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Molecular modeling on DNA cleavage activity of seryl-histidine and related dipeptide

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Abstract—Molecular interaction between seryl-histidine (or seryl-histidine related dipeptides) and DNA model molecule 5′-TpTpdC-3′ was studied by a molecular docking procedure. The key structural features of DNA cleaving activity were investigated.

Previous studies in our laboratory have shown that dipeptide seryl-histidine (Ser-His) and related oligopeptides can cleave multiple categories of natural substrates, such as DNA, protein, and the ester *p*-nitrophenyl acetate (*p*-NPA), with the hydrolysis mechanism.[1,2] It has proved that the hydroxyl functional group of serine and the imidazole functional group of histidine, which serve as a nucleophile and an electrophile, respectively, are the requisite groups for cleavage.[3–5] Our research demonstrated that the function of Ser-His was similar with that of diverse enzymes, such as serine protease and elastase, in which the active sites are also mainly composed by serine and histidine. Ser-His, as an enzyme mimics, is attracting more and more attention for its special biological functions.[6,7] In this article studies have been performed on DNA model molecule 5′-TpTpdC-3′ in complexes with Ser-His dipeptide or other modified dipeptides exhibiting different cleaving activities. This work intends to reveal the key structure features for DNA cleavage activity. The tested peptides along with their DNA cleavage activities are listed in Table 1.

All molecular modeling studies were performed on a Silicon Graphic O2 computer running Tripos SYBYL 6.7 software.[8] The Tripos force field implemented in SYBYL was used. All atomic charges were computed using Gasteiger–Huckel method. Amide bond was set to be *trans*.

Table 1. Tested dipeptides and DNA cleavage activities

Peptide	DNA cleavage activity[8]
Ser-His	+++++[a]
Cys-His	++
Thr-His	±
Asp-His	–[a]
Ser-Arg	–
His-Ser	–

[a] '–' Stands for no detectable cleavage activity; each '+' stands for approximately 20% of Ser-His cleavage activity. '±' stands for marginally detectable cleavage activity.

Conformational searches were performed on interested peptides with the use of random search module implemented in SYBYL. For each search, 1000 starting structures were generated and minimized until the gradient was less than 0.05 kcal/mol/Å^{-1}. All heavy atoms were used for comparing a minimized structure with all previous unique minima, and structures were considered the same unless the least squares superimposition of the compared atoms found one or more pairs of equivalent atoms separated by more than 0.2 Å. Duplicated structures as defined above were recorded by hits times, and the MAX-HITS number was set to 6. Conformations with energy greater than 10 kcal/mol above the global minimum were discarded. The rotatable bonds were changed randomly during the search.

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The structures of the located typical low energy conformations of the Ser-His and related peptides are shown in Figure 1.

Conformational analysis shows that the stable conformations of Ser-His or related dipeptides can adapt ether folded or extended shapes, implying that Ser-His and related dipeptides are very flexible. The flexibility of the dipeptides provides them with the ability to complexes with a wide range of substrates that possess complementary structures. On the other hand, the flexibility of the dipeptides prevent them from forming complexes with substrates firmly or specifically because they are not rigid enough. This may be one of the reasons that the DNA cleavage by Ser-His is buffer dependent, and that buffer molecules such as citric acid/citrate or Tris can inhibit the activity of Ser-His by competitively interacting with DNA.[5,9]

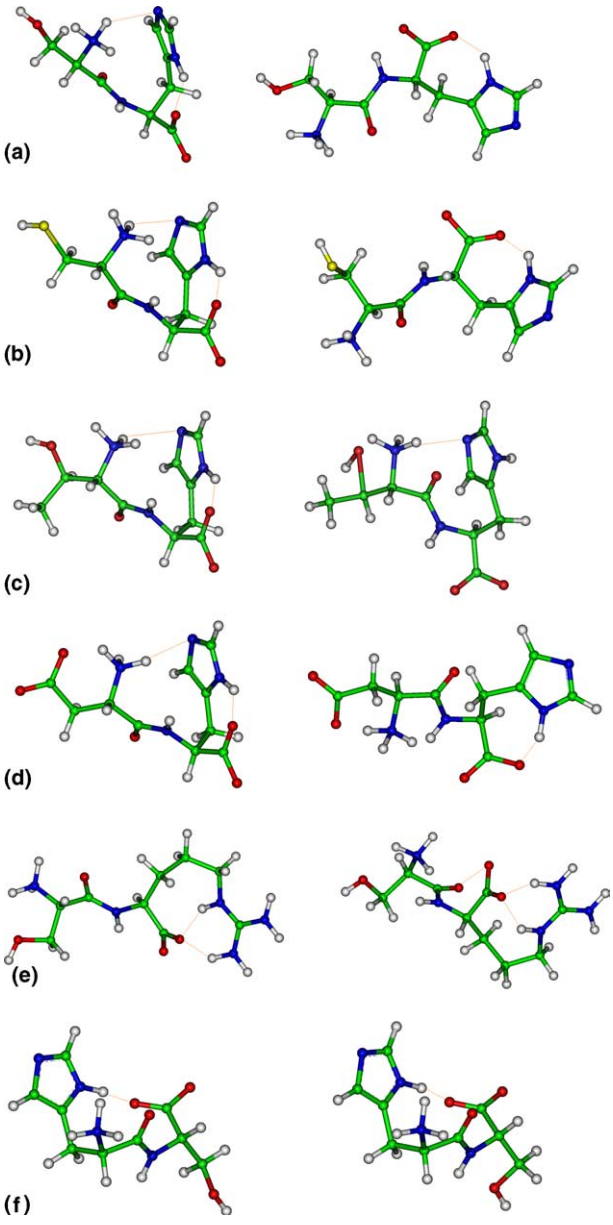

Figure 1. The structures of the located typical low energy conformations of the Ser-His and related peptides: (a) Ser-His, (b) Cys-His, (c) Thr-His, (d) Asp-His, (e) Ser-Arg, and (f) His-Ser. H-bonds are marked by yellow lines.

To reveal the key structure features for DNA cleavage, a series of three-dimensional structure of peptide···5′-TpTpdC-3′ complexes has been constructed using the molecular docking program FLEXIDOCK encoded in SYBYL; and the binding energy between each pair of these peptides and the oligonucleotide has been calculated with Tripos force field. The standard B-DNA double helix of 5′-TpTpdC-3′ was built as model DNA molecule by BUILT/BIOPOLYMER module implemented in SYBYL, and was fixed in space. In contract, the structures of dipeptides were chosen from the correspondent global minima, and allowed to be relaxed flexibly during docking procedure. The rotations were applied to all rotatable bonds of dipeptides. Initial binding geometries between each peptide and 5′-TpTpdC-3′ were scanned and selected by using DOCK module implemented in SYBYL. Then, the docking studies were manually carried out with the FLEXIDOCK algorithm of SYBYL using Tripos force field, which integrates the van der Waals, electrostatic, torsional, and constraint energy terms. The binding energy between DNA and each dipeptide was estimated by simply subtracting the conformational energy of the free DNA and the isolated peptide. The global minimum energy obtained was used as the conformational energy of the isolated peptide. The binding energy was calculated with equation:

$$E_{\text{binding}} = E_{\text{complex}} - (E_{\text{peptide}} + E_{\text{DNA}})$$

where E_{complex} is the total energy of the complex derived from docking, E_{peptide} is the energy of the global minimum conformation of dipeptide, and E_{DNA} is the total energy of the free oligonucleotide 5′-TpTpdC-3′, $E_{\text{DNA}} = 351$ kcal/mol. The binding energies of tested peptides with 5′-TpTpdC-3′ calculated according to above equation are summarized in Table 2. The major structure features in the complexes are summarized in Table 3. The structures of the complexes chozen through performing FLEXIDOCK are displayed in Figure 2.

All the peptides that can cleave DNA have negative binding energies. The higher the cleaving activity, the lower the binding energy value. For example, Ser-His with highest DNA cleavage activity correspondents to a lowest binding energy value (−65 kcal/mol), whereas dipeptide Asp-His, with no detectable cleavage activity, correspondents to a positive binding energy value (26 kcal/mol). Therefore a negative binding energy value

Table 2. DNA cleavage activities and calculated binding energies of tested peptides with 5′-TpTpdC-3′ (kcal/mol)

Peptide	DNA cleavage activity	E_{peptide}	E_{complex}	E_{binding}
Ser-His	+++++	−14	272	−65
Cys-His	+++	−10	283	−58
Thr-His	±	−16	292	−43
Asp-His	−	−46	331	26
Ser-Arg	−	−50	280	−21
His-Ser	−	−8	300	−43

Table 3. The major structure features in docked complexes

Peptide	DNA cleavage activity	Dist. (Å)[a]	Binding points[b]
Ser-His	+++++	3.7	α-NH_3–$(PO_4)_1$ (2H-bonds) OH–$(PO_4)_1$ C(O)NH–$(PO_4)_2$ Imidazol–$(PO_4)_2$
Cys-His	+++	3.6	α-NH_3–$(PO_4)_1$ SH–$(PO_4)_1$ C(O)NH–$(PO_4)_2$ Imidazol–$(PO_4)_2$
Thr-His	±	3.1	α-NH_3–$(PO_4)_1$ OH–$(PO_4)_2$
Asp-His	–	—	α-NH_3–$(PO_4)_1$
Ser-Arg	–	4.9	α-NH_3–$(PO_4)_1$ NH=$C(NH_2)_2$–$(PO_4)_2$
His-Ser	–	5.4	α-NH_3–$(PO_4)_1$ (2H-bonds) C(O)NH–$(PO_4)_2$

[a] Distance between nucleophile O(S) atom of C-terminus side chain and P atom of DNA.
[b] $(PO_4)_1$ and $(PO_4)_2$ stand for the two neighbor phosphates.

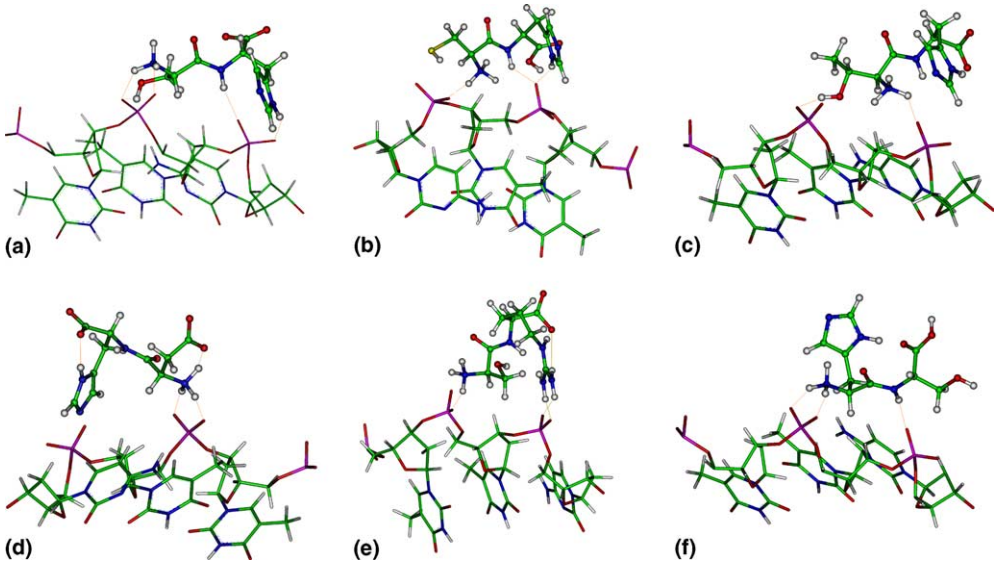

Figure 2. The docked structure of tested peptide binding with 5′-TpTpdC-3′ located by performing FLEXIDOCK: (a) Ser-His, (b) Cys-His, (c) Thr-His, (d) Asp-His, (e) Ser-Arg, and (f) His-Ser. H-bonds are marked by yellow lines.

between peptide and DNA is necessary for DNA cleavage. However, a negative value of binding energy does not guarantee the ability of DNA cleavage. For instance, dipeptides Ser-Arg and His-Ser, have no detectable DNA cleavage activity though their binding energy with DNA are negative. This could be because no effect of solvent was considered during our docking procedures owing to computational limitation. Therefore, a negative $E_{binding}$ is not a sufficient criterion.

Molecular recognitions between tested dipeptides and 5′-TpTpdC-3′ are also very important for DNA cleavage. The structures of the complexes in Figure 2 reveal some structure features for their binding ability. In case of Ser-His and Cys-His, the distances between the nucleophile O or S atom of peptide N-terminus side chain and phosphate P atom of DNA in their complexes (O–P: 3.7 Å and S–P: 3.6 Å, respectively), are both within a contactable range for the O (S) atom conducting nucleophilic attack to the phosphor. This may lead to a formation of pentacoordinate phosphotriester transition state followed by a cleavage of the ester bond, and therefore result in the DNA cleavage.[10–14] For Ser-Arg and His-Ser, the O–P distances in their complexes (4.9 and 5.1 Å, respectively), are beyond the nucleophilic attacking range, not to mention cleavage activity. Therefore, the contactable distance between the nucleophile O (S) atom of peptide C-terminus side chain and phosphate P atom of DNA in a complex is one of key structure features for cleavage activity. However, in the case of Thr-His–5′-TpTpdC-3′, although the distance between the O atom of seryl hydroxy and phosphate P atom of DNA is within the nucleophilic attacking range (3.1 Å), Thr-His exhibits only marginally detectable cleavage activity. Therefore, a contactable O–P distance is also a required but not sufficient criterion.

Another key structure feature is that the dipeptide must bind to two neighboring phosphates in the DNA backbone simultaneously (Table 3). For Ser-His–5′-TpTpdC-3′, the Ser moiety binds to one phosphate through H-bonds and electrostatic interaction, while the His moiety bonds to the other phosphate. The α-amino group of seryl forms two H-bonds with the two oxygen atoms of the phosphate, and the hydroxyl group of seryl forms one H-bond with one oxygen atom of the same phosphate. It seems that Ser uses its own α-amino group as a general base to improve the nucleophilicity of the hydroxyl group; there are two other H-bonds between the imide or imidazole group of His moiety and another phosphate, which provide more recognition sites for constructing the complex. The interaction may play an essential role in keeping the binding geometry and facilitate nucleophilic attack by bringing the DNA closer. The low cleavage activity of Thr-His could be because there are only two H-bonds between the Ser moiety and phosphates, and no close contact between imidazole moiety and phosphate.

In conclusion, we used a molecular modeling method to investigate the key structure features for DNA cleavage. A negative binding energy, a contactable O–P distance, and a multiple binding sites are all very important structure features. Novel nonpeptide L/D serine/histamine amides designed under the guidance of above theoretical results had shown high DNA cleavage activities.[15]

Acknowledgements

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