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Construction and Analysis of Monomobile DNA Junctions†

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ABSTRACT: Immobile DNA junctions are complexes of oligomeric DNA strands that interact to yield branched structures in which the branch point cannot migrate. This is achieved by minimizing the sequence symmetry in the flanking arms, so that base pairs lock at the branch site. Here, we report the design, synthesis, and analysis of two semimobile junctions, structures in which a controlled extent of branch point migratory freedom is deliberately introduced. We have constructed two minimally symmetric four-arm semimobile junctions from synthetic deoxy 17-mers. These junctions, termed "monomobile", contain a single pair of base pairs (A-T or C-G) which can migrate at the site of branching, while the rest of the junction is immobile. We have demonstrated by gel electrophoresis techniques that these junctions form and that they have the predicted 1:1:1:1 stoichiometry. We have compared these junctions with the immobile junction on which they are based, by means of hydroxyl radical protection experiments. From these data, both migratory conformers can be seen to coexist in solution. The semimobile junction with the C-G base pair has the same crossover and stacking pattern observed for the immobile junction, while the junction with the A-T base pair has the opposite pattern. We conclude that crossover and stacking patterns are a direct consequence of the base pairs which flank the junction. In addition, the data indicate that the crossover pattern biases for these junctions are much greater than are the migratory biases.

Genetic recombination involving two DNA duplexes is one of the fundamental processes that generates genetic diversity. Central to duplex exchanges is the Holliday (1964) branched intermediate, formed from four strands of DNA. In naturally occurring recombination, the two double helices which combine to form the Holliday intermediate are identical, or nearly so, thus yielding a structure with 2-fold sequence symmetry. Such an intermediate is unstable and can isomerize to change the particular bases that are paired, as shown in Figure 1. This isomerization process relocates the site of branching and is therefore known as "branch point migration". Branch point migration has been demonstrated experimentally (Lee et al., 1970; Broker & Lehman, 1971; Kim et al., 1972), the rate has been measured (Thompson et al., 1976; Warner et al., 1978, Courey & Wang, 1983), and aspects of the process have been modeled (Meselson, 1972; Robinson & Seeman, 1987).

An understanding of the details of branch point migration is critical if we are to comprehend the detailed chemistry of recombination. This is a difficult process to treat experimentally, since the symmetry of the system renders the entire complex unstable. It has been possible to study the structural features of branched DNA by constructing sets of oligodeoxynucleotides with minimal sequence symmetry (Seeman, 1981, 1982; Seeman & Kallenbach, 1983). Junctions with no sequence symmetry at the site of branching are called immobile junctions. In previous studies, we have demonstrated the formation of immobile junctions with three or four arms (Kallenbach et al., 1983a,b; Ma et al., 1986). A particular immobile four-arm junction, J1, has been explored in depth: Its DNA structure remains primarily B-form (Seeman et al., 1985; Marky et al., 1987), and all bases, including those that flank the junction, are paired (Wemmer et al., 1985). Recently, by hydroxyl radical protection experiments (Churchill et al., 1988), J1 has been shown to be 2-fold symmetric; it appears to consist of two stacked helical domains, such as the parallel (Sigal & Alberts, 1972) or antiparallel helices shown in Figure 2. This is in agreement with the work of Cooper and Hagerman (1987), who have shown that the six pairs of arms of a junction similar to J1 are not equivalent in their electrophoretic mobilities.

In order to study the details of the branch point migration process, it is necessary to relax the constraints on symmetry minimization about the branch site. A branched junction that

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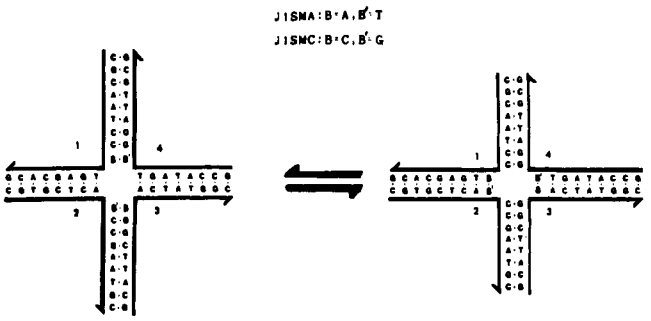

FIGURE 1: The semimobile junctions J1SMA and J1SMC. The parent sequence for the junctions shown here is the immobile junction J1, whose sequence is drawn explicitly. The extra base added to each strand of J1 is indicated in the center of the junction as B or its complement B′. For J1SMA, B = A and B′ = T; for J1SMC, B = C and B′ = G. The base pairing is indicated by the dots between the base pairs. Branch point isomerization is shown. The 2-fold sequence symmetry of naturally occurring Holliday intermediates permits many steps of this process, rather than the single step allowed for the monomobile junctions shown here. The equilibrium illustrated shows how the pairing of the central bases can switch between "horizontal" pairs on the left and "vertical" pairs on the right. The conformer on the left has nine horizontal base pairs in the vertical stacks and eight vertical base pairs in the horizontal stacks, while the conformer on the right has eight base pairs in the vertical stacks and nine in the horizontal stacks. The strand numbering used throughout the text is indicated.

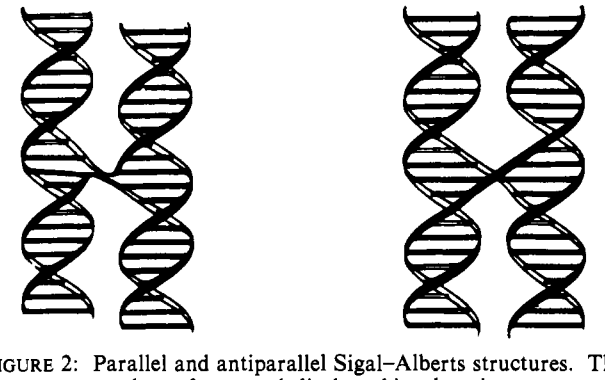

FIGURE 2: Parallel and antiparallel Sigal–Alberts structures. The two structures shown form two helical stacking domains, as proposed by Sigal and Alberts (1972). The two helix axes are antiparallel in the structure on the left. The two double-helical domains there are related by a 2-fold axis perpendicular to the plane of the paper. The parallel Sigal–Alberts structure is shown on the right. The 2-fold axis relating the parallel helical domains lies between the axes, within the plane of the paper. The antiparallel form of the structure may be converted to the parallel form by rotation of one helix 180° about a horizontal axis within the plane of the paper. These drawings were made with 10.5-fold DNA as a basis.

permits a limited amount of migration to occur at its center is termed "semimobile" (Seeman, 1981, 1982), to indicate that it possesses some degree of migrational capability. Constraints on the design of semimobile junctions have been described previously (Seeman, 1981, 1982). Semimobile junctions have been formed by other groups as approximations to immobile junctions (Mizuuchi et al., 1982; Hsu & Landy, 1984; Gough & Lilley, 1985). These have been constructed from large restriction fragments, with six or more mobile base pairs at the junction. Recently, Evans and Kolodner (1987) have constructed a synthetic semimobile junction, in an attempt to create a substrate for a junction resolvase. It seems best for high-resolution physical studies to examine a single migrating base pair in a junction constructed from oligodeoxynucleotides. Such a junction is termed "monomobile". This system permits characterization of a single transition between precisely defined isomers in a molecule small enough for signals from the branch site itself to be a significant fraction of the whole. Furthermore, this system makes it possible to explore sequence-specific effects in a systematic way.

Here, we describe the synthesis and characterization of two semimobile junctions based on the sequence of the immobile junction J1. In the first example, J1SMA, we have inserted an A at the branch sites of the first and third strands and a T at the branch sites of the second and fourth strands. In the second junction, J1SMC, we have inserted a C in the first and third strands and a G in the second and fourth strands (Figure 1). We demonstrate the formation of these junctions from their constituent heptadecamers and show that they possess the intended stoichiometry. We have characterized their frictional properties and their thermal transition profiles.

Finally, we have explored the susceptibility of these junctions to cleavage by hydroxyl radical [generated by the reaction of iron(II)–EDTA with hydrogen peroxide]: We find by this experiment that both expected migratory conformers are present in solution for each junction. The hydroxyl radical protection studies suggest that these junctions form stacked helical structures, just as the parent immobile junction J1 does (Figure 2). J1SMC is similar to J1, in that strands 2 and 4 cross over between helices, while strands 1 and 3 remain helical. In contrast, J1SMA has the opposite pattern, with strands 1 and 3 crossing over, while strands 2 and 4 are helical.

We interpret this result to mean that the pattern of crossover and stacking seen in Holliday-type junctions is a direct consequence of the sequence which flanks the branch site. In addition, the data indicate that there are two distinct isomerization processes in these semimobile junctions: the first, the crossover isomerization which results in the helix–helix stacking we have detected in J1; the second, the branch migration reaction itself. From our results, the free energy difference for the crossover isomerization appears to be greater than the free energy difference for the migration isomerization.

Materials and Methods

Design, Synthesis, Purification, and Formation of Junctions. The advantages of creating a semimobile junction from a well-characterized immobile junction (J1) are apparent. The ease and relatively low expense of automatic oligodeoxynucleotide synthesis combine with these considerations to militate for the initial attempts to form a semimobile junction as a simple insertion in J1, rather than a completely redesigned junction. The criterion number is the shortest segment in a strand which must be unique (Seeman, 1981, 1982). This number is 5 for the monomobile junctions J1SMA and J1SMC, although the criterion number for J1 is 4. While a criterion number of 5 affords the opportunity for competitive binding from lengths of up to four, rather than three, base pairs, no unexpected products are observed.

Individual strands are synthesized and desupportylated using routine phosphoramidite procedures (Caruthers, 1982) on an Applied Biosystems 380B automatic DNA synthesizer. Deprotection of bases is accomplished by treatment with 30% ammonium hydroxide overnight at 55 °C. Strands are dried in a rotary evaporator, coevaporated twice with absolute ethanol, and dissolved in distilled water. The solution is heated to 50 °C and injected on a Varian 5000 HPLC equipped with a Macherey-Nagel 60-7 Nucleogen column heated to 50 °C. The A solution is 10 mM Tris, pH 7.1, containing 4 M urea, and the B solution is 1.25 M NaCl in A. Strands are eluted with a linear gradient (1%/min) of B, precipitated with absolute ethanol, and dialyzed extensively against double-distilled water. A pure sample yields a single band on a denaturing gel. Except as otherwise noted, all oligodeoxynucleotide complexes are formed by dissolving individual strands in 66

mM Tris·HCl, pH 7.6, 11 mM $MgCl_2$, and 1 mM EDTA, heating to 70–100 °C, and slowly cooling to anneal.

Native Polyacrylamide Gel Electrophoresis. All native gels reported here are run at 4 °C. Unless otherwise noted, all native gels contain 20% polyacrylamide [19:1 acrylamide:bis(acrylamide)]. The running buffer consists of 40 mM Tris·HCl, 20 mM acetic acid, 2 mM Na_2EDTA, and 12.5 mM magnesium acetate (TAEMg). The sample buffer consists of equal volumes of TAEMg and glycerol containing 0.2% each of Bromphenol Blue and Xylene Cyanol FF tracking dyes. Gels are stained in 1:1 formamide:water containing 0.01% Stainsall dye. All native gels are run on Hoefer 600 electrophoresis units at 10 V/cm, cooled by a circulating bath.

Melting Experiments. Thermal transition profiles are obtained by monitoring absorbance at 260 nm on a Shimadzu UV-240 recording spectrophotometer. Temperature is controlled by a circulating bath, and the temperature is raised by 1 °C every 10 min.

Hydroxyl Radical Protection Experiments. Individual strands of J1 are radioactively labeled with ^{32}P ([γ-^{32}P]ATP, 3000 Ci/mmol, Amersham), by use of T4 polynucleotide kinase (P-L Biochemicals) and are additionally gel purified from a 20% denaturing polyacrylamide gel. Each of the labeled strands [approximately 1 pmol in 50 mM Tris·HCl (pH 7.5) containing 10 mM $MgCl_2$] is annealed to an excess (90 pmol) of the unlabeled complementary strand or to a mixture (90 pmol each) of the other three strands or is left untreated for the control. The samples are annealed by heating to 100 °C for 2 min and cooling slowly to 4 °C. Hydroxyl radical cleavage of the double-strand and junction samples for all strands takes place at 4 °C for 2 min, as published previously (Tullius & Dombroski, 1985), except for changes in the final concentrations of the reagents [100 μM iron(II), 200 μM EDTA, and 0.15% H_2O_2]. The reaction is stopped by addition of thiourea. The sample is lyophilized, dissolved in formamide/dye mixture, and loaded directly onto a 20% polyacrylamide/8 M urea sequencing gel. The gel is run for 4 h at 35 W, dried, and exposed to X-ray film for about 25 h. The gels are scanned with a Joyce-Loebl Chromoscan 3 densitometer. Positions of cleavage are identified by reference to adjacent gel lanes containing products of A+C rapid sequencing reactions (Bencini et al., 1984) run on each labeled strand.

Results

The electrophoretic mobility of a nucleic acid oligomer in nondenaturing gels is a function of its size, shape, and extent of base pairing (Fangman, 1978; Sealey & Southern, 1982). Figure 3a shows a photograph of a native gel containing equimolar mixtures of the various components of J1SMA, while Figure 3b shows a similar gel for J1SMC. The first four lanes of each gel contain the individual heptadecamer strands, the next four lanes contain the four possible triplet mixtures of strands, the next lane contains the tetrameric junction complex, the next four lanes contain the adjacent pairs of strands, and the last two lanes contain the two pairs not designed to associate as partial Watson–Crick double helices. Single strands on these gels migrate more rapidly than dimers of adjacent strands. However, the diagonal pair combinations migrate as monomers, indicating that they do not associate with each other. As was noted with J1 (Kallenbach et al., 1983a), the three-strand complexes are unstable and tend to migrate as mixtures of trimers and dissociation products. In both cases, the complete tetrameric junction complex migrates as a unique band, with a much lower mobility than any of the other combinations. Kallenbach et al. (1983a) have pointed

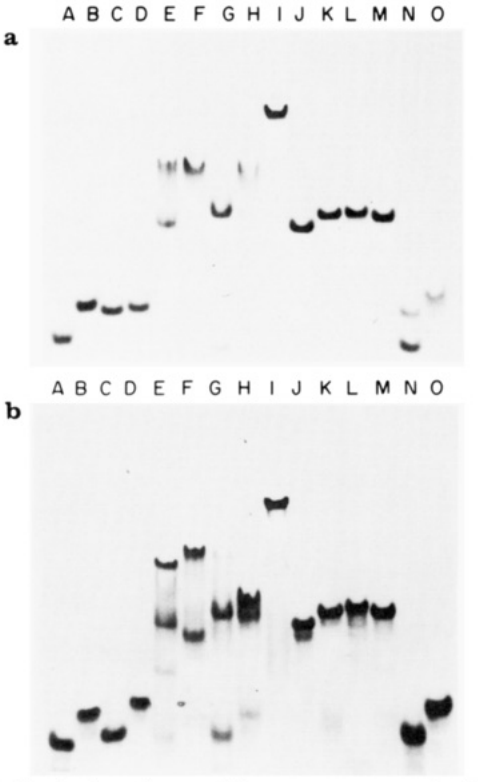

FIGURE 3: Equimolar mixtures of the component strands of J1SMA (a) and J1SMC (b). Shown are photographs of native gels in which the junction components have been combined in all possible equimolar combinations to illustrate their association under conditions of electrophoresis. Lane A–D contain strands 1–4, respectively. Lanes E–H contain the triples 123, 412, 341, and 234, respectively. Lane I contains the tetrameric junction complex. Lanes J–M contain the adjacent pairs 12, 23, 34, and 41, repectively. Lane N contains the opposite pair 13, and lane O contains the opposite pair 24. Note that the complete junction migrates as a single distinct band of lower mobility than any other species.

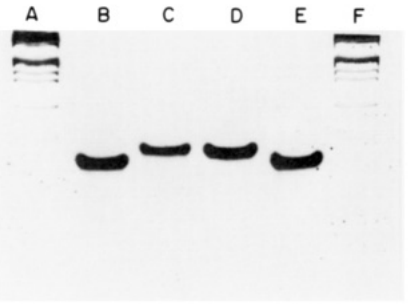

FIGURE 4: Comparison of gel mobilities of junctions and linear standards. Shown is a photograph of a 20% native gel illustrating the relative mobilities of J1, J1SMA, J1SMC, and a *Hae*III restriction digest of bacteriophage ϕX174. Lanes A and F contain the restriction digest, the smallest visible band of which corresponds to a 72-mer linear duplex fragment. Lanes B and E contain J1. J1SMC is in lane C, and J1SMA is in lane D. Note that the two semimobile junctions run with similar mobility.

out that this implies that the junction is a single closed complex whose first strand pairs with its last strand and does not consist of a series of unclosed complexes (e.g., 1:2:3:4:1:2:3...).

Figure 4 compares the gel mobilities of the monomobile junctions with that of J1, and with those of a set of DNA fragments produced by *Hae*III digestion of ϕX174 DNA. The two semimobile junctions, designed to contain 34 base pairs, migrate slightly faster, on a 20% native gel, than the 72-mer band of the restriction digest. The monomobile junctions migrate slightly slower than the immobile junction J1, which has been shown to contain 32 base pairs (Wemmer et al.,

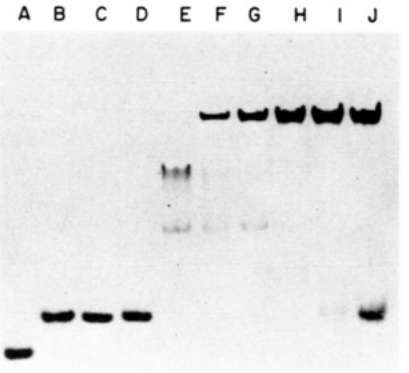

FIGURE 5: Stoichiometry of J1SMA and J1SMC. We illustrate a typical experiment to establish the 1:1:1:1 stoichiometry of the semimobile complexes. Strand triple 123 of J1SMA is titrated with strand 4. Lanes A–D correspond to strands 1–4, respectively. Lane E contains the triple, while lane F contains the triple plus 25% of the missing strand. Note the appearance of the missing junction band. Lane G contains 50% of the missing strand, and lane H contains a 1:1:1:1 mixture. Note the disappearance of the triple. Lane I contains 150% of the fourth strand, and lane J contains double the fourth strand. Note the overflow into a rapidly moving band which corresponds to the excess of the fourth strand. Identical results are obtained for the other permutations of J1SMA and all permutations of J1SMC.

1985). It is impossible to tell from this experiment whether the slight difference in mobility reflects size differences or relative branch point migrational capability.

The stoichiometry of the strands in the tetrameric complexes can be established from the sort of gel electrophoresis experiments shown in Figure 5. This figure illustrates the titration of an equimolar mixture of three strands of a junction with the fourth strand. Each of the four individual strands is run in the first four lanes. Lane E contains the triple 123. Lane F contains the triple plus 25% of strand 4; lane G, the triple plus 50% of strand 4; lane H, a 1:1:1:1 mixture; lane I, the triple plus 150% of the fourth strand; lane J, the triple plus double the fourth strand. The junction band first appears in lane F, and lane H (the 1:1:1:1 mixture) corresponds to the disappearance of the triple. Excess strand in the last two lanes migrates as monomer. We find the same result for the other three cyclic permutations of J1SMA, as well as for all four permutations of J1SMC. This result is similar to that seen for J1 in earlier work (Kallenbach et al., 1983a).

We have also explored some of the physical properties of these junctions. The two panels of Figure 6 illustrate the melting behavior of the two semimobile junctions. This thermal denaturation study monitors hyperchromism at 260 nm [e.g., Freifelder (1976)]. The thermal transition profiles of junction molecules are compared with those of a single strand and of one of the adjacent pairs. Note that in both cases the melting of the junction is more cooperative than the melting of the pair and that melting of the junction results in greater hyperchromicity. The same total strand concentration has been used for each mixture. The behavior observed is again similar to that of J1.

Figure 7 illustrates a Ferguson plot of the electrophoretic mobility versus polyacrylamide concentration for J1, J1SMA, and J1SMC, together with two reference linear DNA duplexes containing 36 and 72 base pairs. A linear Ferguson plot relates the mobility of a macro ion to the retardation coefficient K_R, which provides a measure of the size and shape of the ion:

$$\log M = \log M_0 - K_R T$$

where M is the apparent mobility in a gel of composition T in polyacrylamide, and M_0 is the free mobility of the ion (Rodbard & Chrambach, 1971). The free mobility, M_0, depends on the net charge distribution of the ion (Rodbard & Chrambach, 1971). The data in Figure 7 indicate that the retardation coefficients of DNA junctions differ from those of linear duplexes of any chain length and that those of J1SMA or J1SMC are very close to each other and differ from that of J1. Whether this reflects a greater free volume for the semimobile structures or is a consequence of the dynamics of the isomerization process itself cannot be decided. Whereas the free mobilities of the two linear duplexes are similar, since these lines converge near $T = 0$, the free mobility of the semimobile junctions is different from that of J1, implying

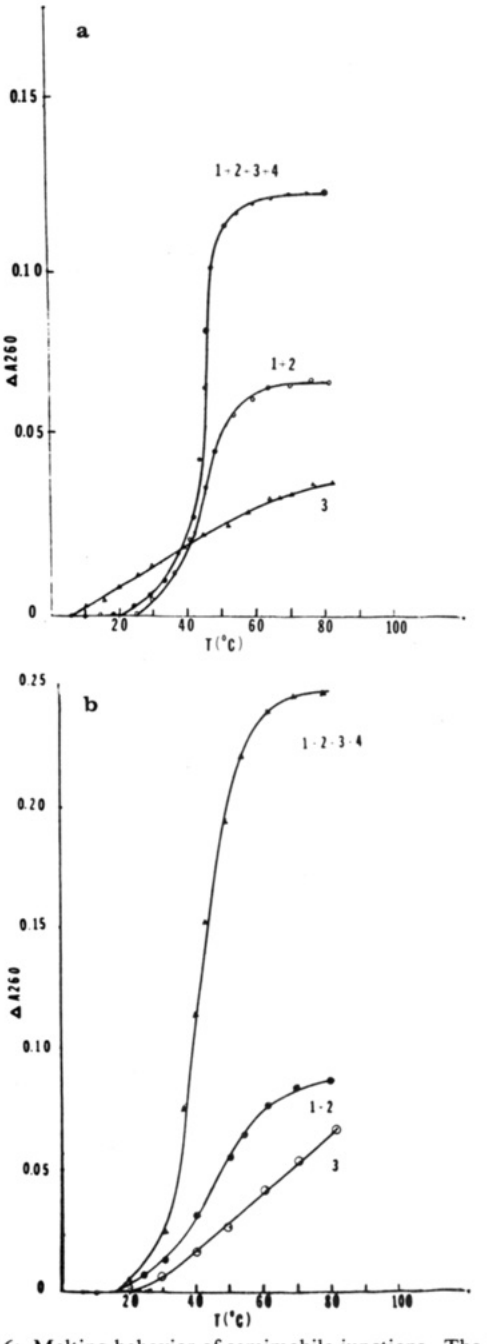

FIGURE 6: Melting behavior of semimobile junctions. The thermal transition profiles for different components of the junctions J1SMA (a) and J1SMC (b) are shown. The relative change in absorbance at 260 nm is measured. In each case, the total strand concentration is 100 μg/mL; thus, 25 μg of each strand is present per milliliter for the junctions (1 + 2 + 3 + 4), 50 μg/mL each of strands 1 and 2 (1 + 2) is present, and 100 μg/mL of strand 3 (3) is present. Note that the junction transition is consistently more cooperative than that of either component. The melting temperatures of the junctions are similar to that measured under similar conditions for J1.

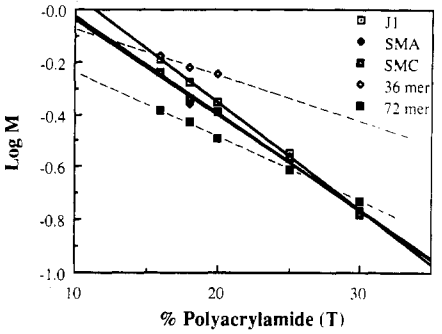

FIGURE 7: Ferguson plot of immobile and semimobile junctions. Gels are cast with different compositions, and the mobilities of the equimolar complexes of J1, J1SMA, andd J1SMC, as well as a 72-mer linear fragment, are compared with respect to the mobility of the reference dye Xylene Cyanol FF. Note that the curves for the junctions are almost exactly coincident. A 36-mer from a previous study (Kallenbach et al., 1983a) was included on the figure for comparison.

some difference in the change density of these structures as well. We have previously noted that formation of J1 requires counterions—either divalent ions or high concentrations of monovalent ions—presumably to screen the repulsion of four phosphates in close proximity at the branch point (Kallenbach & Seeman, 1986). Figure 7 suggests that the effective repulsion at the corresponding sites in J1SMA or J1SMA is lower than that in J1, but still greater than that in duplex DNA.

From the foregoing data, we conclude that we have formed branched DNA junctions from the component strands of J1SMA and J1SMC. However, these data alone do not constitute proof that these junctions are semimobile, rather than immobile. In order to ascertain whether these junctions contain both alternative migratory conformers, we have performed protection experiments which reveal the population-average structure of the junction. In the presence of hydrogen peroxide, iron(II)–EDTA ($[Fe(EDTA)]^{2-}$) attacks DNA through the generation of hydroxyl radical, which diffuses and abstracts a hydrogen atom from deoxyribose, resulting in strand cleavage (Hertzberg & Dervan, 1984). This type of experiment has been used to determine the helical twist of DNA bound to a calcium phosphate substrate (Tullius & Dombroski, 1985), to determine the DNA contact points of cro protein and λ C1 repressor (Tullius & Dombroski, 1986), and to characterize the bending of kinetoplast DNA (Burkhoff & Tullius, 1987).

Recently, we have used this reagent to show that the equilibrium solution structure of the immobile junction J1 is at most 2-fold symmetric; the four arms form two helical stacking domains, thereby assuming a particular crossover structure (Churchill et al., 1988). To perform this experiment, we radioactively label each strand of J1 and complex it with the other three strands, which are not radioactive. We then compare the solution cleavage pattern of each strand of J1 while in the junction with its solution cleavage pattern when it is complexed with its linear duplex complement. The salient feature of J1 structure revealed by iron(II)–EDTA treatment is strong protection of the two junction-flanking bases on strands 2 and 4. Strands 1 and 3 are only slightly protected at those positions but show significant protection four and five nucleotides 3′ to the junction.

Panels a–c of Figure 8 correspond to the densitometer tracings for the protection experiment gels of J1, J1SMA, and J1SMC, respectively. Inspection of panels a (J1) and c (J1SMC) indicates qualitative similarities between the two junctions: Strands 2 and 4 of J1SMC show marked protection in the vicinity of the junction (nucleotide 9 ± 1), while strands 1 and 3 are only slightly protected there, if at all. Some weak protection is seen at residues 12–14 in strand 3 and at residues 5 and 13 on strand 1. Major protection at the junction only on strands 2 and 4 indicates that this junction, like J1, adopts a conformation in which the four identical strand backbones have assumed an approximately 2-fold symmetric structure. Furthermore, the distribution of possible 2-fold symmetric structures is biased and does not average out to be 4-fold symmetric.

Elsewhere (Churchill et al., 1988), we have analyzed this

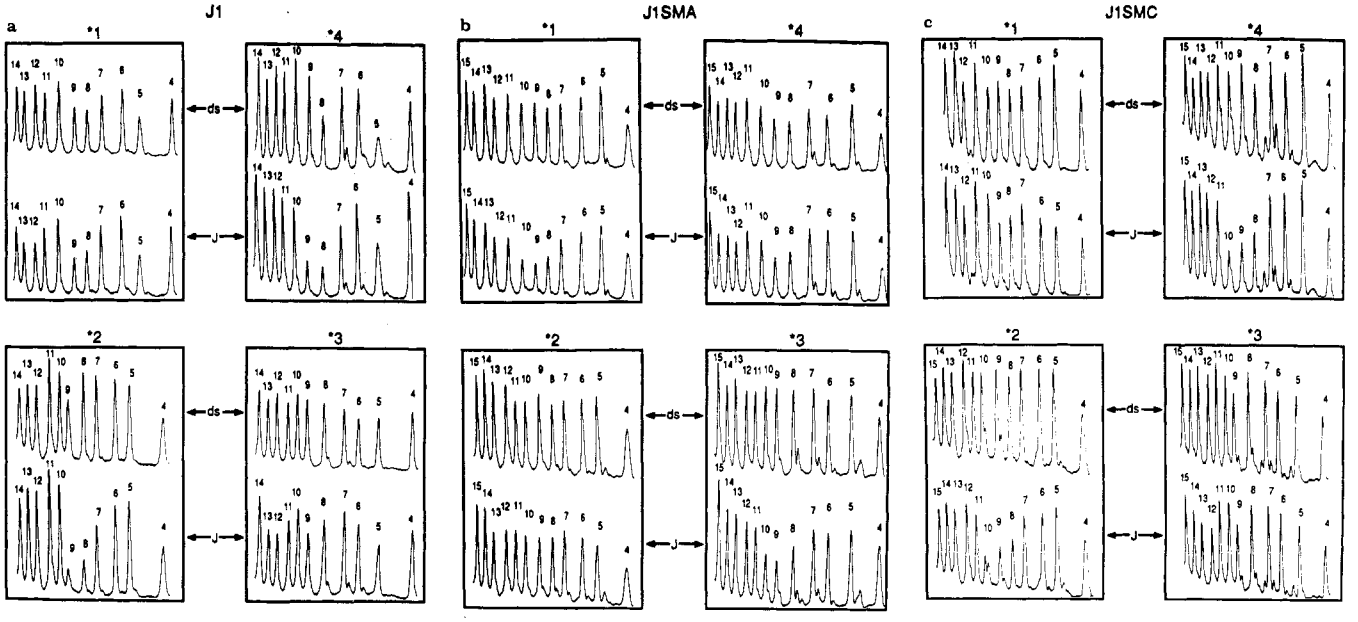

FIGURE 8: Densitometer tracings of hydroxyl radical protection experiments. Panels a–c correspond respectively to protection experiments involving J1, J1SMA, and J1SMC. The cutting pattern for each individual strand when complexed to its complement is indicated by ds, while the cutting pattern for the same strand when complexed in its junction is indicated by J. Residues 8 and 9 flank the branch site for all strands of J1. Note that those two residues are strongly protected on strands 2 and 4 of that junction, but not on strands 1 and 3. Residue 9 corresponds to the migratory base in J1SMA and J1SMC. Thus, residues 8 and 10 are the immobile nucleotides which flank the branch site of those junctions. In J1SMA, residues 8–10 show clear protection on strands 1 and 3 but only weak protection on strands 2 and 4. The J1SMC, residues 8–10 show protection on strands 2 and 4, like J1, but only weak protection on strands 1 and 3.

type of pattern and concluded that it corresponds to a structure in which the protection results from two strands occluding each other to form either a parallel Sigal–Alberts (Sigal & Alberts, 1972) structure or its antiparallel variant. Both these structures contain two strands that are minimally perturbed from a helical conformation and two strands that form a crossover structure (Figure 2). Each unperturbed helical strand spans two stacked arms (with roughly collinear axes) to form a single helical stacking domain. The formation of particular stacking domains is equivalent to taking on a particular crossover isomer. The designation of parallel or antiparallel refers to the relative orientation of the axes of the two helical domains. In the antiparallel structure, and two-fold symmetry axis relating the helical domains is perpendicular to the two helix axes. In the parallel structure, the two-fold symmetry axis is parallel to the axes of the domains. It is not possible to determine from these data whether J1SMC forms a parallel or antiparallel structure. However, the protection seen several residues 3′ to the branch site suggests that the axes of the two helical domains cannot be far from coplanar, or these residues would not be occluded.

One might expect an equilibrium experiment to reveal a slightly different cleavage pattern for a semimobile junction as compared to an immobile junction. In particular, one would expect somewhat weaker protection in the region of the junction but that it should be extended over a slightly larger range of nucleotides, corresponding to the presence of both migratory conformers. This is indeed what we see in the cleavage pattern of J1SMC. Three nucleotides are protected, rather than two, but not quite to the extent seen with J1. Within the resolution of the experiment, the weakening of protection is significant and of roughly equal extent on both sides of the mobile base. We conclude that both migratory conformers of J1SMC are present in roughly equal quantities.

Inspection of Figure 8b reveals a qualitatively similar pattern for J1SMA, with a major difference and surprise: the protection pattern is reversed. Thus, strands 1 and 3 show significant protection in the vicinity of the junction, while strands 2 and 4 are only marginally affected at that location. Very small changes (about four bases 3′ to the junction) can again be noted on the two strands that have virtually unperturbed junction regions. A small degree of protection is again present at residue 5 of one of these chains (2), but not on the other. The three-base protection noted at the junction for strands 1 and 3 indicates that J1SMA is also semimobile, as far as an equilibrium experiment can determine: both conformers are present in roughly equal amounts. The reversal of protection pattern from J1SMC and J1 indicates that the sequence at the junction is critical to the structure of the junction: It determines which way the crossover structure and stacking domains will form.

Discussion

We have constructed two monomobile junctions and have demonstrated that both migratory conformers exist for each of the junctions under the conditions of our protection experiments (4 °C, 10 mM Mg^{2+}). This is all that can be ascertained from a protection experiment involving the equilibrium structure. It remains for us to demonstrate that the two migratory conformers are truly in dynamic equilibrium with each other. Those experiments are in progress.

The migratory equilibrium and the stacking domain or crossover equilibrium are two different phenomena, each of which is critical to the chemistry of genetic recombination. We have shown that J1 and now J1SMA and J1SMC assume structures that appear to combine their four arms into two helical stacking domains. In principle, each junction could accomplish this in two different ways: strands 1 and 3 are continuous, while strands 2 and 4 cross over; alternatively, strands 1 and 3 cross over, while strands 2 and 4 are continuous. Nevertheless, the bias for one crossover structure over another appears to be quite strong under our conditions; in all three cases one crossover or stacking structure predominates. This behavior is very different from the predictions one would make from the known thermodynamics of base stacking in linear duplex DNA (Breslauer et al., 1986). Calculations using these parameters suggest an equilibrium constant near unity for the two alternative stacking (or crossover) structures in the case of J1 (48:52); the equilibrium constant is rigorously unity for monomobile junctions if a linear duplex stacking model is used. We must conclude that the stacking of "faulted" dinucleotides in junctions is quite different from the stacking of linear duplex DNA.

An important result of the current study is the clear demonstration that the stacking domains and crossover structures are sequence dependent, since J1SMA has the opposite structure from J1SMC or J1. It is important to point out that crossover biases will exist in naturally occurring junctions, except at those sequences which have 4-fold symmetry at the site of branching. Thus, only dinucleotides of the sequences CpG, GpC, ApT, or TpA can flank the branch site without a possible crossover bias arising. It is possible that these stacking and crossover biases influence the action of resolving enzymes, such as endonuclease VII (Mizuuchi et al., 1982; Lilley & Kemper, 1984; Jensch & Kemper, 1986; C. J. Newton, J. E. Mueller, B. Kemper, R. P. Cunningham, N. R. Kallenbach, and N. C. Seeman, unpublished results), or similar activities (de Massey et al., 1984; Symington & Kolodner, 1985; West & Korner, 1985), thereby directing the resolution of junction into products.

The branch point migratory event is also characterized by an equilibrium constant. This equilibrium constant will also be a function of the sequences that surround the site of branching before and after migration. In previous work [e.g., Meselson (1972), Thompson et al. (1976), Warner et al. (1979), and Robinson and Seeman (1987)] this equilibrium constant has been assumed to be approximately unity. While we cannot definitely assign a value to this constant for either J1SMA or J1SMC, the protection data can be interpreted as representing a ratio of the two conformations not much different from 1. Refinement of this estimate awaits further studies.

It is clear that the chemistry of recombination is highly dependent upon the interplay of the two equilibria that we have considered. The sequence dependence of branch point migration can determine which sites a Holliday structure can reach once the strand interchange has been accomplished. The sequence dependence of the crossover equilibrium, on the other hand, may affect resolution of the Holliday complex and thus the products of the recombination event. Insofar as our experiments can ascertain, the sequence-dependent bias of the crossover equilibrium is much greater than the sequence-dependent bias of the migratory equilibrium.

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