Mismatches in DNA: Measurement of Reduced Duplex Stability using ^{1}H N.M.R. Spectroscopy

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The destabilisation of a double-stranded DNA fragment, which results when substitution of a single nucleotide introduces a G · T base pair, is determined from an equilibrium monitored using imino proton resonances in the n.m.r. spectrum in H_2O.

Base pairing is an essential feature of the double helical structures formed by polynucleotides. Precise complementarity between adenine and thymine or uracil, and guanosine and cytosine, is central to template-directed synthesis; the results of hydrogen bonding between other bases in DNA, termed mismatches, are potentially mutagenic.[1]

Duplexes containing mismatches are significantly destabilised compared with their correctly paired parents,[2,3] the extent being dependent on the base composition and sequence of the nucleic acid as well as on the type and location of the mismatch.[4] Quantitative understanding of these relationships is relevant to studies both *in vivo* and *in vitro* of mutagenesis involving natural and chemically modified bases.

Present investigations are limited to examining the temperature dependence of helix dissociation in mismatch-containing oligonucleotides[2—5] but this frequently complex and indistinct transition is not suitable for reliably quantifying the small differences between related species. A more useful index of destabilisation is the difference in free energy between a correctly paired oligomer duplex and its mismatch-containing analogue.

Such species are present when two oligomers, differing in sequence at a single position, compete for a third which is the exact complement to one of them (Scheme 1). The position of equilibrium can be determined using ^{1}H n.m.r. spectroscopy, comparing the intensities of imino proton resonances from individual duplexes. This is possible since, by contrast to those from non-exchangeable protons, signals from the imino protons are unique to each duplex and exchange rapidly with solvent water when the base pairs are disrupted.[6]

Signals in this remote region of the spectrum (δ 12—14) are well dispersed; in addition to characteristically different chemical shifts associated with each type of base pair, protons at the core of the helix are particularly sensitive to ring current shielding effects of the bases. This property is well illustrated in the present example (Figure 1). The duplexes are necessarily of very similar sequence yet resonances representative of the individual components can be distinguished in spectra of their mixtures. The control (Figure 1b), in which the two double-stranded species are present in equal concentrations, enables normalisation of the measured signal intensities whose proportions may be distorted *inter alia* by relaxation effects. In the present case corrections are small.

Well below the melting temperature of the duplexes it is assumed that the template strand (heptamer 'A') is entirely occupied by one or other of its complements, and knowing the initial concentration of the heptamers, those of each of the equilibrium components may be calculated. Repeating the experiment with increasing concentrations of the competing strand 'C' enables the equilibrium constant to be determined graphically (Figure 2).

$$A \cdot B + C \rightleftharpoons A \cdot C + B$$

$$K = \frac{[A \cdot C][B]}{[A \cdot B][C]}$$

A d(GCCGCCA)
B d(TGGCGGC)
C d(TGGTGGC)

Scheme 1

The values of ΔG determined in this experiment (Figure 2) are rather lower than that reported for the same mismatch compared to a G · C base pair in an A–T-rich hexadecamer (12.7 kJ mol^{-1} at 298 K). The difference may represent a sequence-dependent effect but detailed comparison is pre-

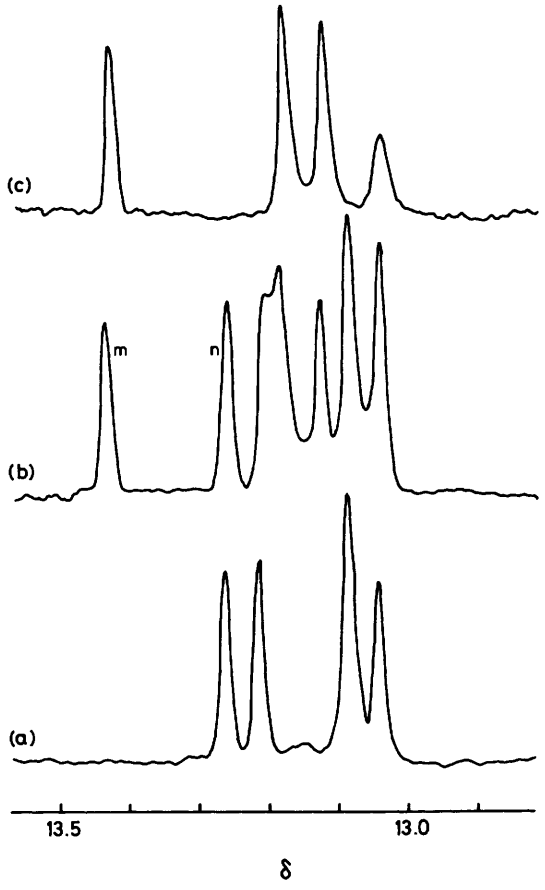

Figure 1. 500 MHz ^{1}H n.m.r. spectra of the duplexes (*ca.* 5 × 10^{-4} M) at 288 K in H_2O–D_2O (9 : 1) containing 0.1 M sodium phosphate, pH 7.0; chemical shifts are downfield of internal trimethylsilyl propionate. (a) A · B; (c) A · C; (b) A · B + A · C, 1 : 1. Spectra were obtained using a Bruker AM 500 instrument operating with quadrature detection in the Fourier transform mode; the carrier was placed downfield of the region of interest and the H_2O signal suppressed using a '1–1 hard pulse';[7] final digital resolution was 0.94 Hz/point. Oligomers were prepared using the solid phase triester method[8] and purified to homogeneity by DEAE cellulose chromatography and h.p.l.c.

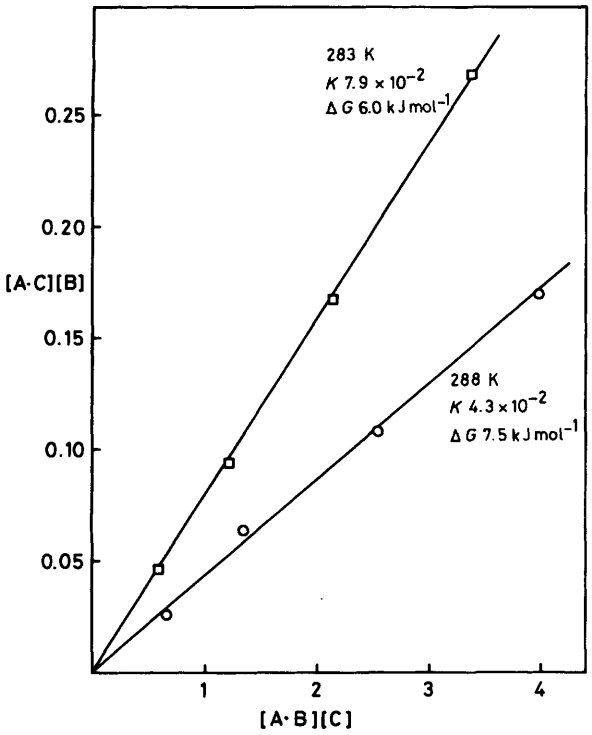

Figure 2. Plots of [A · C][B] *vs.* [A · B][C] at 283 and 288 K for [A] : [B] : [C] ratios of 1 : 1 : 1; 1 : 1 : 2; 1 : 1 : 4; and 1 : 1 : 8; concentrations in arbitrary units. Mixtures were made up from stock solutions of individual oligomers standardised by the absorbance at 260 nm and corrected for hypochromicity. The ratio [A · B] : [A · C] was determined from the area of peaks m and n in Figure 1.

cluded by the dissimilarities both in the DNA and the techniques employed.

The large temperature dependence seen here is probably due to premelting phenomena which affect each duplex differently; it is a consequence of structural differences between the two analogous duplexes. These differences are important in considering the dependence of the stability of mismatch-containing duplexes on sequence and require detailed examination, but incorporation of a mismatch is more likely to result in local perturbations than a gross alteration of the conformation of the double helix.

This novel technique enables straightforward measurement to be made of the difference in free energy between double-stranded oligonucleotides with a common single-strand component. It is likely to be generally useful in the study of mismatches, RNA–DNA hybrids, and the base-pairing properties of chemically modified bases: with judicious choice of sequence, application to longer oligomers is feasible.

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References

1 L. A. Loeb and T. A. Kunkel, *Annu. Rev. Biochem.*, 1982, 29.
2 D. J. Patel, S. A. Kozlowski, L. A. Marky, J. A. Rice, C. Broka, J. Dallas, K. Itakura, and K. J. Breslauer, *Biochemistry*, 1982, **21**, 437.
3 T. A. Millican, G. A. Mock, M. A. Chauncey, T. P. Patel, M. A. W. Eaton, J. Gunning, S. D. Cutbush, S. Neidle, and J. Mann, *Nucleic Acids Res.*, 1984, **12**, 7435.
4 N. Tibanyenda, S. H. De Bruin, C. A. G. Haasnoot, G. A. Van der Marel, J. H. van Boom, and C. W. Hilbers, *Eur. J. Biochem.*, 1984, **139**, 19.
5 B. L. Gaffney, L. A. Marky, and R. A. Jones, *Biochemistry*, 1984, **23**, 5686.
6 C. W. Hilbers and D. J. Patel, *Biochemistry*, 1975, **14**, 2651.
7 G. M. Clore, B. J. Kimber, and A. M. Gronenborn, *J. Magn. Reson.*, 1983, **54**, 170.
8 M. J. Gait, H. W. D. Matthes, M. Singh, B. S. Sproat, and R. C. Titmas, *Nucleic Acids Res.*, 1982, **10**, 6243.