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Supramolecular Assembly-Enabled Homochiral Polymerization of Short $(dA)_n$ Oligonucleotides

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A goal of supramolecular chemistry is to create covalent polymers of precise composition and stereochemistry from complex mixtures by the reversible assembly of specific monomers prior to covalent bond formation. We illustrate the power of this approach with short oligomers of deoxyadenosine monophosphate ($(dA)_n3'p$), $n \geq 3$, which form supramolecular assemblies with cyanuric acid. The addition of a condensing agent to these assemblies results in their selective, non-enzymatic polymerization to form long polymers (e.g., $(dA)_{100}3'p$). Significantly, mixtures of D- and L-$(dA)_5 3'p$ form homochiral covalent polymers, which demonstrates self-sorting of racemic monomers and covalent bond formation exclusively in homochiral assemblies.

Supramolecular assemblies that mimic biology and act as templates for chemical reactions are of current interest.[1] Reactions in assembled systems offer several distinct advantages over those that occur in isotropic solution, including increased local concentrations of substrates,[2] and control over the regioselectivity of a reaction by pre-aligning substrates prior to covalent bond formation.[3] These features are especially valuable for the covalent polymerization of self-assembling monomers on a pre-formed template; a process Nature utilizes to replicate nucleic acids. Despite their potential utility, examples of precisely engineered programmable supramolecular assemblies that promote the formation of well-defined covalent polymers are surprisingly rare.[4]

We recently reported that deoxyadenosine monophosphate (dAMP) and cyanuric acid (CA) assemble into supramolecular polymers in aqueous solutions.[5] The Sleiman[6] and Krishnamurthy[7] laboratories have described the CA-assisted assembly of oligo(dA) into long one-dimensional nanofibers. Although some details of these assemblies remain to be determined, there is agreement that CA and dA are present within these assemblies in a 1:1 molar ratio, with each CA H-bonded to two adenine bases, and *vice versa*.[6-7] Here, we report that assemblies comprised of short dAMP oligomers with CA can be converted non-enzymatically into long linear covalent polymers upon the addition of 1-[(3-dimethylamino)propyl]-3-ethylcarbodiimide (EDC), a water-soluble condensing agent known to promote ligation (i.e., phosphodiester bond formation) between oligonucleotides assembled as duplexes.[8] Specifically, 3'-phosphorylated homo-dA oligonucleotides, such as $(dA)_5 3'p$, assembled in a buffer solution with excess CA are converted to long polymers (i.e., $(dA)_{>100}3'p$) by the addition of EDC (Figure 1a, 1b). Significantly, mixtures of D- and L-$(dA)_5 3'p$ oligomers self-sort into assemblies with CA, resulting in the formation of covalent homochiral polymers.

AFM analysis reveals the morphology of supramolecular assemblies formed from $(dA)_5 3'p$ oligomers and CA. High aspect-ratio fibers result from a mixture of CA (25 mM) and $(dA)_5 3'p$ (500 μM) that are micrometer in length with a height of ca. 2 nm (Figure 1c). The formation of supramolecular assemblies in solution was confirmed by temperature dependent circular dichroism (CD) spectral analysis (Figure 1d). When measured at 4 °C, the CD spectrum of the mixture has strong negative bands with maxima at 214 and 252 nm that disappear when the mixture is warmed to 50 °C. These observations are essentially identical to those previously reported for the assembly of $(dA)_{15}$ with CA,[6] and thus signify the formation of self-assembled structures from $(dA)_5 3'p$ and CA.

The ability of $(dA)_5 3'p$-CA supramolecular assemblies to enable oligonucleotide polymerization was tested by the addition of EDC (250 mM) to solutions of these assemblies at 4 °C. Products of this reaction were analysed by HPLC and by ^{32}P-post-labeling PAGE. The HPLC chromatogram of the reaction mixture from the $(dA)_5 3'p$-CA assembly after 24 h reveals successful polymerization (Figure 1f), $(dA)_5 3'p$ oligonucleotides are condensed into long linear $(dA)_n$ polymers, a result confirmed by PAGE analysis (Figure 1g). No polymerization is observed under identical conditions in the absence of CA. Instead, the only product observed is that from the cyclization of $(dA)_5 3'p$ (Figure 1e). Clearly, supramolecular assembly promotes polymerization by locally concentrating and orienting

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the $(dA)_5$3'p oligonucleotides in a favourable geometry for bond formation while simultaneously suppressing cyclization.

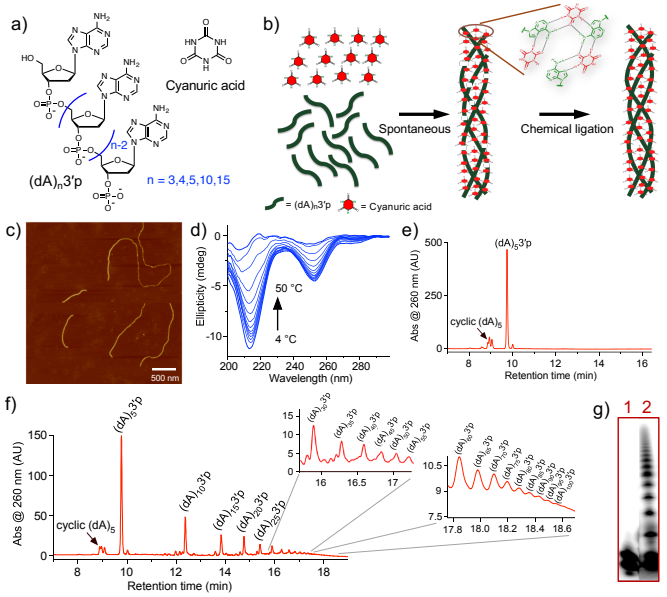

Figure 1. a) Chemical structure of $(dA)_n$3'p and cyanuric acid. b) Schematic representation of cyanuric acid mediated supramolecular polymerization of $(dA)_n$3'p oligomers. c) AFM images of $(dA)_5$3'p-CA assembly and d) Temperature dependent CD spectra (500 μM $(dA)_5$3'p, 25 mM CA, 100 mM HEPES, pH 6.8, 10 mM $MgCl_2$). e) Ion exchange chromatogram of products of $(dA)_5$3'p-CA reaction with EDC. f) Ion exchange chromatogram of products of $(dA)_5$3'p–CA assembly polymerization reaction (500 μM ($(dA)_5$3'p, 100 mM HEPES, pH 6.8, 10 mM $MgCl_2$, 250 mM EDC, 4 °C). g) ^{32}P–post-labelled PAGE data of $(dA)_5$3'p (Lane 1) and $(dA)_5$3'p–CA assembly (Lane 2) polymerization reaction products.

The polymerization reaction of $(dA)_5$3'p-CA assemblies is relatively rapid and remarkably robust. Under the conditions described above, conversion greater than 50 % is observed after 3 h (Figure S1). Further, neither the assembly nor the polymerization reaction is especially sensitive to the specific buffer used or to divalent cation concentration (Figures S2 and S3). In fact, polymerization occurs with good yield in the absence of both buffer and Mg^{2+} ions in aqueous solution ($(dA)_5$3'p 500 μM, CA 25 mM, pH ~4.4) (Figure S4).

The effect of varying the CA concentration on supramolecular assembly formation in solution for samples containing a fixed $(dA)_5$3'p concentration of 500 μM was monitored by CD spectroscopy. There is no evidence of assembly for a sample with 2.5 mM CA (1:1 CA:dA), but stable assemblies are evident for 12.5 mM CA (5:1 CA:dA) and assembly efficiency increases when the CA concentration is increased to 25 mM CA (10:1 CA:dA) (Figure S5). This behaviour is consistent with the report of Sleiman and co-workers who showed that supramolecular assemblies formed by oligo(dA) and CA have a CA:dA stoichiometry of 1:1, but samples containing oligo(dA) concentrations in the micromolar range require a large excess of CA for full assembly.[6] For samples containing 500 μM $(dA)_5$3'p, we observe a dramatic change in the CD spectra upon increasing the CA concentration from 10 mM to 12.5 mM indicating that the minimum assembly concentration, MAC, of CA is ca. 12.5 mM for the $(dA)_5$3'p-CA supramolecular assembly (Figure S5).

Polymerization reactions with 500 μM $(dA)_5$3'p showed a similar dependence on relative CA concentration. No polymerization is observed for the 1:1 sample (2.5 mM CA), but long polymers are formed for the 5:1 sample (12.5 mM CA). The efficiency of polymerization improves for the 10:1 sample (25 mM CA), but no further increases in polymerization efficiency are observed for samples with greater CA concentrations for this sample series with 500 μM $(dA)_5$3'p (Figures S6 and S7). Similarly, we investigated the assembly and polymerization reaction for a constant 10:1 molar ratio of CA:dA while varying the $(dA)_5$3'p concentration from 100 to 500 μM. CD analyses of these samples revealed assembly for $(dA)_5$3'p concentrations of 200 μM or higher (Figure S8). Likewise, the addition of EDC resulted in polymerization when the $(dA)_5$3'p concentration is above 200 μM (Figures 2a and 2b). These observations of concentration-dependent polymerization and CD spectra confirm the conclusion that polymerization occurs only when $(dA)_5$3'p and CA are assembled.

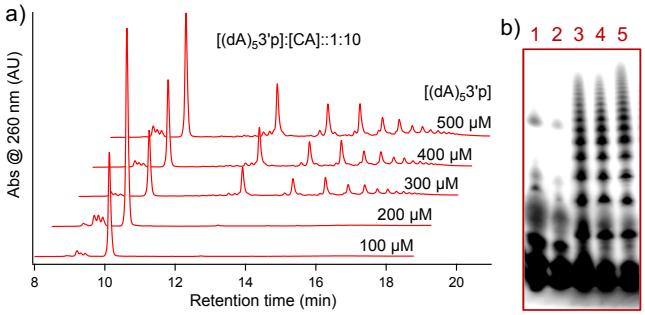

Figure 2. a) Ion exchange chromatograms of: $(dA)_5$3'p-CA polymerization reactions at various $(dA)_5$3'p concentrations (100 to 500 μM) all with 10 equivalents of CA, and after 24 h of reaction (10 mM $MgCl_2$, 100 mM HEPES, pH 6.8, 250 mM EDC, 4 °C). b) Image of gel from ^{32}P post-labeled PAGE analysis of the same reactions analyzed in (a). Lanes 1, 2, 3, 4, 5 correspond to products from reactions containing 100, 200, 300, 400, 500 μM $(dA)_5$3'p, respectively.

The polymerization of $(dA)_n$ oligomers is not restricted to the pentanucleotide $(dA)_5$3'p. The effect of initial oligonucleotide length on assembly and polymerization was explored using samples of $(dA)_n$3'p with n = 1 to 5, 10, and 15. Solutions of each oligomer, at a concentration required to form stable assemblies with CA (in a constant CA:dA ratio of 10:1) were analysed after 24 h of reaction (100 mM HEPES, pH 6.8, 250 mM EDC, 4 °C). The mononucleotide $(dA)_1$3'p and the dinucleotide $(dA)_2$3'p do not polymerize to a significant extent (Figures S9 and S10). For $(dA)_1$3'p, the pyrophosphate-liked dimer, 5'-dA3'pp3'dA-5', is the main product, which is consistent with results from numerous previous attempts to achieve nonenzymatic nucleotide condensation even in the presence of an oligonucleotide template with complementary nucleobases.[9] HPLC analysis (Figure S9) shows that $(dA)_n$3'p oligomers with $n \geq 3$ polymerize well. They exhibit varying degrees of polymerization with gradually decreasing yields as a function of length, as is typical of polymerization reactions.

The effect on polymerization of moving the phosphate group from the 3'- to the 5'-position was also examined. Samples containing 500 μM of 5'p$(dA)_5$ and 25 mM of CA were analysed by HPLC after 24 h of reaction with EDC (Figure S11).

Polymerization occurred with 5'p$(dA)_5$ but with lower efficiency than is observed for the 3'-phosphorylated oligomer, which is consistent with previous findings for EDC-driven reactions with duplex DNA.[8]

The assembly-mediated polymerization reaction of $(dA)_5$3'p is remarkably selective. We found that a single mispair caused by substituting a dG or dC for a dA in $(dA)_5$3'p disrupts assembly and completely inhibits polymerization (Figure S13). Finally, we examined the ability of an assembled 5'-phosphate RNA oligomer to polymerize. In solution, 5'p$(A)_5$ at 500 μM concentration assembled readily with 5 mM CA (relative to nucleobase) and the subsequent addition of EDC caused polymerization, but less efficiently than for 5'p$(dA)_5$ (Figure S12). Clearly, non-enzymatic assembly driven polymerization of $(A)_n$ oligomers is a general, selective, and robust process.

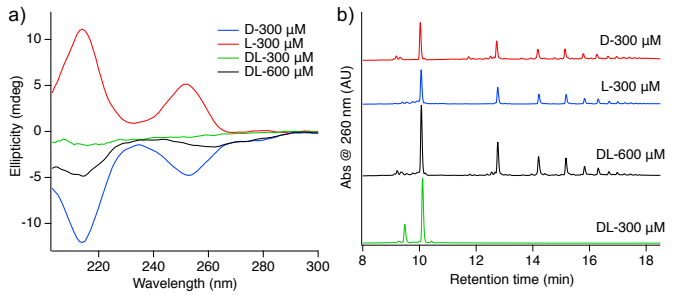

Figure 3. a) CD spectra of D-$(dA)_5$3'p-CA and L-$(dA)_5$3'p-CA assemblies. D-300 μM: 300 μM D-$(dA)_5$3'p-CA and 15 mM CA; L-300 μM: 300 μM L-$(dA)_5$3'p-CA and 15 mM CA; DL-300 μM: 150 μM D-$(dA)_5$3'p, 150 μM L-$(dA)_5$3'p and 15 mM CA; DL-600 μM: 300 μM D-$(dA)_5$3'p, 300 μM L-$(dA)_5$3'p and 30 mM CA. All samples 10:1 in CA:dA molar ratio, 100 mM HEPES, pH 6.8, 10 mM $MgCl_2$, 4 °C. The mixed CD spectrum is not identically zero possibly because the synthetically produced L-enantiomer is less pure than the natural D-enantiomer. b) Ion exchange chromatograms of polymerization reactions for samples defined in (a) after addition of 250 mM EDC and incubation for 24 h at 4 °C.

Nucleic acids are chiral, and we investigated the potential for $(dA)_5$3'p assembled with CA to undergo self-sorted chiral polymerization. Orgel demonstrated that incorporation of the "wrong" (opposite) enantiomer in a growing nucleic acid polymer chain efficiently terminates the growth process.[10] A clear advantage of supramolecular assembly prior to covalent polymerization is that the opposite enantiomer can be readily exchanged for the "right" one under thermodynamic control.

We previously reported that supramolecular polymers formed from chiral or achiral stacked hexad rosettes self-segregate into homochiral domains.[11] This behaviour shows that homochiral assemblies of a similar system are more thermodynamically stable than their heterochiral alternatives.[12] If $(dA)_5$3'p-CA assemblies also undergo a self-sorting process, a mixture of the enantiomeric L-$(dA)_5$3'p and D-$(dA)_5$3'p oligomers with CA would form homochiral assemblies and subsequent covalent bond-forming reactions would produce homochiral polymers. This possibility was tested by examining the CD spectra at 4 °C of D- and L- enantiomers of $(dA)_5$3'p separately and as mixtures (300 μM of each, total oligomer concentration of 600 μM) with excess CA (30 mM, 10 equivalents (Figure 3a). As expected, the assemblies formed from opposite $(dA)_5$3'p enantiomers with CA have oppositely signed spectra with characteristic Cotton bands at 213 and 252 nm. In contrast, the CD spectrum of the mixture shows only weak bands. Significantly, a solution of 150 μM of each enantiomer and 15 mM of CA, which is below the MAC (220 μM of $dA_5$3'p at 10 molar equivalents of CA), shows no meaningful CD spectrum even though the total oligomer concentration (300 μM) is above the MAC. This observation suggests that self-sorting of enantiomers occurs. HPLC analyses of the results of adding EDC to these samples are shown in Figure 3b. As expected, this reaction gives essentially identical polymerization results for the D- and L-enantiomers and, significantly, a similar result is observed for the reaction of the racemic mixture when the concentration of each enantiomer is above the MAC. However, when the concentration of the individual enantiomers is below the MAC, but the total concentration is above the MAC, no polymerization is observed, an observation which indicates that polymerization requires assembly with homochiral self-sorting.

Confirmation of homochiral polymerization of mixtures of D- and L-$(dA)_5$3'p-CA assemblies was obtained by carrying out experiments in the presence of oligomers lacking terminal phosphate groups. The absence of a terminal phosphate group will not affect assembly with CA. Of course, without a terminal phosphate group oligonucleotide polymerization is not possible. Thus, if assemblies were to form that contain both oligomer enantiomers, the inclusion of one enantiomer lacking a phosphate group will inhibit polymerization of both oligomers. We carried out polymerization reactions using equal concentration (300 μM) mixtures of L-$(dA)_5$3'p and D-$(dA)_5$, which lacks a terminal phosphate group. The results show that polymerization of L-$(dA)_5$3'p proceeds normally and is uninhibited by the presence of D-$(dA)_5$ (Figure 4a). In contrast, the reaction of assemblies formed from a homochiral mixture of D-$(dA)_5$3'p and D-$(dA)_5$ oligomers with EDC gives dimers $(dA)_{10}$ and trimers $(dA)_{15}$ lacking phosphate end groups, which inhibits further polymerization (Figure 4b). Finally, we observed that one or two L-sugars in an otherwise homochiral D-$(dA)_5$3'p oligomer is sufficient to stop polymerization (Figures 4c, d). Evidently, the inclusion of a single "mismatch" is sufficient to disrupt assembly and polymerization. These experiments confirm our hypothesis that assembly would efficiently separate oligonucleotides based on their chirality and thereby enable homochiral polymerization.

Identifying methods for the polymerization of nucleotides and short oligonucleotides to form longer nucleic acids is an ongoing challenge. The pioneering work of the Orgel,[10, 13] and Szostak[14] laboratories has revealed a number of the problems specifically associated with template mediated polymerization of activated nucleotides. In principle, the formation of long polymers requires the availability of long templates,[15] which presents a "chicken-and-egg" problem. Similarly, the non-templated synthesis is plagued by cyclization of activated oligonucleotides,[16] which constrains non-enzymatic reactions. Specific process have been reported that resolve some of these issues including the use of mineral surfaces,[17] lipid assemblies,[18] stacking interactions,[19] base-intercalating molecules,[16a] condensing buffers,[20] diamidophosphate[21] and liquid crystalline assemblies.[22] In the case of liquid crystals, Bellini and co-workers recently reported an assembly-promoted

non-enzymatic polymerization reaction for short DNA and RNA oligomers,[23] but very high oligonucleotide concentrations (>100 mg/mL) are required.

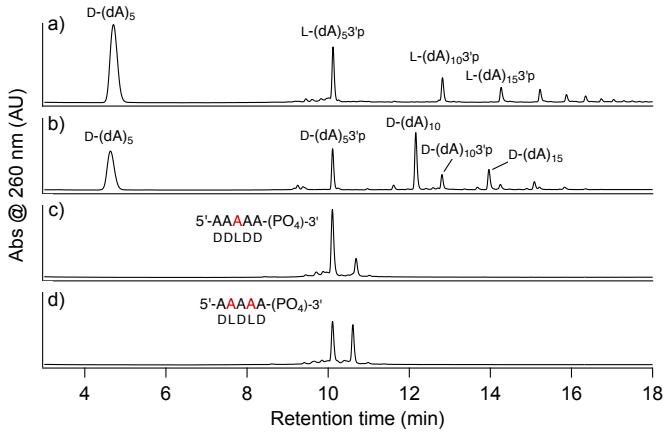

Figure 4. Ion exchange chromatograms of reactions products for various stereoisomer/phosphorylated forms of dA pentanuleotides with CA and 250 mM EDC after 24 h at 4 °C. a) 300 μM D-$(dA)_5$, 300 μM L-$(dA)_5$3'p. b) 300 μM D-$(dA)_5$, 300 μM D-$(dA)_5$3'p. (c) 300 μM D-$(dA)_2$-L-dA-D-$(dA)_2$. d) 300 μM D-dA-L-dA-D-dA-L-dA-D-dA. All reaction solutions contained 30 mM CA, 100 mM HEPES, pH 6.8, 10 mM $MgCl_2$.

Conclusions

The findings reported here provide proof-of-principle for a new approach to the synthesis of nucleic acid polymers. The efficient, selective non-enzymatic polymerization of low concentrations of short $(dA)_n$ oligomers in supramolecular assemblies with CA simultaneously overcomes the need for long template strands, avoids cyclization and provides for chiral self-sorting. This mechanism provides a facile error correction process that may be relevant for understanding the origins of nucleic acids. Of course, assemblies enabling the emergence and evolution of functional nucleic acids must have been composed of mixed nucleotides rather than simple homo-adenine sequences. The results presented here support the powerful idea that supramolecular assembly guided covalent polymerization is a process that can be used for the synthesis of complex functional materials.

Conflicts of interest

There are no conflicts to declare.

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