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Directed DNA Metallization

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DNA is an outstanding material for the preparation of nano- and microscale assemblies,[1−3] which are believed to have potential for the construction of nanoelectronic devices. DNA metallization procedures were developed in order to increase the conductivity of subsequent DNA nanostructures, thereby enabling their use as molecular wires. The metallization process, which involves the chemical reduction of DNA-complexed metal salts (e.g., Ag,[2a−d] Pd,[4a] Pt,[4b] and Cu[4c]), results in uniformly metallized DNA architectures. For the construction of DNA-based electronic devices, however, a more selective protocol that allows sequence-selective metallization of DNA is required.[2f] In this communication, we report a simple method to direct the metallization process to specific DNA strands or stretches of DNA. Current protocols for nonspecific silver deposition of DNA strands involve either photoreduction (254 nm) of Ag(I) ions complexed to DNA[5] or chemical reduction of Ag(I) ions by glutaraldehyde-modified DNA.[2] Both procedures provide uniformly metallized DNA. We reasoned that it may be possible to program the metallization process to occur only on selected DNA strands if one could specifically label DNA with aldehyde groups.

Since the construction of nanodevices requires the preparation of long DNA double-stranded scaffolds from several to hundreds of nanometers in length,[1] we surmised that selective aldehyde labeling could be achieved via an enzymatic method. Subsequently, we devised the two-step protocol depicted in Figure 1. In the first step, DNA polymerases are used to introduce acetylene reporter groups into selected genes via the enzymatic incorporation of 5-position-modified pyrimidine nucleoside triphosphates, such as **1** and **2** (Figure 2).[6] The second step involved reaction of the acetylene reporter groups with aldehyde azides using the Cu(I)-catalyzed Huisgen 1,3-cycloaddition "click reaction".[7] As a consequence of this derivatization process, the selected genes are now adorned with aldehyde functions. Although the direct incorporation of an aldehyde-modified triphosphate analogue provides a more expeditious route toward aldehyde-modified DNA, their enzymatic incorporation provided mixed results.

To evaluate how efficiently both compounds were accepted by various polymerases, we used the *polη* and *polH* genes from yeast and human cDNA as suitable template DNA strands.[8] These genes were initially isolated, then cloned into a plasmid, and subsequently used for the PCR.[9] Amplification of both the *polη* and *polH* genes using standard PCR conditions with a mixture of triphosphates (dATP, dCTP, dGTP, and **1** or **2**) afforded, for both triphosphates (**1** and **2**), full-length amplicons (∼2142 bp for human *polη* and 318 bp for yeast *polη*) (Figure 3a).[9] In experiments where triphosphate **1** replaced dTTP, full-length amplicons were readily obtained using a variety of commercially available high-fidelity polymerases. For compound **2**, however, full-length amplicons of suitable quantity were only obtained when the *Pwo* polymerase was

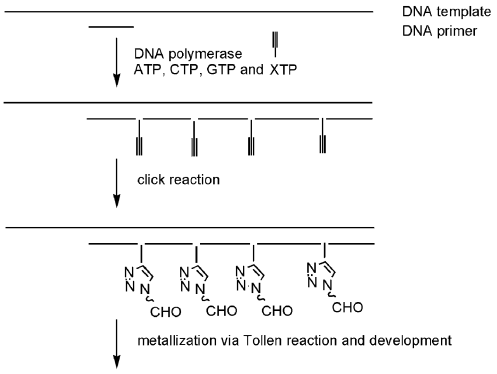

Figure 1. Schematic depiction of the selective metallization process.

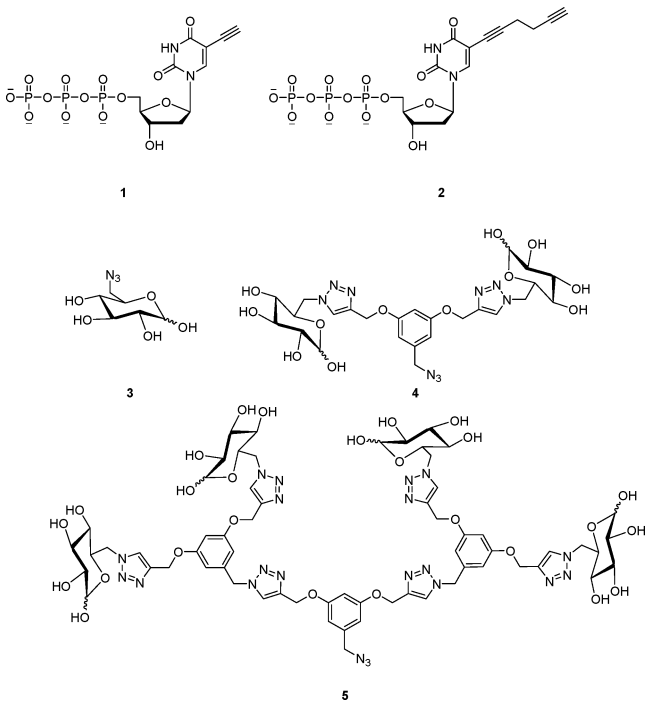

Figure 2. Depiction of molecules **1**−**5** used for this study.

used. In both examples, the class B *Pwo* polymerase provided the highest yields of PCR products. Enzymatic digestion of the acetylene-decorated *polη* gene amplicon incorporating **2** and subsequent HPLC and mass spectrometric analysis confirmed the complete replacement of thymidine by the nucleoside **2**.[9]

In addition, the acetylene-modified DNA strands can also be used as template strands. PCR amplicons generated with **1** or **2** could be used as templates for the PCR using either the four natural triphosphates or a triphosphate mixture containing, again, **1**/**2** instead of dTTP. Sequencing of the PCR products obtained with the natural

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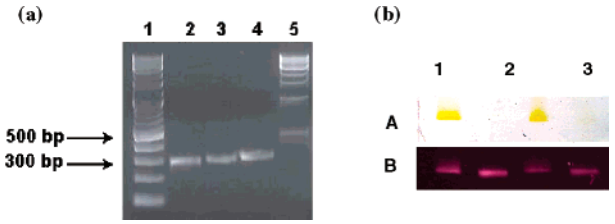

Figure 3. PCR assays (*Pwo* pol.) of the *polη* gene from yeast (318 bp). (a) Lanes 1 and 5: DNA ladder (NEB 2-log; 0.1−10.0 kb). Lane 2: Positive control (using dTTP). Lane 3: PCR using **1**. Lane 4: PCR using **2**. (b) PCR assay incorporating triphosphate **2** [lane 1, 7.0 ng; lane 3, 3.5 ng DNA loadings] and dTTP [lane 2, 7.0 ng; lane 4, 3.5 ng DNA loadings]. Gel A corresponds to treatment with a Tollens solution followed by development, whereas gel B corresponds to treatment with the fluorescent stain SYBR Green II.

set of triphosphates provided the correct base sequence, reflecting the high fidelity of the polymerases when incorporating **1** or **2**.[9] We then investigated the compatibility and efficiency of the click reaction between acetylene-modified DNA prepared using **1** and **2** and the galactose azide **3**, containing the aldehyde as a protected hemiacetal. Clicking onto synthetic oligodeoxyribonucleotides (ODNs) comprising one or several consecutive alkyne-modified nucleosides **1** revealed incomplete conversion by MALDI-TOF.[9] This is most likely attributed to the steric shielding of the acetylene by the DNA backbone. In contrast, the yield of click product of ODN's comprising the more flexible alkyne with azide **3** was always quantitative even in ODN examples incorporating the nucleoside **2** in six consecutive positions.

We then investigated whether the Ag deposition process can be directed to just aldehyde-modified DNA. To this end, nonacetylene-modified DNA (318 nucleobases) and its DNA cognate prepared with either **1** or **2** were loaded onto a 5% TBE−urea polyacrylamide gel (Figure 3b). The click reaction was then performed directly on the gel by agitation of the DNA-containing gel in a 1:1 MeOH:H_2O solution comprising **3**, $CuSO_4$, and a reducing agent (e.g., TCEP or sodium ascorbate). Washing the gel with an aqueous $Ag(NH_3)_2OH$ solution (Tollens reagent) and subsequent development of the Ag(0) nuclei with a typical developer solution (citric acid and formaldehyde) furnished, only in the case of the aldehyde-modified DNA strands, yellow/brown spots on the gel, indicative of Ag deposition (Figure 3b, Gel A). The whole process of the Tollens reaction and development is performed in just 30 and 3 min, respectively. To prove that the unmodified DNA is indeed present on the gel, we repeated the experiment but now treated the gel with SYBR Green II (Figure 3b, Gel B). This time, both aldehyde-modified and natural DNA bands were observed. The click reaction followed by Ag deposition is highly efficient because the galactose-modified 318-mer DNA was detectable by eye down to 1.3 ng.[9] The click reaction using **1**-modified DNA is less efficient, in agreement with the data from the small oligonucleotides, as indicated by the lower detection sensitivity of only 3.5 ng. When the aldehyde density on the DNA was significantly increased, by the use of azide containing dendrimers **4** and **5**, the detection limit of **2**-modified DNA was reduced to 0.9 and 0.5 ng, respectively, indicative of an increase in the amount of Ag(0) deposition around the modified DNA

We finally investigated the structure of the metallized DNA using AFM.[9] Nonmodified DNA gave under our conditions no metal deposition, in line with the gel electrophoresis results. Sugar-modified DNA, however, exhibited Ag(0) deposition after limited exposure to the Tollens reagent and a subsequent development process, therefore proving that Ag(0) deposition is indeed localized along the sugar (aldehyde)-modified DNA. Further confirmation of the Ag(0)-templating properties of sugar-modified DNA was demonstrated by an increase in DNA diameter as a function of the development time.

In conclusion, we have developed an efficient and selective method for the deposition of Ag(0) around aldehyde-modified DNA. The modification involves incorporation of acetylene-containing nucleotide triphosphates using DNA polymerases followed by a click reaction that can be efficiently performed directly on a polyacrylamide gel. Using this method, Ag(0) deposition can be confined only to the modified DNA. The ability to insert the acetylene labels enzymatically offers the possibility to exploit the arsenal of molecular biological tools in order to construct conductive DNA nanodevices. Experiments to ascertain whether these metallized DNA constructs conduct electricity are currently underway.

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Supporting Information Available: Information concerning the preparation of **1** and **2**, the PCR assays, the enzymatic digest of modified PCR fragments, "on the gel" and ODN clicking efficiency and Ag deposition procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) For further details, please consult the Supporting Information.

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