A temperature dependent transition in the Pribnow box of the trp promoter

Jean-François Lefevre, Andrew N. Lane and Oleg Jardetzky*

Stanford Magnetic Resonance Laboratory, Stanford University, Stanford, CA 94305, USA

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Proton NMR spectra of the trp operator-promoter (sequence CGTACTAGTT.AACTAGTACG) show selective changes in chemical shift and relaxation rates over the range of temperature 0–45°C for the non-exchangeable protons of A11 and A12 only. These bases are in the centre of the Pribnow box. The changes imply that at least three conformational states become significantly populated in this range of temperature, and probably involve a change in the propellor twists of A11 and A12 for one transition, and changes in the helical twist and local pitch for the other. As (1) mutations in the Pribnow box that destroy the TAA sequence impair promoter activity, and (2) the abortive initiation assay for RNA polymerase shows a transition near 20°C, we propose that the observed conformational transitions in the trp promoter are an essential feature of good promoters.

Promoter *Conformation change* *DNA* *NMR*

1. INTRODUCTION

The Pribnow box (consensus sequence TAATAT) found at the −10 position of prokaryotic promoters is likely to be involved in the formation of the active complex, also called the 'open' state [1,2]. In this state, an open site covering about 10 base pairs from the centre of the Pribnow box is formed [3]. It has been observed for some promoters that the formation of the open state is highly dependent on the temperature [4,5], with the mid-point lying between 15 and 25°C. we report here conformational transitions, observed by NMR, localised to the Pribnow box region of the trp operator-promoter, which has the sequence TTAACT [6]. These transitions occur in the temperature range 10–30°C, and so may be related to promoter activity.

2. MATERIALS AND METHODS

Trp operator DNA (sequence CGTACTAGTT.AACTAGATACG) which contains the Pribnow box (underlined [6]) was purchased from PL Biochemicals, and used without further purification. The DNA was lyophilised from 99.77% D_2O containing 10 mM sodium phosphate, 100 mM NaCl, pD* 8.5, and redissolved in 100% D_2O.

Proton NMR spectra were recorded at 500 MHz on a JEOL GX 500 instrument, and were referenced to internal 2,2′-dimethylsilapentane-5-sulphonate. Driven, truncated Nuclear Overhauser Effects (NOEs) were measured according to [7].

3. RESULTS AND DISCUSSION

3.1. *Effect of temperature on chemical shifts*

Our findings can be summarised as follows. The chemical shifts of all of the non-exchangeable protons of the bases are dominated by ring current of the neighbouring bases [8]. As the temperature is increased, the chemical shifts generally change monotonically, by about 0.06 ppm over the range 0–45°C (increasing shielding for AH2 and decreasing shielding for A and GH8). This is a known effect [9] which can be attributed to a combination

* To whom correspondence should be addressed

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of a change in the bulk diamagnetic susceptibility, and to a generalised decrease in the helical twist. However, the chemical shifts of A11 and A12 behave differently. The chemical shift of A11 H2 changes 0.17 ppm, whereas that of A11 H8 goes through a maximum. As the slopes of chemical shift vs temperature for all of the H8 protons except those of A11 and A12 are identical, and because the slope for A11 H8 at the extremes of the temperature range approaches that of the other bases, it is possible to calculate the excess chemical shift at each temperature. This is shown in fig.1. Clearly, the protons of A11 and A12 experience changing environments as the temperature is raised. The observation of a maximum for A11 H8 cannot be explained by a more extensive unwinding in this region, but requires a second conformational change that acts in the opposite direction. Hence, there must be at least three conformational states accessible to this small region of the molecule over this range of temperatures.

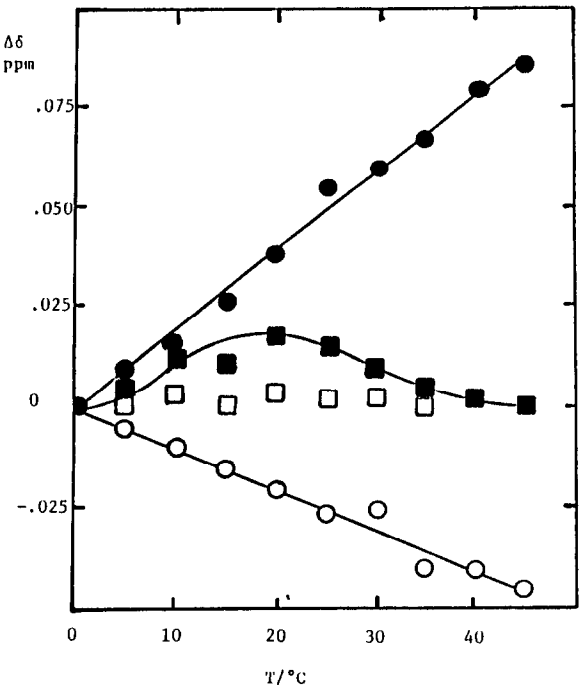

Fig.1. Dependence of chemical shifts on temperature. The chemical shifts were taken from spectra recorded at 500 MHz as described in section 2. The excess chemical shift was calculated by subtracting the background contribution determined as described in the text. (■) H8 of A11, (□) H8 of A12, (●) H2 of A11, (○) H2 of A12.

In fact, a more detailed analysis, which will be presented elsewhere, shows that three states gives an adequate description of the data, and allows us to calculate the enthalpy changes of the transitions and the populations of the states at any temperature.

3.2. *Dependence of the distance between A11 H2 and A12 H2 on temperature*

The distance between two nuclei can be estimated using the nuclear Overhauser effect, especially when there are only two spins to consider. This is so for A11 H2 and A12 H2, as they are isolated from the other protons in the molecule. For a macromolecule, the initial slope of the build-up of the NOE in truncated NOE experiments is given by [9]:

$$\sigma = (\alpha/10r^6)\tau_R \quad (1)$$

where σ is the cross relaxation rate constant, α is a molecular constant whose value is 5.693×10^{-37} $cm^6 \cdot s^{-2}$, and τ_R is the overall tumbling time of the molecule. Provided that τ_R can be measured or estimated, measurement of σ allows the internuclear separation to be calculated. We have previously measured the tumbling time as a function of temperature, and have shown that it obeys the viscosity law (Lane, Lefevre and Jardetzky, unpublished). The dependence of the NOE for A11 H2 to A12 H2 on the irradiation time at different temperatures is shown in fig.2B. The slopes of these lines are the cross relaxation rate constants. These were converted into apparent distances according to eqn 1. The main point is that at 5°C, the apparent distance is about 3.3 Å, decreasing to 3 Å at 25°C, and increasing again to about 3.4 Å at 40°C. To account for these large changes, it is necessary to postulate that at least three structural parameters are varying. Thus, a change in the helical twist of 10° changes the distance by only 0.2 Å, while a change in the local pitch of 0.2 Å produces a change of only 0.17 Å. Together, they might account for the change from one state to the next. The most likely structural parameter to account for the change in distance in the opposite direction is the propellor twist, or roll. A change of 10° in the propellor twist is sufficient to change the distance by 0.5 Å.

The rate of exchange between the conformations

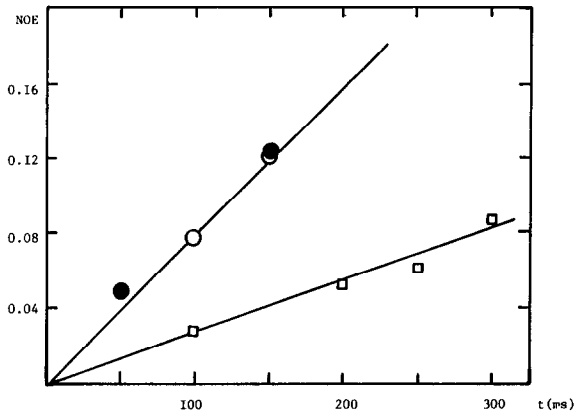

Fig.2. Build up of the NOE from A11 H2 to A12 H2. NOEs were determined at different irradiation times and different temperatures according to [7]. (○) 5°C, slope = 0.84 s^{-1}, (●) 25°C, slope = 0.8 s^{-1}, (□) 35°C, slope = 0.27 S^{-1}.

can be assessed from the dependence of the line width of A11 H2 on temperature. The line widths of all protons other than A11 H2 decrease monotonically as the temperature is raised, according to the viscosity law. The line width of A11 H2, however, first increases to a maximum at about 30°C, and then decreases (not shown). The maximum increase in line width is about 11 Hz. Detailed calculations (to be presented elsewhere) show that most of this increase in line width must be due to a contribution from exchange among the conformations. At the mid-points of the transitions, the exchange rate constants must be about 1000 s^{-1}.

3.3. *The biological relevance of the conformational transitions*

We note that the conformation changes are restricted to the trinucleotide sequence TAA in the Pribnow box. The sequences TAC and TAG do not show this behaviour, at least in this temperature range. Now the consensus sequence of the Pribnow box is TAATAT [10]; it is possible that the conformational features associated with TAA are essential for an efficient promoter. Initiation of transcription is at least a two-step process, consisting of an initial, rapid binding reaction (formation of the closed complex), followed by an isomerisation of the RNA polymerase-promoter complex to the active, or open state [11,12]. The isomerisation of the polymerase-promoter complex is characterised by a transition at about 20°C. We note, too, that the isomerisation measured in the abortive initiation assay is fastest for those Pribnow boxes that contain TAA; mutations that destroy this sequence have smaller isomerisation rate constants (isomerisation limited) [12]. For example, the lac P promoter is isomerisation limited, and its Pribnow box has the sequence TATAGT. In contrast, the lac UV5 promoter has the sequence

Table 1

Sequences of Pribnow boxes and isomerisation rate constants

Promoter	Sequence	Isomerisation rate constant		Ref.
		Predicted	Observed	
λPR	GATAATG	high	high	12
tet	TTTAATT	high	high	11
lacP	TATGTTG	low	low	12
lacUV5	TATAATG	high	high	12
T7A1	GATACTTA	high	high	12
trp	GTTAACT	high	nd	–
trpR	TATCGTA	low	low*	13

The isomerisation rate constant is obtained from the approach to the steady state in the abortive initiation assay for RNA polymerase [10–12]. The classification high or low is according to [12]. nd. not determined. *the trp R promoter is weak in vivo, but the isomerisation rate constant has not been determined

TATAAT, and isomerises relatively rapidly. It is possible to predict the relative rate constant for the isomerisation process on the basis of the presence of TAA or TAT (or their complements) in the Pribnow box. Table 1 presents the predicted rate constants, with the observed values, according to the classification of Hawley et al. [12]. The good agreement between the prediction and the observation lends support to our proposal. A critical test will be the measurement of the isomerisation rate constant for the trp promoter.

In conclusion, we have shown that there are important conformational transitions in the Pribnow box associated with the sequence TAA, which produces a conformational state from which transcription is rapidly initiated. This is the first direct evidence for a specific geometric feature of DNA that is involved in function and specific recognition by a DNA binding protein.

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