson, T.J.: CLUSTALW: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. **22**, 4673–80 (1994)
12. Koradi, R., Billeter, M., Wuthrich, K.: Related Articles, MOLMOL: A program for display and analysis of macromolecular structures. J. Mol. Graph. **14**, 51–5 (1996)
13. Laskowski, R.A., MacArthur, M.W., Moss, D.S., Thornton, J.M.: PROCHECK: a program to check the stereochemical quality of protein structures. J. Appl. Cryst. **26**, 283–91 (1993)
14. Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J.: Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. **25**, 3389–02 (1997)
15. Huber R., Kurr, M., Jannasch, H.W., Stetter, K.O.: A novel group of abyssal methanogenic archaebacteria (Methanopyrus) growing at 110-degrees-C. Nature (London) **342**, 833–36 (1989)
16. Kurr, M., Huber, R., Konig, H., Jannasch, H.W., Fricke, H., Trincone, A., Kristjansson, J.K., Stetter, K.O.: Methanopyrus kandleri, gen. and sp. nov. represents a novel group of hyperthermophilic methanogens, growing at 110°C. Arch. Microbiol. **156**, 239–47 (1991)