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Design and synthesis of DNA-tethered ruthenium complexes that self-assemble into linear arrays

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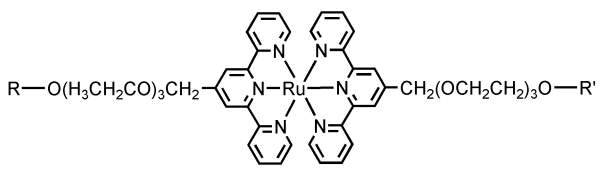
Ruthenium(II) bis(terpyridine) complexes have been prepared with two triethylene glycol linkers to which DNA sequences have been attached; hybridization at various complex ratios results in linear arrays of varying lengths.

Self-assembly by DNA hybridization has been used to create complex connectivities[1] notably those using immobile junctions[2,3] or dendrimers,[4] to aggregate gold nanoparticles,[5,6] and in limited cases to tether metal complexes.[7–11] One preliminary report describes the use of Fe(III) to organize DNA–ligand assemblies in solution.[10] A number of ruthenium complexes, including those composed of terpyridines,[12–14] have the potential to be used as nanoelectronic or optical devices and in some cases have been designed to self-assemble.[13] Nucleic acids have the potential to guide the self-assembly of a variety of hybrid materials, as well as to precisely space a series of such metal-based nanodevices at regular intervals in a linear array. We describe here the synthesis of two Ru(II) bis(terpyridine) complexes tethered to DNA sequences and their self-assembly into linear arrays.

A simple self-complementary DNA sequence should spontaneously assemble by hybridization to form long linear arrays, but it is difficult to control the length of such assemblies. An alternative approach uses two building blocks, each consisting of a metal complex tethered to two DNA sequences (Fig. 1). A pair of such conjugates can be prepared such that the DNA sequence of one (A-complex) is complementary to the second (B-complex). In the two extreme cases, in which the ratio of A : B or B : A is 2 : 1 or greater, a distribution of products result with the simple A_2B or B_2A trimer as a major component. As the ratio of complexes approaches 1 : 1 longer and longer arrays should result.

For the preparation of such linear arrays, we chose a bis(terpyridine) Ru(II) complex as the metal center, a complex that was likely to remain stable during the synthesis and deprotection of the desired DNA sequences. The synthesis of the Ru complex began with a terpyridine ligand tethering a tri(ethylene glycol) linker prepared essentially as described[15] for the corresponding compound tethering a di(ethylene glycol) linker. The linker was designed to provide some flexibility and distance between the metal complex and the DNA duplex formed upon hybridization. In order to best control the asymmetric nature of the final DMT-protected phosphoramidite derivative of the metal complex, it was simplest to initially prepare the monomer $Ru(tpy)Cl_3$ terpyridine ligand with attached linker and react this material with the terpyridine ligand tethering a DMT-protected linker. The product of this reaction contained both terpyridine ligands but only one of the linkers carried the DMT protecting group. The DMT-protected complex was converted to the corresponding phosphoramidite without difficulty and used directly for DNA conjugate synthesis.



A-complex R = R' = 5'-ACG CCG CTA TTA TCG CCG CA–

B-complex R = R' = 5'-TGC GGC GAT AAT AGC GGC GT–

Fig. 1 Sequence of two Ru(II) bis(terpyridine)–DNA complexes designed to form long linear arrays.

From this procedure two stable ruthenium complexes were prepared, each tethering two DNA sequences. When the sequences are complementary, self-assembly should result. DNA strand polarity will also affect the nature of the assembly process. In this case we chose to make symmetric conjugates, those in which both DNA strands are tethered by their 3′-termini to the ruthenium complex. To prepare the target conjugate it was necessary to initiate the DNA synthesis using a 5′-bound nucleoside and elongate the strand in the 5′→3′ direction using reverse nucleoside phosphoramidites (3′-DMT, 5′-phosphoramidite). After incorporation of the Ru(II) bis(terpyridine) complex, the final DNA strand was synthesized in the conventional manner. After conjugate assembly and deprotection, the products were isolated by HPLC. Radiolabeling of each conjugate followed by PAGE analysis resulted in a single band.

We prepared two conjugates each containing two identical 20-mer DNA strands tethered to a central ruthenium(II) bis(terpyridine) complex such that the sequence of each conjugate was complementary to the other (Fig. 1). In one case the sequences both terminated in dA (A-complex) while in the second the sequences terminated in dT (B-complex). The T_m value for the simple 20-mer duplex (25 mM PIPES, pH 7.0, 100 mM NaCl, 10 mM $MgCl_2$) was 75.4 °C. The A-complex plus two equivalents of the complementary 20-mer resulted in nearly the same T_m (74.9 °C) as did the B-complex plus two equivalents of its complementary 20-mer (T_m = 74.2 °C). Equivalent amounts of the A- and B-complex also resulted in essentially the same T_m(74.9 °C). These experiments suggest that the duplexes linking the ruthenium complexes are not destabilized by the presence of the metal center and are capable of forming linear arrays with thermal stabilities determined simply by the thermal stability of the linking DNA duplex.

Linear arrays of the ruthenium-DNA conjugates were prepared simply by mixing the A-complex and the B-complex under conditions that promote hybridization. Control of the length of the arrays can in principle be accomplished simply by controlling the ratio of the two complexes as noted above. With one complex in excess concentration over the other, no significant high molecular weight array results. When the two complexes are mixed at a 1 : 1 ratio, then linear arrays of essentially infinite length are possible. An alternative approach would employ the uncomplexed but complementary 20-mers as array terminators. Even small amounts of the complementary 20-mer should result in assembly termination at relatively short lengths of hybridized complex.

To probe the assembly of these linear arrays two experiments were performed. In the first varying ratios of the A-complex and B-complex were mixed, heated, cooled and analyzed by nondenaturing PAGE (Fig. 2). Lanes 2 and 8 contain the A- and B-complexes, with excess complementary 20-mer. The bands in

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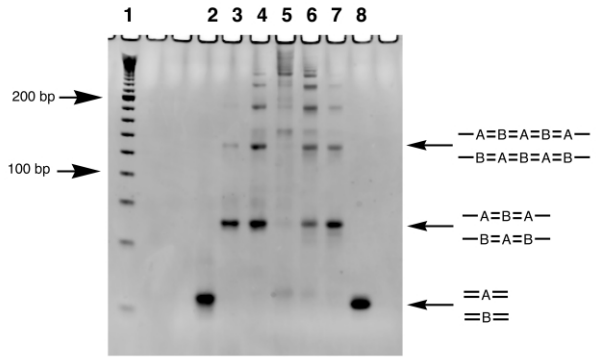

Fig. 2 Nondenaturing PAGE analysis of hybridization products of A- and B-complexes (Fig. 1). Lane 1: 20 bp ladder, Lane 2: A-complex + excess complementary 20-mer, Lanes 3–7: A-complex + B-complex in ratios of 4 : 1, 2 : 1, 1 : 1, 1 : 2, 1 : 4, Lane 8: B-complex + excess complementary 20-mer (visualized by EtBr).

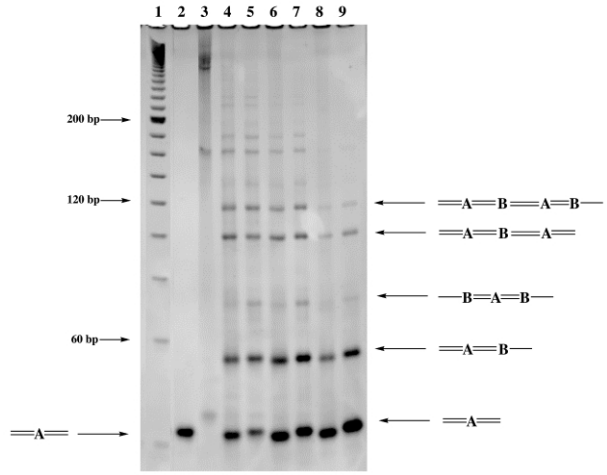

Fig. 3 Nondenaturing PAGE analysis of hybridization products of A- and B-complexes (Fig. 1). Lane 1: 20 bp ladder, Lane 2: A-complex + excess complementary 20-mer, Lane 3: 1 : 1 ratio of A- and B-complex, Lanes 4, 6, 8 contain the 1 : 1 complex plus increasing amounts of the simple 20-mer complementary to the A-complex, Lanes 5, 7, 9 contain the 1 : 1 complex plus increasing amounts of the simple 20-mer complementary to the B complex, only the former complexes are labelled (visualized by EtBr).

these lanes (having two 20 bp arms) have mobilities slightly reduced relative to the 40 bp standard. This difference can likely be explained on the basis of the Ru(II) cation and additional mass of the ligand and linkers. Lanes 3–7 contain varying ratios of the A- and B-complexes. There are virtually no single-stranded complexes in these lanes. There are also no dimers since the smallest hybridization product with one complex in excess over the other would the ABA or BAB trimer. In both cases a small migration anomaly (observed sequence length/ actual sequence length) relative to the 20 bp standard is observed. Additional bands are also present in all the lanes with excess A- or B-complex and these likely correspond to the products $A(BA)_nBA$ and $B(AB)_nAB$. At a complex ratio of 1 : 1 virtually all of the smaller hybridization products were absent and all of the material migrates as high molecular weight assemblies (Lane 5, Fig. 2).

In a second experiment, varying amounts of the complementary 20-mers were added to the 1 : 1 mixture of A- and B-complexes (Fig. 3). With increasing amounts of either of the complementary 20-mers the size of the linear hybridization products decreased. The number of hybridization products is also increased since after the hybridization to form the BA (AB) dimer, this dimer can then hybridize to the corresponding complementary 20- mer or complementary complex. One increases the size of the hybridization product by 20 residues, the other by 40 residues (compare =A=B– with –B=A=B– in Fig. 3. Since the complementary 20-mer is present in excess, the former tends to dominate the latter.

Using these procedures it will be possible to use DNA hybridization to place metal complexes at regular distances in linear arrays.

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