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NMR Study of Consensus DNA-binding Site for *Arabidopsis thaliana* Class I Transcription Factor AtTCP1

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Abstract The TCP domain is a DNA-binding domain present in plant transcription factors and has a similar structural feature to the bHTH motif of eukaryotic transcription factors. The imino proton exchange study has been performed for the DNA duplex containing the consensus DNA-binding site for the AtTCP11 transcription factor. The first two base pairs in the consensus 5'-GTGGG-3' sequence are relatively very unstable but lead to greater stabilization of the neighboring two G•C base pairs. These unique dynamic features of the five base pairs in the consensus DNA sequence might play crucial roles in the effective DNA binding of the AtTCP11 protein.

Keywords NMR, DNA binding, Hydrogen exchange, TCP transcription factor

Introduction

The TCP domain is a DNA-binding domain present in plant transcription factors and its name comes from the first three characterized members of the transcription factor family: TB1 from maize, CYC from antirrhinum, and PCF from rice.[1,2] The TCP domain has a similar structural feature to the bHTH (basic helix-turn-helix) motif of eukaryotic transcription factors, which consist of two putative

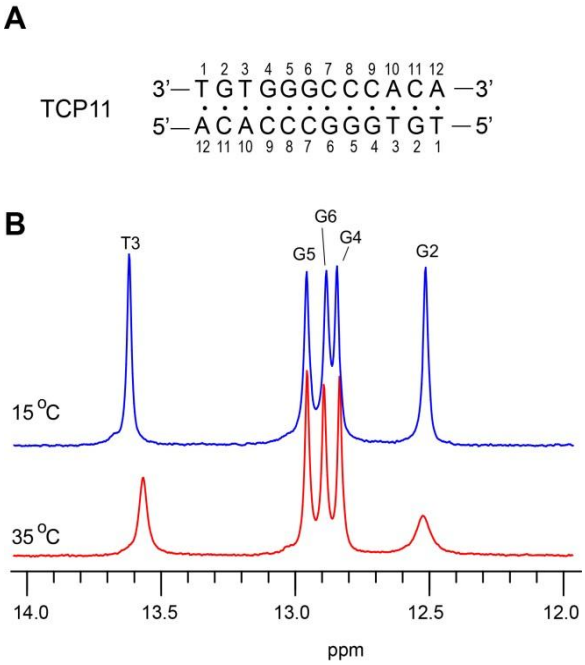

Figure 1. (A) DNA sequence context of the TCP11 DNA duplex. (B) 1D imino proton spectra of the TCP11 DNA duplex in 90% H_2O/10% D_2O buffer at 15 °C (top) and 35 °C (bottom).

α-helices connected by a loop with a basic N-terminal region. The TCP transcription factors can be divided into two classes, named I and II based on sequence homology within the TCP domains.[2] Recently, it was reported that the Arabidopsis class I TCP proteins interact with a dyad-symmetric sequence composed of two GTGGG half-sites.[3]

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AtTCP11 protein is a developmental regulator that influences the growth of leaves, stems, and petioles.[3] AtTCP11 shows a different DNA binding specificity with a preference for the sequence GTGGGCCNNN.[3] To understand the DNA binding mechanism of AtTCP11 protein, the imino proton exchange rates were measured for the DNA duplex containing the consensus DNA-binding site for the AtTCP11 transcription factor (referred to as TCP11 duplex, Fig. 1A). This study indicates that unique feature of base-pair stability of the TCP11 DNA duplex might correlate with its specific binding affinity for AtTCP11 protein.

Experimental Methods

The All DNA oligonucleotides were purchased from M-biotech Co. (Seoul, Korea). The oligonucleotides were purified by reverse-phase HPLC and desalted by Sephadex G-25 column. DNA duplexes were dissolved in an NMR buffer (90% H_2O/10% D_2O solution containing 10mM sodium phosphate (pH 8.0) and 100mM NaCl). NMR experiments were carried out on a Agilent DD2 700 MHz spectrophotometer (GNU, Jinju) equipped with *z*-axis pulsed-field gradient cold probe. 1D NMR data were processed and analyzed with the program FELIX2004 (FELIX NMR, San Diego, CA) or VNMRJ (Agilent, Santa Clara, CA) and 2D data were processed with the program NMRPIPE[4] and analyzed with the program Sparky.[5] To measure the hydrogen exchange rates of the imino protons, water magnetization transfer experiments were performed using delay times ranging from 5 to 100 ms.[6] The imino hydrogen exchange rate constants (k_{ex}) were determined by fitting the data to Eq. (1):[6]

$$\frac{I(t)}{I_0} = 1 - 2\frac{k_{ex}}{(R_{1w} - R_{1a})}(e^{-R_{1a}t} - e^{-R_{1w}t}) \quad (1)$$

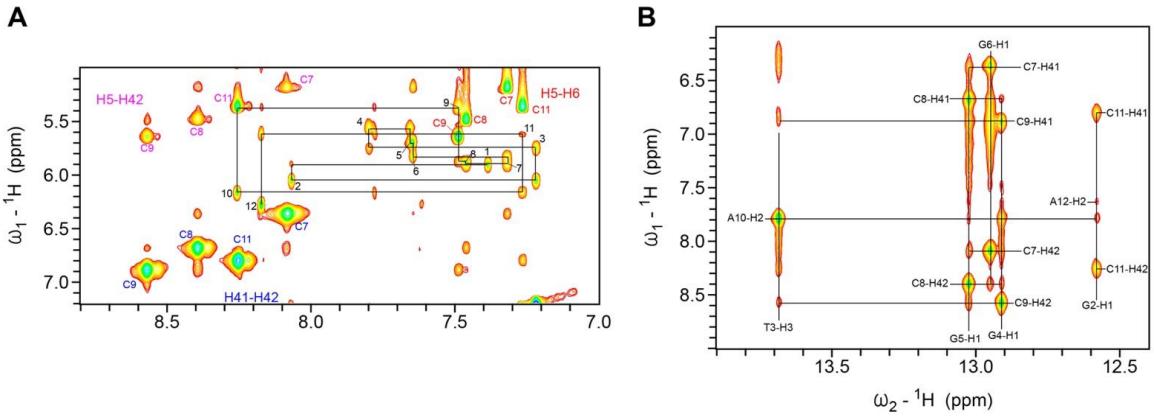

Figure. 2. Expanded NOESY (250 ms mixing time) contour plots of the TCP11 duplex in 90% H_2O/10% D_2O buffer at 15 ^oC. (A) A typical region (base to H1′ protons) in the sequential NMR connectivity. (B) NOE cross-peaks between the G-imino and C-amino protons of the G·C base pairs and those between T-imino and A-H2 protons of the A·T base

where R_{1a} and R_{1w} were the independently measured the imino proton and water, respectively, and I_0 and

Table 1. Chemical shifts of proton resonances in the TCP11 DNA duplex.

Residue	Imino/Amino	H2/H5/Me	H6/H8	H1′	H2′/H2″
T1	n.d.[1]	1.56	7.39	5.90	1.94/2.28
G2	12.58	–	8.07	6.05	2.77/2.85
T3	13.69	1.42	7.22	5.75	2.04/2.39
G4	12.91	–	7.80	5.56	2.62/2.70
G5	13.02	–	7.66	5.70	2.56/2.71
G6	12.95	–	7.65	5.82	2.51/2.65
C7	6.36/8.09	5.20	7.32	5.90	2.09/2.44
C8	6.68/8.39	5.48	7.46	5.87	2.10/2.41
C9	6.88/8.57	5.64	7.59	5.37	2.05/2.35
A10	–	7.78	8.26	6.16	2.66/2.80
C11	6.80/8.25	5.36	7.27	5.62	1.88/2.24
A12	–	7.63	8.17	6.27	2.42/2.60

[1]Not determined.

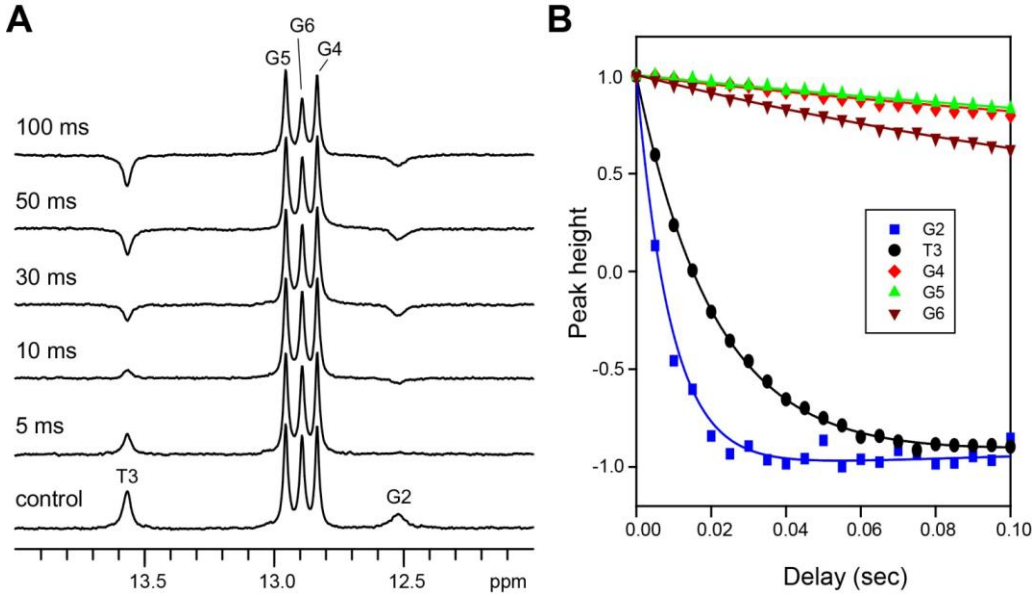

Figure. 3. (A) 1D imino proton spectra of the water magnetization transfer experiments for the TCP11 duplex at 35 °C. The delay times between the selective water inversion and acquisition pulse are indicated on the left of spectra. (B) Relative peak height [I(t)/I_0] in the water magnetization transfer spectra for the imino protons as a function of delay time. Solid lines indicate the best fitting of these data using Eq. (1).

and are the apparent longitudinal relaxation rates of $I(t)$ are the peak intensities of the imino proton in the

water magnetization transfer experiments at times zero and *t*, respectively.[6]

Results and Discussion

Two-dimensional NOESY spectra of the TCP11 duplex in 90% H_2O/10% D_2O buffer solution containing 10 mM sodium phosphate (pH 8.0) and 100 mM NaCl were acquired at 15 ^oC with 250 ms mixing times. The non-exchangeable base and sugar protons were assigned according to their intra-residue and sequential NOE connectivities (Fig. 2A). The exchangeable protons were assigned by the strong G-imino to C-amino or T-imino to A-H2 NOE cross peaks in the NOESY spectra (Fig. 2B). The chemical shifts of the proton resonances of the TCP11 duplex are given in Table 1. Fig. 1B shows the imino proton spectra of TCP11 duplex acquired at 15 and 35 ^oC. All imino protons except that of the terminal T1·A12 base pairs exhibit sharp resonances at 15 ^oC (Fig. 1B). However, all G2 and T3 imino proton resonances were significantly broadened at 35 ^oC, indicating instabilities of the G2·C11 and T3·A10 base pairs (Fig. 1B).

Table 2. Exchange rate constants, k_{ex}, (s^{-1}) of the TCP11 duplex at 35 ^oC

Base pair	Imino proton	k_{ex}
G2·C11	G2	109.1±0.7
T3·A10	T3	46.5±0.2
G4·C9	G4	1.09±0.05
G5·C8	G5	1.01±0.09
G6·C7	G6	2.43±0.03

The exchange rate constants of the imino protons for the TCP11 duplex were determined by water magnetization transfer method at 35 ^oC. Some imino protons show large differences in peak intensities as a function of delay time after water inversion (Fig. 3A). The most rapidly exchanging G2 imino proton shows negative peak at short delay times (10 ms in Fig. 3A). Similarly, the T3 imino proton also rapidly exchanges with solvent water and show negative peak at delay time of 30 ms, whereas the G5 resonance, which is the slowest exchanging imino proton, shows still positive up to 100 ms. The relative peak intensities of the water magnetization transfer for the imino proton resonances of the TCP11 duplex at 35 ^oC are plotted as a function of delay time in Fig. 3B. Table 2 shows the k_{ex} data of the imino protons of the TCP duplex determined by fitting to Eq. (1). The G5 imino proton is the slowest exchanging proton (k_{ex} of 1.01±0.09 s^{-1}), indicating that the G5·C8 base pair is the most stable base pair in the TCP11 duplex. The G2 imino proton next to the terminal T1·A12 base pair has the largest exchange rate constant of any nonterminal base pairs (k_{ex} of 109.1±0.7 s^{-1}). The T3 imino proton has larger k_{ex} value (46.5±0.2 s^{-1}) than central G imino protons. The G4 imino proton next to the T3·A10 base pair has k_{ex} values of 1.09±0.2 s^{-1} (Table 2). This result indicates that the G4·C9 base pair is unusually very stable even though this base pair has the neighboring unstable T3·A10 base pair. Interestingly, the central G6·C7 base pair, which has two neighboring G·C base pairs, has 2-fold larger k_{ex} value compared to the G4·C9 and G5·C8 base pairs (Table 2).

In summary, we determined the k_{ex} values of the imino protons in the AtTCP11 consensus DNA sequence using NMR spectroscopy. The first two base pairs (G2·C11 and T3·A10) are relatively very unstable but lead to greater stabilization of the neighboring two G·C base pairs. In addition, the final G·C base pair in the consensus DNA sequence has 2-fold larger k_{ex} value compared to the other G·C base pairs These unique dynamic features of the five base pairs in the consensus 5'-GTGGG-3' sequence might play crucial roles in the effective DNA binding of the AtTCP11 protein. Thus, this hydrogen exchange study can explain why the five conserved base pairs of the TCP11 binding site.

Acknowledgements

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