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ORIGINAL PAPER

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Molecular modeling of diastereoisomeric aggregates of L/D ser/histamine amide with 5'-TpTpdC-3'

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Abstract Novel nonpeptide serine/histamine amides (**1**: L-Ser-Hism, **2**: D-Ser-Hism) with potent DNA cleavage activity were designed. Conformational analysis and docking study were carried out in an attempt to understand the DNA cleavage mechanism of the designed enantiomeric nonpeptides. First, the most stable conformers of the designed amides were obtained from the conformational analysis by random search. Next, the three-dimensional structures of L-Ser-Hism···5'-TpTpdC-3' and D-Ser-Hism···5'-TpTpdC-3' complexes were built using molecular docking techniques. The docked diastereoisomeric aggregates show that both L-Ser-Hism and D-Ser-Hism bind to two neighboring phosphates in the 5'-TpTpdC-3' backbone through H-bonds. This binding mode suggests a possible phosphodiester bond hydrolysis mechanism. In addition, the binding energies of two constructed complexes were also calculated with the Tripos force field. It indicates that the binding ability between L-Ser-Hism and 5'-TpTpdC-3' is stronger than that of D-Ser-Hism, suggesting a stronger DNA cleavage activity of L-Ser-Hism than that of D-Ser-Hism. The results agree with our experimental DNA cleavage assays. Supplementary material is available for this article if you access the article at http://dx.doi.org/10.1007/s00894-002-0114-9. A link in the frame on the left on that page takes you directly to the supplementary material.

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Introduction

The discovery of artificial nucleic acid cleavage agents in functional mimics of RNase has been of great interest for several decades. [1, 2, 3, 4, 5, 6] A number of small molecules have been shown to hydrolyze RNA as probes in RNA structure studies. However, hardly any small organic molecules that cleave DNA via a hydrolysis mechanism have been reported. Therefore, the discovery of such organic molecules that can cleave DNA following a hydrolysis mechanism is a stimulating and exciting challenge.

Early work in our laboratory showed that both DNA and RNA were cleaved by *N*-phosphoryl serine in a saturated aqueous solution of histidine, but not by the phosphoryl serine alone. [7] Since our previous studies demonstrated that *N*-phosphoryl amino acids can catalyze a self-assembly reaction to form homo- or hetero-oligopeptides, [8, 9, 10, 11] it was proposed that Ser–His derived from *N*-phosphoryl serine coupled with histidine or its *N*-phosphoryl derivative might be responsible for the DNA and RNA cleavage. This led to the discovery of dipeptide Ser–His and related oligopeptides that can cleave DNA via the hydrolysis mechanism. It was also shown that the hydroxyl functional group of the *N*-terminal amino acid serine and the imidazole functional group of histidine are necessary for cleavage. [12, 13] Following these results, novel nonpeptidic serine/histamine amides (**1**: L-Ser-Hism, **2**: D-Ser-Hism) that possess the essential structural features for cleavage were designed. We found that both designed enantiomers were capable of cleaving DNA. [14] In this paper, we report the results of a conformational analysis and molecular docking study on DNA cleavage activity of the designed ser/histamine amides **1** and **2** in an attempt to understand the structure information of the DNA cleavage mecha-

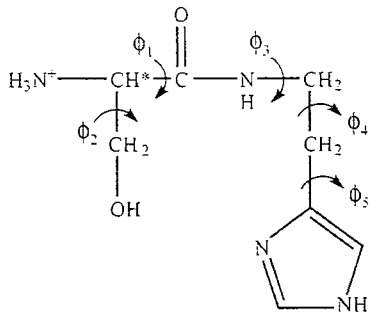

Fig. 1 Chemical structure of the designed serine/histamine amide. **1**: L-Ser-Hism, **2**: D-Ser-Hism. Rotatable bonds are marked in ϕ_1, ϕ_2, ϕ_3, ϕ_4, ϕ_5, respectively

nism. The conformational analysis was carried out with a random search, and the docking study was performed with a molecular docking program. Standard B-DNA double helix 3'-TpTpdC-5' was built manually as a mimic of DNA. By using the global minimum energies of the designed amides, the binding energy between DNA mimics and the amides were estimated and compared with DNA cleavage assays. Chemical structure of the designed serine/histamine amide is shown in Fig. 1.

Methods

Conformational searches and molecular docking were performed with Sybyl [15] version 6.7 on an SGI O2 R10000 workstation. The Tripos force field implemented in Sybyl was used. All atomic charges were computed using the Gasteiger–Huckel method. Amide bonds were set to be *trans*.

Conformational searches

Conformational searches were performed using the random conformational search method implemented in Sybyl. For each search, 1,000 starting structures were generated and minimized until the gradient was less than 0.05 (kJ mol^{-1})/$Å^{-1}$. All heavy atoms were used for comparing a minimized structure with all previous unique minima; structures are considered the same unless the least squares superimposition of the compared atoms finds one or more pairs of equivalent atoms separated by more than 0.2 Å. Duplicated structures as defined before and those with energy greater than 10 kJ mol^{-1} above the global minimum were discarded. The MAX-HITS number was set to 6. The torsional angles defined in Fig. 1 were changed randomly during the search.

Molecular modeling

The standard B-DNA double helix of 5'-TpTpdC-3' was built by the BUILT/BIOPOLYMER module implemented in SYBYL and fixed in space. The conformation of L-Ser-Hism (**1**) and D-Ser-Hism (**2**) were relaxed flexibly, and rotations were applied to all rotatable bonds indicated in Fig. 1.

Initial binding geometries for each L-Ser-Hism···5'-TpTpdC-3' and D-Ser-Hism···5'-TpTpdC-3' were scanned and set using the DOCK module implemented in Sybyl. Then, the docking study was carried out manually with the FlexiDock algorithm of SYBYL. The Tripos force field, which incorporates the van der Waals, electrostatic, torsional and constraint energy terms, was used. The binding energy between DNA and the designed amide was estimated by simply subtracting the conformational energy of the free DNA and the isolated **1** or **2** from that of the complex. The global minimum energy obtained by the conformational analysis was used as the conformational energy of the isolated **1** or **2**. The binding energy was calculated with equation:

$$E_{binding} = E_{complex} - E_{ligand} - E_{DNA}$$

where $E_{complex}$ is the total energy of the complex, E_{ligand} is the energy of the global minimum conformation of **1** or **2**, E_{DNA} and is the total energy of the free oligonucleotide. In the actual derivation of the binding energy, the conformational energy of DNA was not subtracted because it was the same for both cases.

DNA cleavage assays

0.043 $\mu g/\mu l$ of λ-DNA containing 10 mM of compound **1** or **2**, was incubated at 50 °C for a period of time in 40 μl Briton-Robinson (BR) buffer (120 mM). Samples containing 17% of DNA loading buffer were subjected to agarose gel (0.6% in 1×TAE) electrophoresis.

Results and discussions

Conformational searches

Random searches were performed on designed amides **1** and **2**. The energy of the most stable conformation located is 3.28 kcal mol^{-1}, with 11 hit times for **1** and that of 3.28 kcal mol^{-1}, with 12 hit times for **2**. The structures of the most stable conformations of the two enantiomers located are shown in Fig. 2.

Molecular docking

As an aid in attempting to obtain structural insight into the possible DNA cleavage mechanism of the designed amides, the three-dimensional structures of L-Ser-Hism···5'-TpTpdC-3' and D-Ser-Hism···5'-TpTpdC-3' complexes were constructed using the molecular docking program FlexiDock encoded in Sybyl, and the binding energies between these two amides and the oligonucleotide were calculated with the Tripos force field. The docked structures of **1** and **2** binding with oligonucleotide 5'-TpTpdC-3' are shown in Fig. 3.

Both **1** and **2** bind to two neighboring phosphates in the DNA backbone through H-bonds. The hydroxyl group

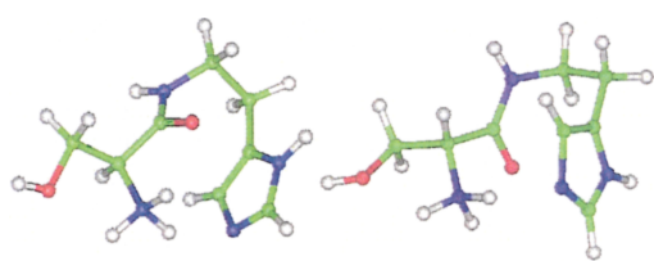

Fig. 2 The most stable conformations of designed enantiomers located by random search. **1**: L-Ser-Hism (E=3.28 kcal mol^{-1}, *left*), **2**: D-Ser-Hism (E=3.28 kcal mol^{-1}, *right*)

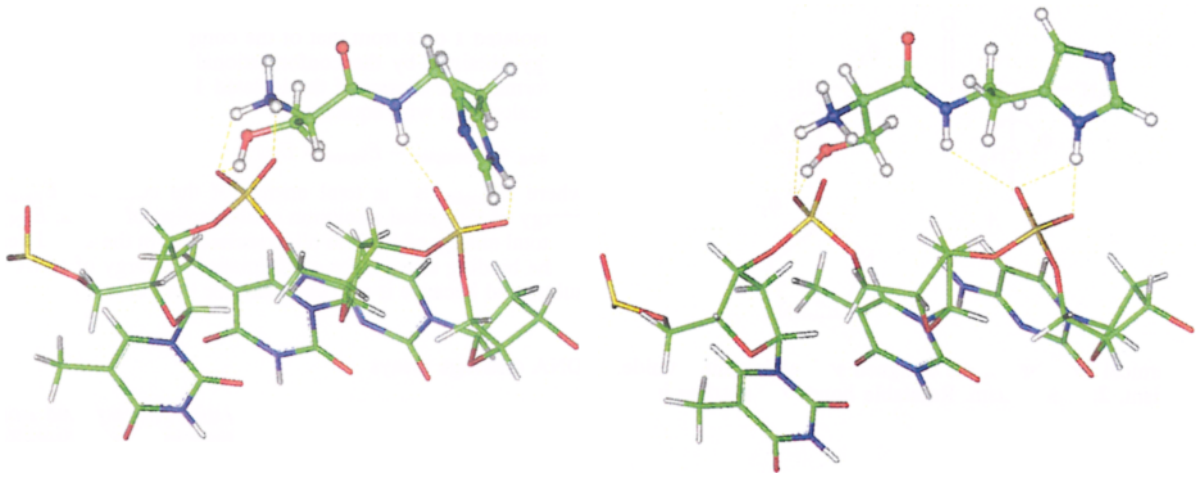

Fig. 3 Docking structures of **1** and **2** binding with oligonucleotide: L-Ser-Hism···5'-TpTpdC-3' (*left*), D-Ser-Hism···5'-TpTpdC-3' (*right*). Hydrogen bonds are shown in dotted lines. Only one strand of the oligonucleotide is shown for clarity

and ammonium group of seryl bind to one phosphate, while the imide group and imidazole ring bond to another phosphate. In the complex of L-Ser-Hism···5'-TpTpdC-3', the ammonium group of seryl forms two H-bonds with the two oxygen atoms of the phosphate, the hydroxyl group of seryl forms one H-bond with one oxygen atom of the phosphate. The distance between the oxygen atom of the hydroxyl group and the phosphorus atom is 3.6 Å, which is within the distance range for a nucleophilic attack on phosphorus by oxygen. This may thus lead to formation of a pentacoordinate phosphotriester transition state followed by a cleavage of the ester bond and result in the DNA cleavage. [16] There are two other H-bonds between the Hism moiety and another phosphate, which provide more recognition sites for constructing the complex. This may play an essential role in keeping the binding geometry and facilitate nucleophilic attack by bringing the DNA closer. In the complex of D-Ser-Hism···5'-TpTpdC-3', the ammonium group and the hydroxyl group of seryl form H-bonds with the oxygen atom of the phosphate. The distance between the oxygen atom of hydroxyl group and the phosphorus atom is 3.8 Å, which is also within the distance range for nucleophilic attack of the phosphorus by hydroxyl. There are three H-bonds formed between the Hism moiety and the other phosphate. These will also facilitate the nucleophilic attack on the phosphorus by the oxygen atom of the hydroxy group. In conclusion, the binding mode of the docked complexes suggests a possible phosphodiester bond hydrolysis mechanism.

The binding energies calculated for these two complexes are not identical. **1** is calculated to be about 12.3 kcal mol^{-1} more strongly bound than **2**, indicating that **1** is more specific in recognizing DNA than **2**.

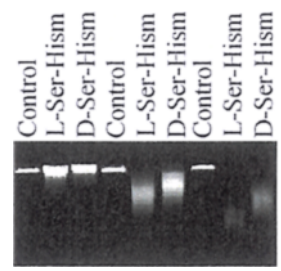

Fig. 4 DNA cleavage of the amides L-Ser-Hism (**1**) and D-Ser-Hism (**2**)

DNA cleavage effect

The DNA cleavage of the amides **1** and **2** is shown in Fig. 4. Both enantiomeric amides **1** and **2** at a concentration of 10 mM in BR buffer at 50 °C exhibited significant DNA cleavage ability. After 12–24 h of incubation, it was found that λ-DNA was cleaved by both. The cleavage effect was even more significant after 36 h of the incubation, and the cleavage activity of **1** is somewhat stronger than that of **2**.

Conclusion

This paper examines the interactions of designed non-peptide serine/histamine amides (**1** and **2**) with DNA using a molecular modeling approach. The diastereoisomeric aggregates of **1** and **2** with 5'-TpTpdC-3' were constructed by means of the FlexiDock method. The results demonstrated that the designed amides and 5'-TpTpdC-3' are linked through a net of H-bonds. This special structure feature is favorable for nucleophilic attack on the backbone phosphodiester by the hydroxyl group of designed amides, suggesting a possible hydrolysis DNA cleavage mechanism. The DNA cleavage

activity of the designed amides was also tested by means of gel electrophoresis. The DNA cleavage assay demonstrated the DNA cleavage ability of the designed amides and also showed that **1** is more active than **2** in DNA cleavage, which is consistent with the docking experiments.

Supplementary material

The coordinates of structures shown in Figs. 2 and 3 in pdb format are available in the supplementary material.

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