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Molecular Modeling on the Recognition of Wobble DNA Including G:T Mismatched Pairs by Two Structures of Chiral Metal Complex Δ,Λ-$[Ru(phen)_2hpip]^{2+}$

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In this work, the recognition of DNA including G:T mismatched pairs by the two different structures of $[Ru(phen)_2hpip]^{2+}$ was firstly studied with molecular modeling respectively. The results revealed that all of the four chiral isomers of the two structures could recognize the mismatched DNA from the minor groove orientation especially and the interaction was enantioselective and sitespecific. The two left isomers were more preferential than the right ones. Especially, the structure II which had much lower energy after interacting with DNA was the advantaged structure. Detailed energy analysis indicated that the steric interaction in the process of the complex inserting base stack determined the recognition results and the electrostatic interaction made an effect to some extent.

Keywords metal complex, molecular modeling, steric collision, electrostatic interaction

Introduction

In recent years, people studied the interaction between transitional metal polypyrimidine complex and DNA more deeply, and it has been a very active subject in the bioinorganic chemistry field.[1-3] The octahedron polypyrimidine ruthenium(II) complex was extensively used as DNA structural probe, photic switch, cleavage reagent and electron transition that was conducted by DNA.[4] These micromolecules, which could specially recognize DNA, lay a solid foundation for new medicine to choose gene. We already successfully simulated the recognition interaction of some chiral metal complex with sheared DNA including G:A mismatches.[5-8] We have not found any report about complexes recognizing G:T. But G:T mismatched pair was quite stable and difficult to recognize, and also could lead to some molecular diseases. Consequently in this work, we firstly studied the recognition of DNA including mismatched G:T pairs by $[Ru(phen)_2hpip]^{2+}$[phen＝1,10-phenanthroline, hpip＝2-(2-hydroxyphenyl) imidazole (4,5-f)(1,10)-phenanthroline].

G:T pair takes Wobble form,[9] where the purine base is migrated to the minor groove of DNA and pyrimidine base is located at the major groove. There are two hydrogen bonds between the purine and pyrimidine bases (Figure 1).

Solvent molecules bridges nucleobases from major and minor grooves respectively, making the mismatch stabler, approximate to normal A:T pair. But it just distorts DNA double helix slightly. Then, most of polymerizing enzyme could avoid mismatch pair breezily and continue extension. The mismatch may escape from testing and departing from polymerizing enzyme complex.[10] Hence it is important to recognize this kind of mismatch.

$[Ru(phen)_2hpip]^{2+}$ is often used as a DNA structural probe. The bidentate ligand hpip has an aromatic heterocyclic plane and can be inserted into and stacked between two base pairs of double helical DNA. Hpip

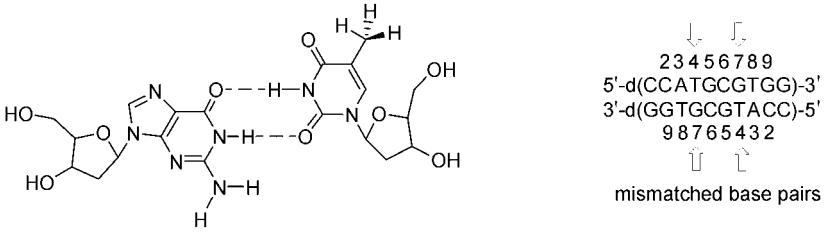

Figure 1 Hydrogen bonds between G and T and the mismatched DNA series.

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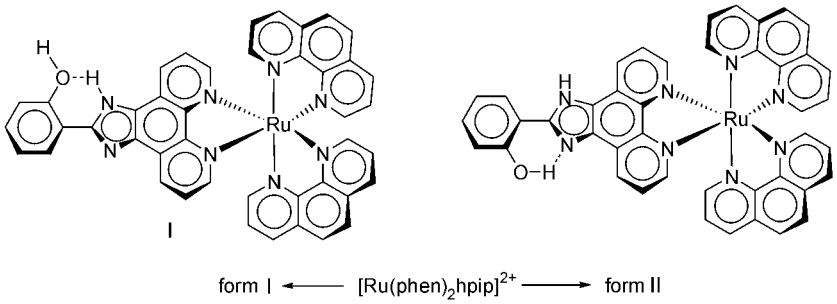

Figure 2 Two structures of Δ-$[Ru(phen)_2hpip]^{2+}$.

has two kinds of vibrational planar structures that can be distinguished by the different hydrogen bonds. Based on this, there are two kinds of $[Ru(phen)_2hpip]^{2+}$ (Figure 2). The potential energy of the two structures was −153.96 and −149.34 kcal/mol (−646.63 and −627.23 kJ/mol) respectively after calculation. System could offer about 29.0 kJ/mol (approximate 7 kcal/mol) energy at room temperature. The complex could rotate freely in isolated circumstance because imidazole and benzene were linked by C—C single bond. But when the ligand hpip was inserted into DNA base stack whether in form I or form II, the steric hindrance and conjugated stack would stabilize the structure and it could not be changed to another. Thus, when DNA binding interaction was studied, the differences of recognition results brought by the two different resonant structures must be considered.

Calculation method

All of molecular modeling work was performed in SGI workstation with Insight II software package. The metal complex $[Ru(phen)_2hpip]^{2+}$ was constructed in the builder module and its molecular dynamics was calculated with DISCOVER 98 program in ESFF force field. The structures of conformers were optimized and the model with lowest potential energy was selected. The mismatched DNA 5′-d$(CCATGCGTGG)_2$-3′ (Figure 1) was downloaded from the National Center for Biotechnology Information.[11] The metal ions and all H_2O molecules of the model were eliminated and all bond and atom types were modified according to the normal bonding form of DNA molecule. Then the DNA structure was optimized under AMBER force field. The complex was optimized in vacuum, but considering the stable function of water to the structure of DNA, the DNA molecule and all the models including DNA were optimized in aqueous solution. In the whole process, the default parameters of the program were used.

Electroneutrality of each docked structure was achieved with the addition of 16 Na^+ counterions in the model of complex-mismatched DNA association by standard procedures to balance the phosphate anions on the DNA and the positive charges of metal Ru(II). At the beginning of optimization and energy minimization, the steepest descent method was used until the RMS derivation was less than 5.0 kcal/mol (21.0 kJ/mol). Then it was switched to conjugate gradient method automatically by the DISCOVER 98 program. When the RMS derivation was less than 0.5 kcal/mol (2.1 kJ/mol), optimization and energy minimization were stopped.

Many work teams indicated that one of the recognition interaction manners between transition metal complex including planar aromatic heterocyclic ligands and mismatched DNA was classical intercalation.[12,13] Each isomer was docked manually into the DNA base stack between every double base pair except the terminal regions of mismatched DNA, and intercalations took place in the major groove and minor groove respectively. At beginning, the hpip plane was placed nearly parallel to the base pair plane (perpendicular to DNA helix axis) and just out of the DNA helix. This point was regarded as the first checkpoint and its intercalation depth was defined as 0 nm. Then, Ru(II) complex was docked into base stack until the hpip ligand was intercalated to the base stack entirely. The checkpoint was selected for every 0.2 nm, and the intercalation depths were thus defined as 0.2, 0.4 nm, *etc*. Then based on the potential energy distribution, the optimal interaction model could be acquired for every isomer and DNA. The interaction was investigated between the four chiral isomers of two structures of $[Ru(phen)_2hpip]^{2+}$ and the Wobble DNA respectively. Though the hpip ligand was asymmetric, considering the DNA sequence was quite symmetric, all the sites were not calculated repeatedly.

Results and discussion

All the calculated results are shown in Tables 1—4. The other data with unit of kcal/mol (1 kcal/mol=4.2 kJ/mol) were the potential energy of system after the complex intercalated to DNA. The data of optimal intercalation depth were boldfaced in order to distinguish with others. Before intercalation, the energy of the isolated DNA was 2599.9 kcal/mol (10751.6 kJ/mol).

From the intercalation results, the process of recognition of Wobble DNA by $[Ru(phen)_2hpip]^{2+}$ was clearly found to show obvious grooveselectivity, enantioselectivity and sitespecificity. Detailed energy analysis displayed that the steric interaction in the intercalating process determined the recognition results and the electrostatic interaction made an effect to some extent.

Table 1 Potential energy of Λ-$[Ru(phen)_2hpip]^{2+}$ (form I) binding with Wobble DNA (kcal/mol)

Intercalation depth/nm	Major groove							Minor groove						
	C_2A_3	A_3T_4	T_4G_5	G_5C_6	C_6G_7	G_7T_8	T_8G_9	C_2A_3	A_3T_4	T_4G_5	G_5C_6	C_6G_7	G_7T_8	T_8G_9
0.0	2423.0	2407.1	2423.2	2419.4	2408.7	2420.3	2423.4	2420.4	2413.9	2406.7	2419.8	2423.0	2412.0	2419.3
0.2	2393.3	2384.5	2382.9	2383.4	2372.0	2385.3	2393.3	2392.0	2371.4	2372.4	2383.9	2394.7	2379.9	2391.3
0.4	2384.3	2381.8	2367.0	2364.2	2348.9	2376.0	2366.2	2387.8	2364.0	2358.7	2388.8	2381.3	2382.1	2383.8
0.6	2379.6	2365.8	2366.2	2344.4	2346.1	2379.8	2358.2	2391.7	2360.2	2340.7	2380.4	2361.1	2369.1	2370.1
0.8	2384.7	2334.9	2359.7	**2322.6**	2335.1	2364.6	2356.8	2385.8	2348.0	2324.1	2366.1	2332.2	2366.8	2369.3
1.0	2368.3	2333.9	2346.2	2324.8	2334.9	**2344.5**	2353.3	**2374.1**	2316.1	2313.1	2363.9	2329.7	2361.1	2384.9
1.2	2343.1	**2329.7**	**2331.3**	2343.2	2328.1	2353.8	2331.9	2385.7	**2314.2**	**2292.9**	2349.2	**2325.0**	2356.9	2375.6
1.4	**2329.0**	2348.8	2342.3	—	**2323.9**	2351.5	**2315.3**	—	2327.2	2300.2	**2348.1**	2325.2	**2348.4**	2358.5
1.6	2339.6	2362.9	2342.6	—	2330.1	2372.2	2316.6	—	—	2298.9	2365.9	2327.3	2355.4	**2352.9**

Table 2 Potential energy of Δ-$[Ru(phen)_2hpip]^{2+}$ (form I) binding with Wobble DNA (kcal/mol)

Intercalation depth/nm	Major groove							Minor groove						
	C_2A_3	A_3T_4	T_4G_5	G_5C_6	C_6G_7	G_7T_8	T_8G_9	C_2A_3	A_3T_4	T_4G_5	G_5C_6	C_6G_7	G_7T_8	T_8G_9
0.0	2417.5	2415.0	2412.6	2415.9	2412.2	2418.5	2426.4	2402.4	2417.3	2402.6	2315.6	2410.8	2409.7	2415.2
0.2	2395.1	2386.6	2380.1	2392.0	2368.7	2394.5	2378.3	2364.5	2376.9	2374.3	2389.4	2386.5	2373.6	2401.5
0.4	2385.7	2384.9	2367.7	2387.4	2352.5	2391.6	2359.8	2362.1	2372.8	2359.5	2391.2	2379.8	2375.3	2369.8
0.6	2384.1	2372.1	2370.8	2372.1	2354.4	2394.8	2346.4	2367.9	2368.7	2348.5	2384.2	2365.7	2364.5	**2359.4**
0.8	2381.8	**2366.7**	2373.0	2357.5	**2337.9**	2372.6	2349.2	2364.6	2362.6	2334.7	2387.7	2355.6	2352.0	2366.0
1.0	2384.3	2370.1	2350.7	**2350.8**	2344.5	**2371.4**	2350.5	2360.2	2344.6	2322.6	2377.2	2354.9	2334.8	2387.6
1.2	**2375.5**	2372.7	**2343.2**	2359.1	2351.7	2388.7	2335.9	2347.1	2331.1	2315.5	2382.9	2361.4	2318.8	2387.8
1.4	2385.5	2385.5	2357.2	—	2357.3	2409.8	**2321.7**	2342.4	**2321.0**	2318.7	2372.8	**2342.7**	**2310.0**	—
1.6	2391.6	—	2356.8	—	2357.9	—	2325.3	**2340.3**	2343.3	**2302.7**	**2351.3**	2350.8	2321.3	—

Table 3 Potential energy of Λ-$[Ru(phen)_2hpip]^{2+}$ (form II) binding with Wobble DNA (kcal/mol)

Intercalation depth/nm	Major groove							Minor groove						
	C_2A_3	A_3T_4	T_4G_5	G_5C_6	C_6G_7	G_7T_8	T_8G_9	C_2A_3	A_3T_4	T_4G_5	G_5C_6	C_6G_7	G_7T_8	T_8G_9
0.0	2432.8	2419.2	2419.3	2440.1	2439.1	2432.3	2440.9	2434.8	2428.2	2420.7	2426.9	2428.7	2427.6	2432.6
0.2	2399.6	2401.0	2388.4	2421.8	2403.6	2413.9	2411.8	2402.1	2402.7	2386.2	2400.7	2397.1	2394.6	2401.4
0.4	2391.6	2409.3	2374.5	2397.8	2379.7	2398.9	2384.7	2398.1	2397.7	2377.3	2387.5	2382.5	2390.5	2386.0
0.6	2389.8	2374.0	2368.8	2374.9	2357.5	2372.1	2381.6	2388.1	2389.2	2363.3	2370.1	2375.8	2387.2	2371.5
0.8	2381.5	**2361.8**	2353.6	2361.2	2349.3	**2349.7**	2359.9	2379.9	2366.2	2338.5	2352.1	2360.9	2386.0	**2355.0**
1.0	2374.9	2376.4	2326.9	**2359.2**	2351.3	2350.2	2323.2	2377.8	**2342.2**	2316.6	2347.6	2351.7	2387.3	2360.2
1.2	2349.6	2365.1	**2310.8**	2364.0	2344.9	2351.2	**2299.3**	2363.0	2343.4	2309.8	**2346.4**	**2319.1**	2364.0	2368.4
1.4	**2332.2**	—	2312.3	2371.2	2339.5	2370.8	2301.8	**2350.9**	2361.4	2296.4	2356.1	2332.7	**2358.2**	—
1.6	2337.3	—	2318.4	2392.9	**2336.6**	—	2309.8	2359.3	—	**2282.6**	—	—	2371.7	—

Table 4 Potential energy of Δ-$[Ru(phen)_2hpip]^{2+}$ (form II) binding with Wobble DNA (kcal/mol)

Intercalation depth/nm	Major groove							Minor groove						
	C_2A_3	A_3T_4	T_4G_5	G_5C_6	C_6G_7	G_7T_8	T_8G_9	C_2A_3	A_3T_4	T_4G_5	G_5C_6	C_6G_7	G_7T_8	T_8G_9
0.0	2435.2	2425.7	2433.1	2440.7	2435.3	2439.7	2436.5	2437.0	2433.1	2431.2	2433.1	2429.6	2432.3	2427.1
0.2	2402.1	2391.0	2394.6	2408.7	2393.0	2409.9	2392.4	2402.9	2399.8	2401.2	2399.8	2402.1	2380.5	2413.8
0.4	2385.0	2388.2	2380.3	2400.5	2370.7	2405.9	2378.0	2400.4	2380.8	2384.6	2379.9	2390.5	2372.0	2388.7
0.6	2381.3	2385.4	2372.0	2394.5	2358.6	2382.5	2376.4	2397.4	2383.8	2365.1	2363.7	2362.1	2366.2	2376.6
0.8	2381.3	**2366.3**	2363.0	2386.6	2356.8	**2366.7**	2365.5	2383.7	2358.6	2340.3	2347.4	2349.2	2349.8	**2358.6**
1.0	2377.4	2369.9	2342.2	2369.5	2358.8	2370.4	2346.3	2380.2	2337.1	2321.5	2333.0	2340.3	2332.3	2383.0

Continued

Intercalation depth/nm	Major groove							Minor groove						
	C_2A_3	A_3T_4	T_4G_5	G_5C_6	C_6G_7	G_7T_8	T_8G_9	C_2A_3	A_3T_4	T_4G_5	G_5C_6	C_6G_7	G_7T_8	T_8G_9
1.2	2364.4	2371.2	**2341.0**	2357.9	2363.9	2378.4	2331.3	2374.4	**2327.0**	2305.9	**2325.0**	2323.7	2317.7	—
1.4	**2347.7**	2386.0	2356.0	2364.2	**2346.1**	2397.9	2318.5	2379.7	2331.6	2292.1	2339.3	**2314.7**	**2306.4**	—
1.6	2355.3	—	—	**2352.7**	2347.0	—	**2307.1**	**2367.7**	2335.9	**2288.6**	—	2318.9	2314.0	—

Grooveselectivity

This result was consistent with other work teams to intercalate from minor groove orientation preferentially. This selectivity was due to the steric interaction. There were more base pairs and phosphor frameworks in major groove, and when hpip ligand was inserted into DNA from this orientation, the steric collision between ancillary ligands phen and nucleobases was very strong and they were easy to collide with phosphor frameworks. Instead of this, when hpip was inserted from minor groove, the metal complex was consistent with the helix orientation of phosphor frameworks. It could avoid collision to decrease steric interaction. Furthermore, the stretch orientation of bases was another important reason of the groove-selectivity. The bases in the Wobble DNA studied stretched from minor groove to major groove (Figure 3).

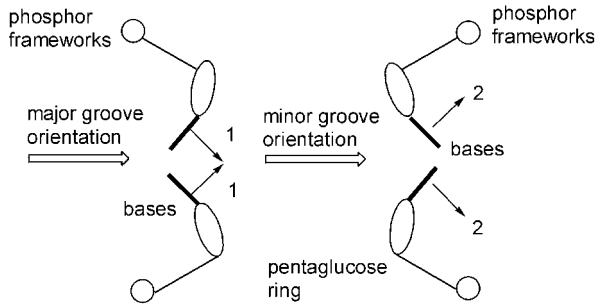

Figure 3 Steric collision when the complex was intercalated to DNA from major and minor groove.

When the complex was inserted into base stack from major groove orientation, the base in the intercalation region would move to minor groove because of steric interaction (arrow 1). This change made DNA structure more crowded. But the base moved to major groove (arrow 2) when complex was inserted to DNA from minor groove. It did not lead to more crowded structure because of the orientation of bases stretched and it was much more expansive in major groove. The complex encountered stronger steric resistance in major groove but much weaker in minor groove, and correspondingly the complex selected minor groove.

Enantioselectivity

Both of the two structures of $[Ru(phen)_2hpip]^{2+}$ selected their left isomer optimally to intercalate to DNA from minor groove orientation. When Λ-$[Ru(phen)_2hpip]^{2+}$ was inserted into DNA, the ancillary ligand phen in the tail of complex faced to pyrimidine bases T_4 and C_6. Oppositely, when Δ-$[Ru(phen)_2hpip]^{2+}$ inserted into DNA, phen faced to purine bases G_7 and G_5 (Figure 4). The purine bases would bring much stronger steric collisions than the pyrimidine bases because bases G and A were larger than C and T. Consequently, based on steric interaction, the left isomer was thought much more optimal than the right one.

Detailed energy analysis

Table 5 described detailed energy distribution for interaction of the complex-DNA system in optimal intercalation depth in minor groove orientation. In the table, Total means total energy that is the sum of Nonbond and Internal. Nonbond means nonbond energy, which describes the steric interactions and is the sum of the VDW and Electr. VDW means Van de Waals energy,

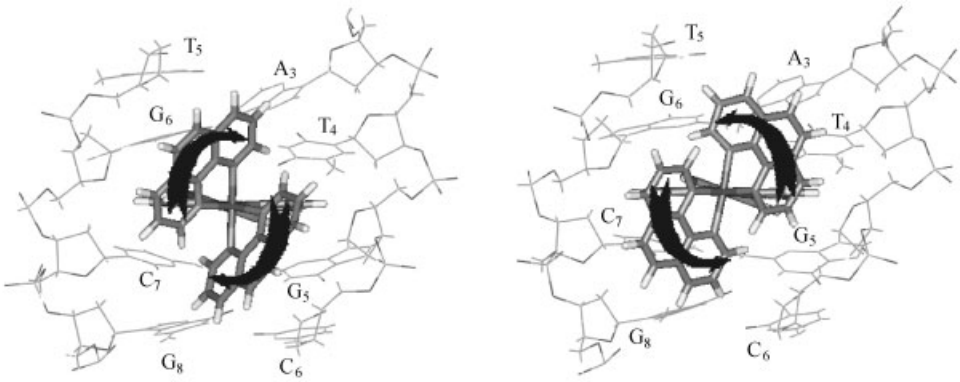

Figure 4 Steric collision between phen and DNA to intercalate from minor groove (left : Λ- and right: Δ-).

Table 5 Detailed energy distribution for interactions of complex Λ-$[Ru(phen)_2hpip]^{2+}$ in minor groove orientation (kcal/mol)

Intercalation region	Form I					Form II				
	Total	Internal	Nonbond	VDW	Electr	Total	Internal	Nonbond	VDW	Electr
C_2G_9/A_3T_8	2374.1	560.0	1814.2	−1.3	1815.5	2350.9	575.5	1775.5	−11.7	1787.1
A_3T_8/T_4G_7	2314.2	568.2	1745.9	−9.1	1755.1	2342.2	570.0	1772.1	−14.2	1786.3
T_4G_7/G_5C_6	**2292.9**	**567.2**	**1725.7**	**0.5**	**1725.2**	**2282.6**	**581.2**	**1701.3**	**−6.5**	**1707.9**
G_5C_6/C_6G_5	2348.1	596.8	1751.3	−6.4	1757.7	2346.4	589.2	1757.3	−2.1	1759.3
C_6G_5/G_7T_4	2325.0	565.4	1759.5	−10.3	1769.9	2319.1	565.1	1754.0	−11.3	1765.3
G_7T_4/T_8A_3	2348.4	586.4	1762.0	−13.7	1775.7	2358.2	587.1	1771.1	−10.7	1781.8
T_8A_3/G_9C_2	2352.9	576.1	1776.8	5.3	1771.4	2355.0	549.1	1805. 9	−0. 3	1806.1

Electr means electrostatic energy. Internal energy term describes the bond properties. The unit of all data is kcal/mol.

As showed, the Non-bond energy was larger than Internal item, which was consistent with our discussion above. Within Nonbond energy term, electrostatic energy was 100—1000 times larger than VDW energy, which meant that VDW influenced result lightly. In general, electrostatic energy determines the energy of whole system. For Λ-$[Ru(phen)_2hpip]^{2+}$ with form II, at optimal intercalation depth, its Internal energy was 14.0 kcal/mol (58.8 kJ/mol) higher but Non-bond energy was 24.4 kcal/mol (102.5 kJ/mol) lower than Λ-$[Ru(phen)_2hpip]^{2+}$ with form I. That is to say, although the π-π stack was sparser between hpip and bases of Λ-$[Ru(phen)_2hpip]^{2+}$ with form II, it encountered much weaker steric collision and matched with DNA system much better than Λ-$[Ru(phen)_2hpip]^{2+}$ form I. All of these made the total energy decrease.

Changes of DNA

The structure of DNA was found changed seriously after interaction with complexes. The distance between two base layers at the intercalation site was enlarged once, but there were not obvious changes at the other sites. Both the two forms of $[Ru(phen)_2hpip]^{2+}$ selected T_4G_5 from minor groove orientation optimally to interact with DNA. But there were also some differences among these models after interaction. The two most optimal models are shown in Figure 5.

In order to hold the hpip ligand, the average distance between T_4A_7 and G_5T_6 was found to be increased to about 0.637 (Λ-) and 0.603 nm (Δ-) respectively when hpip interacted with DNA at the site of T_4G_5. The hpip ligand located in the middle of the two base layers, which was approximate 0.3185 and 0.3015 nm respectively. Both of the two distances were less than the size 0.34 nm between two standard bases, resulting in the much tighter π-π stack than that before hpip ligand intercalation.

Conclusion

Based on the modeling work and result analysis above, some following conclusions could be obtained: (1) The metal complex has two kinds of structures and both of them could recognize the Wobble DNA including G:T mismatched pairs with groove-selectivity, site-specificity and enantioselectivity. Every isomer selected minor groove orientation to recognize the Wobble DNA. The left isomers were much more optimal than the right ones. Both of the two kinds of Λ-$[Ru(phen)_2hpip]^{2+}$ could recognize the site T_4G_7/G_5C_6 that was border upon mismatched pair in mismatched DNA. (2) After detailed energy analysis, the steric interaction was found to determine the recognition results mainly and the electrostatic interaction was to make an effect to some extent. (3) To compare the two kinds of structures of the metal complex, the system energy of complex in form II-DNA was also found to be lower than the form I-DNA and such form was the optimal structural form. System could offer about 7 kcal/mol (29 kJ/mol) energy at room

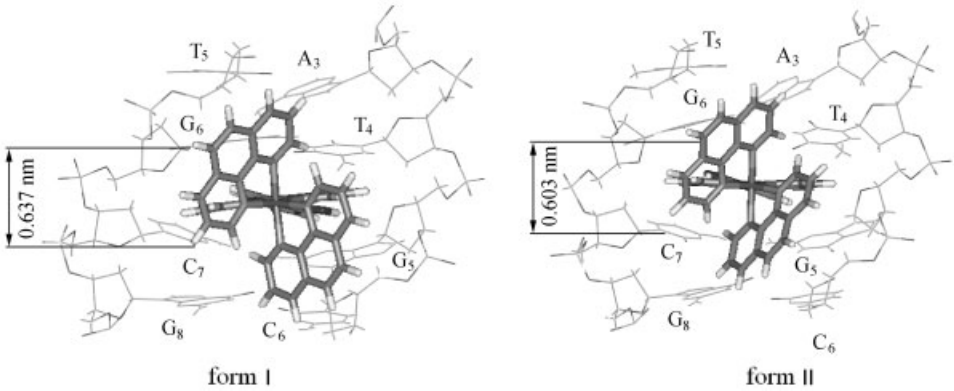

Figure 5 Changes of the distance between two bases after intercalation of Λ-$[Ru(phen)_2hpip]^{2+}$ complex.

temperature. The energy difference of two kinds of complex to interact with DNA respectively was up to 10 kcal/mol (42 kJ/mol). Certain satisfied experiment results could be predicted and such conclusion has some reference value for searching recognition probe of G:T mismatch and surveying even diagnostic molecular diseases.

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