Rapid Cleavage of Oligodeoxyribonucleotides from *cis*-Diol-Bearing Universal Polymer Support

by **Pradeep Kumar** and **Kailash C. Gupta***

Nucleic Acids Research Laboratory, Institute of Genomics and Integrative Biology (formerly Centre for Biochemical Technology), Mall Road, Delhi University Campus, Delhi-110007, India (fax: +91 11 7667471; e-mail: kcgupta@cbt.res.in)

Two sets of conditions have been proposed for the cleavage of oligodeoxyribonucleotides from *cis*-diol-bearing polymer supports and their complete deprotection. The first condition involves the use of spermine in the presence of Zn^{2+} ions in aqueous NH_3 at 60° for 1 h, while the second one employs the use of 0.5M LiOH in conjunction with 3.5M Et_3N/MeOH at 75° for 40–60 min, or under microwaves for 5–8 min, depending on the protection employed for nucleic bases.

Introduction. – Oligonucleotides and their modified analogues are of special interest in molecular biology and medicinal chemistry because of their use as diagnostic agents, probes and primers for gene sequencing, and their potential application as therapeutics [1]. The last decade has seen tremendous improvements in the synthesis of these molecules [2–6]. However, there still remain some problems to be addressed by the nucleic-acid chemists. One of the problems arisen because of the use of various types of phosphoramidite synthons carrying base-labile and conventional protecting groups for nucleic bases and 2′-OH function in ribonucleosides, which necessitates a large number of pre-derivatized polymer supports. Some attempts have been made to address this problem. *Gough et al.* [7] were the first to propose a universal support based on uridine nucleoside. In a subsequent publication [8], they reported a modified approach for the preparation of a universal support by the use of an adapter, 2′(3′)-O-benzoyluridine-5′-O-cyanoethyl-*N,N*-diisopropylphosphoramidite coupled to a standard T-support. The longer time required to cleave oligomers from these supports makes them non-compatible to modern oligonucleotide synthesis. Moreover, these supports employ nucleosidic material that does not incorporate in oligonucleotide chains and hence goes waste. Non-nucleoside-based universal supports [9–11] have also been proposed, but the cleavage of oligomers from them is equally time-consuming, which restricts their use for DNA and RNA synthesis by using synthons carrying conventional and base-labile protecting groups for nucleic bases. Most of the universal supports reported to date are based on the *cis*-diol system and require considerably longer cleavage times for the release of oligonucleotides.

In a recent study, *Komiyama* and *Yoshinari* [12] reported the role of diamines and oligoamines, particularly ethylenediamine, for the efficient hydrolysis of RNA under physiological conditions. Encouraged by this study, *Azhayev* [13] incorporated an ethylenediamine residue in the ribose-like linker to enhance the elimination of the terminal phosphodiester along with the sugar originally linked to the universal support.

He proposed a set of deprotection conditions and claimed the compatibility of this universal support for the preparation of all common types of oligonucleotides including unusual base-labile nucleoside units.

More recently, *Earnshaw* and *Gait* [14] reported the cleavage of hairpin ribozyme by a polyamine, spermine, in the presence of a divalent metal ion, Mg^{2+}. They have also reported the cleavage of hammerhead ribozyme under the same conditions. We presume that the cleavage of oligonucleotides from *cis*-diol-bearing universal support very much resembles the cleavage of hairpin or hammerhead ribozymes.

We, therefore, propose to study the cleavage of oligonucleotides from 1,4-anhydroerythritol bearing universal support and using spermine in the presence of a divalent metal ion. However, spermine-assisted deprotection of oligonucleotides from the universal support in the presence of Mg^{2+} did not work at all. Therefore, we tried a number of metal ions, *viz.*, Ni^{2+}, Co^{2+}, Cu^{2+}, Zn^{2+} *etc.* in place of Mg^{2+}. Out of these, Zn^{2+} was found to be the best, which was used in all the studies.

In a very recent study, *Surzhikov et al.* [15] reported the use of a reagent consisting of 0.5M aq. LiOH (30 µl) and 3.5M Et_3N (in MeOH; 300 µl) for the deprotection of oligonucleotides at 75° for 60 min. The reagent seems to be quite attractive, since it accelerates the rate of deprotection of oligonucleotides. It was, therefore, thought to study the cleavage of oligodeoxyribonucleotides using this reagent at 75° as well as under microwaves, making the cleavage and deprotection process very fast.

Results and Discussion. – In the present communication, we have tried to develop a one-step cleavage method for oligodeoxyribonucleotides from *cis*-diol-based universal support and their complete deprotection. While designing the strategy, the following points were kept in mind , *viz.*, *i*) the deprotection should be rapid, *ii*) it should not involve multiple steps, and *iii*) the final product after deprotection should match the corresponding standard oligonucleotides deprotected under standard conditions. Taking these points into consideration, we describe here two sets of conditions for the rapid cleavage of oligonucleotides from *cis*-diol-group-bearing universal polymer support **1** and simultaneous removal of protecting groups from nucleic bases and internucleotide phosphates. The structure of the universal support is based on the 1,4-anhydroerythritol system, as depicted in *Fig. 1*. One of the OH groups of 1,4-anhydroerythritol was protected by a dimethoxytrityl (DMTr) group, and the other one was attached to polymer support *via* a succinate linkage. The universal polymer support was functionalized as reported earlier [10c] with DMTr loading of *ca.* 40 µmol/g of the support.

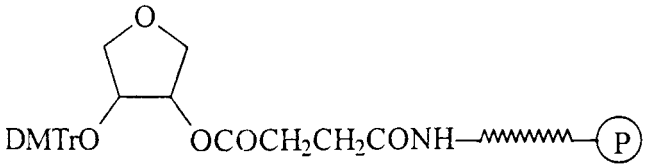

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Fig. 1. *Structure of the universal polymer support*

To test the deprotection conditions, a number of oligonucleotides, *viz.*, d(TTT), d(TTT TTT TTT T), d(CTC TCT CTC TCT CTC), and d(TAA AGG AAT CAA GGT CTG G), were assembled on the universal support by using conventional and labile nucleosidephosphoramidite synthons. The synthesis was carried out on *Pharmacia-LKB Gene Assembler Plus* at 0.2 -μmol scale according to the standard protocol of the manufacturer [16]. The coupling efficiency based on released DMTr cation was found to be >98% in all cases, including oligomers synthesized on standard supports. To avoid the modification of deoxycytidine during deprotection, the exocyclic amino group of dC was protected by an Ac group. Recently, it has been reported by *Polushin et al.* [17] that the use of amines for the deprotection of oligonucleotides modifies deoxycytidines carrying benzoyl protecting groups for their exocyclic amino functions. The same oligomers were also assembled on the standard polymer support for comparison. d(TTT) was selected as a model sequence to study the cleavage kinetics from universal support. Support-bound d(TTT) was divided into portions, taken up in different vials, and subjected to the proposed sets of deprotection conditions.

The first set of deprotection condition employs the use of spermine and $ZnCl_2$ in aq. NH_4OH. Following time and concentration kinetics, the oligonucleotides were cleaved from universal support in 1 h at 60° with spermine (1M) and $ZnCl_2$ (0.5M) in aq. NH_4OH (condition *A*). *Fig. 2* shows the elution profile of crude d(TTT) deprotected from universal support following the proposed condition *A* and co-injected with the standard d(TTT) deprotected under standard deprotection condition [5]. The inset of the figure shows the MALDI-TOF spectra of d(TTT). In case of mixed-base oligonucleotides synthesized with synthons carrying conventional protecting groups for nucleic bases, an

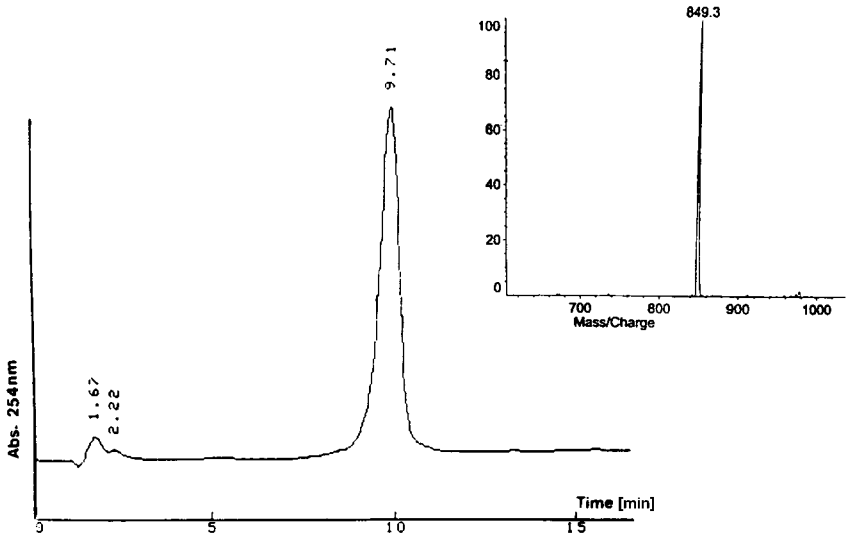

Fig. 2. *RP-HPLC Elution profile of crude d(TTT) deprotected from universal support following condition* A *and co-injected with standard d(TTT) deprotected under standard conditions.* HPLC Conditions: column, *Lichrosphere RP-18*; gradient, 0–25% *B* in 25 min; flow rate, 1 ml/min; solvent *A*, 0.1M $AcONH_4$, pH 7.1, solvent *B*, MeCN; AUFS, 0.05. *Inset:* MALDI-TOF-MS of d(TTT)

extended deprotection procedure was followed, *i.e.*, aq. NH_4OH containing spermine (1M) and $ZnCl_2$ (0.5M), 8 h, 60°.

Similarly, support-bound oligomer d(TTT) was exposed in a mixture of aq. LiOH and Et_3N, either at 75° for 60 min (condition *B1*), or under microwaves for 5–8 min (condition *B2*; 5 min were required in case of labile protecting groups, while 8 min were required for the removal of conventional protecting groups from nucleic bases, as well as simultaneous removal of internucleotide phosphate protecting groups). The fully deprotected oligonucleotides were obtained quantitatively from the support. All these oligomers were desalted, concentrated in the same manner as described in the *Exper. Part*, and analyzed by RP-HPLC. Further, the identity of the oligomers was established by co-injecting them with standard oligomers. The integrity of the cleaved oligonucleotides from universal support was finally characterized by MALDI-TOF-MS analysis.

Fig. 3 shows the HPLC elution profile of a co-injection of deprotected d(TTT) using condition *B2* (0.5M LiOH (30 µl) and 3.5M Et_3N (300 µl), and exposed to microwaves for 8 min) and the standard d(TTT). The support-bound oligomer sequence d(TAA AGG AAT CAA GGT CTG G) was cleaved under condition *A* (aq. NH_4OH containing spermine (1M) and $ZnCl_2$ (0.5M) at 60° for 1 h). The corresponding chromatograph is shown in *Fig. 4*. The inset of the figure shows the MALDI-MS of the same oligomer.

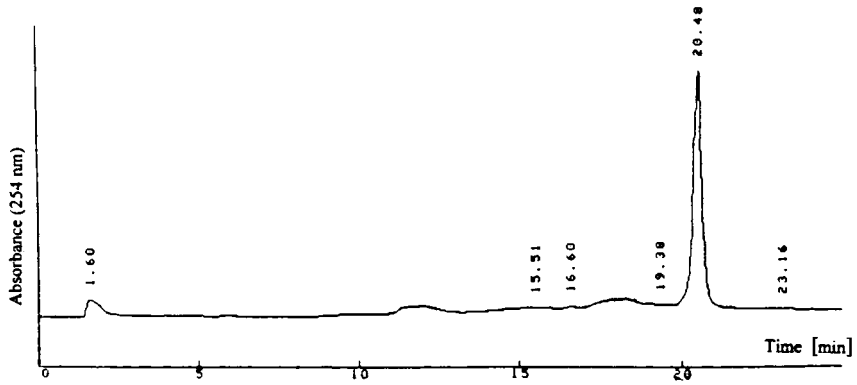

Fig. 3. *RP-HPLC Elution profile of crude d(TTT) deprotected from universal support using condition* B2 *and co-injected with standard d(TTT) deprotected under standard conditions.* HPLC conditions: column, *Lichrosphere RP-18*; gradient, 0–15% *B* in 25 min; flow rate, 1 ml/min; solvent *A*, 0.1M $AcONH_4$, pH 7.1, solvent *B*, MeCN; AUFS, 0.05.

The financial support from the *Department of Science and Technology*, New Delhi, under Indo-Israel project, is gratefully acknowledged.

Experimental Part

1. *General.* Long-chain alkylamine-controlled pore glass (LCAA-CPG), 4-(dimethylamino)pyridine, and spermine were purchased from *Sigma Chemical Co.* (St. Louis, MO). 1,4-Anhydroerythritol was obtained from *Aldrich Chemical Co.* (USA). All other chemicals, reagents, and solvents were purchased from local suppliers and purified prior to their use. TLC was performed on silica gel *60F-254* plates (*Merck*, India) and compounds

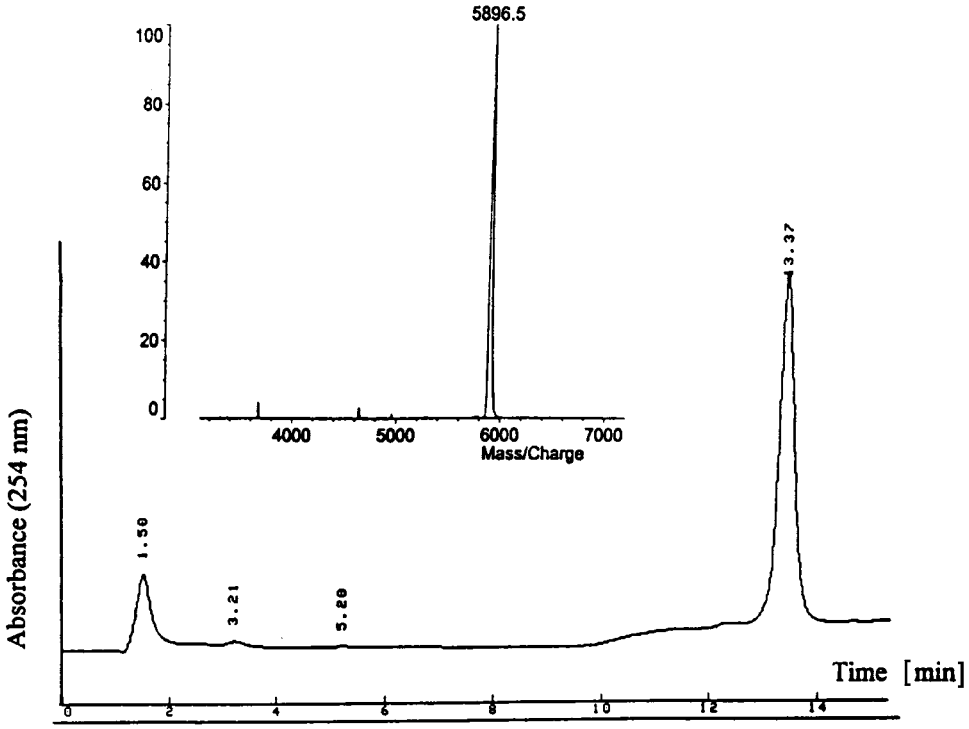

Fig. 4. *RP-HPLC Elution profile of mixture of crude d(TAA AGG AAT CAA GGT CTG G) deprotected under the proposed condition* A *as well as standard condition*. HPLC Conditions: column, *Lichrosphere RP-18*; gradient, 0–50% *B* in 25 min; flow rate, 1 ml/min; solvent *A*, 0.1M $AcONH_4$, pH 7.1, solvent *B*, MeCN, AUFS, 0.05. *Inset:* MALDI-TOF-MS of d(TAA AGG AAT CAA GGT CTG G).

were detected under short wavelength UV light. Oligonucleotide synthesis was carried out at 0.2 -μmol scale on a *Pharmacia LKB Gene Assembler Plus* according to the standard phosphoramidite chemistry. High-performance liquid chromatography (HPLC) was performed on *Shimadzu LC-4A* and *LC-10A* fitted with a variable detector (set at 254 nm). Anal. HPLC was carried out on RP (ODS) columns (*Lichrosphere RP-18*) supplied by *Merck*, Germany. Further characterization was carried out by use of MALDI-TOF-MS (*SEQ IV*, *Kratos*, UK). 3-Hydroxypicolinic acid (HPA) and 2,4,6-trihydroxyacetophenone (THAP) were used as a matrix for long and short oligonucleotides, respectively.

2. *Preparation of* cis-*Diol Group-Bearing Universal Polymer Support.* Universal polymer support **1** was prepared according to the procedure reported earlier [10c] from our laboratory. The loading on the functionalized polymer support was *ca.* 40 μmol/g of support.

3. *Oligonucleotide Synthesis.* A number of oligonucleotides, *viz.*, d(TTT), d(TTT TTT TTT T), d(CTC TCT CTC T), and d(TAA AGG AAT CAA GGT CTG G), were assembled on the universal polymer support **1**. The synthesis was carried out at 0.2 -μmol scale with both conventional (dA^{bz}, dC^{ac}, dG^{ibu}) and base-labile nucleoside-phosphoramidites (dA^{pac}, dC^{ac}, dG^{pac}) protections for nucleic bases. The corresponding oligonucleotides for comparison purposes were also synthesized on the standard polymer supports. The coupling efficiencies in both cases were found to be comparable.

4. *Deprotection and Purification of Oligonucleotides.* Cleavage of oligonucleotide chains from the support and removal of protecting groups from the exocyclic amino groups of the nucleic bases and internucleotidic phosphates were achieved following one of the conditions given below. *Condition* A: To the support-bound oligomer, in a deprotection vial, aq. NH_4OH containing spermine (1M) and $ZnCl_2$ (0.5M) were added. The suspension was kept at 60° for 1 h, followed by concentration in a speed vac and desalting on RP silica. The

oligomer was eluted with 30% MeCN in $AcONH_4$ buffer, pH 7.1. The solvent was removed, and the oligomer was analyzed on RP-HPLC.

Condition B1: The support-bound oligomer, in a deprotection vial, was treated with a mixture of 0.5M LiOH (30 µl) and 3.5M Et_3N (300 µl) at 75° for 60 min. The mixture was neutralized with AcOH followed by usual workup as described in condition *A*.

Condition B2: The support-bound oligonucleotide was suspended in a mixture of 0.5M LiOH (30 µl) and 3.5M Et_3N (300 µl) and exposed to microwaves for 8 min (80×6 s) in case of conventional protecting groups bearing oligonucleotides, and for 5 min (50×6 s) in case of labile protecting groups. After each exposure, the mixture was cooled in ice-cold H_2O. Standard oligonucleotides were deprotected according to a procedure described in [5], desalted, and concentrated in the same manner as discussed above.

The deprotected and desalted oligomers were analyzed by RP-HPLC. Further characterization was carried out by MALDI-TOF-MS analysis.

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Received May 13, 2002