Symposium date: 27th September 2009

doi:10.1093/nass/nrp137

Docking simulation of polyamines on a kissing-loop RNA dimer

Misaki Imai[1*], Daisuke Chikatsu[1], Emire Inomata[1], Tairo Oshima[2] and Gota Kawai[1*]

[1]Department of Life and Environmental Sciences, Chiba Institute of Technology, 2-17-1 Tsudanuma, Narashino-shi, Chiba 275-0016 and [2]Institute of Environmental Microbiology, Kyowa-kako Company, 2-15-5 Tadao, Machida, Tokyo 194-0035, Japan

ABSTRACT

Polyamines, especially branched polyamines such as tetrakis(3-aminopropyl)ammonium (Taa), stabilize the tertiary structure of RNA molecules.

In this study, we examined the polyamine binding site of the HIV-1 dimerization initiation site (DIS) in the kissing-loop dimer by the docking simulation. It was found that Taa binds predominantly to the kissing loop interaction site of DIS.

INTRODUCTION

Polyamines are known to stabilize the structure of nucleic acids in living cell. Especially, Tetrakis(3-aminopropyl)-ammonium (Taa) from *Thermus thermophilus* has branched structure and is thought to stabilize the tertiary structure of RNA molecules (Fig.1A) (1). Taa has been shown to stabilize the tRNA (1) as well as an RNA kissing dimer of the dimerization initiation site (DIS) from the HIV-1 genomic RNA (to be published).

HIV-1 DIS is a highly conserved sequence in the 5'-leader of the HIV-1 genomic RNA. As shown in Fig. 2, DIS has a nine nucleotide loop with a 6 nt self-complementary sequence and forms a metastable kissing-loop dimer that is converted into a stable extended-duplex dimer. It is known that HIV-1 genomic RNA forms a dimer in two steps and it is believed that the two conformations of genomic RNA dimers are corresponding to the two types of dimers of DIS. However, the mechanism of the conformational conversion is not known yet.

As described above, our previous study by gel shift assay suggested that Taa stabilized the kissing-loop dimer of DIS. Furthermore, Taa stabilizes the kissing-dimer more efficiently than caldopentamine (Cdp) which is a liner polyamine also found in *T. thermophilus* (Fig. 1B). In order to elucidate how Taa stabilizes the kissing-loop dimer, we examined the binding pocket of the kissing-loop dimer and, then, docking simulation was applied for the polyamines and the DIS kissing-loop dimer system. It was found that polyamine predominantly bound to the kissing-loop interaction site of DIS.

RESULTS AND DISCUSSIO N

All results shown here were obtained by a simulation software ICM-pro (Molsoft L.L.C.), which is known as BioPackage in Japan.

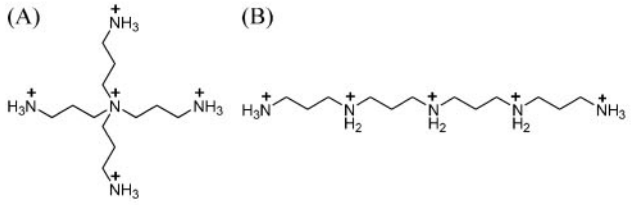

Fig. 1 Polyamines found in *Thermus thermophilus* (1). (A) Taa and (B) Cdp.

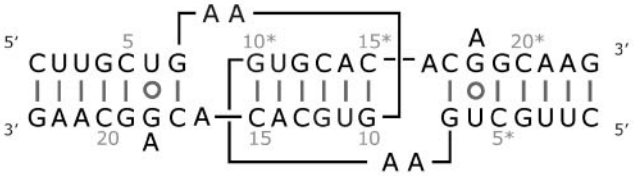

Fig. 2 Secondary structure of the HIV-1 DIS kissing-loop dimer. The position 18 is A or G depending the HIV-1 strain.

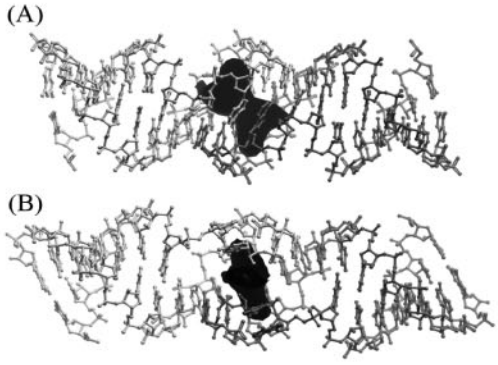

Fig. 3 Predicted binding pockets of the DIS kissig-loop dimers. (A) Solution structure (PDB ID: 2D1B) and (B) crystal structure (PDB ID: 1XPE). The position 18 is G and A for the solution and crystal structures, respectively.

Binding pocket of the HIV-1 DIS kissing-loop dimers

Several structures of HIV-1 DIS have been registered in Protein Data Bank (PDB). In this simulation, we used two different structures as the receptor; a solution structure (39 nt, PDB ID: 2D1B) and a crystal structure (23 nt, PDB ID: 1XP7) (2, 3). These two structures are different to each other in the conformation of A8 and A9. For the solution structure, a 23 nt region corresponding to the crystal structure was used for the analysis.

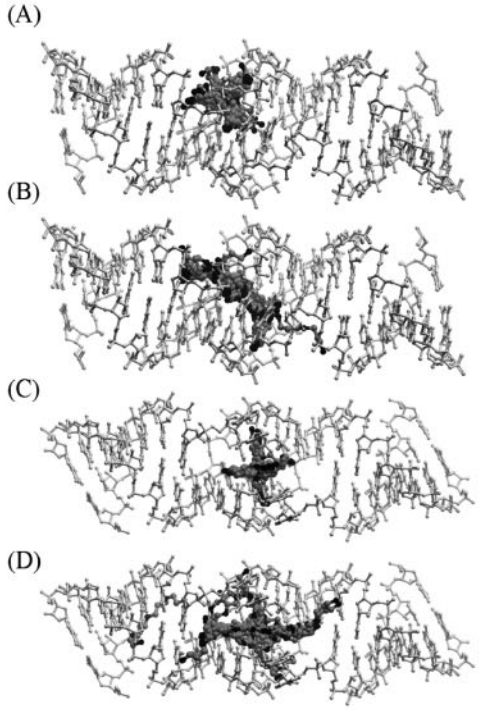

Fig. 4 Result of the docking simulation. Taa (A, B) and Cdp (C, D) for ligand and the solution structure (A, C) and the crystal structure (B, D) for acceptor. For each combination, results of 30 trials were superimposed.

For both structures, binding pockets were predicted inside the kissing-loop interaction site; major groove of the intermolecular stem (Fig. 3). These results suggest that the kissing-loop interaction site can be the binding site of the polyamines and such interaction may stabilize the kissing-loop dimer.

Docking simulation of polyamines and the HIV-1 DIS kissing-loop dimers

Two different polyamines, Taa and Cdp (Fig. 1), were used as the ligand. The solution and crystal structures of the kissing-loop dimer described above were used as the acceptor.

In most cases, polyamines bind to the binding pocket for each combinations of polyamine and DIS. Taa was found to bind specific position for each the structure (Fig. 4A, C). However, the positions are slightly different to each other. This is probably due to the difference of the RNA structures and further analysis such as molecular dynamics simulation may be required. Cdp also bound to the binding pocket (Fig. 4B, D). However, because of the linear shape, the edge of the Cdp molecule sometimes reached the stem region. Again, the bound positions are slightly different between the two RNA structures.

These results suggest that polyamines, especially Taa, bind the kissing-loop interaction site and stabilize the DIS kissing-loop dimer.

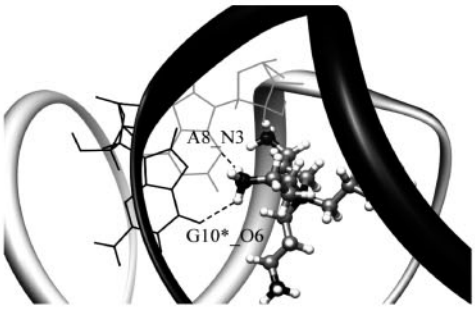

Fig. 5 Interaction between Taa and HIV-1 DIS kissing-loop dimer.

Predicted interaction between Taa and the HIV-1 DIS kissing-loop dimer

It was suggested by the docking simulation that Taa interacted with the neigh boring flanking bases, especially G10. It is notable that the smaller polyamine, spermidine, also binds to the bulge-out residues in the RNA molecule (4). Furthermore, the amino groups of polyamine interacted with the phosphate groups of RNA backbone as well as the nitrogen and oxygen atoms of the bases. Probably, the amino groups of Taa suppress the repulsion among the phosphate groups of RNA backbone and stabilize the kissing-loop structure. The interaction between amino groups of Taa and the nitrogen and oxygen atoms of flexible residues, such as G10 and A8, in RNA may also stabilize the structure (Fig. 5). To clarify the interaction between DIS and polyamine precisely, further analyses including the NMR and the MD simulation are in progress.

CONCLUSION

Present analyses clearly indicated that Taa binds to the kissing-loop interaction site and stabilizing the interaction.

REFERENCES

1. Terui. Y., Ohnuma. M., Hiraga. K., Kawashima. E., Oshima. T. (2005) *Biochem. J.*, **388**, 427–433.
2. Baba. S., Takahashi. K., Noguchi. S., Takaku. H., Koyanagi. Y., Yamamoto. N., Kawai. G. (2005) *J. Biochem.*, **138**, 583–592.
3. Ennifar. E., Dumas. P. (2006) *J. Mol. Biol.*, **356,** 771–782.
4. Higashi, K., Terui, Y., Inomata, E., Katagiri, D., Nomura, Y., Someya, T., Nishimura, K., Kashiwagi, K., Kawai, G., Igarashi, K. (2008) *Biochem. Biophys. Res. Commun.*, **370**, 572–577.

*Corresponding author. E-mail:
gkawai@sea.it-chiba.ac.jp