A nuclear Overhauser enhancement study on the imino proton resonances of a DNA pentadecamer comprising the specific target site of the cyclic AMP receptor protein in the *ara* BAD operon

Angela M. Gronenborn, G. Marius Clore, Michael B. Jones* and Joseph Jiricny*+

Division of Physical Biochemistry, National Institute for Medical Research, Mill Hill, London NW7 1AA, and **Chemistry Laboratory, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, England*

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A 500 MHz ^{1}H-NMR study on a synthetic DNA pentadecamer comprising the specific target site of the cAMP receptor protein in the *ara* BAD operon is presented. Using pre-steady state NOE measurements, unambiguous assignments of all the imino proton resonances and associated adenine (H2) resonances are obtained. From the NOE data interbase pair interproton distances involving the imino and adenine (H2) protons are determined. It is shown that these distances are very similar to those expected for classical B DNA (RMS difference of 0.5 Å), but are significantly different from those expected for classical A DNA (RMS difference of 1.1 Å)

Synthetic oligonucleotide *Imino proton* *NOE* *Interproton distance* *CRP specific site*

1. INTRODUCTION

The cAMP receptor protein (CRP) regulates the transcription of at least 20 genes including all catabolite repressible operons [1,2]. The cAMP · CRP complex binds to specific DNA target sites located at the 5′ end of each gene it regulates. In some cases this interaction stimulates transcription as in the case of the *lac* [3] and *ara* BAD [4] operons, whereas in other cases it represses transcription as in the case of its own structural gene [5] and the *ompA* gene [6]. At present the molecular mechanism of the interaction of the cAMP · CRP complex with specific DNA target sites is unknown although it has been shown that the interaction induces a B to C transition in the structure of the DNA without changing the handedness of the helix, namely right handed [7–9]. As an initial step in studying the structural aspects of this interaction it is essential to investigate the isolated components of the system. In this respect the crystal structure of the cAMP · CRP complex has been solved at 2.9 Å resolution [10,11], and NMR studies on CRP and its N-terminal core αCRP as well as on their interaction with cyclic nucleotides have been carried out [12–14]. We extend here the solution NMR studies to the synthetic DNA pentadecamer

5′ A A A G T G T G A C G C C G T 3′ (+)
3′ T T T C A C A C T G C G G C A 5′ (−)

+ Present address: Friedrich Miescher Institut, Postfach 273, CH 4002 Basel, Switzerland

Abbreviations: CRP, cAMP receptor protein of *Escherichia coli;* cAMP, adenosine cyclic 3′,5′-phosphate; NOE, nuclear Overhauser enhancement or effect

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comprising the specific target site for CRP in the *ara* BAD operon [4,15]. Using the nuclear Overhauser effect (NOE) to demonstrate the proximity of protons in space [16], we have employed the same sequential assignment procedure that has been applied so successfully to tRNAs [17–22] and more recently to DNA oligonucleotides [23–26] to assign unambiguously all detectable imino protons and associated adenine H2 protons. From the pre-steady state NOE data interproton distances involving the imino and adenine H2 protons are calculated and compared to those expected for regular right-handed B and A DNA helices.

2. EXPERIMENTAL

Both strands of the DNA pentadecamer were synthesized from suitably protected nucleosides using the solid support phosphotriester method (on controlled pore glass) and purified by ion exchange chromatography using a Partisil 10 SAX column essentially as in [27]. After desalting and lyophilization, equal amounts of the two pentadecamers were taken up in a buffer comprising 90% H_2O/10% D_2O, 100 mM KCl, 5 mM potassium phosphate (pH 6.6) and 0.01 mM EDTA. The concentration of each strand was 0.8 mM.

NMR spectra were recorded on a Bruker AM500 spectrometer using the time shared hard 1-1 observation pulse (θ_x–τ–θ_x) for water resonance suppression [28,29] with the carrier placed 3048 Hz downfield from the water resonance, a delay τ of 160.5 μs, a total flip angle ($2\theta_x$) of 90°, an acquisition time of 0.188 s (8196 data points and a spectral width of 21 739 Hz) and a relaxation delay of 0.5 s. The NOEs were observed by directly collecting the difference free induction decay (FID), by interleaving 16 transients after saturation for 0.5 s of a given resonance with 16 transients of off resonance irradiation (also applied for 0.5 s), negating the memory between 16 transient cycles. Prior to Fourier transformation, the free induction decays were subjected to data shift manipulation to reduce further the water resonance and thereby eliminate baseline distortions [28,30,31], and then multiplied by an exponential equivalent to a line broadening of 5 Hz. Chemical shifts are expressed relative to 4,4-dimethylsilapentane-1-sulphonate.

It will be noted that the integrated intensities of the exchangeable proton resonances are not all equal due to both chemical exchange and amplitude distortion arising from the nature of the 1-1 excitation pulse. Consequently, the NOE magnitudes were obtained by dividing the intensity of the observed peak, say x, as measured from the difference spectrum, by the total intensity of peak x measured from the difference spectrum in which peak x is irradiated. The estimated relative error in the NOE magnitudes is $\leqslant \pm 0.15$.

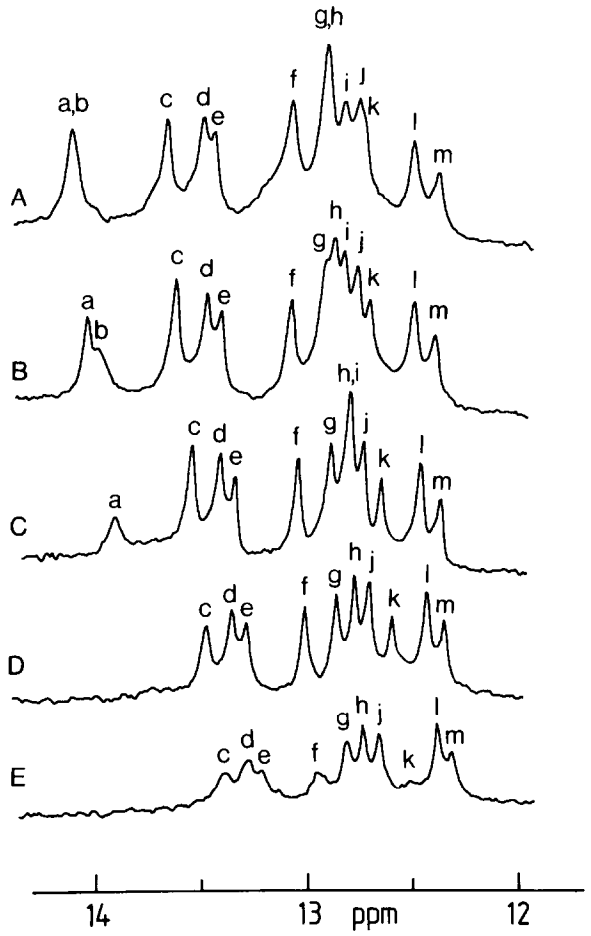

Fig. 1. Temperature dependence of the imino proton resonance region (12–14 ppm) of the 500 MHz ^{1}H-NMR spectrum of the pentadecamer. (A) 0°C, (B) 15°C, (C) 30°C, (D) 45°C, and (E) 60°C. Experimental conditions: 0.8 mM (per strand) pentadecamer, in 90% H_2O/10% D_2O, 100 mM KCl, 5 mM potassium phosphate pH 6.6 and 0.01 mM EDTA. 400 transients were recorded for each spectrum.

3. RESULTS AND DISCUSSION

Fig.1 shows the temperature dependence of the imino proton resonance region of the double stranded pentadecamer over the 0–60°C range. At the lowest temperatures, 13 distinct resonances are seen grouped into two sets of peaks: the low-field set comprising resonances a–e are assigned to the imino proton resonances of AT base pairs, and the high-field set comprising resonances f–m are assigned to the imino proton resonances of the GC base pairs. This assignment of resonance type is based on the large body of data available on the location of Watson-Crick hydrogen-bonded imino proton resonances [17–26,32]. The two imino protons of the terminal AT base pair at both ends of the pentadecamer are not visible due to kinetic fraying. As the temperature increases successive resonances or groups of resonances broaden and finally disappear owing to rapid exchange with solvent water. At 25°C resonance b broadens and disappears; this is followed at 40°C by resonances a and i. Taken together with the pentadecamer sequence, these findings enable one to assign resonances b and a to the T(H3) imino protons of the $A_{2+}T_{14-}$ and $A_{3+}T_{13-}$ base pairs respectively, and resonance i the G(H1) imino proton of the $G_{14+}C_{2-}$ base pair.

A more rigorous approach to the assignment of the imino proton resonances is based on NOE measurements. This requires no assumption about the resonance frequencies of particular types of imino protons and no previous knowledge of the thermal melting properties, and permits the complete assignment of the imino proton resonance region of the spectrum. For short irradiation times, the pre-steady state NOE observed on resonance i following irradiation of proton j, N_{ij}, is given by

$$N_{ij} \sim \sigma_{ij} t \qquad (1)$$

where t is the length of the selective irradiation pulse and σ_{ij} the cross-relaxation rate between protons i and j [33,34]. σ_{ij} is inversely proportional to $1/r_{ij}^6$ so that distance ratios or distances, if one distance is already known, can be obtained from the equation

$$r_{lk}/r_{ij} = (\sigma_{ij}/\sigma_{lk})^{1/6} = (N_{ij}/N_{lk})^{1/6} \qquad (2)$$

providing the correlation times of the two interproton distance vectors are the same. Because of the r_{ij}^{-6} dependence of σ_{ij}, effects are only detectable between protons separated by $\leqslant 5$Å. Consequently, irradiation of the imino proton resonance of base pair i will result in small interbase pair NOEs on the imino and adenine H2 protons of the adjacent base pairs, $i-1$ and $i+1$, on either side. In addition large intrabase pair NOEs will be observed between the T(H3) imino proton and the A(H2) proton in an AT base pair, and between the G(H1) imino proton and the amino protons of C or G in a GC base pair (providing, in the latter case, the amino protons are not broadened beyond detectability by exchange with solvent water). The selective irradiation pulse used in the quantitative pre-steady state NOE measurements reported here was 0.5 s, and control experiments using different irradiation times for a few selected resonances indicated that eq. 1 was valid at this irradiation time.

Fig.2 illustrates the application of the NOE method of assignment to the imino proton resonances of the pentadecamer. Irradiation of peak c (fig.2B, 15°C) results in a large intrabase pair NOE on the non-exchangeable A(H2) resonance 1 and small interbase pair NOEs on the adjacent imino proton resonances j and l. Irradiation of the A(H2) resonance 1 (fig.2C, 15°C) then results in a large intrabase pair NOE on the imino proton resonance c as well as small interbase pair NOEs on the adjacent imino proton resonances j and l. Finally irradiation of peak l (fig.2D, 15°C; fig.2E, 0°C) results in a large intrabase pair NOE on the exchangeable amino proton resonance o and small interbase pair NOEs on the imino proton resonances c and d and the A(H2) resonances 1 and 2. Extending these measurements by systematically irradiating all imino, amino and A(H2) resonances in turn permit the unambiguous assignment of these resonances as illustrated in terms of the NOE flowchart in fig.3.

The pre-steady state NOEs that could be quantitated are given in table 2 at two temperatures, 0 and 15°C. It will be noted that the magnitude of the intrabase pair NOEs between the T(H3) and A(H2) protons at 15°C is about half that at 0°C, although the distance between these two protons in a Watson-Crick AT base pair is 2.9 Å. This is due to the slower tumbling (i.e. longer correlation time) of the pentadecamer at the lower

Table 1

Values of the pre-steady state NOEs (0.5 s pre-irradiation time) observed for the pentadecamer together with the interproton distances calculated from them and the corresponding interproton distances protonexpected for classical B and A DNA[a]

	NMR				Fibre diffraction r_{ij}(Å)	
	0°C		15°C			
	% NOE	r_{ij}(Å)	% NOE	r_{ij}(Å)	B DNA	A DNA
(A) Intrabase pair						
T(H3)-A(H2)						
(reference distance)[a]		2.9		2.9	2.9	2.9
$A_{3+}T_{13-}$	−19		−13			
$T_{5+}A_{11-}$	−22		−15			
$T_{7+}A_{9-}$	−35		−15			
$A_{9+}T_{7-}$	−32		−14			
G(H1)-amino[b]					2.6/2.4	2.6/2.4
$G_{8+}C_{8-}$			−33	2.5		
$C_{12+}G_{4-}$		−35	2.5			
(B) Interbase pair						
T_{13-}(H3)-G_{4+}(H1)	− 4	3.8	−5	3.4	3.4	3.7
G_{4+}(H1)-T_{5+}(H3)	− 4	3.9	−6	3.4	3.5	3.4
G_{4+}(H1)-A_{11-}(H2)	− 5	3.7	−	−	3.8	4.5
T_{5+}(H3)-G_{6+}(H1)	− 8	3.4	−6	3.4	4.1	5.0
A_{11-}(H3)-G_{6+}(H1)	− 7	3.5	−5	3.5	4.3	5.3
G_{6+}(H1)-T_{7+}(H3)	− 6	3.9	−5	3.5	3.5	3.4
G_{6+}(H1)-A_{9-}(H2)	− 8	3.7	−2	4.1	3.8	4.5
T_{7+}(H3)-G_{8+}(H1)	− 6	3.9	−3	3.4	4.1	5.0
A_{9-}(H2)-G_{8+}(H1)	−12	3.5	−4	3.6	4.3	5.3
G_{8+}(H1)-T_{7-}(H3)	− 4	4.1	−6	3.4	3.7	4.3
G_{8+}(H1)-A_{9+}(H2)	− 8	3.7	−6	3.4	3.9	4.6
T_{7-}(H3)-G_{6-}(H1)	− 7	3.7	−4	3.6	3.5	3.4
A_{9+}(H2)-G_{6-}(H1)	− 9	3.6	−6	3.4	3.8	4.5
RMS difference (Å)						
NMR(0°C) − NMR(15°C)			0.4			
NMR(0°C) − B_FDNA			0.5			
NMR(15°C) − B_FDNA			0.5			
NMR(0°C) − A_FDNA			1.1			
NMR(15°C) − A_FDNA			1.1			
B_FDNA − A_FDNA			0.7			

[a] The interproton distances for classical B and A DNA are derived from the fibre diffraction data in [35]. NMR interproton distances are calculated from the NOE data using eq. 2 with the intrabase pair distance (2.9 Å) and NOE between the T(H3) and A(H2) protons of an AT base pair as an internal reference (see text for discussion). The relative error in the measured NOE values, $\Delta N/N$, is $\leq \pm 0.15$, and assuming an error of ± 0.1 Å in the reference distance, the error in the calculated interproton distances is $\leq \pm 0.2$ Å

[b] The distances between the G(H1) imino proton and the hydrogen-bonded amino protons of G and C in a GC base pair are 2.4 and 2.6 Å, respectively

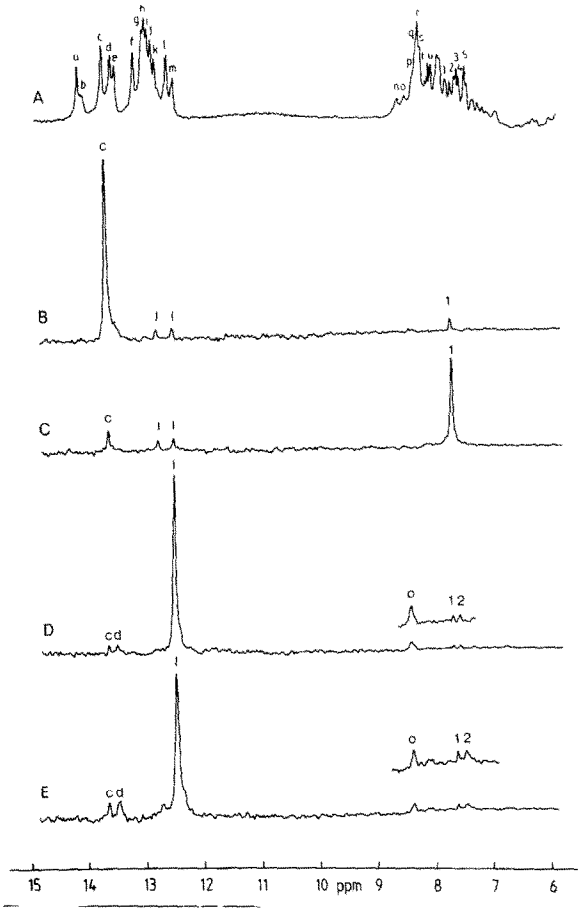

Fig. 2. Pre-steady state NOE measurements on the pentadecamer in 90% H_2O/10% D_2O. (A) The 500 MHz ^{1}H-NMR spectrum between 6 and 15 ppm with the exchangeable imino and amino proton resonances labelled a–m, and o–u, respectively, and the non-exchangeable A(H2) proton resonances labelled 1–4. Difference spectra (off-resonance minus on-resonance pre-irradiation) following pre-saturation for 0.5 s of (B) the T_{7-} (H3) resonance (peak c), (C) the A_{9+}(H2) resonance (peak 1), and (D) and (E) the G_{8+}(H1) resonance (peak l). The temperature was 15°C for spectra A–D and 0°C for spectrum E. The assignments of the other peaks seen in the difference spectra are given in fig. 3. Note that a decrease in intensity of a particular resonance is seen as a positive peak in the difference spectrum. The experimental conditions are the same as those in fig. 1. 400 and 6400 transients were recorded for the reference and difference NOE spectra, respectively.

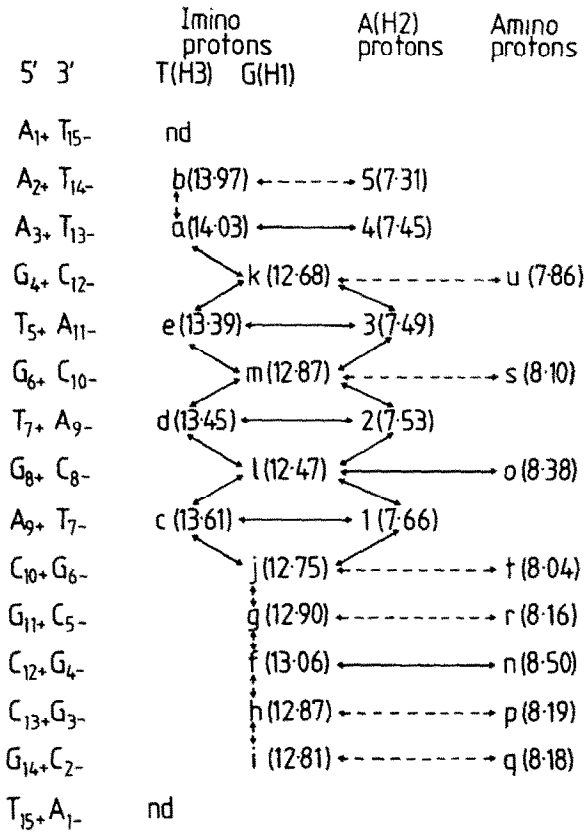

Fig. 3. Flow chart of the observed NOEs together with the resonance assignments. The continuous lines (——) represent quantified NOEs; the dashed lines (– – –) represent NOEs that could not be quantified. The imino, amino and A(H2) resonances are labelled a–m, o–u, and 1–5, respectively. Chemical shifts (ppm) at 15°C are given in parentheses. nd, not detectable (due to kinetic fraying).

temperature. It can also be seen that whereas the magnitude of the NOEs between these two protons is approximately the same for the different AT base pairs at 15°C, significant differences are observed at 0°C; namely, at 0°C the AT base pairs at the end of the pentadecamer have smaller NOE values than those in the middle. This can be attributed to a more marked degree of mobility of the residues at the ends of the pentadecamer relative to those at the centre at 0°C.

To calculate interproton distances we have made use of eq. 2 using the distance (2.9 Å) and NOE between the A(H2) and T(H3) protons of the AT base pairs as an internal reference. In the case of the data at 15°C, all intrabase pair NOEs between these two protons had a value of $15 \pm 2°$. In the case of the data at 0°C, the separation between the

imino and A(H2) protons of AT base pair i from the imino protons of the adjacent GC base pairs, $i-1$ and $i+1$, was calculated using the value for the intrabase pair NOE between the T(H3) and A(H2) protons of AT base pair i as the reference. Assuming an error of ± 0.1 Å in the reference distance and a relative error of $\leq \pm 0.2$ in the measured NOE values, the error in the computed distances is $\leq \pm 0.2$ Å. The interproton distances obtained in this manner are given in table 1 and compared to those expected for classical B and A DNA based on the fibre diffraction data in [35]. As can be seen from table 1, the interproton distance data at both temperatures are very close to those expected for B DNA with a root mean square (RMS) difference of ~ 0.5 Å but significantly different from those expected for A DNA (RMS difference ~ 1.1 Å). This finding is in complete agreement with the CD spectrum of the pentadecamer (not shown) which is characteristic of B DNA. Also noteworthy is that the present data do not provide any indication of substantial propellor twisting of any of the base pairs of the pentadecamer, since this would result in a significantly shorter interbase pair distance between the A(H2) and G(H1) protons than between the T(H3) and G(H1) protons of adjacent AT and GC base pairs [24-26].

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