Oligonucleotide Synthesis Using Solution Photogenerated Acids

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Received July 31, 1998

A greater challenge than gene sequencing is to uncover sequence information, to relate these messages to the mechanisms of the various biological processes, and to develop means for monitoring and controlling such processes. To achieve these goals, an efficient approach is to assemble oligonucleotide arrays (ONAs) containing a variety of addressable sequences for high throughput applications in genetic, biomedical, and biochemical areas.[1] An increasing number of methods for the preparation of ONAs have been reported.[2] A critical issue for achieving automated massive ONA synthesis is to be able to control each individual reaction at a specific address on a supporting surface containing multiple reactive sites. To date, light-directed synthesis using photomask-guided photolithography has been used to produce DNA ONAs on a planar solid surface.[2f,g,3] In a typical process, each step of the synthesis, or the addition of a monomer containing a 5′-O-photolabile protection group, is preceded by the cleavage of a photolabile protection group on the 5′-O position of the growing chain. Thus, whether a reaction occurs at a specific site is modulated by light irradiation and photomask patterning, a complicated and costly process not accessible to a vast majority of research and applied laboratories. We report herein a new solution photochemistry based on the concept of using in situ photogenerated reagents to affect otherwise conventional reactions. Central to this approach is the use of light to convert an inactive compound to an active reagent that is necessary for the subsequent reaction. This concept has been the foundation of the imaging industry and modern microelectronic processes for the fabrication of solid state computer chips and devices.[4] We now demonstrate that similar light-controlled reactions can be applied to those occurring in solution, such as oligonucleotide syntheses.

The key component to our new approach is the use of an acid precursor that can be converted in situ into an acid by a solution photolytic process (Scheme 1, A and B). This photogenerated acid (PGA) cleaves the acid-labile 4,4′-dimethoxytrityl (DMT) group on the 5′-O position of growing oligonucleotide chains to give free 5′-OH groups and thus, permits synthesis of oligonucleotides using conventional chemistry, such as the phosphoramidite chemistry using 5′-O-DMT protected nucleophosphoramidites.[5]

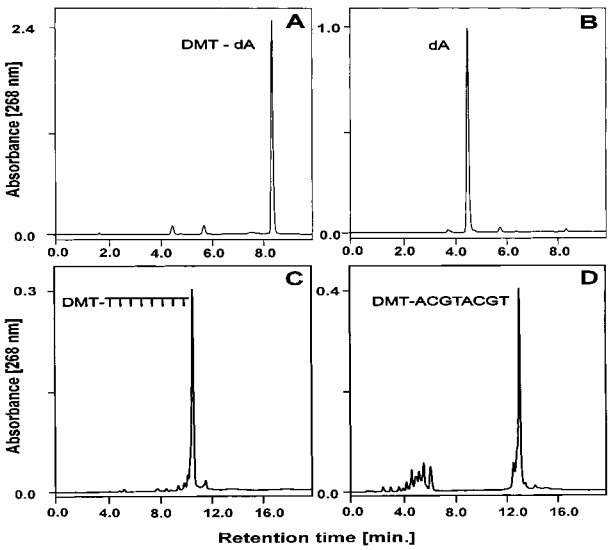

Figure 1. HPLC profiles of the photocontrolled reactions using PGA as the detritylation reagent. (A) 5′-O-DMT-dA on CPG in the presence of UVI-6974 without light irradiation. (B) The same reaction as in A but with light irradiation (yield 98.7%). The relative peak intensities between DMT-dA and dA were due to the difference in injection sample sizes. (C) DMT-TTTTTTTT (97% stepwise yield) and (D) DMT-d(ACGTACGT) (92% stepwise yield) synthesized using PGA as the detritylation reagent. HPLC conditions: C_8 column (Rainin, 4.6 × 250 mm, 5 μm), or C_{18} column (Waters, 10 × 100 mm, 10 μm); solvent 1 was CH_3CN and solvent 2 was 0.1 M triethylammonium acetate (TEAA); pH ≈ 6.5. Experimental details are provided in Supporting Information.

We have first investigated whether the PGA compounds are suitable for solution reactions of nucleosides (experimental details are provided in Supporting Information). In a typical experiment, 5′-O-DMT protected nucleosides attached to CPG (controlled porous glass, CPG Inc.) were treated with a CH_2Cl_2 solution containing mixed triarylsulfonium hexafluoroantimonate[6,7] (ssb). The solution in a Pyrex glass test tube was irradiated with light at 365 nm for 0.5−3 min in a dark room. The colorless solution turned into orange, indicating cleavage of 5′-O-DMT and the formation of DMT^+. The CPG was then washed with CH_2Cl_2 and CH_3CN. The nucleosides were deprotected and cleaved from CPG after treatment with concentrated NH_4OH. The results of these reactions were analyzed using HPLC on a C_{18} reverse phase column by both separate and co-injections. The UV profiles of the interested peaks were compared with those obtained from authentic samples (Supporting Information). The HPLC profiles for DMT-dA and PGA deprotection of dA (98.7% yield) are displayed in Figure 1, A and B. Similar results were obtained for the PGA deprotection reactions of 5′-O-protected dC, dG, T, and U.[8] These experiments demonstrate that (a) a PGA in solution efficiently cleaves 5′-O-DMT to give 5′-OH groups; (b) all

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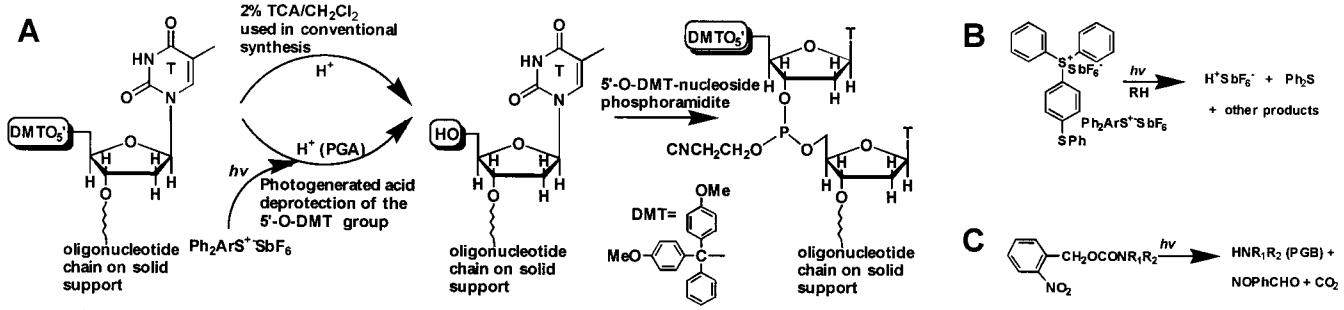
(7) Photochemical agents were from Secant Chemicals Inc. and Midori Kagaku Co. Triarylsulfonium hexafluoroantimonate belongs to a family of onium salts, which undergo photodecompositions, either directly or sensitized, to form free radical species and then mainly diarylsulfide and H^+. These photolytic intermediates and products have been extensively used in cationic- and radical-catalyzed polymerizations for high-resolution microimaging photolithograpy (Scheme S1, Supporting Information).

(8) The respective yields are (% of HPLC peak integration): dC 95.4, dG 99.0, T 99.8, U 99.0. Control reactions were run in parallel, which included the reactions using UVI-6974 without light irradiation or a conventional acid [2% trichloroacetic acid (TCA) in CH_2Cl_2]. The no-light reactions ensured no pre-detritylation occurred, and the TCA reactions generated reference compounds for comparison with the PGA detritylation products.

10.1021/ja9827191 CCC: $15.00
Published on Web 11/20/1998

Scheme 1[a]



[a] A. 5′-O-DMT deprotection using a photogenerated acid (PGA) or a conventional acid and the coupling of nucleoside monomers to the growing oligonucleotide chains. B. Reaction scheme of photolytic acid generation from triarylsulfonium hexafluoroantimonate. Other products include reduced aromatic compounds and thioether compounds. RH is a hydrogen donor.[4,7] C. Representative reaction of photolytic base generation from (2-nitrobenzyl)oxycarbonyldialkylamine. R_1 and R_2 are substituent groups, such as R_1, $R_2 = (CH_2)_5$.[11]

protected nucleosides examined (6-*N*-benzoyl-dA, 4-*N*-benzoyl-dC, 2-*N*-isobutyryl-dG, T, and RNA U) are stable under the optimized photolytic deprotection conditions; (c) the PGA chemistry is compatible with what are used for oligonucleotide synthesis. Additionally, our preliminary data indicate that other PGA compounds, such as triarylsulfonium hexafluorophosphate, 2,1,4-diazonaphthoquinone sulfonates, and perhalogenated triazine, are also potential candidates for PGA deprotection reactions (Scheme S1, Supporting Information).

A number of oligonucleotides have been synthesized (Figure 1, C and D, Table S2, Supporting Information) using a PGA, such as ssb, as the detritylation agent and standard phosphoramidite chemistry. Using standard CPG derivatized with nucleosides (~0.2 μmol) and 5′-O-DMT protected phosphoramidite monomers,[5] the syntheses were carried out on a DNA synthesizer (Perspective Expedite 8909) with minor modifications in the reagent delivery path and the reaction protocols to allow UV irradiation during the detritylation step. A solution of ssb in CH_2Cl_2 was placed in the supply bottle (now kept away from light) normally used for that of 2% TCA/CH_2Cl_2. A UV lamp was mounted next to the CPG column and turned on during detritylation (365 nm, 1−3 min). Excessive solvent wash was applied after the detritylation reaction. Other steps of the synthesis were identical to those used in routine oligonucleotide synthesis (Table S1, Supporting Information), and the stepwise yields were monitored by online DMT^+ color assay. Upon completion of the synthesis, oligonucleotides were cleaved from CPG using concentrated NH_4OH, and the results were examined using the C_{18} reverse phase HPLC. The representative HPLC profiles for the T_8 and d(ACGTACGT) octamers are displayed in Figure 1C and D.[9] In our initial attempts, we have found that a significant amount of truncated sequences coexisted with the full-length sequence (e.g., T_2, T_3, and T_n ($n < 8$) coexisted with T_8). This was likely due to temporarily 5′-OH blocking by an unknown reaction species, since PGA detritylation was complete (null DMT^+ level using TCA following PGA deprotection). This problem was overcome by extended solvent wash. Presently, efforts are being made to optimize the light-controlled synthesis by varying the designs of the instrument/reaction vessel and reaction conditions, such as PGA used, PGA concentrations and co-reagents (sensitizers and radical stabilizers), and light irradiation times. Side reactions are being analyzed.

The synthesis of oligonucleotides using solution PGA under light-controlled conditions serves as a novel example for a new type of reaction that will occur in solution upon the activation of photogenerated reagents, such as PGA and photogenerated base (PGB)[10] (Scheme 1C). A major advantage of these photoreactions is their otherwise use of conventional conditions and reactants, whereas the existing photocontrolled reactions require reactants protected with photolabile groups.[2f,g,3,11] Therefore, the applications of these photoreactions are limited by the availability of suitable compounds and their costs. Additionally, the reactions demonstrated herein appear to be more efficient (the activation of UVI-6974 in oligonucleotide synthesis used less than 0.13 J compared to at least ~1.65 J used for photocleavage of the 5′−O-photolabile groups[2g]) and are less likely to suffer from side reactions.[12] Furthermore, the advancement of the solution photocontrolled reactions should benefit from the extensive lists of chemicals already existing for microimaging processing and from the understanding of the mechanisms of the corresponding photolytic polymer reactions.

In the long term, the photocontrolled reactions described here should have immense potential in the development of not only ONAs, but also a broad range of micromolecular arrays (MM-chips) by solid surface parallel synthesis. The chemistry is to be used in combination with an optical control device,[13] which can project light onto a multiple of confined, selected sites to switch on photocontrolled reactions. Therefore, efficient stepwise syntheses of a plurality of molecules of diverse chemical structures can be achieved. Since only conventional chemistry is used, the applications can be easily extended to the photocontrolled syntheses of RNA, oligonucleotide analogues, peptides, oligosaccharides, or varieties of molecules. The availability of these chemistries should lay down the foundation for a flexible and economic MM-chip technology that will make the preparation of versatile MM-chips a routine procedure. This will in turn, greatly accelerate the discoveries in various scientific fields.

Acknowledgment. This work is in part, supported by the Robert A. Welch Foundation (E-1270). The authors thank Dr. Xiaochuan Zhou (Xeotron Co. and University of Michigan) and Dr. Charles J. Scanio (Secant Chemicals Inc.) for many valuable discussions, Yogesh Sanghvi (Isis Pharmaceuticals Inc.) for ESMS analysis, and Secant Chemicals Inc. (MA) and Midori Kagaku Co., Ltd. (Japan) for generous donations of the photochemical reagents.

Supporting Information Available: Experimental details (10 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

JA9827191

(9) The stepwise yields of the photocontrolled synthesis of DNA oligonucleotides containing 3−12 residues are in the range of 88−97% (calculated from HPLC peak integration, Table S2, Supporting Information). Polyacrylamide gel electrophoresis was used to examine the quality of CGAAAGC and T_8 sequences synthesized using PGA or TCA. These crude materials exhibited the expected mobility and high purity (Figure S3, Supporting Information). HPLC retention times of some PGA synthesized oligonucleotides, such as the four DNA trimers (dA_3, dC_3, dG_3, and T_3), were calibrated by those of the sequences made using standard conditions. Furthermore, selected sequences were detritylated, and the formation of the correct sequences was confirmed by HPLC comparison with those of oligonucleotides regularly synthesized. Mass analyses were also obtained for the four trimers, confirming the formation of the expected sequences (Supporting Information).

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