Review

Kinking of DNA and RNA by base bulges

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Base bulges are features of nucleic acid structure where a duplex section is interrupted by one or more formally single-stranded bases on one strand that are unopposed by bases on the other strand. Base bulges are very common features of folded RNA structures, where they present highly recognizable features for specific protein binding; a good example is the binding of the human immunodeficiency virus transactivator protein Tat to the three-pyrimidine bulge in the response element TAR (1, 2). Bulges may be created in heteroduplex DNA arising from recombination between imperfectly homologous sequences, where they become features to be recognized by the repair machinery. In general, base bulges in nucleic acids are an important feature in the repertoire of folding elements, and one that can be exploited for specific recognition by proteins of various kinds.

It is clear that base bulges have a rather major effect on local geometry, because their inclusion brings about a significant destabilization of DNA or RNA duplexes (3–6). Analysis of the molecular geometries of branchpoints within nucleic acids in solution provides an interesting challenge. Because of the elongated, sequential construction of nucleic acids, both short-range and long-range information is required, and neither alone can fully define the structure of the molecule. NMR can provide a wealth of information on the stereochemistry of nucleic acids, but the information is essentially short-range. The absence of longer distances makes global features such as kinks difficult to estimate with assurance. For these reasons a combined approach exploiting a number of different biophysical methods has evolved for the analysis of these features.

One of the simplest techniques used to study branched nucleic acid structures is gel electrophoresis, yet the approach is perhaps surprisingly powerful. The mobilities of duplex species that are bent or kinked in some manner are retarded in polyacrylamide gels, as is well known in the case of DNA that is curved by the presence of phased oligoadenine tracts (7, 8). Although the physical basis of this effect is not fully understood, these experiments can usually be set up so that the relative mobilities of a series of related molecules are compared. The electrophoretic mobilities of DNA (9–11) and RNA (12, 13) duplexes are reduced by the inclusion of bulged bases. The magnitude of the retardation depends on the size of the bulge (Fig. 1A) and the sequence of the bulge; purine bulges generate a greater retardation than pyrimidine bases (9, 11, 13). Such effects suggest that the bulge changes the trajectory of the helix axis by introducing either a kink or a point of flexibility. The latter is excluded by introducing two bulges into a molecule and varying the spacing between them.

Directed kinking would lead to a fixed dihedral angle between the outer arms of the structure that would vary with the spacing between the two bulges, whereas isotropic flexibility would give much less dependence on spacing. It has been found both in DNA and in RNA that electrophoretic mobility varies sinusoidally with the spacing between the bulges (Fig. 1B), indicating that the bulge generates a directed kink in the helical axis. Such data have provided estimates of the helical period of RNA and RNA-DNA hybrid helices (12, 14, 15). The strong phase dependence of electrophoretic mobility clearly excludes isotropic flexibility, although some anisotropic flexibility could still be present. Opposing an As bulge in an RNA duplex by adenine bases readily leads to increased mobility; the addition of just two opposing adenine bases is sufficient to restore normal electrophoretic mobility, suggesting that defined axial kinking is cancelled by the extra bases (16).

The axial kinking caused by base bulges has been confirmed spectroscopically by fluorescence resonance energy transfer (FRET). This method has some real advantages for the study of branched nucleic acid species of various kinds and has been undergoing something of a renaissance in the last few years. Fluorophores can be attached to the ends of individual helices, and end-to-end distances can be estimated from the transfer of the excitation energy by dipolar coupling between the transition moments of dyes with overlapping emission and excitation profiles. If the mobility of the dyes allows orientation effects to be neglected, then the efficiency of energy transfer should increase as the distance between the dyes becomes shorter. Upon inclusion of base bulges into DNA and RNA duplexes, the end-to-end distance is found to shorten, giving increased energy transfer between dyes attached to the two 5' termini (17) (Fig. 2). The profiles of

Abbreviations: FRET, fluorescence resonance energy transfer; TEB, transient electric birefringence.
Fig. 2. Kinking of DNA and RNA duplexes by base bulges is revealed by FRET. The distance between donor (D) and acceptor (A) fluorophores attached to the 5' termini of the component strands of bulged duplexes becomes shorter if the axis is significantly kinked by the presence of a bulge. Upon excitation of the donor, the efficiency of energy transfer to the acceptor by dipolar coupling depends on the inverse sixth power of the distance between them, and hence should increase with such kinking. A series of 18-bp DNA and RNA duplexes with $A_n$ bulges ($n = 0$ to 9) were prepared with fluorescein (donor) and tetramethylrhodamine (acceptor) attached to the 5' termini of the two component strands, and FRET efficiency was determined spectroscopically. The plot shows that for both DNA and RNA duplexes, there is a shortening of the end-to-end distance as the bulge size increases to $A_7$.

FRET efficiency against bulge size are very similar for both DNA and RNA, indicating similar kinking processes despite the differences in geometry of DNA and RNA helices. FRET efficiency increases up to a bulge size of seven adenine bases, and it has been estimated that the bend angle for a DNA duplex containing a central $A_7$ bulge is of the order of 90°. Beyond the $A_7$ bulge the FRET efficiency decreases with bulge size, while electrophoretic mobility continues to decrease uniformly. This clearly suggests that the two techniques may not be sensitive to exactly the same structural aspects in some circumstances.

Estimates of angles of axial kinking in bulged RNA molecules have recently been derived by a hydrodynamic method, transient electric birefringence (TEB). Zacharias and Hagerman (13) studied the kinking of RNA duplexes containing either $A_n$ or $U_n$ bulges ($n = 1$ to 6). They concluded that there was a linear dependence of axial kinking with bulge size and estimated a bend angle of 93° for the duplex containing an $A_n$ bulge. The molecules containing the $U_n$ bulges were less kinked when compared with the corresponding $A_n$ bulged molecules, and an interesting effect of magnesium ions was observed for the $U_n$ series. Both the FRET and the TEB experiments provide a calibration of gel electrophoretic mobilities which can be used to assess other sequences.

Electron microscopy provides a potentially rather direct way to examine helical kinking, and several bulged DNA molecules have been studied in this manner.

Griffith and coworkers (18) visualized a three-pyrimidine bulge generated by heteroduplex formation between wild-type and mutant cystic fibrosis genes by conventional electron microscopy methods (18), revealing images of kinked DNA fragments. To minimize any possible distortion due to binding to a support, a 100-bp DNA fragment containing a central $A_7$ bulge was examined by cryo-electron microscopy in vitrified solution (J. Bednar, J. Dubochet, A. I. H. Murchie, and D. M. J. L., unpublished data). Many images of kinked DNA fragments were obtained (Fig. 3), with an average included angle around 85°. This value was in good agreement with that derived from the FRET data, and in the same range as that observed for the $A_6$ bulge by TEB. The width of the distribution found by cryo-electron microscopy indicated a degree of flexibility at the bulge site.

There is general agreement between techniques on the global shape of DNA and RNA molecules containing base bulges—that the bulges introduce a defined kink into the helical axis. To study the structure at a more detailed stereochemical level other methods must be employed, and most information has come from the use of NMR. Studies of single-base bulges have indicated that in most cases the unpaired base is stacked within the helix (3, 19–24), although pyrimidine base bulges within A+T-rich regions were found to be extrahelical (4, 25). There have been two NMR studies of multiple-base bulges in DNA, both of which found that the extra bases were stacked into the helix continuously with the flanking DNA (26–28). However, the geometry of the nonbulged strand was distorted opposite the location of the bulge, which might represent the principal focus of the axial kink. In one study (28) the loss of a thymine imino resonance

Fig. 3. Visualization of bulge-containing DNA duplexes by cryo-electron microscopy. A solution of 120-bp DNA duplexes containing central $A_7$ bulges was vitrified by rapid cooling. The unstained image shows a number of L-shaped DNA molecules, corresponding to centrally kinked duplexes. Since the orientation of the molecules in the vitrified solution is random, the projected angle of kinking can be apparently increased or reduced for a given species. (Bar = 50 nm.)
suggested that an A-T base pair immediately adjacent to an U₃ bulge was disrupted. By contrast, in a study of a different sequence, flanking G-C base pairs were found to be intact (27), indicating that the geometry in and around the bulge is sequence-dependent.

Probably the best studied bulged RNA structure is the human immunodeficiency virus TAR sequence, which contains a U₃ bulge (29). In the absence of a ligand the three uracil bases were stacked into the helix. This would be consistent with the axial kinking suggested by gel electrophoretic retardation (16). However, the NMR studies indicated that there was a pronounced change in the conformation upon binding of arginine, as very simple model for the Tat protein. The uridine bases became extrahelical, one of them participating in the formation of a U:A-U base triple, although this latter feature has been disputed in another study (F. Aboul-ela and G. Varani, personal communication).

The exploitation of features such as base bulges by RNA-binding proteins is not surprising. The geometry of the A-form helix in RNA makes access to the major groove rather difficult, and the local geometry of the base bulge introduces a kind of Achilles heel into the underlying base sequence. Hence much of specificity of RNA–proteins binding involves the structure of the RNA target, and the conformation of these features and their recognition properties are intimately connected with the biological function of these important molecules.

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