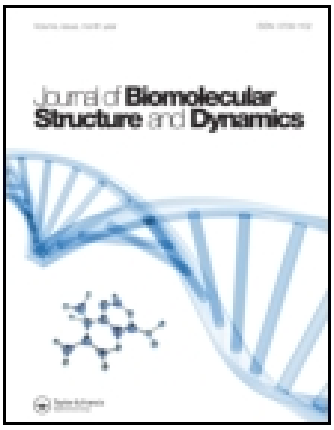

Journal of Biomolecular Structure and Dynamics
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tbsd20

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Published online: 21 May 2012.

To cite this article: M. S. Madhusudhan & Saraswathi Vishveshwara (1998) Modeling of Angiogenin - 3′-NMP Complex, Journal of Biomolecular Structure and Dynamics, 16:3, 715-722, DOI: 10.1080/07391102.1998.10508283

To link to this article: http://dx.doi.org/10.1080/07391102.1998.10508283

Journal of Biomolecular Structure & Dynamics, ISSN 0739-1102
Volume 16, Issue Number 3, (1998)

Modeling of Angiogenin - 3′-NMP Complex†

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http://www.albany.edu/chemistry/sarma/jbsd.html

Abstract

Angiogenin belongs to the Ribonuclease superfamily and has a weak enzymatic activity that is crucial for its biological function of stimulating blood vessel growth. Structural studies on ligand bound Angiogenin will go a long way in understanding the mechanism of the protein as well as help in designing drugs against it. In this study we present the first available structure of nucleotide ligand bound Angiogenin obtained by computer modeling. The importance of this study in itself notwithstanding, is a precursor to modeling a full dinucleotide substrate onto Angiogenin. Bovine Angiogenin, the structure of which has been solved at a high resolution, was earlier subjected to Molecular Dynamics simulations for a nanosecond. The MD structures offer better starting points for docking as they offer lesser obstruction than the crystal structure to ligand binding. The MD structure with the least serious short contacts was modeled to obtain a steric free Angiogenin - 3′ mononucleotide complex structure. The structures were energetically minimized and subjected to a brief spell of Molecular Dynamics. The results of the simulation show that all the ligand-Angiogenin interactions and hydrogen bonds are retained, redeeming the structure and docking procedure. Further, following ligand - protein interactions in the case of the ligands 3′-CMP and 3′-UMP we were able to speculate on how Angiogenin, a predominantly prymidine specific ribonuclease prefers Cytosine to Uracil in the first base position.

Introduction

Modeling of protein - ligand interaction is a challenging problem in computational biology. In this study we have modeled a mononucleotide ligand binding to Angiogenin. Angiogenin is a 14KD monomeric protein, structurally and sequentially similar to bovine pancreatic ribonuclease (RNase A). Though most of the RNase A active and binding sites are conserved in Angiogenin, it has a very weak RNase activity (10^5 times weaker than RNase A in terms of specificity constant) (1). This weak activity seems to be crucial for the main biological function of Angiogenin - angiogenesis. A dual site model for angiogenesis based on biochemical evidences proposes that along with the RNase active and binding sites, an endothelial cell binding site is also vital for the proteins activity (2). These sites have been identified in the structures of Bovine and Human Angiogenins (3-6). There are not any structures of Angiogenin complexed with its substrates or their analogs. A ligand bound structure of Angiogenin would lead to a more comprehensive approach to designing drugs and inhibitors against Angiogenin, a key ingredient in the proliferation of cancer cells.

Superimposing the ligand onto the crystal structure of Angiogenin from a crystal structure where RNase A is complexed to the ligand leads to a sterically unfavourable structure (3) (Figure 1). Even though there is good conservation between Angiogenin and RNase A, especially in the active and binding site regions, the ligand encounters obstructing residues. The chief obstructer being E118, whose side-chain passes through the base of the ligand. In a recent study we explored the conformational versatility of uncomplexed, native Human and Bovine Angiogenins, by subjecting

†This article is dedicated to David Beveridge on the occasion of his 60th birthday.

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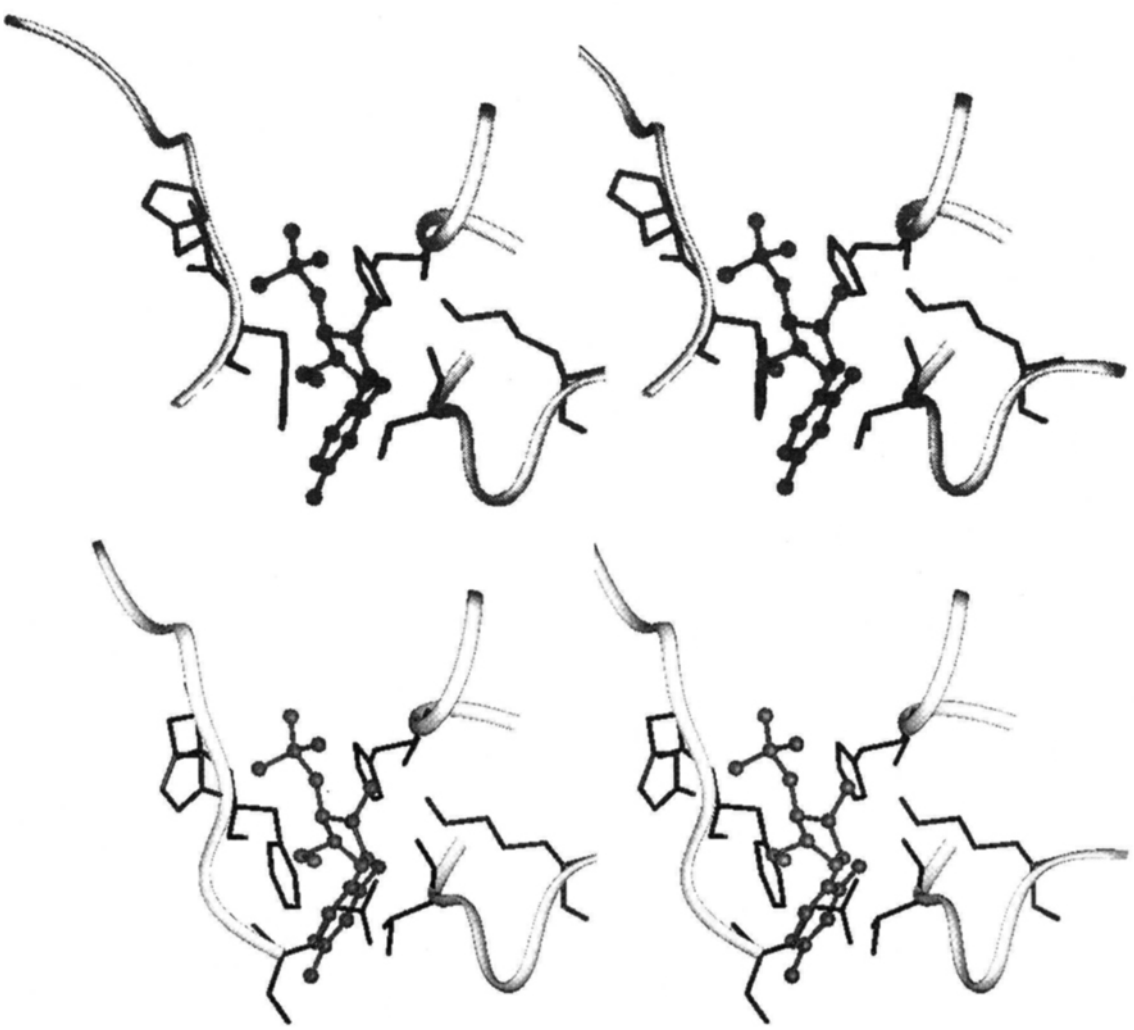
their crystal structures to a nanosecond of Molecular Dynamics (MD) (7). The proteins took on no such conformation that would allow steric hindrance free ligand binding. The seriousness of the bad contacts as compared to the crystal structure were however alleviated. We therefore took one of the MD structures as the starting structure for docking studies. The main chain of the C-terminus was modeled to alternate conformations to abet ligand docking. This was partially inspired from MD studies which showed large RMS fluctuation of the C-terminal segment. Using a combination of MD and systematically altering the side chain and main chain torsion angles of obstructing residues of the protein along with manipulating the ligand position we have arrived at a structure of Angiogenin that accommodates a mononucleotide ligand in a steric free conformation. Most importantly this conformation retains most of the RNase A - ligand like interactions and hydrogen bonds.

Methods

Docking Procedure

The crystal structure and snapshots from the MD simulations were superimposed on the RNase A - 3'-CMP complexed structure (pdb code IRPF (12)). We considered 100 snapshots from the MD simulation and the MD average structures(7) for superposition. Only select active and binding site residues (H14,T45, K41, and H115) were considered for superposition. All superimpositions were done using Kearsley's method (8). Severe short contacts are obtained when 3'-CMP is superposed on the X-ray structure (Figure 1). These short contacts arose chiefly due to the residue E118 of the C-terminus. The seriousness of the short contacts reduced



1a

1b

Figure 1: Stereo plot of the active site of a) RNase A - 3'CMP complex crystal structure and b) 3'CMP position superimposed on the X-ray structure of Bovine Angiogenin. The ligand is shown in Ball and stick representation and the protein residues His14, Lys41, Thr45, His115, Phe116 and Glu118 in stick representation. The residues are labeled in Figure 3b.

in the MD structure, but were not completely eliminated. One of the structures, which had the least steric contact with the ligand, was considered for further modeling. From the selected complex structure, a systematic conformational search was carried out to remove short contacts by varying the active site residues F116 and H14 side chain torsion angles in steps of 4 degrees and the orientation of the C- terminal segment starting from the 118th residue (ψ of 117) in steps of 1 degree. We were able to group the contact free conformations into two distinct ranges of parameters. Of these two ranges only one was selected for further investigations for reasons discussed in the results section.

In a representative structure of the selected range, the ligand position was translated in all the three directions in steps of 0.1Å to optimize protein-ligand hydrogen bonds and their distances.

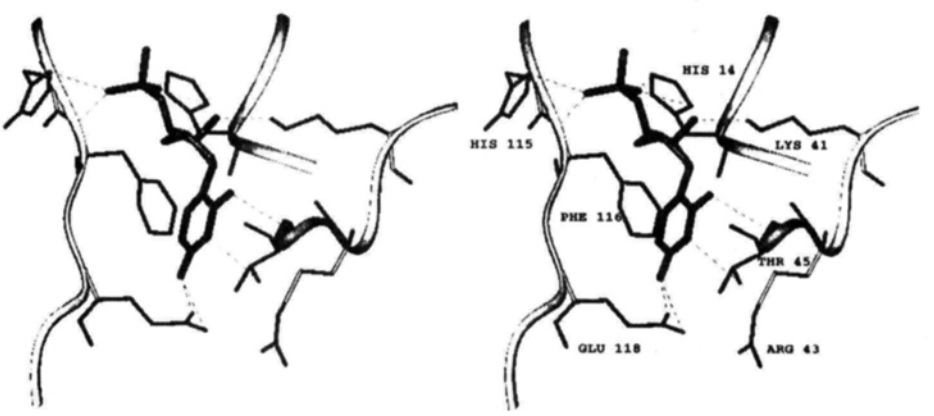
The best-bound structure was energy minimized and subjected to molecular dynamics simulations.

Molecular Dynamics Protocol

The protocol used for Molecular Dynamics was similar to the one used earlier and reported elsewhere (7). The protein was put in the centre of a box with dimensions (58.4 × 52.5 × 42.5) Å^3. 3246 waters solvated the Angiogenin - 3′-CMP complex while 3189 waters solvated the 3′-UMP complex. The modeled structure was first subject to 1000 steps of energy minimization. The minimized structure was then equilibrated in the NVT ensemble for 20ps by coupling it to a heat bath (9) at 100 and 200° C for 4ps each and at 300° C for 12ps. NVE ensemble dynamics was then carried out for a further 80ps. PME (10) was used to evaluate electrostatics and non-bonded interactions were calculated within a cut-off of 12Å. Minimization and MD were carried out using the AMBER4.1 suite of programs and the Amber4.0

Figure 2: Superimposition of the C$^\alpha$ traces of the Bovine Angiogenin crystal structure and the modeled structure used in the docking studies. The dashed line connects the residues E118 of both structures, separated by a distance of 3.27Å.

3a



3b

Figure 3: Stereo plot of a) modeled Angiogenin-3′CMP complex and b) active site details of a). The ligand is represented in thick lines and the protein residues in thin lines.

force field parameters (11) on an SGI power challenge parallel computing machine. All modeling and analysis programs were developed by the group and are written in FORTRAN 77.

Results and Discussion

3'-CMP-Angiogenin Complex

a) Docking

As mentioned earlier, the direct superposition of 3'-CMP structure from the RNase A-CMP complex(3) onto the X-ray structure of bovine Angiogenin led to severe steric clashes and the seriousness of the short contact reduced when the Angiogenin structures from MD simulation were considered. An exploration of the conformations of the C-terminal segment and the active-site residues gave rise to contact free positions of 3'-CMP retaining many of the RNase A-ligand hydrogen bond interactions. The results of this search are presented in Table I. An important feature is that it was necessary to move the C-terminal segment to remove the short contact between E118 and the ligand. A small shift of this segment however was sufficient to achieve this goal. Although the search yielded another C-terminal conformation, which gave rise to contact free ligand position, changing the value of ψ117 around 180° places the C-terminal segment in an orientation which is drastically different from that of the original structure. Hence this conformation was not considered for further investigation. A comparison of the x-ray and the modeled structures is given in Figure 2. Stereo plots of the docked 3'-CMP-Angiogenin complex in full view and the active site are shown in Figures 3a and 3b respectively. As a consequence of modeling the C-terminus, the hydrogen bond that held together the side chains of E118 and T45 is broken. This in turn frees the side chains of these two residues to interact with the ligand through hydrogen bonds.

Table I

Conformational changes modeled in Angiogenin steric hinderance free docking of ligand 3'CMP.

	ψ Asp 117	χ^1 His 14	χ^1 Phe 116
X-ray structure	96.0°	-61.8°	-179.6°
Starting MD structure	153.3°	-54.2°	99.9°
Modeled structure I*	169 .3° to 179.3° (173.3°)	-28.5° to- 52.5° (-40.5°)	-137.7° to- 205.7° (-171.7°)
Modeled structure II	336° to 350°	"	"

*The allowed range of parameters is given and the values in paranthesis were chosen for minimization and then subjected to MD simulations.

b) MD Simulation of the Complex

MD simulations for 100 ps was carried out on the docked 3'-CMP-Angiogenin complex. The ribose of the ligand fluctuated between C2' exo (starting confornmation) and C3' endo during MD simulations. The glycosidic torsion angle χ^1 fluctuated about 15° about the starting structure value of -166°. The variations in different parts of the protein structure with respect to the x-ray structure and the Angiogenin MD structure chosen for docking are presented in the RMSD plots (Figure 4).

The most significant fluctuation can be seen at the C-terminal segment. The backbone of this segment in general has moved by about 2-3Å with respect to both X-ray and MD structures. The residues 118-120 have moved by about 3Å and 4Å away from the MD and the X-ray structures respectively. The loop regions contribute significantly to the RMS fluctuation. It is interesting to note that many of the solvent exposed basic residues (18K, 35R, 38R, 51K, 67R and 90R) exhibit large RMSD. The residue 67R is a part of RGD segment implicated in receptor binding (4) and may interact with the second base of a dinucleotide

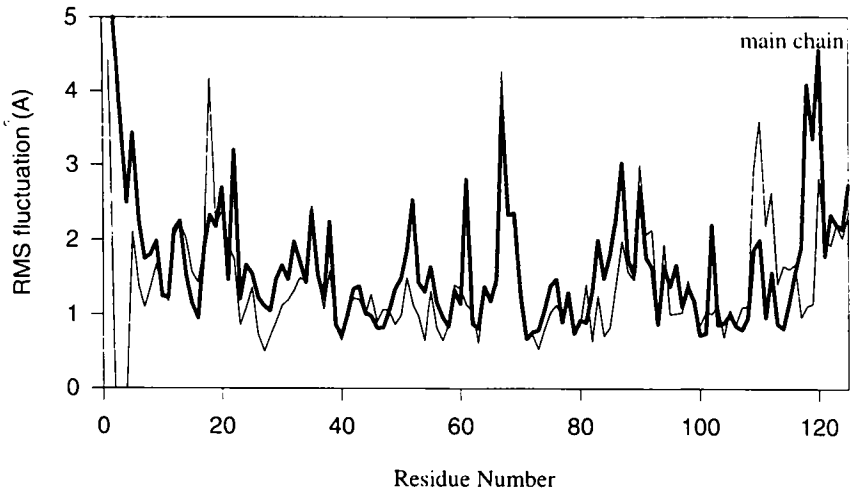


Figure 4: Residue-wise RMS fluctuations of Angiogenin 3′CMP complex with respect to minimized modeled structure (thin line) and with respect to the crystal structure (thick line).

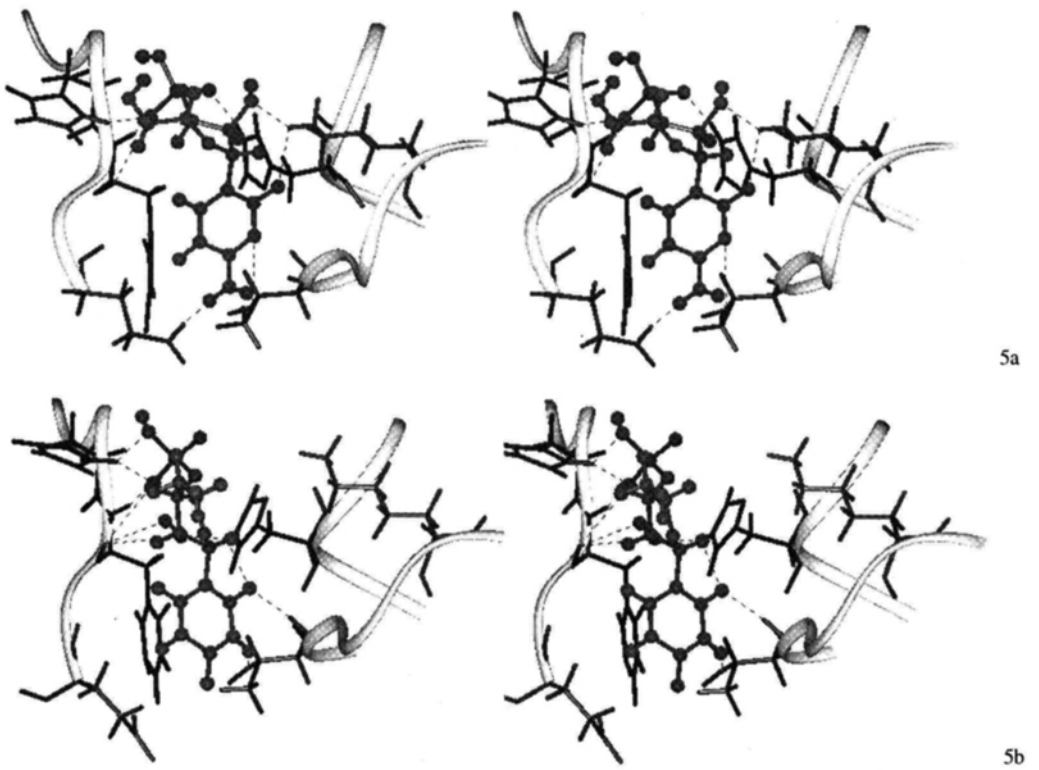


Figure 5: Stereo plot of the active site in a typical structure obtained during MD simulation of a) Angiogenin - 3′CMP complex and b) Angiogenin - 3′UMP complex. The ligand is shown in a thicker stick representation than the protein residues. The residues are labeled in Figure 3b.

ligand. The side chain of R67 hydrogen bonds to the side chain of E109, a residue also known to interact with the second nucleotide of the ligand. During the course of dynamics of the native protein there was a stable hydrogen bond between the side chain of R67 and the side chain of D117. This is now broken during the MD simulation of the Angiogenin-3′-CMP complex. A stereo view of a typical snapshot of the active site from the 3′-CMP-Angiogenin simulation is shown in Figure 5a. The trajectories of the hydrogen bonds and their stability can be seen in Figure 6. All the hydrogen bonds found in the case of RNase A-3′-CMP complex (12) are also formed in the complex with Angiogenin. T45 backbone N and the side-chain Oγ1 atoms form hydrogen bonds respectively with O2 and N3 atoms of the base. The interaction of 2′-OH of ribose with Nζ of K41 and OP2 with Nε2 of H14 is retained in the same way as in RNase A. The phosphate interaction with H115 and F116 are also retained. The basic difference is that the N4 of base in RNase A interacts with S123 via water mediated hydrogen bond, whereas the once obstructing E118 now interacts directly with this N4 group of the base. The aromatic ring of F116 interacted with the base of the nucleotide as in the case of RNase A.

3'-UMP- Angiogenin Complex

Docking of 3'-UMP to Angiogenin was initially effected by the same procedure as that of 3'-CMP by changing the -NH2 group on C4 of the base to =O. Although this gave rise to a contact free position for 3'-UMP as in the case of 3'-CMP, MD simu-

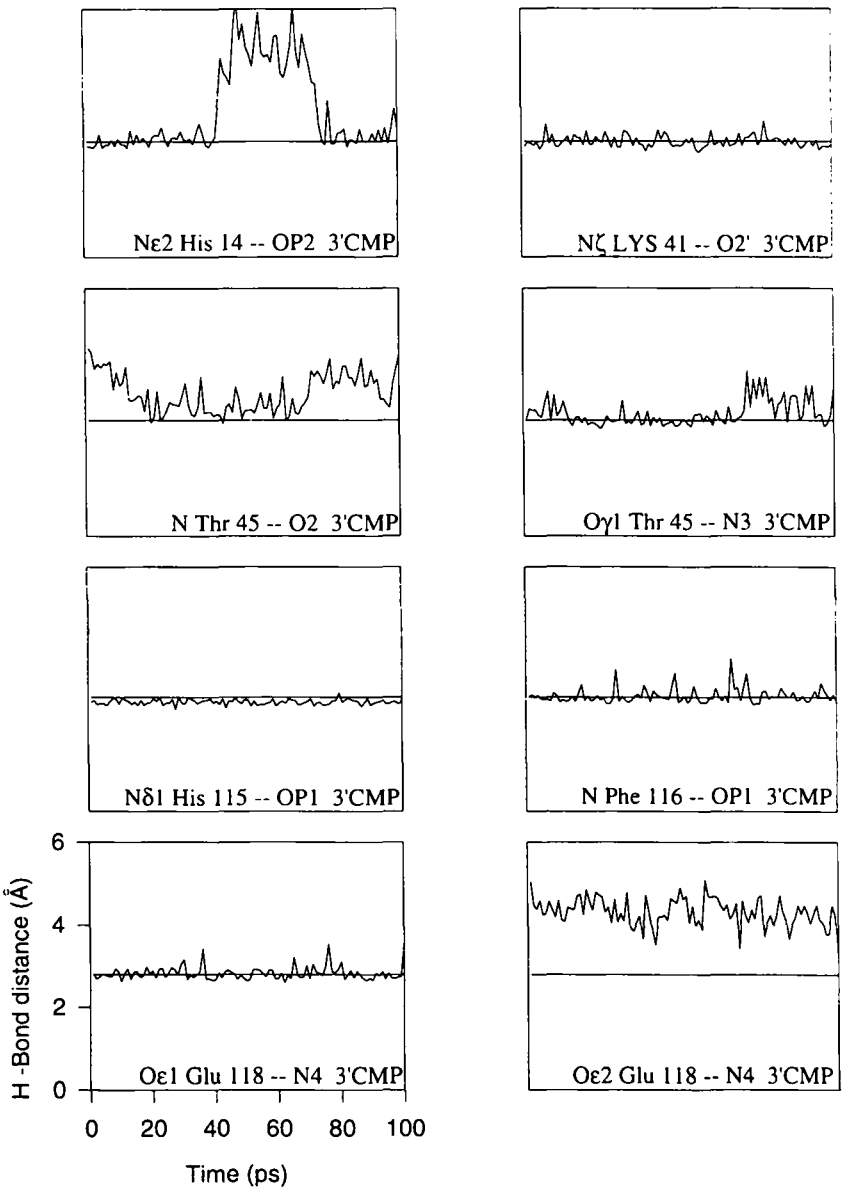


Figure 6: Hydrogen bond trajectories between Angiogenin and 3'CMP.

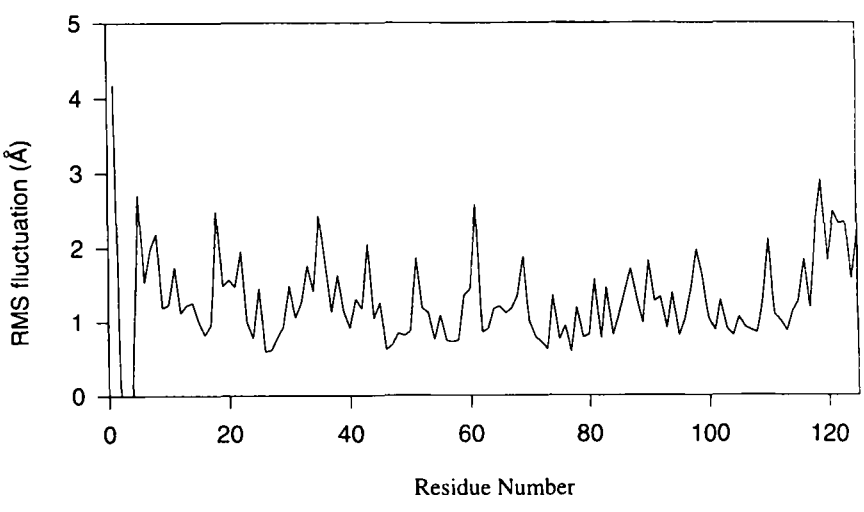


Figure 7: Residue-wise RMS fluctuations of Angiogenin 3'UMP complex with respect to minimized modeled structure.

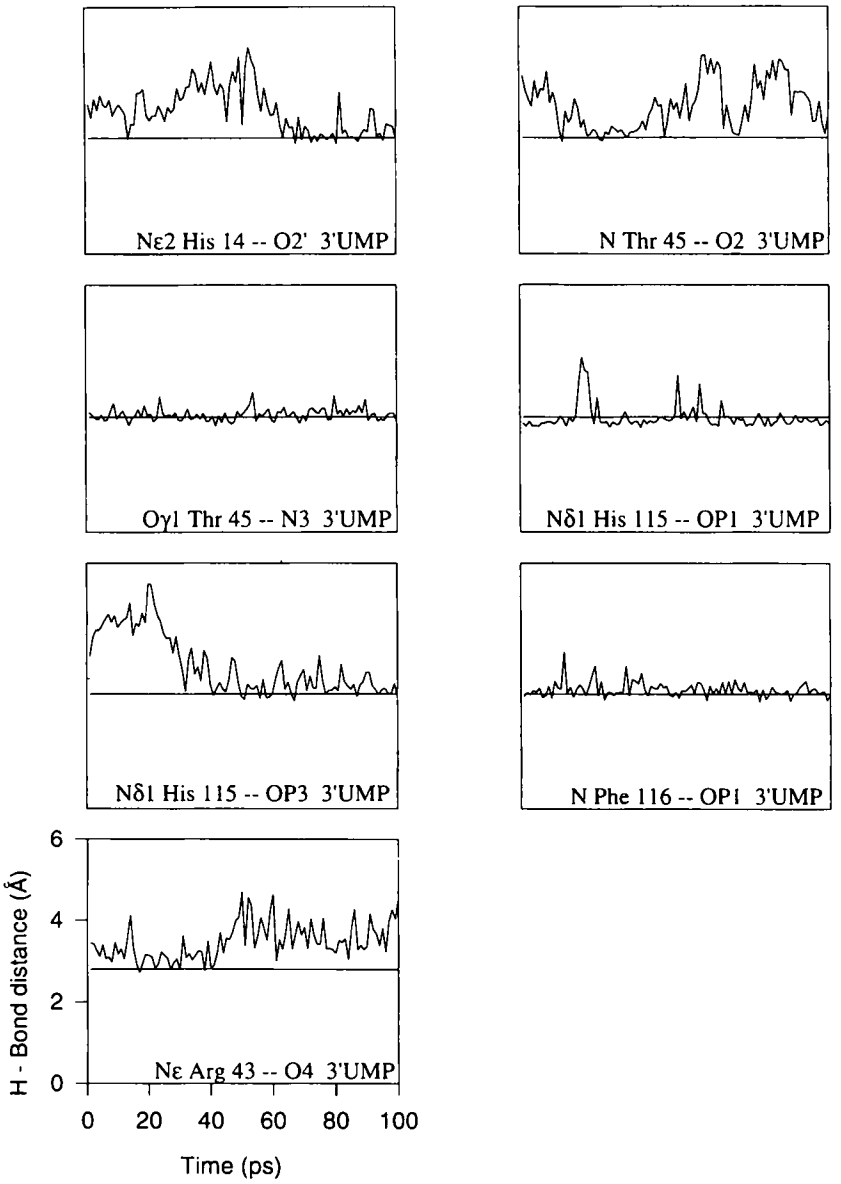


Figure 8: Hydrogen bond trajectories between Angiogenin and 3′UMP.

lation of this modeled complex did not proceed beyond 40ps. An analysis of the final structure of this simulated complex indicated the movement of the ligand from the active site, disrupting most of the good protein-ligand hydrogen bonds. Another simulation was set up by using a starting Angiogenin conformation which was obtained after 2ps of dynamics of the 3′-CMP complex simulation. The MD simulation of this complex proceeded without any difficulties. A stereo plot of the active site of a typical structure obtained during this simulation is shown in Figure 5b. The ligand assumed approximately the same conformation as in the case of 3′-CMP complexed structure simulation. However the average value of the glycosidic torsion angle was 20° away from that of the starting structure value. The residue-wise RMSD of the complex with the starting structure is given in Figure 7. Unlike the 3′-CMP complex simulation there are no distinct peaks in the RMS fluctuation plot. This could have implications on the binding of dinucleotide ligands. In the case of 3′-CMP the RGD loop showed large fluctuations hinting at conformational diversity of the region and one of these conformations might interact with the second nucleotide base. In the case of 3′-UMP the range of conformations sampled by this loop is much smaller than in the 3′-CMP complex. The protein-ligand hydrogen bond trajectories given in Figure 8 further the base specificity argument. E118, which in the case of 3′-CMP hydrogen bound to N4 of the base, does not in the case of 3′-UMP bind to the equivalent O4 position. Although both bases can be fitted in the same location of the active site, their

electrostatic interactions are different. This observation is in accord with experimental data that show Angiogenins' preference to Cytosine over Uracil in the first base position to be about one order of magnitude (in units of specificity constant) (13).

Conclusions

We have for the first time modeled a nucleotide bound structure of Angiogenin. The structures extracted at various time points of the simulation of the free native Angiogenin proved to be a better starting structure for docking than the crystal structure. This starting structure was further modeled to obtain a steric free docking of the ligand by changing the conformation of the C-terminal and the side chains of a few obstructing residues. The models were subjected to energy minimization and 100ps of MD. The docked ligands 3′-CMP and 3′-UMP retained most of the RNase A - ligand type of interactions and Hydrogen bonds. T45 Oγ1 hydrogen bound to N3 of the base, Nε2 and Nd1 of His 14 and His 115 respectively had interactions with the phosphate oxygens and the side chain of Lys 41 hydrogen bound to the ribose. In addition to these interactions, Glu 118 (a residue whose side chain sterically obstrucuts ligand in the unmodeled structure) is hydrogen bound to N4 of the 3′-CMP base. This interaction is absent with the equivalent atom O4 of the 3′-UMP ligand. This difference in hydrogen bonding could play a major role in substrate specificity of the protein. Our results concur with experiments that suggest better binding to Cytosine than to Uracil.

The fluctuation of the RGD loop during the course of dynamics of 3′-CMP complexed modeled structure could have implications on second nucleotide binding as this loop region lies in the approximate region where RNase A has its second nucleotide binding sites. The role of water mediated interactions, longer simulations and dinucleotide docking could give further leads to the mechanism of Angiogenin's activity.

Acknowledgements

Saraswathi Vishveshwara has a long association with Professor David L. Beveridge as his graduate student and as a visiting scientist. It is indeed a great pleasure to thank Professor Beveridge for initiating us to the field of Biocomputing and for his constant encouragement over the period of years which has enabled us to carry out state of the art computing in our laboratory. We thank the Supercomputer Education and Research Centre of the Indian Institute of Science for computing facilities

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Date Received: September 28, 1998

Communicated by the Editor Ramaswamy H. Sarma