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Actinomycin D binds to single stranded DNA oligomers which contain double GTC triplets

Weihong Du [a,b,1], Ling Wang [a,1], Juan Li [a], Baohuai Wang [c,1], Zhifen Li [c], Weihai Fang [a,*]

[a] *Department of Chemistry, Beijing Normal University, Beijing 100875, China*
[b] *Department of Chemistry, Renmin University of China, Beijing 100872, China*
[c] *Institute of Physical Chemistry, Peking University, Beijing 100871, China*

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Abstract

The present work carried out a study on the interactions between Actinomycin D (ActD) and some single-stranded DNA oligomers, which contain double GTC triplets separated by TTT sequence. The interactions of drugs with DNA oligomers were investigated by UV, circular dichroism (CD) spectroscopy and isothermal titration calorimetry (ITC). The results indicate that ActD binds to the single stranded DNA oligomers in the fold back binding model as supported by added A/T base at DNA strand terminal which facilitates the formation of hairpin. The apparent binding constant K_b, the apparent binding molar enthalpy ΔH^0 and other thermodynamic data were obtained. The binding affinities are sequence dependent and related to the length of DNA strand. And the higher molar binding enthalpy indicates that the binding process is enthalpy driven.

Keywords: Actinomycin D; Single stranded DNA; GTC triplets; Thermodynamics

1. Introduction

Actinomycin D (ActD) [1] is one of the most studied anticancer drug that specifically inhibits the elongation phase of transcription. It acts by intercalating to duplex DNA GpC and certain GpG sites via its phenoxazinone chromophore [2,3] (Fig. 1). Recently, ActD was revealed to bind strongly to some non-GpC containing sequences and certain sequence specific single stranded DNA, which assists development of drugs against HIV and other retrovirus because the replication of their single stranded RNA genomes proceeds through a single stranded DNA intermediate [4–7]. It is very interesting that ActD has greater binding affinity to 5′-GXC/CYG-5′ than classical GpC sites, especially for T/T (X/Y) mismatch [8]. For single stranded DNA, some kind of hairpin model has been suggested earlier but in quite different sequence contexts [9–11]. Chou et al. found an unusual interaction mode for the intercalated drug ActD to single stranded DNA 5′-GXCACCGYC-3′, this hairpin central Watson–Crick base pair X/Y was looped out and displaced by the ActD chromophore [12]. Furthermore, Chen et al. found GTC binding site gave rise to a fold back binding mode for sequence specific single stranded DNA [13].

Although some sequence specific DNA oligomers were reported to investigate the interaction between ActD and DNA, the mismatch sequence was not utilized to reveal the binding enthalpy and entropy characteristics of the ActD affinity. We here study the single stranded DNA oligomers which contain double GTC triplets separated by TTT triplets. These oligomers are listed in Table 1. Strand 1 differs from strand 2 on the central triplets. Strands 3, 4 and 5 differ from each other on the order of A/T base flanking the central binding site sequence 5′-GTCTTTGTC-3′. Strand 6 is chosen for a comparison with published work.

As having been known, the TTT sequence is always located at the top of hairpin and GTC/GTC mismatch is of strong ActD binding affinity. The purpose of this research is to present thermodynamic data for the interactions of ActD with those single stranded DNA oligomers and find out some kind of intrinsic factor on the binding affinity of ActD with the specific DNA

* Corresponding author. Tel.: +86 10 58805382; fax: +86 10 58802075.
E-mail address: fangwh@bnu.edu.cn (W. Fang).
[1] These authors contribute equally to this paper.

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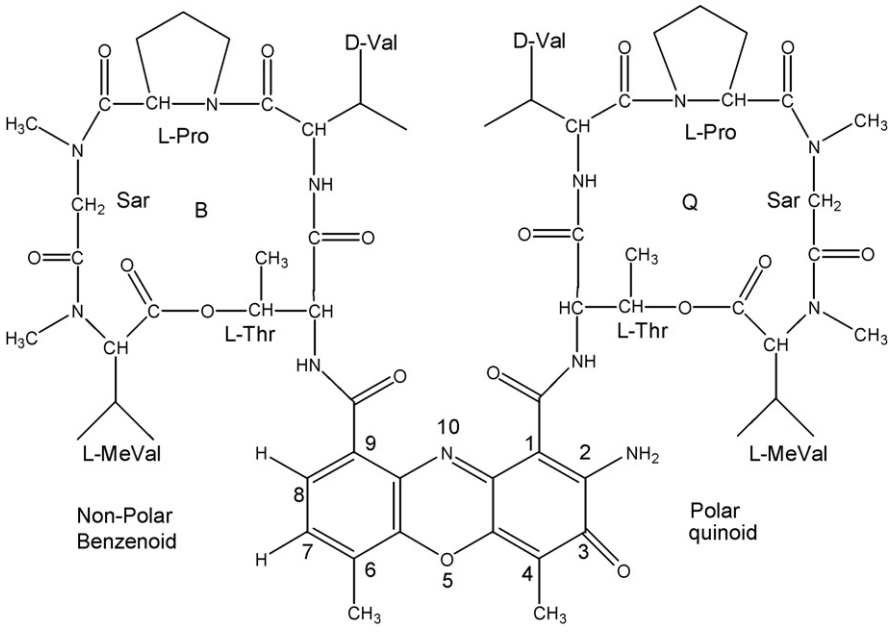

Fig. 1. Chemical structure of actinomycin D.

sequence flanked on either side by the nucleotides T or A (see strands 3, 4 and 5). The apparent binding enthalpy and the apparent binding free energy were obtained in this work. And understanding the ActD binding to non-GpC containing DNA sequences may be important in regulation of processes that require single stranded DNA as intermediates.

2. Experimental

2.1. Materials

The ActD and the synthetic DNA oligomers were purchased form Sangon Biological Engineer, China. ActD was used without further purification. DNA oligomers were purified by PAGE and lyophilized twice before use. Concentrations of the ActD and DNA solutions were determined as reported [13]. Stock solutions for DNA and ActD were prepared by dissolving sample in 10 mM potassium phosphate buffer of pH 7.0 containing 0.2 M NaCl and 0.1 mM EDTA.

2.2. Apparatus and procedure

UV absorption spectra were recorded with a Cintra 10e UV spectrometer. Data were collected from 500 nm to 350 nm. Absorbance spectral titration was carried out by starting with a 10 μM ActD solution of 2 ml in cell and following by progressive additions of the oligomers stock solution at equal time intervals according to the ratio of DNA to ActD from 0 to 2.

Table 1
Single stranded DNA oligomers and their abbreviation

d (GTCACCGTC)	Strand 1
d (GTCTTTGTC)	Strand 2
d (TGTCTTTGTCA)	Strand 3
d (ATGTCTTTGTCAT)	Strand 4
d (AATGTCTTTGTCATT)	Strand 5
d (TGTCTTTTGTC)	Strand 6

The circular dichroism (CD) spectra were recorded at room temperature with a CD6 Spectropolarimeter, Jobin Y von (38640 Claix, France). The data were collected from 300 nm to 220 nm with a scan speed of 0.5 nm/s.

The measurements of the heat of mixing DNA oligomer and ActD were carried out with the isothermal titration calorimetry (ITC) from CSC4200 (Provo, UT). The instrument was electrically calibrated by means of a standard electric pulse as recommended by the manufacturer. Titrations were carried out at 298 K by starting with ActD solution followed by progressive additions of the DNA oligomers stock solution at equal time and volume intervals.

3. Results

3.1. Binding stoichiometry of drug to DNA strand

The ActD binds to strands 3, 4 and 5 with 1:1 drug to strand binding stoichiometry. Representative absorption spectral alterations during a typical titration are illustrated in Fig. 2A for the system of ActD and strand 3. ActD exhibits an absorption maximum near 440 nm in buffer solution without DNA. Progressive additions of DNA stock solution result in considerable hypochromic effects on account of the binding of DNA to ActD. Absorbance differences between 425 nm and 475 nm during absorption spectral titrations were used to obtain the binding isotherms and were shown in Fig. 2B. The choice of 425 nm was based on the fact that the absorbance change at this wavelength was slightly larger than that of 440 nm upon DNA binding and that of 475 nm was to mimic the baseline value [8]. The absorbance differences of two wavelengths

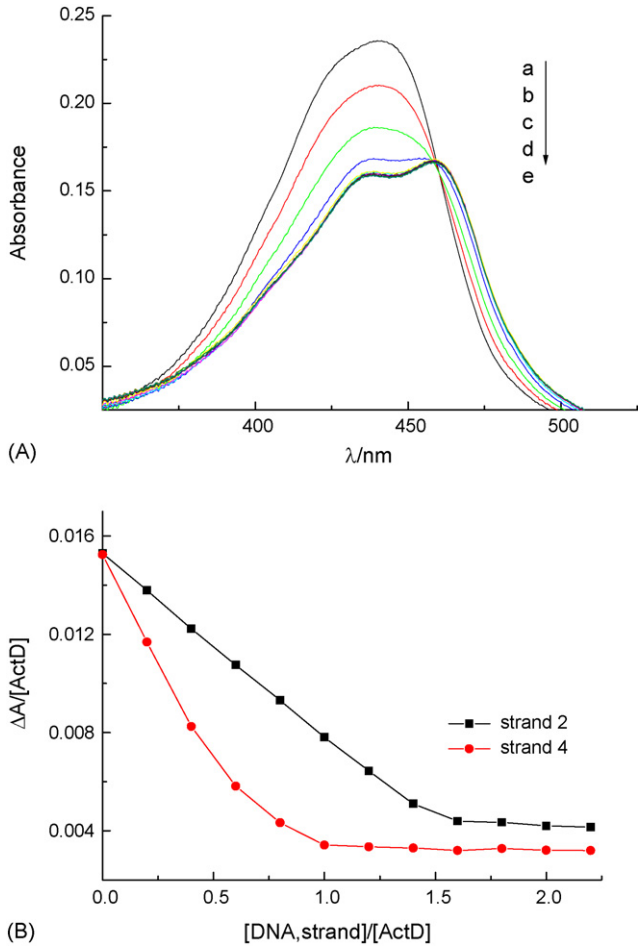

Fig. 2. (A) Typical spectral alterations during absorption spectral titrations as illustrated by the ActD and strand 3 system. For the concentrations of solutions a to e, see text. (B) Comparison of equilibrium binding isotherms for ActD binding to DNA. ΔA is the absorbance difference between 425 nm and 475 nm. Both ActD and DNA strand concentrations are in μM. All spectra titrations start with solutions containing 10 μM ActD.

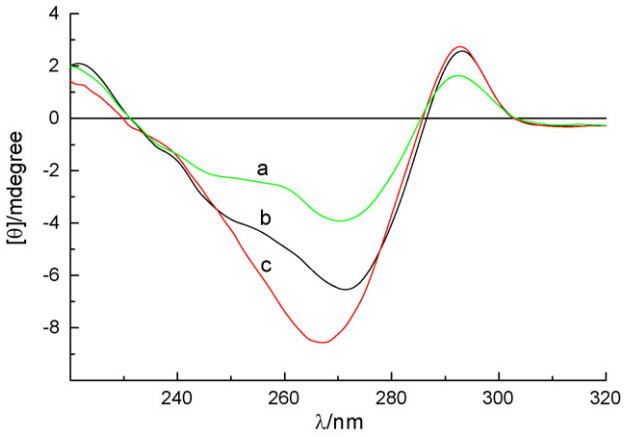

Fig. 3. The different CD spectra of 120 μM DNA in the presence of 60 μM ActD by subtracting the absorption of pure ActD and DNA oligomers contributions. All CD measurements were made at 298 K using cylindrical cells of 1-mm path length. The data were collected from 300 nm to 220 nm with a scan speed of 0.5 nm/s. Labels a, b and c were for the systems of ActD with strands 2, 3 and 4, respectively.

should minimize the errors due to baseline fluctuations during titrations.

The saturation patterns of these isotherms in Fig. 2 suggest that strands 3 and 4 bind strongly to ActD via 1:1 binding mode. But the ActD binding affinity to strand 2 whose terminal bases are removed from strand 3 is considerably weakened due to decreasing hypochromic effects and unideal stoichiometry. The binding affinity of drug to strand 1 was detected for comparison with the sequence 5′-GTCAC-CGAC-3′ [12]. From the apparent binding constants and their stoichiometry (Table 2), strand 1 shows a weaker binding affinity to ActD than that of 5′-GTCACCGAC-3′ due to displacing A8 with T8 in strand 1. Both strand 1 and strand 2 show reduced binding affinity than that of strand 3. Strand 5 is detected to understand if longer A/T base paring at terminal helps high binding affinity. UV titration curve of strand 5 is similar with that of strand 3. Despite UV spectra may carry out the analysis of binding study, thermodynamic parameters are obtained in this research by ITC method since ITC gives direct results of the apparent binding constant and the apparent binding enthalpy.

3.2. DNA conformational change induced by ActD

The ActD binding to DNA also induces a substantial CD spectral alteration in the DNA absorption spectral region. Thus, induced CD spectral characteristics can also provide some indications in conformational change of complex. Distinct CD spectral characteristics are induced by ActD binding to DNA strands 2, 3 and 4 as illustrated by their different CD spectra in Fig. 3. The CD spectra were obtained by subtracting out the absorption of both pure oligomer and ActD contributions. Negative CD maximum near 267 nm was induced by ActD binding to strand 4, whereas negative maximum near 270 nm and slight shoulder around 260 nm were apparent for ActD bind-

Table 2
Thermodynamic parameters of ActD binding to DNA oligomers at 298 K

DNA	n[a]	K_b (μM^{-1})	ΔH^0 (kJ mol^{-1})	ΔG^0 (kJ mol^{-1})	$T\Delta S^0$ (kJ mol^{-1})
Strand 1	0.62 ± 0.03	2.80 ± 0.15	−51.8 ± 1.2	−36.8 ± 0.1	−15.2 ± 1.3
Strand 2	0.73 ± 0.04	5.84 ± 0.09	−67.8 ± 0.9	−38.6 ± 0.1	−27.1 ± 1.0
Strand 3	1.08 ± 0.02	115.8 ± 0.12	−93.0 ± 0.4	−45.9 ± 0.2	−47.0 ± 0.6
Strand 4	1.02 ± 0.03	22.8 ± 0.21	−105.1 ± 0.5	−42.1 ± 0.3	−63.1 ± 0.8
Strand 5	1.16 ± 0.05	6.11 ± 0.22	−117.8 ± 0.7	−38.7 ± 0.2	−70.1 ± 0.9
Strand 6	1.01 ± 0.04	22.0 ± 0.16	−91.8 ± 0.3	−42.4 ± 0.1	−49.4 ± 0.4

[a] n = [ActD]/[DNA, strand].

ing to strand 2 and strand 3. Positive CD maxima of DNA at 280 nm in the absence of ActD moved to 290 nm in the presence of ActD. The results indicate very strong DNA conformational change after ActD binds to DNA. The prominent presence of the 270 nm absorbance in different CD spectra suggests that it may be characteristic of the binding at the GTC/GTC site, highlighting the effect of the looped-out T-base.

3.3. Thermodynamic properties of ActD binding to DNA

ITC was used to determine the binding thermodynamic parameters: the apparent binding enthalpy ΔH^0, the apparent binding constant K_b, and the stoichiometry n. The ITC titration curve for ActD binding to strand 4 at 298 K is shown in Fig. 4. Fig. 4A shows the trace recorded for each injection made at equal time intervals. After each titration, an exothermic heat effect was observed. The area of each peak was integrated and corrected by the heat effect of buffer as background. And Fig. 4B shows the fit of integrated heat to a titration curve calculated on the basis of the single binding site model for strand 4.

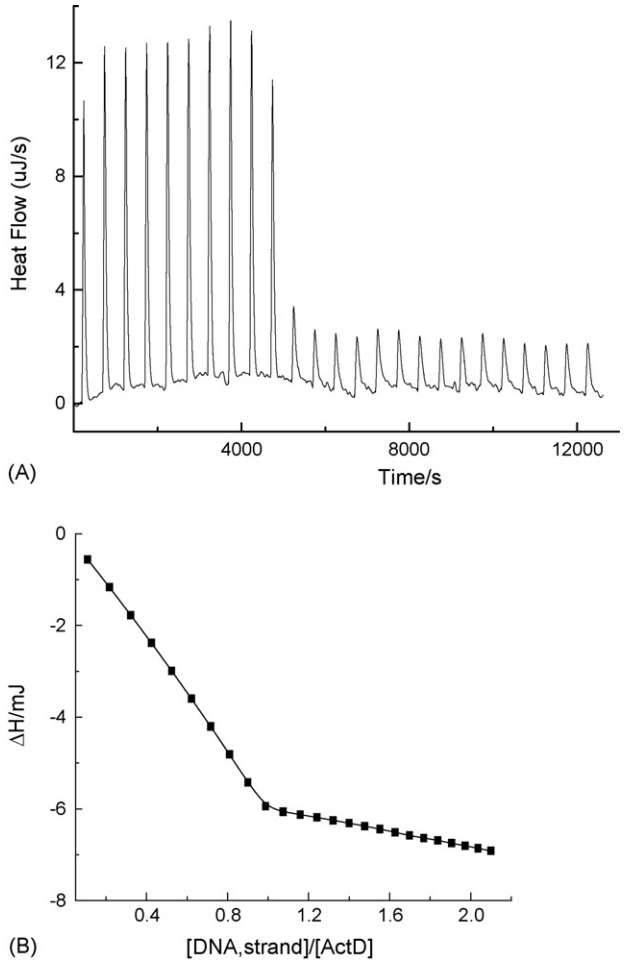

Fig. 4. (A) Typical ITC profile of the binding process between the ActD and strand 4 at 298 K. For each injection, 10 μL, 380 μM DNA was titrated into the cell containing 50 μM ActD. (B) The fit of integrated heat to a titration curve calculated on the basis of the single binding site model.

By fitting the titration curve with a nonlinear least-squares method, the apparent binding enthalpy ΔH^0, and the apparent binding constant K_b of ActD with DNA can be estimated with the assumption of an independent binding site model. The apparent free energy (ΔG^0) is obtained from the equation $\Delta G^0 = -RT \ln K_b$, in which K_b is the apparent binding constant. The ΔS^0 function is calculated from the standard thermodynamic relation $\Delta G^0 = \Delta H^0 - T\Delta S^0$. The results are summarized in Table 2. The provided values are the average of at least three experiments.

4. Discussions

All of the DNA oligomers display greater binding affinities to ActD than classical GpC sequences. The apparent binding molar enthalpies increase subsequently with the added DNA strand terminal base number. It indicates from the stoichiometry that an expected fold back binding mode is supported, except for the short strands 1 and 2. That means one single stranded DNA binds to one ActD molecule in the form of hairpin, at 5′-GTC/GTC-3′ binding site.

The binding constant of strand 3 to ActD is much larger than other strands. Compared with strand 3, strands 4 and 5 show reduced binding affinities. It is possible that terminal A/T base pairing leads to space encumbrance during drug-DNA binding, and the slight contribution of duplex formation. It is also seen from CD spectrum that for strand 4, the binding of ActD induces a strong CD spectral change in DNA absorption region since

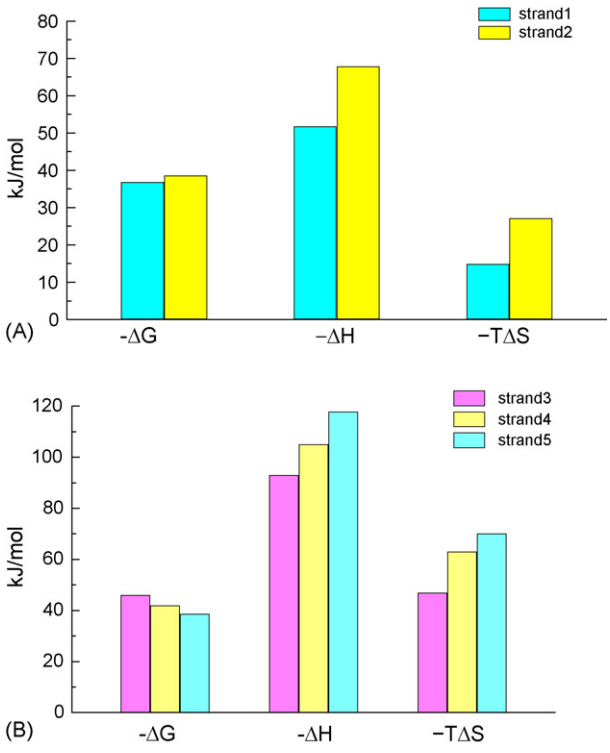

Fig. 5. Comparison of the enthalpic and entropic contributions to the binding free energy of ActD bound to the DNA oligomers at 298 K, where $\Delta G = \Delta H - T\Delta S$. Strand 1 and strand 2: upper panel A; strand 3, strand 4 and strand 5: bottom panel B.

longer DNA strand contributes at this point, but thermodynamic data show the binding affinity of strand 4 to drug is lower than that of strand 3, possibly by added A/T base at DNA strand terminal.

Thermodynamic data are important for disclosing the binding affinity of drug to DNA oligomers [14,15]. To consider flanking series effects, these thermodynamic results are similar to what has been observed, but the apparent binding constants and the apparent binding molar enthalpies are much larger than that of ActD binding to duplex DNA which contains only classical GpC sites [14]. That means the 5′-GTC/GTC-3′ binding site supplies much larger space for drug intercalation, and the binding affinity increases dramatically. Fig. 5 gives a comparison of the data ΔH^0 with $T\Delta S^0$ for all investigated systems. With change in DNA series from strand 1 to strand 5, the ΔH^0 is larger than the $T\Delta S^0$ throughout. It reveals that the binding process is remarkably enthalpy driven. It can be seen that with added A/T base, strand 5 leads to increasing molar enthalpy but decreased binding constant.

For strands 3, 4 and 5, we have to consider the contribution of enthalpy from Watson–Crick base pairing when DNA oligomers fold back and bind to ActD. In order to make it clear, the apparent binding enthalpy of ActD to the sequence 5′-TGTCTTTTGTC-3′ (strand 6), though the sequence is same as former work [13], was determined to identify if the larger molar enthalpy is mostly from binding or from base pairing, since strand 6 could reveal the source of apparent binding molar enthalpy without A/T base pair included. Our data show a good agreement of the binding constant ($K_b = 22.0 \times 10^6\ M^{-1}$) with what has reported [13] and the apparent binding molar enthalpy ΔH^0 is 91.8 kJ mol^{-1}, close to what we obtained in present work for strands 3 and 4. That means the apparent molar enthalpies are primarily from the contribution of ActD binding to single stranded DNA.

In conclusion, the ActD has a great binding affinity to single stranded DNA oligomers which contain double GTC triplets, and the binding affinity is quite sequence dependent. All results support the fold back binding mode for strands 3, 4 and 5. And the larger molar binding enthalpy indicates that the binding process is enthalpy driven.

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