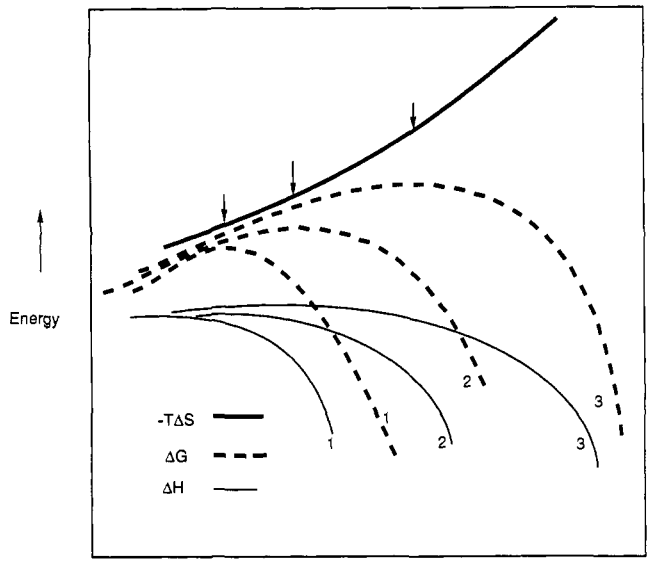

Figure 1. Schematic diagram of energy terms as functions of reaction coordinate for a series of three entropy-controlled cycloadditions with little or no enthalpic barrier, presented in the manner of Houk.[6] In each case, the value of $-T\Delta S$ at the transition state (maximum of ΔG) is marked with an arrow.

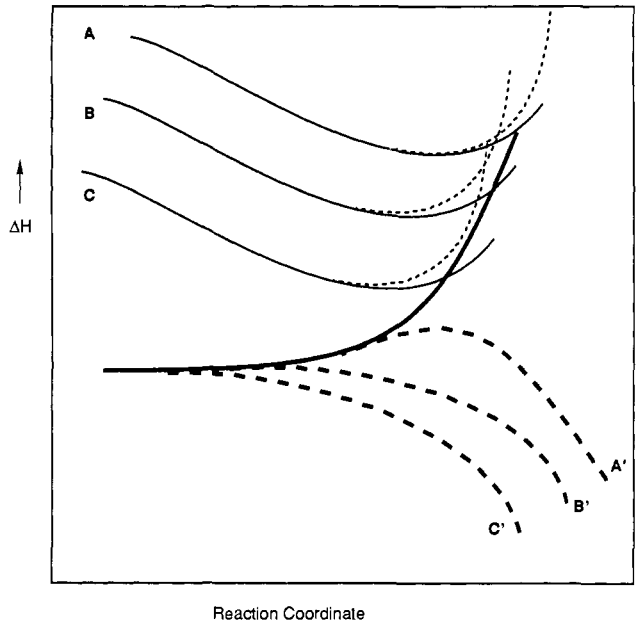

Figure 2. Schematic diagram of configurational interaction of ground level (heavy solid line) with charge-transfer configurations of decreasing energy A, B, and C to produce ground state reaction profiles A′, B′, and C′, respectively.

and the other is entropy controlled? A detailed treatment remains to be given, but we point out that just this result can be deduced qualitatively as a corollary from Houk's model[6] for entropy control, according to which the contribution of the $-T\Delta S^{\ddagger}$ term to $\Delta G^{\ddagger}$ depends on the position of the transition state (maximum in ΔG) along the reaction coordinate. In the ordinary Diels–Alder series, all of the reactions have substantial enthalpic barriers, but to a good approximation, the reaction exothermicities hardly vary. Therefore, by the Hammond postulate, the Diels–Alder transition states all should have about the same location, and hence all should have about the same value of $-T\Delta S^{\ddagger}$. The major contributor to $\Delta\Delta G^{\ddagger}$ thus will be the $\Delta\Delta H^{\ddagger}$ term.

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In contrast, the diyl cycloadditions have little or no enthalpic barrier, but in terms of Figure 1, the variation in their relative rates must result from shifts of the position of the transition state along the reaction coordinate, which in turn produces a monotonic variation of $-T\Delta S^{\ddagger}$.[6] What causes these shifts? For didactic convenience, we offer an interpretation based on a configuration interaction (CI) treatment[2c,d,7,8] of cycloadditions, although the same result would be obtained in any formalism that takes into account the idea[6] that the more reactive the system, the faster the drop of the floor of the potential energy pass. In the CI formalism, the wave function for the transition state can be described as the result of quantum mechanical mixing of ground and excited, notably charge transfer (CT), wave functions (Figure 2). The deeper the CT energy minimum, the stronger the interaction with the ground level. With reference to Figure 2, one sees that this will cause a steeper descent of the ΔH profile to lower energy, which in turn, as Figure 1 shows, will result in a shift to an earlier, lower maximum in ΔG. In this view, a single physical basis underlies both the changes of transition-state energy that produce enthalpy control of the Diels–Alder sequence and the shifts of transition-state location that produce entropy control of the biradical addition sequence.

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Nonenzymatic Ligation of Oligodeoxyribonucleotides on a Duplex DNA Template by Triple-Helix Formation

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We report that a double-stranded DNA template can direct the sequence-specific formation of a phosphodiester linkage between oligodeoxyribonucleotides in aqueous solution by juxtaposing the termini head-to-tail in a triple-helical complex (Figure 1).[1] A triple-stranded complex can be formed when a pyrimidine oligodeoxynucleotide binds in the major groove of duplex DNA parallel to the purine Watson–Crick strand. In the complex, sequence specificity for the condensation of oligodeoxynucleotides

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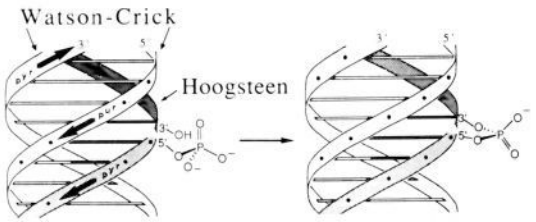

Figure 1. Ligation of a 3′-hydroxyl and an activated 5′-phosphate of two oligonucleotides is directed by a double-stranded template through formation of adjacent triple-helical complexes.

is directed by Hoogsteen base pairing.[6-11]

A double-stranded template, 37 base pairs in length, and two pyrimidine oligodeoxynucleotides, 15-mers A and B, were synthesized by chemical methods and purified by gel electrophoresis (Figure 2). Oligodeoxynucleotides A and B are complementary in a Hoogsteen sense to adjacent unique 15-bp sequences on the double-stranded template. In a triple-stranded complex, the 3′ end of A would be proximal to the 5′ end of B (Figure 2). Oligodeoxynucleotide B was phosphorylated at the 5′ end with polynucleotide kinase and [γ-^{32}P]ATP and incubated in the presence of A (0.7 μM concentration) and the duplex DNA template (0.15 μM concentration). The 5′-terminal phosphate of B was activated in situ with BrCN, imidazole, and $NiCl_2$.[4] After 9 h (20 °C), the reaction products were separated by denaturing polyacrylamide gel electrophoresis and visualized by autoradiography (Figure 3A). Condensation of 15-mers A and (^{32}P)B in the presence of duplex template was apparent on the autoradiogram by the production of a radioactive 30-mer in 40% yield (lane 6).[12] The product of the reaction can be identified as a 30-mer by comparison of its electrophoretic mobility with that of an authentic 5′-phosphorylated 30-mer having the sequence expected for the head-to-tail A–B condensation product (lane 11). Within experimental limits, no condensation product ($\leq$1%) is apparent in the absence of A (lane 9) or in the absence of template under the same conditions (lane 3). No product ($\leq$1%) is detected after incubation of A, B, and the pyrimidine strand of the template alone (lane 5); however, some product (15% yield) is found after incubation of A, B, and the purine strand of the template (lane 4).

The requirement for A in the coupling reaction to produce 30-mer is consistent with condensation of B exclusively with A.

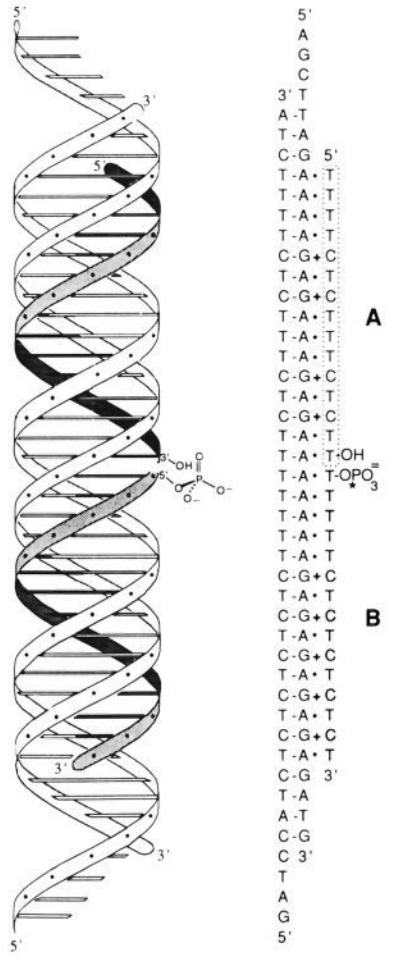

Figure 2. In the triple-stranded complex, the pyrimidine Hoogsteen strands (A and B) are the condensing oligonucleotides (shaded). The 3′ terminus of oligonucleotide A is proximal to the 5′ terminus of oligonucleotide B. Both are parallel to the purine template strand.

No self-condensation of B is evident. Optimal formation of the 30-mer occurs when both strands of the DNA duplex template are present. Remarkably, condensation is observed in the presence of A and the purine strand of the template alone, even though the condensing oligodeoxynucleotides are not complementary to the purine template in an antiparallel Watson–Crick fashion. Condensation could result from the accommodation of a minimum of four base mismatches necessary for Watson–Crick hybridization or the formation of a double-stranded DNA complex in which the purine and pyrimidine strands are parallel to each other.[13]

If the double-stranded template is directing condensation of oligodeoxynucleotides A and B, the 30-mer produced will have a 5′-hydroxyl, a 3′,5′-phosphodiester at position 15–16, and a nucleotide sequence corresponding to a head-to-tail alignment of A and B (Figure 2). To analyze for product composition, the 30-mer produced in the reaction was purified by gel electrophoresis and digested with calf spleen phosphodiesterase, a 5′-exonuclease that degrades DNA terminated by 5′-hydroxyl.[14] After treatment of the condensation product with this phosphodiesterase, only a trace of the 30-mer is visible and most of the radioactive label is found in mononucleotides (Figure 3B, lane 2). The susceptibility of the reaction product to degradation by the exonuclease indicates that it possesses a 5′-hydroxyl. The release of mononucleotides

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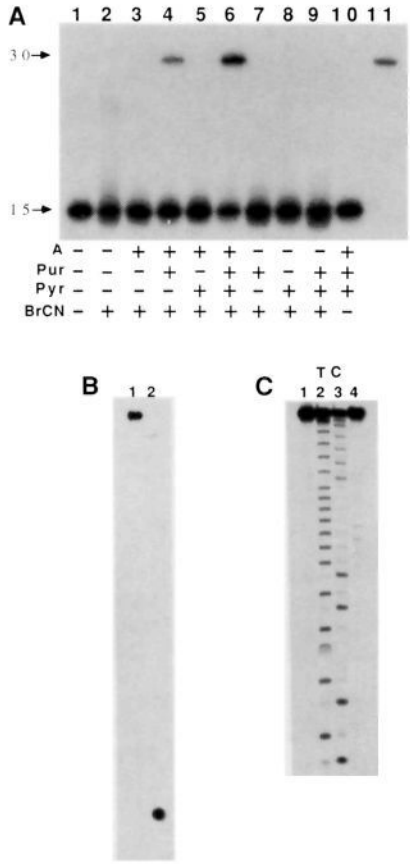

Figure 3. Condensation of oligonucleotides A and (^{32}P)B and product analysis; autoradiograms of high-resolution, 20% polyacrylamide denaturing gels: (A) Production of a radioactive 30-mer by template-directed condensation of 15-mers A and (^{32}P)B. Lanes 1–10: all reaction mixtures contain 5′-^{32}P-phosphorylated B. Activation of the 5′-phosphate of B was initiated by addition of a freshly prepared solution of BrCN (0.4 M) to the reaction mixtures. Reaction mixtures initially contained 40 mM BrCN, 20 mM imidazole·HCl (pH 7.0), 20 mM $NiCl_2$, and 100 mM NaCl in a total volume of 30 μL. In control experiments, BrCN, oligonucleotide A, and one or both strands of the template were omitted from the reaction. After a 9-h reaction time (20 °C), the DNA was precipitated with ethanol, and the precipitates were dissolved in buffer and loaded on the gel. Lane 11: authentic synthetic 30-mer with the sequence 5′-$T_4(CT)_2T_2(CT)_2T_6(CT)_5$-3′ phosphorylated at its 5′ end with polynucleotide kinase and [γ-^{32}P]ATP. (B) Enzymatic hydrolysis of the condensation product by treatment with calf spleen phosphodiesterase. Lane 1: condensation product. Lane 2: condensation product after treatment with 4 μg of calf spleen phosphodiesterase (Boehringer Mannheim) at 37 °C for 8 h. (C) Sequencing analysis of the 5′-phosphorylated condensation product. Lane 1: purified, ^{32}P 5′ end-labeled condensation product. Lane 2: T-selective chemical cleavage reaction ($KMnO_4$).[15] Lane 3: C-selective cleavage reaction (hydrazine, NaCl).[16] Lane 4: 5′ end-labeled condensation product treated with 4 μg of calf spleen phosphodiesterase at 37 °C for 8 h.

containing most of the radioactive label and the absence of a radioactive 16-mer corresponding to enzymatic termination at the coupling site indicate that the predominant linkage formed in the condensation reaction is a 3′,5′-phosphodiester bond.

For sequencing analysis, the condensation reaction was carried out with oligodeoxynucleotide B phosphorylated by using unlabeled ATP. The A–B product was then purified by gel electrophoresis and treated with polynucleotide kinase and [γ-^{32}P]ATP. The ^{32}P 5′ end-labeled product was isolated by gel electrophoresis and subjected to chemical sequencing reactions specific for C and T (Figure 3C, lanes 2 and 3).[15,16] The sequence of the 30-mer verifies the condensation of the 3′ terminus of A with the 5′ terminus of B. Finally, the 5′-phosphorylated product was treated with calf spleen phosphodiesterase and shown to be resistant to degradation, confirming that the exonuclease had little endonuclease or 3′-exonuclease activity (Figure 3C, lane 4).

In conclusion, double-stranded DNA can serve as a template to align reactive termini of oligonucleotides and promote their condensation. Within the context of self-assembling chemical systems for macromolecular information transfer, this conversion of sequence information is not self-replicating.[17] Triple-helix-directed ligation can create sequences that are neither identical nor complementary in a Watson–Crick sense to the template, but rather *new sequences* of nucleic acids.

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Chelation Enhanced Fluorescence Detection of Non-Metal Ions

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Several groups have reported observing fluorescence enhancements in nonaqueous solutions upon the binding of some metal ions to conjugate fluorescent probes, probes in which the metal ligand is not an integral part of the fluorophore substructure.[2] Conjugate probes offer great potential as reporter molecules in that the known selectivities of azacrown and cryptand binding sites can, in principle, be applied unaltered to the design of fluorescent probes with similar selectivities and large signal ranges. Recently, we have found that anthrylazamacrocycles demonstrate both chelation-enhanced fluorescence (CHEF) and chelation-enhanced quenching (CHEQ) upon metal ion binding in 100% aqueous solution.[3] However, both conjugate and integral fluorescent probes have been applied to date almost exclusively to the detection of metal ions.[4] We now report the first observation of large CHEF effects on the binding of anions to anthrylpolyamine conjugate probes, which serve to "signal" molecular recognition events involving carboxylate, sulfate, and phosphate groups. Moreover, we propose intracomplex protonation of a

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