was produced before generation of $e_s^-$. On the basis of the CV behavior for each reduction, the only soluble species were the −1 and −2 ions; all other anions were either insoluble or partially soluble. Note that in the LiI CV, all of the reduction waves were about equally spaced in potential, while wave V in Figure 1B is shifted to a less negative potential, probably because of the precipitation of $K_5C_{60}$ from soluble $C_{60}^{4-}$. The potentials of the different waves in both $NH_3$/LiI and $NH_3$/KI solutions and a comparison to those in other solvents are given in Table I.[14]

These results support the strong dependence of the redox chemistry of $C_{60}$ on both solvent and cation of the supporting electrolyte and demonstrate that electrochemical reduction to the −6 state can be accomplished in liquid $NH_3$ in the presence of $K^+$. Electrolysis of $C_{60}$ slurries by $e_s^-$ generated electrochemically is quantitative and provides a useful new approach in the preparation of $C_{60}$ compounds and films.

**Acknowledgment**. The support of this research by a grant from the National Science Foundation (CHE 8901450) is gratefully acknowledged.

## Ligand-Promoted Dimerization of Oligonucleotides Binding Cooperatively to DNA

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*Received September 18, 1992*

In biological systems, specific protein–DNA interactions are often modulated by ligands as in the regulation of gene expression.[1] Many transcription factors are active as dimers, but inactive as monomers. In at least one case, dimerization is regulated by an additional protein cofactor.[2] We recently described the design of a triple helical complex comprising two oligonucleotides which bind adjacent sites on DNA cooperatively through dimerization.[3] If dimerization could be controlled by additional ligands, such an artificial nucleic acid system would be analogous to the naturally occurring inducible protein systems. We now report that the stability of the dimerization domain can be enhanced by a small molecule, echinomycin (E). The strengths of ligand-promoted control of oligonucleotides binding cooperatively to DNA are quantitated by affinity cleaving.

Our design begins with a Y-shaped structure consisting of two oligonucleotides (Figure 1).[3] Each of these oligonucleotides contains a recognition domain (11 or 15 bases) and a dimerization domain (5 bases) separated by a one-base linker.[3] Site-specific DNA recognition is achieved through specific Hoogsteen hydrogen bonding and local triple helix formation (T·AT and C+GC triplets)[4] while dimerization occurs through Watson–Crick hydrogen bonding. The stability of the Watson–Crick dimerization domain can be controlled both by length and/or by the addition of sequence-specific DNA-binding peptides or proteins. In order to minimize dimerization in the absence of ligand, a 5-bp mini-helix was used (Figure 1). To obtain ligand-mediated control of dimerization, a specific 4-bp recognition sequence for the DNA-binding drug echinomycin (E) was incorporated into the dimerization domain. Echinomycin (E), a bis-intercalating minor

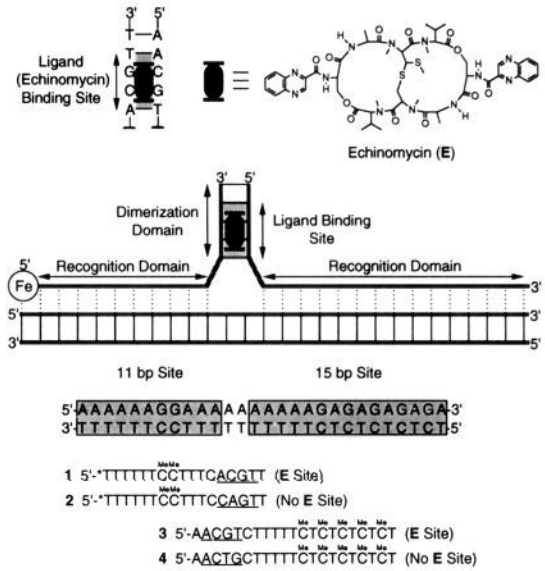


**Figure 1**. Schematic representation of a Y-shaped nucleic acid complex composed of two triple helix forming oligonucleotides which dimerize through formation of a small segment of Watson–Crick double helical DNA. Contained within the mini-helix is a high-affinity site for the DNA-binding molecule, echinomycin (E).

groove-binding molecule, is known to bind sequence specifically to the 4-bp site 5′-ACGT-3′ and stabilize duplex DNA formation at micromolar concentrations (Figure 1).[5-8] Echinomycin (E) binding should therefore augment the stability of the 5-bp dimerization domain and increase the affinity of the resulting dimeric oligonucleotide complex for its specific DNA target site.

Four oligonucleotides, **1**–**4**, were synthesized to test this design (Figure 1). Oligonucleotides **1** and **3** contain the echinomycin recognition sequence 5′-ACGT-3′ in the dimerization domain while **2** and **4** do not. The modified base thymine–EDTA (T*) was incorporated at the 5′-termini of oligonucleotides **1** and **2**, each targeted to the 11-bp site, to allow analysis of site-specific binding by the affinity cleaving method.[4a,9] The binding affinities of oligonucleotide pairs **1**,**3** and **2**,**4** in the presence and absence of echinomycin (E) were measured.

Affinity cleavage experiments were performed on a $^{32}$P-end-labeled restriction fragment (852 bp) containing the adjacent 11- and 15-bp target sites (Figure 2).[3] Reaction of oligonucleotide–EDTA·Fe **1** (100 nM) with the target DNA (pH 7.0, 37 °C) alone or in the presence of echinomycin (E) results in little cleavage (Figure 2, lanes 2 and 3). A reaction of **1** in the presence of **3** (1.0 $\mu$M)[10] affords a modest increase in cleavage, revealing that some cooperativity occurs even with a 5-bp dimerization domain (lane 4). Upon addition of echinomycin (E) (50 $\mu$M) to the reaction containing oligonucleotides **1** and **3**, a dramatic increase in cleavage occurs (lane 5). This demonstrates that the small ligand E significantly enhances the affinity of oligonucleotides **1** and **3** for its site. Only minimal cleavage is obtained in the reaction containing **2**, **4**, and E (lane 10), demonstrating that this result depends on a sequence-specific ligand-binding site within the dimerization domain. For comparison, significant cleavage is obtained in a reaction containing **2** and **4** when the concentration of **2** is raised by a factor of 10 from 100 nM to 1.0 $\mu$M (lane 11). This demonstrates that oligonucleotide **2** binds

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(10) At 1.0 $\mu$M concentration, oligonucleotides **3** and **4** should nearly saturate their cognate binding sites (>90% occupancy).[11] Similarly, echinomycin at 50 $\mu$M concentration should be saturating.[8]

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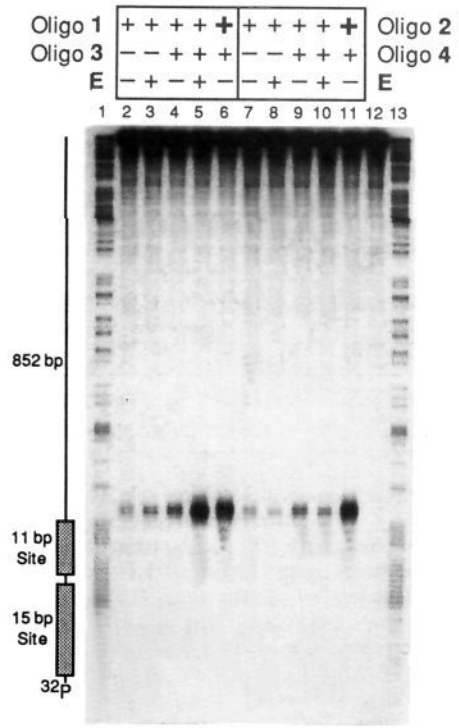


**Figure 2.** Autoradiogram of a denaturing polyacrylamide electrophoresis gel of reaction products from affinity cleavage reactions with the oligonucleotides described below and a $^{32}$P-labeled restriction fragment from pMD5556 containing the target site shown in Figure 1. The radiolabeled restriction fragment was prepared by digestion of pMD5556 with *Eco*RI followed by incubation with [$\alpha$-$^{32}$P]dATP and [$\alpha$-$^{32}$P]TTP in the presence of Sequenase. Digestion with *Xmn*I and subsequent gel purification of the 852-bp fragment yielded the DNA used in the experiments below. Affinity cleavage reactions were performed by preincubating the desired oligonucleotides together with Tris–acetate, pH 7.0 (25 mM), NaCl (10 mM), spermine (1 mM), $Fe(NH_4)_2(SO_4)_2{\cdot}6H_2O$ (2 $\mu$M), sonicated calf thymus DNA (100 $\mu$M in bp), MeOH (10% v/v), 30 000 cpm $^{32}$P-labeled DNA, and 50 $\mu$M echinomycin (E), where noted below, in a volume of 40 $\mu$L at 37 °C for 1 h. The cleavage reactions were initiated by addition of dithiothreitol (4 mM, final concentration) and allowed to react for 6 h at 37 °C, at which time they were terminated by NaOAc/EtOH precipitation. The samples were washed with 70% EtOH, dissolved in $H_2O$, dried in vacuo, dissolved in formamide–TBE loading buffer, heated to 95 °C for 3 min, and subjected to electrophoretic separation on an 8% denaturing polyacrylamide gel (19:1, monomer:bis). Except where noted below, oligonucleotides **1** and **2** are used at 100 nM, oligonucleotides **3** and **4** at 1 $\mu$M, and E at 50 $\mu$M. Lanes 1 and 13, adenine-specific sequencing reaction;[13] lane 2, oligonucleotide **1**; lane 3, **1** with E; lane 4, **1** and **3**; lane 5, **1** and **3** with E; lane 6, **1** (1 $\mu$M) and **3**; lane 7, **2**; lane 8, **2** with E; lane 9, **2** and **4**; lane 10, **2** and **4** with E; lane 11, **2** (1 $\mu$M) and **4**; lane 12, no oligonucleotide.

and cleaves as well as **1** under similar conditions in spite of its insensitivity to E (lane 6).

To determine the magnitude of ligand-mediated control, the quantitative affinity cleavage titration[11] was used to obtain binding isotherms and equilibrium binding constants for **1** alone, **1** in the presence of 1.0 $\mu$M **3**, and **1** in the presence of 1.0 $\mu$M **3** and 50 $\mu$M E (Figure 3). These experiments yielded values of $(3.0 \pm 0.4) \times 10^5$, $(1.3 \pm 0.2) \times 10^6$, and $(4.1 \pm 1.4) \times 10^6$ $M^{-1}$, respectively. This analysis demonstrates that the 5-bp dimerization domain, created by the addition of **3**, increases the affinity of **1** for its site 4.3-fold. Addition of echinomycin to the dimeric **1**,**3** system further enhances the binding of **1** by a factor of 3.2. We conclude that ligand-mediated control of dimerization in cooperatively binding oligonucleotides to single sites on double helical

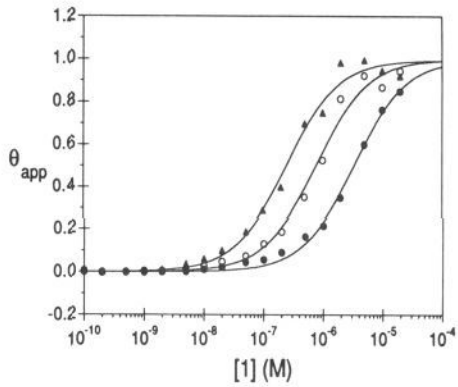


**Figure 3.** Binding isotherms obtained for oligonucleotide **1** alone, in the presence of 1.0 $\mu$M **2**, or in the presence of 1.0 $\mu$M **2** and 50 $\mu$M E, using the quantitative affinity cleavage titration method.[11] These experiments were performed as described in Figure 2 with the modifications described below. In each experiment, [**1**] was varied from 100 pM to 20 $\mu$M and the $[Fe(NH_4)_2(SO_4)_2]$ was varied such that the [Fe(II)]/[**1**] ratio was fixed at 2.0. Reactions were preincubated for 24 h prior to initiation with DTT. After electrophoresis, the dried gels were exposed to photostimulable storage phosphorimaging plates, the plates were visualized using a Molecular Dynamics 400S PhosphorImager, and the cleavage and background intensities for each reaction were obtained. A theoretical binding curve, $I_{fit} = I_{sat}K[\mathbf{1}]/(1 + K[\mathbf{1}])$, where $I_{sat}$ is the apparent maximum cleavage intensity and $K$ the equilibrium association constant, was used to fit the experimental data ($I_{exp}$) using $I_{sat}$ and $K$ as adjustable parameters.[11] At least three complete data sets were used to determine each association constant. For the data shown above, $I_{exp}$ was divided by $I_{sat}$ to obtain $\theta_{app}$ (fractional saturation). Data for **1** alone is shown by ●, **1** and **3** by ○, and **1**, **3**, and echinomycin by ▲; each data point represents the average of three or four individual measurements.

DNA is attainable, and we envision the design of further ligand-promoted intermolecular nucleic acid assemblies.

**Acknowledgment.** This work was supported by the National Institutes of Health (GM-35724) and a Damon Runyon–Walter Winchell Cancer Research Fund postdoctoral fellowship for M.D.D.

## Quantum Mechanical Tunneling in the Ene Reaction of Triazolinedione with Sterically Hindered Alkenes

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*Received July 24, 1992*

The reaction of *N*-phenyl-1,2,4-triazoline-3,5-dione (PTAD) with alkenes recently has received considerable mechanistic attention.[1] For example, recent stereoisotopic studies and direct spectroscopic observations[2] of this reaction have established the formation of an aziridinium imide intermediate.[1a,d,e,g]

In the present communication we present results that show hydrogen tunneling in the ene reaction of PTAD with tetramethylethylene-$d_6$ (TME-$d_6$, **1**) and 2,2,7,7-tetramethyl-4-*cis*-

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