Available online at www.sciencedirect.com
SCIENCE DIRECT®
Thermochimica Acta 416 (2004) 59–63
thermochimica acta
www.elsevier.com/locate/tca

Interaction of harmine with oligonucleotide d(GTGCAC)$_2$

Weihong Du, Baohuai Wang, Zhifen Li*

Institute of Physical Chemistry, Peking University, Beijing 100871, PR China

Received 15 October 2002; received in revised form 20 November 2002; accepted 25 December 2002

Available online 22 January 2004

Abstract

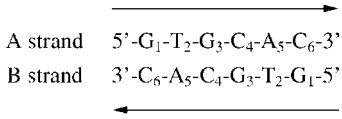
This work carried out a study on the interaction between harmine and oligonucleotide d(GTGCAC)$_2$. The binding thermodynamic parameters were obtained by ITC method. Harmine binds to oligonuleotide duplex d(GTGCAC)$_2$ in a fashion of intercalation mode and specific interaction and nonspecific interaction exist in the binding process. ^{1}H NMR experiments indicate that oligonucleotide d(GTGCAC)$_2$ is a generally self-complementary B-type duplex molecule, and harmine binds to DNA duplex d(GTGCAC)$_2$ at the side of methoxyl group preferentially in a mode of intercalation.

Keywords: Harmine; Intercalation; Oligonucleotide d(GTGCAC)$_2$; Thermodynamic

1. Introduction

Many biological responses caused by mutagenic and antitumor substances are often associated with the binding of small molecules (ligand) with DNA [1,2]. Therefore, an enormous amount of researches has been conducted on the binding of small ligands to DNA in physical chemistry, molecular pharmacology and carcinogenesis. Quantitative binding parameters are essential for the design of sequence-specific ligand for biological applications [3].

In this paper the binding specificity of β-carboline alkaloid, harmine with hexamer oligonucleotide d(GTGCAC)$_2$ was examined by ITC, CD, and NMR. Harmine, a tricyclic fused planar aromatic compound, potentially binds in a fashion of intercalation into DNA [4]. It possess some pharmaceutically potential biological activities of anticancer, hallucinogenic [5] and monoamine oxidase inhibition [6]. Though the interaction of harmine with natural DNA was reported [7,8], the specific binding site of harmine to DNA is still unknown. A selection of hexamer oligonucleotide d(GTGCAC)$_2$ as a target will help further understanding about the relationship of interaction between molecular structure of ligand and specific DNA sequence. The duplex is as follows:



2. Experimental

2.1. Materials

Single strand oligonucleotide d(GTGCAC) was purchased from Shanghai Shenggong Biological Technology Company. It was synthesized by phosphodiester method (without phosphatic group at 5′ end and 3′ end) with its purity up to 95% (PAGE electrophoresis). Harmine hydrochloride was from Sigma. Other chemicals were of analytical reagent grade. Redistilled water were used in all experiments.

2.2. Sample preparation

Double strand d(GTGCAC)$_2$ was obtained by regular renaturation experiments [9]. The buffer system was phosphate buffer of pH 6.1, composed of 10 mM Na_2HPO_4–NaH_2PO_4 and 20 mM NaCl. The concentration of DNA was measured by ultraviolet spectroscopy and expressed by that of duplex d(GTGCAC)$_2$. Fresh solution was prepared before each experiment. Harmine was dissolved in the same buffer.

* Corresponding author. Fax: +86-10-62751708.
E-mail address: lizhifen@pku.edu.cn (Z. Li).

0040-6031/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.tca.2003.11.025

2.3. Isothermal titration calorimetry

The measurements of the binding thermodynamic parameters of harmine with DNA duplex d(GTGCAC)$_2$ at 298.2 K were carried out with an isothermal titration calorimeter (ITC) from CSC Company, USA. It was calibrated by means of a standard electric pulse. The 750 μl DNA solution of 5.1×10^{-2} mM in sample cell was titrated with 250 μl harmine solution of 1.73 mM in syringe at per injection of 10 μl by stirring the sample cell at 200 rpm and the experiment was finished by 25 injections. To correct the dilution heat of ligand, the control experiment was also performed at same temperature using similar conditions with buffer only in reference cell. All solutions were degassed to reduce the noise. The heat of each reaction was determined by integration of the peak observed after the contribution from the dilution heat of each injection was subtracted.

2.4. Circular dichroism (CD) spectroscopy

CD spectra of DNA with or without ligands were recorded by a Jasco J-500 automatic-recording spectropolarimeter (Japan) in a quartz cell with a path-length of 1.0 cm. Scanning wavelength was from 210 to 300 nm. The concentration of DNA hexamer was 6.9×10^{-6} M, the concentrations of harmine were 0, 1.7×10^{-5}, and 3.4×10^{-5} M, respectively.

2.5. NMR spectroscopy

1D and 2D COSY and NOSEY ^{1}H NMR spectra for pure harmine solution, pure DNA solution or mixing solution of DNA with harmine of mole ratio 1:1 were recorded on a Bruker Avance DRX-500 500 MHz NMR spectrometer operating at 500 MHz at 288 K. Chemical shifts were referenced to TSP (sodium 3-(trimethylsilyl)-1-propanesulfonate). The 2D COSY and NOESY experiments were acquired according to standard pulse program with the carrier frequency placed on the HDO resonance using 1024 points in t_2 and 512 points in t_1 and a pulse repetition rate of 1.6 s. NOESY spectra were acquired for mixing time values of 200 and 400 ms. A presaturation pulse was utilized to suppress the HDO resonance. 2D NMR spectra were apodized with a 1/4 sine bell, zero filled by $1k \times 1k$ matrix and Fourier-transformed in both dimensions.

For pure oligonucleotide duplex NMR measurement, d(GTGCAC)$_2$ sample was dissolved in 0.5 ml phosphate buffer, repeatedly lyophilized from 99.8% D_2O and then finally dissolved in 99.96% D_2O (Cambridge Scientific) in 5 mm NMR tube. The concentration of DNA duplex was 2 mM. For the study on binding of harmine with DNA duplex, equal moles of d(GTGCAC)$_2$ and harmine were dissolved in 0.5 ml buffer, repeatedly lyophilized from 99.8% D_2O and then finally dissolved in 99.96% D_2O with concentration of 0.8 mM. Other conditions were the same as mentioned above.

Table 1
The binding thermodynamic parameters of harmine with d(GTGCAC)$_2$

n	1.6
K	1.5×10^4
$\Delta H_{298.2\,K}$ (kJ mol^{-1})	−28.5
$\Delta G_{298.2\,K}$ (kJ mol^{-1})	−23.9
$\Delta S_{298.2\,K}$ (J mol^{-1} K^{-1})	−15.4

3. Results

3.1. ITC data

The binding thermodynamic parameters of harmine with oligonucleotide duplex was obtained by ITC experiment data processed by ITC Binkwork Software and listed in Table 1. A plot of cumulative reaction heat versus the amount of harmine is shown in Fig. 1.

3.2. CD spectra

In pH 6.1 phosphate buffer, d(GTGCAC)$_2$ showed a positive peak at 258 nm and a negative peak at 229 nm, which corresponded to the conformation of DNA duplex (Fig. 2a). With the addition of harmine (Fig. 2b and c), the absorption of the two peaks were both increased slightly (Fig. 2).

3.3. NMR spectra

The resonances of the free hexamer oligonucleotide were assigned from NOESY and COSY spectra according to the methods established [10,11]. The results are listed in Table 2.

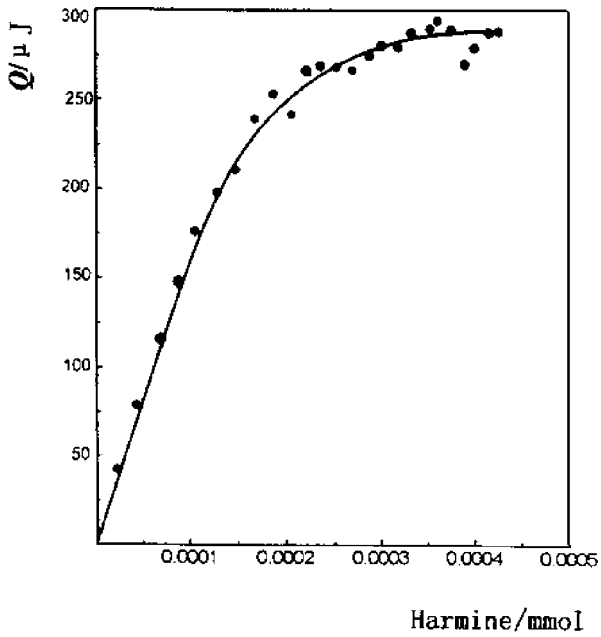


Fig. 1. The plot of cumulative reaction heat of harmine with d(GTGCAC)$_2$ vs. moles of harmine for ITC.

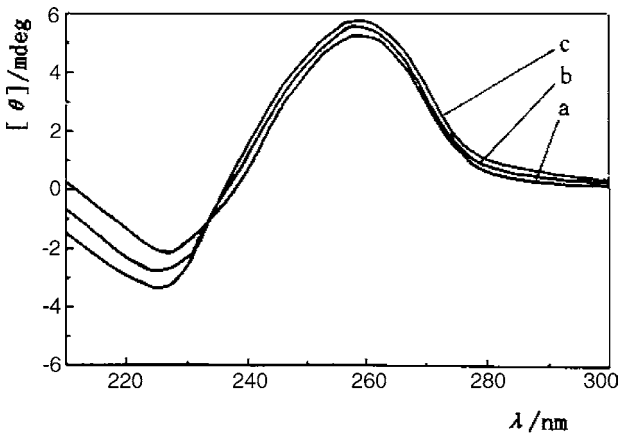


Fig. 2. CD spectra of d(GTGCAC)$_2$ with various concentration of harmine (●): (a) pure DNA; (b) harmine:DNA = 2.5:1; (c) harmine:DNA = 5:1.

Table 2
Nonlabile proton chemical shift of DNA duplex d(GTGCAC)$_2$

	H8/H6	H5/H2/Me	H1′	H2′	H2″	H3′	H4′	H5′/H5″
G_1	7.99		6.03	2.68	2.82	4.83	4.25	3.80, 3.78
T_2	7.37	1.40	5.97	2.25	2.59	4.94	4.28	4.16
G_3	7.93		5.92	2.67	2.76	5.02	4.42	4.12, 4.18
C_4	7.42	5.45	5.66	2.09	2.42	4.85	4.20	–
A_5	8.29	7.87	6.29	2.70	2.90	5.03	4.43	4.13, 4.17
C_6	7.35	5.33	6.08	2.14	2.14	4.50	4.02	4.08, 4.27

Table 3
Nonlabile proton chemical shift of DNA duplex d(GTGCAC)$_2$ in mixing solution

	H8/H6	H5/H2/Me	H1′	H2′	H2″	H3′	H4′	H5′/H5″
G_1	7.90	–	5.92	2.56	2.75	4.82	4.23	3.80, 3.82
T_2	7.35	1.37	5.93	2.21	2.50	4.92	4.23	4.15
G_3	7.85	–	5.80	2.63	2.69	4.99	4.38	4.16, 4.23
C_4	7.37	5.36	5.64	2.07	2.38	4.81	4.18	–
A_5	8.25	7.82	6.20	2.72	2.86	5.00	4.42	4.16
C_6	7.33	5.30	6.02	2.12	2.18	4.48	4.05	4.07, 4.25

2D COSY and NOSEY spectra of 1:1 harmine and oligonucleotide d(GTGCAC)$_2$ solution gave rise to the chemical shifts of short DNA after binding with harmine, which are in Table 3.

The chemical shifts of nonlabile proton of harmine before and after binding to DNA are listed in Table 4.

4. Discussion

From the ITC data, the binding enthalpy change for harmine to d(GTGCAC)$_2$ at 298 K is $-28.5\,kJ\,mol^{-1}$. It is quite close to the value for binding of harmine to calf thymus DNA considered as an evidence for intercalation mode of interaction in literature [7]. The binding number n is 1.6 is the molar ratio of ligand to DNA, indicating that specific

Table 4
Nonlabile protons chemical shift of harmine before and after mixing with DNA duplex

Proton	δ (ppm) (before mixing)	δ (ppm) (after mixing)
H24	6.79	6.50
H25	6.71	6.28
H27	7.93	7.65
H28	7.87	7.56
H23	7.77	7.54
H16, H17, H18 (–OCH_3)	3.88	3.60
H20, H21, H22 (–CH_3)	2.68	2.56

interaction and nonspecific interaction both exist in the binding process of harmine with oligonucleotide d(GTGCAC)$_2$. From Fig. 1, the cumulative reaction heat approached to a limit after enough ligand was added, which indicated the binding process of ligand with DNA was completed.

With the addition of harmine, the absorption of the two peaks of CD were both increased slightly, but the form of the curves kept. It indicated that harmine could induce DNA conformational changed slightly at high mole ratio of harmine to DNA.

Make an inspection on COSY spectrum of d(GTGCAC)$_2$ and expand the region of H1′–H2′, H2″ in Fig. 3, six spin systems are found, and correspond to six nucleotide residues. It indicates that d(GTGCAC)$_2$ is a self-complementary duplex and the two chains from 5′ to 3′ end are totally the same. Proton resonance in twelve nucleotide residues behaves as that in six residues of one chain. It makes spectra simple.

Each base's aromatic resonance displayed a stronger NOE cross peak to its own sugar H2′H2″ resonances than to the

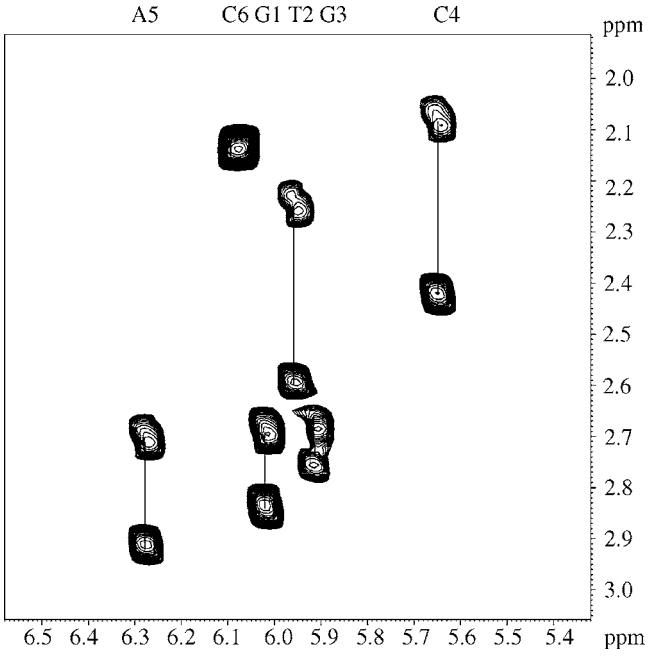


Fig. 3. Expended 2D COSY spectrum of d(GTGCAC)$_2$ in buffer in the range of H1′–H2′,H2′.

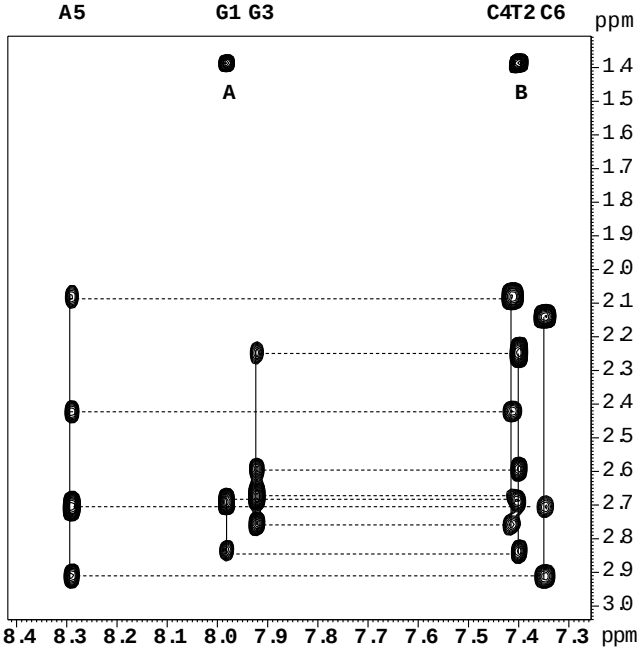


Fig. 4. Expended 2D NOESY spectrum of d(GTGCAC)$_2$ in phosphate buffer in the range of H6/H8–H2′, H2″. Solid and dashed lines illustrate the sequential walks.

sugar of the 5′-flanking residue (Fig. 4), the oligonucleotide d(GTGCAC)$_2$ may be described generally as a B-DNA conformation duplex [12], since the assignment of nonlabile protons resonance follows the regular sequence walks and NOE intensity rule.

Comparing the chemical shift of 11 nonlabile protons of harmine in pH 6.1 phosphate buffer solution with that in 1:1 harmine to DNA mixed solution in same buffer one finds that

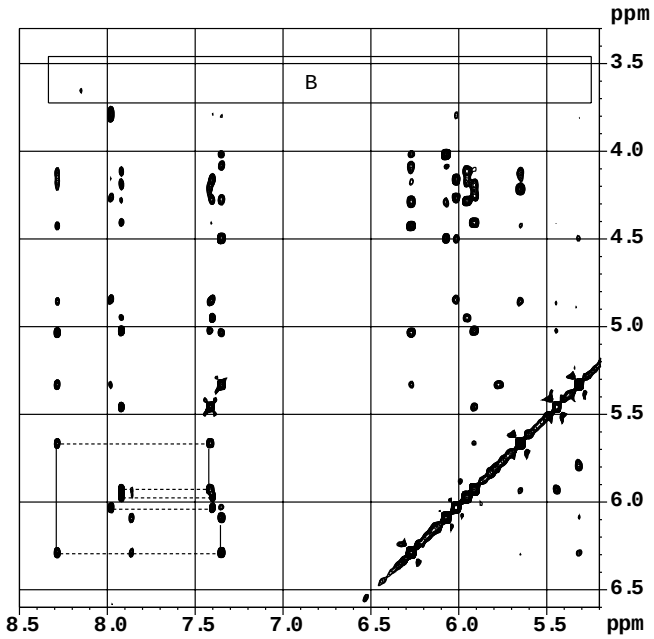


Fig. 5. Expended 2D NOESY spectrum of d(GTGCAC)$_2$ in buffer.

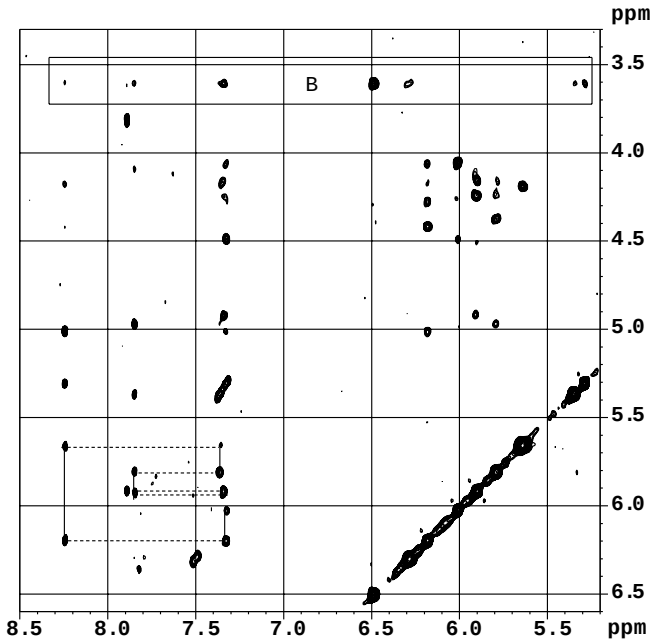


Fig. 6. Expended 2D NOESY spectrum of d(GTGCAC)$_2$ with harmine in mixing solution in the same range as in Fig. 5.

all peaks of protons in harmine shifted to up field (Table 4), especially the aromatic protons shifted up to 0.3–0.4 ppm, and the peaks were broaden. It gives support to the suggestion that harmine binds DNA in the mode of intercalation [13]. However, from 2D NOESY NMR spectrum of DNA with harmine (Fig. 6), in dilute solution DNA sequential link existed similar to that in Fig. 5. But the base's aromatic proton resonance was broaden, which indicated the binding of harmine with DNA.

In expanded 2D NOESY spectrum of d(GTGCAC)$_2$ with harmine, Fig. 6, the NOE cross peaks of harmine methoxyl group with some base's aromatic protons, H6/H8 of A_5, T_2 and C_6 and H5 of C_6 were observed in area B. However, area B in Fig. 5 is clean. This suggested that harmine binds to DNA at the side of methoxyl group preferentially. Furthermore, the chemical shift change for methoxyl group proton is larger than that of methyl group. Although Wang et al. [14] predicted that N11 in six member ring of harmine might be the active site of molecule.

Acknowledgements

This project was supported by the National Natural Science Foundation of China grant No. 20133030.

References

[1] M. Petersen, J.P. Jacobsen, Bioconjugate Chem. 9 (1998) 331–340.
[2] D. Norman, D. Live, M. Sastry, et al., Biochemistry 29 (1990) 2861–2875.

[3] A. Odani, T. Sekiguchi, H. Okada, Bull. Chem. Soc. Jpn. 68 (8) (1995) 2093–2102.
[4] G. Duportail, Int. J. Biol. Macromol. 3 (1981) 188–192.
[5] A. Hoffer, H. Osmond, The Hallucinogens, Academic Press, New York, 1967.
[6] W.M. Mcisaac, V. Estevez, Biochem. Pharmacol. 15 (1966) 1625–1627.
[7] B. Wang, Y. Zhang, M. Yang, P. Miao, K. Wang, Wuli Huaxue Xuebao (Acta Phys. Chim. Sin.) 10 (1994) 82–86.
[8] Z. Taira, S. Kanzawa, C. Dohara, S. Ishida, M. Matsumoto, Y. Sakiya, Jpn. J. Toxicol. Environ. Health 43 (1997) 83–91.
[9] T. Sheng, J. Wang, Biological Chemistry, vol. 1, Advanced Education Press, Beijing, 1990, p. 244 (in Chinese).
[10] D.R. Hare, D.E. Wemmer, S.H. Chou, G. Drobny, B. Reid, J. Mol. Biol. 171 (1983) 319–336.
[11] K. Wuthrich, NMR of Proteins and Nucleic Acids, Wiley/Interscience, New York, 1986, Chapters 11–13.
[12] P.S. Thomas, J.K. Barton, Biochemistry 34 (1995) 15049–15056.
[13] H.P. Hopkins, M. Yang, W.D. Wilson, D.W. Boykin, Biopolymers 31 (1991) 1105–1114.
[14] Z. Wang, J. Hao, D. Tang, X. Lin, S. Zhang, J. Mol. Sci. Int. Ed. 4 (1986) 227–232.