**1H NMR studies of lac-operator DNA fragments**


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**ABSTRACT**

The hydrogen-bonded imino protons of a 14 base pair double-stranded DNA fragment comprising one half of the lac operator of E. coli were investigated by 360 MHz 1H NMR. From combined melting studies of this synthetic 14 b.p. fragment and its two constituent 7 b.p. fragments a nearly complete assignment for the low-field proton resonances was obtained. The experimental spectra are compared with calculated spectra and with the spectrum of a 51 b.p. DNA restriction fragment from E. coli containing the complete lac operator. Structural information on these oligonucleotides is presented. This study is a prerequisite for future 1H NMR investigations of the interaction of the lac operator with the lac repressor.

**INTRODUCTION**

In the Escherichia coli lactose operon the expression of structural genes is negatively controlled by specific binding of the tetrameric lac repressor (MW 156000) to the 20-25 b.p. (base pair) lac operator. The sequence of the operator, as it was determined by Gilbert and Maxam 1 is shown in Fig. 1. An important characteristic of this operator is that it possesses an approximate twofold axial symmetry, a property in common with many other DNA sequences that are recognized specifically by proteins, e.g. the binding sites for lambda repressor and cro in bacteriophage λ and that for the cyclic AMP receptor protein in the lac operon of E. coli. From elegant methylation-protection experiments Ogata and Gilbert 2 showed, that the pattern of operator sites contacting the lac repressor in the complex possesses approximately the same symmetry relations. Furthermore, they showed that this pattern does not change much when lac repressor headpiece (the 51 or 59 amino acid residues long N-terminal tryptic fragment of repressor) is used instead of native repressor for their protection experiments. This suggests that the lac repressor binds with two
(out of its four) headpieces to the lac operator. Apart from these
symmetry arguments, there is additional experimental evidence support-
ing this hypothesis: the quaternary structure and operator binding
characteristics of lac repressor-β-galactosidase chimaeras, low-angle
X-ray data, revealing the spatial arrangement of headpieces on the
lac repressor7 and high-resolution NMR data, probing the changes in
operator structure upon binding 1-2 headpiece molecules. It thus
seems reasonable to model the lac repressor-lac operator interaction
by the binding of one headpiece to half the operator.

On the basis of this hypothesis we have synthesized a 14 b.p.
DNA fragment comprising one half of the operator (fragment AB, Fig. 1).
It is our intention to study the interaction of this fragment with
lac repressor and its isolated headpiece in order to understand the
molecular mechanism of specific protein-DNA interaction.

In this paper we report on 1H NMR studies at different temperatures
of the hydrogen-bonded imino protons of the 14 b.p. fragment AB and
its constituent fragments A and B (see Fig. 1). For assigning the
imino-proton resonances the following properties were used:
1. The AT and GC imino-proton resonances fall in different spectral
regions.9
2. Thermal denaturation ('melting') of short deoxy oligonucleotides
occurs sequentially and starts from the terminal base pairs.10-13

This melting behaviour was independently confirmed in the present study.
3. Partial symmetry in the base-pair sequence, causing protons in near-
ly identical chemical surrounding to resonate at approximately the
same frequency.

The assignment was completed by comparing the spectra of the
separate fragments A and B with that of the larger fragment AB. This

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5'-TGGAATTGTGAGCGGATAACAATTTCA
ACCTAAACACTGGCTATGTTAAAGT
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Fig. 1 Sequence of the E. coli lac-operator as determined by Gilbert
and Maxam. The symmetrical regions around the central base
pair GC 13 are marked by bars. The fragments used in this
study and the numbering of the base pairs, as employed in the
text, are indicated.
approach can only be successful if the structure of the small oligo-
nucleotides does not change much when they are part of the larger
fragment (apart from fraying effects in the terminal base pairs that
become linked together in the larger fragment). As will be discussed
below, this condition is indeed fulfilled. Our approach has resulted
in a nearly complete assignment of the imino proton resonances.

The spectrum of the 14 b.p. lac operator fragment was compared
with a calculated spectrum and with a previously obtained spectrum
of a 51 b.p. restriction fragment comprising the complete lac operator.
The comparison has led to several assignments in the latter spectrum.

The present investigation is a prerequisite for the interpretation
of $^1$H NMR studies of the interaction of lac repressor headpiece with
the operator fragment (manuscript in preparation).

MATERIALS AND METHODS

The DNA fragments were synthesized via an improved phosphotriester
approach. The oligonucleotides were converted into their NH$_4^+$
salts by slow passage through a column (12 x 1 cm$^2$) of Dowex cation-
exchange resin (NH$_4^+$-form); elution was performed with distilled and
de-ionized water. Lyophilization afforded the deoxy oligonucleotides
as white fluffy compounds.

NMR spectra

Samples were prepared by dissolving the appropriate amount of the
complementary single-stranded oligonucleotides (ammonium salt) in 5% 
H$_2$O (Merck), 1 mM 2,2-dimethyl-2-silapentane-5-disodiumsulphonate
(DSS) and were titrated to pH 6 with 1% ammonia. Typically, the samples
were 6 mM in double-stranded fragment and 0.1 M in NH$_4^+$. For one series
of experiments the fragment B was converted to the sodium salt by
passage over Dowex 50 W cation exchanger. After lyophilization the
material was dissolved in 0.01 M sodium cacodylate, 0.1 M NaCl, 1 mM DSS,
5% H$_2$O, pH 5.6.

$^1$H NMR spectra were recorded on a Bruker HX 360 spectrometer
operating in the rapid-scan cross-correlation mode. Typically 1000
scans for each spectrum were accumulated and correlated in a Nicolet
BNC-12 computer. At each temperature the high-field spectrum was
recorded separately to obtain the DSS reference signal. Chemical
shifts are reported in ppm downfield from this resonance.
Nucleic Acids Research

RESULTS AND DISCUSSION

The thymine N3 and the guanine N1 imino protons are involved in hydrogen bonding within the Watson-Crick base pairs AT and GC, respectively. The exchangeable protons can be observed in double-stranded DNA molecules dissolved in water by using 1H NMR. They resonate between 12 and 15 ppm downfield from DSS. Upon thermal denaturation of the double helix these resonances lose intensity, broaden or shift up-field. This can be explained within the following scheme:

\[
\begin{align*}
H & \quad T \quad C \quad W \\
H & \quad T \quad C \quad W
\end{align*}
\]

where H, C and W represent a proton in the helix site (hydrogen-bonded imino proton), in the coil site (non-bonded imino proton in a single-stranded structure) and in the solvent site, respectively. The \( \tau^{-1} \)'s are the transition probabilities per unit time for the transitions indicated in the subscripts.

Two exchange situations are of importance in this case:

a) H - C in slow exchange: the line width of the helix resonance \( \Delta \nu \) is then given by

\[
\Delta \nu = \Delta \nu_H + 1/ \tau_{HC}
\]

where \( \Delta \nu_H \) is the natural line width of the proton in the helix site. Thermal denaturation leads to broadening and disappearance without shift for the low-field imino-proton resonances as is normally observed for central basepairs in a double helix.

b) H - C in fast exchange, C - W in slow exchange, \( \tau_{CW} \ll \tau_{CH}^{-1} \tau_{HC}^{-1} \). The resonance position \( \omega \) is given by

\[
\omega = f_H \omega_H + f_C \omega_C
\]

where \( f \) represents the fraction of protons in the site denoted by the subscript. The line width of this merged resonance is given by

\[
\Delta \nu = f_H \Delta \nu_H + f_C \Delta \nu_C + f_C/\tau_{CW}
\]

where \( \Delta \nu_C \) is the natural linewidth of the proton in the coil site. In this case, thermal denaturation leads to broadening and up-field
shifting of the helix resonance (fraying of terminal base pairs).

In this paper we employ the 'melting' characteristics of the fragments A, B and AB to obtain assignments for the imino-proton resonances. Both exchange situations a) and b) were encountered.

Melting of fragment A

Fig. 2 shows a selection of imino-proton spectra of fragment A. At 0°C a total intensity of seven proton resonances is observed, six of which are well resolved. Around 14 ppm, where AT imino protons are known to resonate, three resonances, one of double intensity, are found, in agreement with the base sequence. Around 13 ppm three resonances are found, arising from GC imino protons. Upon raising the temperature to 16°C a gradual loss of intensity, broadening and

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Fig. 2 360 MHz $^1$H NMR spectra of fragment A at different temperatures. The duplex (ammonium salt, 7.8 mM) was dissolved in H$_2$O containing 5% $^2$H$_2$O and 1 mM DSS. pH was brought to 6.0 with aq. NH$_3$. The crosses indicate positions where melting occurs. Resonances originating from base pairs that are depicted in small capitals have disappeared from the spectrum.
shifting is observed for the two low-field GC resonances, while all other resonances remain unaffected. This shows that melting of the fragment starts at the terminal GC base pairs, in spite of the fact that GC base pairs are more stable than AT base pairs. This behaviour is generally observed for small, double-stranded deoxy oligonucleotides. Therefore, the broadened resonances must be assigned to GC base pairs 1 and 7 (GC 1 and GC 7, see Fig. 1). At 27°C only one GC proton resonance is observed, obviously arising from the GC 2 proton.

One of the four AT resonances broadens around 20°C. Since at least one GC pair is stable at this temperature, it follows that this low-field resonance arises from the AT 6 proton. Due to the partial two-fold symmetry of fragment A the AT 4 and AT 5 protons are very likely to resonate at approximately the same field. Hence, the resonance of double intensity at 13.81 ppm (0°C) can be assigned to these protons. By elimination the remaining resonance at 13.95 ppm is assigned to AT 3.

It is interesting to compare our results with those obtained by Patel and Canuel on the self-complementary GGAATTCC duplex. Due to the symmetry of this fragment only four proton resonances, each of double intensity, were observed. The low field GC proton resonance broadens upon raising the temperature to 20°C and, as in our case, was assigned to the terminal GC pairs. Upon raising the temperature to 32°C all other resonances broaden simultaneously. This lack of sequential melting in the GGAATTCC duplex is due to the protection of the AT region by a symmetrical double block of GC pairs.

By comparing the low-temperature spectrum of this symmetrical fragment with our spectrum of fragment A (0°C) the remaining ambiguity about the GC 1 and GC 7 resonances can be resolved: the low-field resonance at 12.99 ppm must be assigned to GC 1 and, hence, the remaining resonance at 12.84 ppm to GC 7. A summary of these assignments is given in Fig. 6.

Melting of fragment B

Figure 3 shows a series of imino-proton spectra of fragment B (see Fig. 1). At 2°C seven resonances can be observed, two of which are broadened to a large extent. These resonances (indicated with crosses in the figure) are attributed to the terminal AT 8 and GC 14, which are subject to fraying. The remaining AT signal at 14.07 ppm

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is therefore due to the internal AT 10 proton. This is also evidenced by the relatively high thermal stability of this base pair.

In the temperature range 32 – 35°C two GC resonances remain relatively sharp while the AT 10 resonance broadens. Hence these resonances must be assigned to either GC 11 and GC 12 or GC 12 and GC 13. The melting of fragment AB (see below) learns that GC 13 cannot be involved, since this proton resonates at 13.3 ppm (29°C) leading to the assignment of the sharp resonances to GC 11 and GC 12.

The remaining GC resonances, which lose intensity between 13 and 29°C, must be due to GC 9 and GC 13. As argued above, from the comparison with the spectrum of AB it follows that the low-field resonance belongs to GC 13 and, by elimination, the high-field resonance to GC 9. The assignment of the 13.27 ppm resonance to GC 13 is in agreement with its thermal stability: around 29°C GC pairs adjacent to terminal GC pairs are known to melt out (see Fig. 2 and ref. 12). Fig. 4 shows a series of spectra of the same fragment B in 10 mM cacodylate buffer. In contrast with the results discussed above, increase in temperature
The duplex (sodium salt, 4.8 mM) was dissolved in H2O, containing 5% 2H2O, 0.1 M NaCl, 0.01 M sodium cacodylate and 1 mM DSS, pH 5.6.

results in an up-field shift for at least two imino-proton resonances. This is most clearly visible for the GC resonances at 12.4 and 12.8 ppm (3°C). The increase in intensity of the resonance at 13.1 ppm upon raising the temperature to 19°C is very likely to be caused by the up-field shift of the broad resonance just visible at 13.25 ppm (3°C). With this in mind, the assignments following from this melting study match those obtained from the spectral series of Fig. 3. The different response of the imino-proton resonances to melting in the ammonia and cacodylate media (Figs. 3 and 4, respectively) can be attributed to a change in the rate parameter $t_{\text{CW}}^{-1}$. From the Eigen equation $t_{\text{CW}}^{-1}$ we calculate the upper limits $t_{\text{CW}}^{-1} = 5 \times 10^{-5} \text{ kdiff}^{-1}$
and $\tau^{-1} = 2.5 \times 10^{-2} k_{\text{diff}} (\text{s}^{-1})$ in the ammonia and cacodylate media, respectively, where $k_{\text{diff}}$ stands for the diffusion-controlled encounter rate between the iminoproton and the proton acceptor. Therefore it seems likely that the decrease in $\tau^{-1}$ in the cacodylate medium causes the imino-proton resonances of the fraying terminal base pairs to be less broadened than in 0.1 M $\text{NH}_4^+$ (see Eqn. (3)). Apparently the condition for fast exchange between the helix and coil sites is just met for the imino protons of base pairs adjacent to the terminal ones of the fragment in cacodylate (up-field shifting; exchange situation b, see above). It is likely that due to the high ionic strength of the $\text{NH}_4\text{Cl}$ medium a decrease in helix-coil exchange rate occurs for these protons concomitant with an increase in helix stability.$^{10, 11}$ This will lead to broadening without shifting for the imino proton resonances of these basepairs upon an increase in temperature in this medium (exchange situation a, see above). It is not known at present, however, why the imino protons of the terminal GC pairs of fragment A show only minor shifts and broadening, in contrast to the terminal GC protons of fragment B.

**Melting of fragment AB.**

Fig. 5 presents the melting series of the 14-b.p. fragment AB. At low temperature four AT resonances (two of double intensity) and an integrated intensity of eight GC resonances, six of which are well resolved, are observed. At 26°C terminal GC pairs have already melted out (see Fig. 2) and two resonances are seen to broaden, allowing these two to be assigned to the GC 2 and GC 13 protons. Comparison with the spectrum of fragment A (see Fig. 6) learns that the 12.83 ppm resonance must be assigned to GC 2 and, hence, the 13.30 ppm resonance to GC 13. At 38°C the low-field AT resonance at 13.96 ppm loses about half of its intensity, showing that the AT 3 proton belongs to this peak of double intensity. In the temperature range 50 - 56°C the high-field AT resonances lose intensity, while only one GC signal disappears. It follows, that the high-field AT resonances must be assigned to the AT 4, AT 5 and AT 6 protons and the 13.08 ppm resonance (3°C) to GC 12. Furthermore, on basis of symmetry arguments and by comparison with the spectrum of fragment A (see above) the line of double intensity at 13.81 ppm can be attributed to AT 4 and AT 5. Due to overlap at the higher temperatures it cannot be decided which of the two high-field AT resonances visible at 3°C must be assigned.
Fig. 5 360 MHz $^1$H NMR spectra of fragment AB at different temperatures. Duplex concentration, 6.1 mM; pH 6.2. Further experimental conditions as in Fig. 2.

The resonances observable at 60°C are thus unambiguously assigned to the imino protons of the base pairs depicted next to the spectrum (bold capitals). Above this temperature melting can be thought to continue from two sides:

i) If melting continues from the left, the vanishing GC resonance at 12.29 ppm must be assigned to GC 7 and the remaining resonances at 12.64 and 12.93 ppm (3°C) to GC 9 and GC 11. The AT resonance at 13.96 and one of the resonances around 13.65 ppm (3°C) are then assigned, in a pairwise manner, to AT 8 and AT 10.

ii) If melting continues from the right, the vanishing GC resonance
at 12.29 ppm must be assigned to GC 11 and, by elimination, the 12.7 and 12.95 ppm resonances to GC 7 and GC 9. The AT resonances are assigned in the same manner as in case i) above.

These ambiguities can be resolved by comparing the AB spectrum with those of the fragments A and B, as illustrated in Fig. 6. This comparison, however, does not yield assignments for the base pairs AT 6, GC 7, AT 8 and GC 9, since these are central base pairs in the AB fragment, but terminal or next to terminal base pairs in the A and B fragments (see discussion). Thus, half of the intensity of the 14.0 ppm signal can be assigned to AT 10 by comparison with the spectrum of fragment B and the resonance at 12.93 ppm to GC 11. Since this resonance is still relatively sharp at 60°, it follows that melting continues from the "left" (case i), see above. According to case i), then, the high-field resonance at 12.29 ppm belongs to GC 7 and the 12.64 ppm resonance to GC 9.

The arguments that have led to the assignments in the spectra of the three fragments are summarized in table 1. It is evident that melting studies on the 7-b.p. fragments were indispensable for the interpretation of the spectrum of the larger fragment. Surprisingly, however, also some assignments in the spectrum of the smaller fragment B could only be arrived at by comparison with the spectrum of the 14-b.p. fragment! The consistency of the assignments in the spectra of all three fragments is in itself strong evidence for their correctness.

Discussion

In Fig. 6 (left-hand side) the low-temperature spectra of the three fragments are shown and the assignments indicated. The sum (A+B) of the spectra of A and B is included as well. It can be seen, that significant up-field shifts have occurred only for the AT 6, GC 7 and GC 9 protons in AB. The AT 8 proton cannot be incorporated in this comparison due to fraying processes occurring in fragment B.

Also shown in Fig. 6 (right-hand side) is a series of calculated spectra of the three fragments. The agreement is not exceptionally good, partially due to fraying processes, affecting the terminal base pairs in fragment B. However, also in the calculated spectra up-field shifts have occurred only for the AT 6, GC 7, AT 8 and GC 9
Table 1 Summary of the evidence for assignment of imino-proton resonances of DNA fragments A, B and AB

<table>
<thead>
<tr>
<th>Chemical shift* in AB</th>
<th>Assignment</th>
<th>Arguments for assignment in A or B</th>
<th>in AB</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.96</td>
<td>AT 3</td>
<td>by elimination after assigning other three AT resonances</td>
<td>melting (~30°C) and comparison with A</td>
</tr>
<tr>
<td>13.96</td>
<td>AT 10</td>
<td>melting (~32°C)</td>
<td>comparison with B</td>
</tr>
<tr>
<td>13.81</td>
<td>AT 4 and 5</td>
<td>symmetry of AT block (AT 3 to 6)</td>
<td>symmetry of AT block and comparison with A</td>
</tr>
<tr>
<td>13.09, 13.62</td>
<td>AT 6 or 8</td>
<td>absolute assignment impossible due to overlap; comparison with A cannot be used for these central base pairs</td>
<td></td>
</tr>
<tr>
<td>14.03</td>
<td>AT 6</td>
<td>melting (~39°C)</td>
<td></td>
</tr>
<tr>
<td>13.30</td>
<td>GC 13</td>
<td>melting (~29°C) leads to assignment to GC 9 or GC 13; comparison with AB resolves ambiguity</td>
<td></td>
</tr>
<tr>
<td>13.07</td>
<td>GC 12</td>
<td>melting (~35°C) leads to the assignment to GC 11 or 12. From AB position of GC 12 is known</td>
<td>melting (56°C)</td>
</tr>
<tr>
<td>12.93</td>
<td>GC 11</td>
<td>same arguments as for GC 12</td>
<td>melting (~60°C) and comparison with B</td>
</tr>
<tr>
<td>12.75</td>
<td>GC 2</td>
<td>melting (~29°C)</td>
<td>melting (~26°C) leads to assignment to GC 2 or 13. Comparison with A resolves ambiguity</td>
</tr>
<tr>
<td>12.64</td>
<td>GC 9</td>
<td>melting (~13°C) leads to GC 9 or GC 13. Position of GC 13 (13.3 ppm) known from AB</td>
<td>melting (~60°C) and comparison with A leads to assignment to GC 10; comparison with B resolves ambiguity</td>
</tr>
<tr>
<td>12.29</td>
<td>GC 7</td>
<td></td>
<td>melting of AB (~60°C) leads to ambiguity between GC 7 and GC 11. Comparison with B resolves this</td>
</tr>
<tr>
<td>12.84</td>
<td>GC 7</td>
<td>melting of A leads to assignment to GC 1 or GC 7. Comparison with literature (Ref. 18) resolves ambiguity</td>
<td></td>
</tr>
<tr>
<td>12.99</td>
<td>GC 1</td>
<td>same argument as for GC 7 in fragment A</td>
<td></td>
</tr>
</tbody>
</table>

Footnote: Shifts are expressed in ppm relative to DSS and refer to the low-temperature spectra.

protons in AB, when compared with the spectra of A and B. Like in the experimental spectra the largest shift is calculated for the GC 7 resonance. Thus, although there is a one-to-one correspondence of the calculated and experimental spectra, the one-to-one correspondence of the calculated and experimental spectra is rather weak, these calculations show that the comparison between the large and small fragments.
Fig. 6 Left-hand side: low-temperature spectra of fragments A, B and AB (Figs. 2, 3 and 5, respectively) and the sum (A + B) of the spectra of fragments A and B. The assignments are indicated by numbers, which correspond to the base-pair numbering indicated at the top of this figure. Numbers enclosed within brackets indicate pair-wise assignments. The lines between the spectra A + B and AB connect the resonance positions of base pairs 6, 7 and 9, which have undergone extra up-field shifts in AB relative to A and B.

Right-hand side: calculated spectra of fragments A, B and AB and the sum of spectra A and B (A + B). Calculations are based on ring-current shielding effects (according to the formalism of Haigh and Mallion) and atomic diamagnetic anisotropy contributions (according to McConnell). Ring-current intensities and the elements of the susceptibility tensors were obtained from Giessner-Prettre. The intrinsic resonance positions were taken as 14.85 (AT imino protons) and 13.70 ppm (GC imino proton) down-field from DSS. A regular B-DNA structure was assumed.

End effects have to be taken into account, extending up to two base pairs on both sides of the linkage (i.e. base pairs 6, 7, 8 and 9). For the other resonances the correspondence between the peak positions in the large fragment AB and those in the subfragments...
A and B is always well within 0.1 ppm (r.m.s. deviation 0.05 ppm).

In Fig. 7 a comparison is made between the spectrum of the synthetic 14 b.p. operator fragment and that of a 51 b.p. restriction fragment from E. coli containing the complete lac operator. This fragment was employed in a recent NMR investigation into the structural changes that occur in the DNA upon binding to the lac repressor headpiece.

As discussed above the imino-proton resonances of base pairs 3 to 12 in the AB fragment are expected to have the same position in the spectrum of the restriction fragment. It can be seen in Fig. 7 that this is indeed the case, except for the resonance of GC 12. The other lines can all be recognized in the spectrum of the 51 b.p. operator fragment (Fig. 7B).

Structural aspects

In the assignment procedure we used the assumption that the structure of the small 7 b.p. oligonucleotides does not change much...
when they are linked together in the 14 b.p. fragment. Indeed it
is observed (see Fig. 6) that eight of the twelve resonances in the
spectrum of AB have the same chemical shifts as resonances in the
spectra of A and B, while four show substantial differences in position.
Furthermore, this assumption, combined with independent evidence
obtained from melting studies, has led to a consistent assignment
of the imino-proton resonances in all three fragments. Therefore
we feel that our assumption is basically correct and that the 7 b.p.
fragments retain their structure (apart from possible end effects)
when they are part of the 14 b.p. fragment. Very likely, the same
structural correspondence holds for the 14 b.p. fragment in the
51 b.p. restriction fragment. Thus, a partial peak assignment in
the spectrum of the 51 b.p. fragment can be obtained by comparison
with the spectrum of the 14 b.p. fragment.

Apart from being useful to reach NMR assignments the melting
behaviour of these DNA fragments is of interest in itself. The
AT-rich fragment A melts at a considerably lower temperature than
the GC-rich fragment B. Interestingly, the combination of these
two very dissimilar fragments into the fragment AB leads, not only
to an increase in thermal stability, but also to a highly asymmetrical
melting behaviour. At 65°C (see Fig. 5) only the base pairs 9, 10
and 11 have sufficient helix character for their imino protons to
be observable in the NMR spectrum. Although it is realized that the
disappearance of resonances is not only governed by the helix-coil
equilibrium (see Eqs 2 and 4 and ref. 17), it is very likely that
the actual melting of the double helix occurs in the same asymmetrical
manner. The asymmetrical melting is also visible in the behaviour
of the fragments A and B.

Finally, we would like to comment on the temperature behaviour
of fragment AB that can be seen in Fig. 5. Most of the lines show
appreciable up-field shifts before melting sets in. This phenomenon
is most pronounced for the AT protons. Early et al. suggested
that it arises from temperature-induced alterations in DNA structure
taking place predominantly at the AT base pairs. However, it could
also be explained by structural changes in the entire fragment which
affect the AT resonance positions more than those of the GC pairs.
An intriguing possibility is that the effect originates from a
propeller twist between bases in a pair, as was recently observed
It would be reasonable to assume that the average twist angle becomes larger at higher temperatures. For a GC pair, which has three hydrogen bonds, the imino proton will lie at or near the twist axis, so that its intrinsic chemical shift is not strongly influenced. However in a AT base pair it is likely that the axis is shifted away from the imino proton, because of the flattening influence of its two hydrogen bonds. Thus twisting would result in a more pronounced loosening of the AT imino hydrogen bond with a concomitant up-field shift of the imino-proton resonance to its coil position.

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