Rapid conditions for the cleavage of oligodeoxyribonucleotides from cis-diol-bearing universal polymer supports and their deprotection

P. Kumar and K. C. Gupta*

Nucleic Acids Research Laboratory, Centre for Biochemical Technology, Mall Road, Delhi University Campus, Delhi-110 007, India

Received February 15, 1999; Revised and Accepted March 23, 1999

ABSTRACT

Two sets of deprotection conditions have been evolved for the deprotection of oligodeoxyribonucleotides and their cleavage from commercially available cis-diol group-bearing universal polymer supports. In the first case, oligodeoxyribonucleotides anchored on the universal support were subjected to one of the standard deprotection conditions followed by treatment with aqueous 0.5 M sodium chloride + 0.2 M sodium hydroxide solution for 30 min at room temperature. In the second case, oligonucleotides bound to the universal support were treated with methanolic sodium hydroxide solution under microwave radiation to obtain fully deprotected oligomers within 4 min. Under both conditions, the cleavage of oligonucleotides from the support and their deprotection occurred quantitatively without any side product formation. The cleaved oligonucleotides were found to be identical in all respects (retention time on HPLC and biological activity in PCR) to the corresponding standard oligonucleotides.

INTRODUCTION

Synthetic oligodeoxyribonucleotides and their modified analogs have become important tools in modern biological science, diagnostic and therapeutic applications. Chemical synthesis of these molecules has been tremendously simplified in the last 10–15 years. However, one would still require preparation of a large number of pre-derivatized polymer supports (1–9) because of the commercial availability of a variety of phosphoramidite synthons for their assembly. It was, therefore, considered necessary to develop a universal support, which could obviate the need to prepare such pre-derivatized supports. Gough et al. were the first to propose a universal support based on 2-(3′)-O-benzoyluridine-5′-O-succinate attached to controlled pore glass and demonstrated its application for the synthesis of oligonucleotides, using phospho and phosphite triester approaches (10,11). Cleavage and deprotection of the oligomers were effected in two steps, i.e. the usual aqueous ammonia treatment followed by exposure to Pb2+ ions. Recently, they have suggested an alternative route to obtain a universal support by using an adapter, 2′(3′)-O-benzoyluridine-5′-O-cyanoethyl-N,N-disisopropylphosphoramidite, prior to actual synthesis (12). However, the cleavage of oligodeoxyribonucleotides from this support takes 48 h at 65°C in 25 ml concentrated ammonium hydroxide, while in the case of oligoribonucleotides, the same can be achieved in 24 h at 50°C in 25 ml pyridine, concentrated ammonium hydroxide (1:4 v/v). Further attempts (13–16) have been made in the recent past, but none of them were found to be compatible with modern oligonucleotide synthesis.

In this communication, we report rapid and improved conditions for the cleavage of oligonucleotides from cis-diol group-bearing universal supports. We have proposed two sets of conditions, namely a first one involving the use of one of the standard deprotection conditions followed by treatment with aqueous 0.5 M sodium chloride + 0.2 M sodium hydroxide solution for 30 min at room temperature, and a second involving the use of 0.2 M sodium hydroxide (MeOH:water 1:1 v/v, A) for 4 min for oligonucleotides synthesized using base labile synthons or 1.0 M sodium hydroxide (MeOH:water 1:1 v/v, B) for 4 min for oligonucleotides having conventional protecting groups for nucleic bases except G, which carries a phenoxyacetyl protecting group, under microwave radiation. The oligomers cleaved under both conditions were fully characterized by HPLC and PCR and found to be identical to the corresponding standard oligomers.

MATERIALS AND METHODS

Long chain alkylamine–controlled pore glass (LCAA-CPG) (500 Å) and 4-dimethylaminopyridine were procured from Sigma Chemical Co. (St Louis, MO). Other solvents and reagents employed in the present study were purchased from local suppliers and purified prior to their use.

Thin layer chromatography (TLC) was performed on silica gel 60 F-254 plates (Merck, Darmstadt, Germany) and compounds were detected under short wavelength UV light. Proton NMR spectra were recorded on a Perkin Elmer R-32 spectrometer operating at 90 MHz. Chemical shifts are given on a p.p.m. scale and relative to the internal standard tetramethylsilane. The

*To whom correspondence should be addressed. Tel: +91 11 7257 439; Fax: +91 11 7257 471; Email: kcgupta15@hotmail.com
notations used are as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad; Ar, aromatic.

High performance liquid chromatography (HPLC) was carried out on a Shimadzu LC-4A fitted with a variable detector, SPD-2AS (set at 254 nm). Analytical HPLC was performed on reverse phase (C-18) columns supplied by Merck.

Oligonucleotide synthesis was carried out at 0.2 μmol scale following phosphoramidite chemistry on a Pharmacia LKB Gene Assembler Plus following the standard protocol (17).

Preparation of vicinal diol system containing universal support

A number of supports containing vicinal diol systems have been described. Some of them have employed uridine while the others are based on non-nucleosidic material. Universal supports I and II have been functionalized essentially by the reported procedures (10,11,13). Support III, containing 1,4-anhydroerythritol, was prepared by a slight modification of the earlier described procedures (15,18).

In a round-bottomed flask (50 ml), 1,4-anhydroerythritol (10 mmol) was dried by co-evaporation with pyridine (50 ml) and finally suspended in anhydrous pyridine (25 ml). 4,4'-Dimethoxytrityl chloride (5 mmol) and 4-dimethylaminopyridine (2 mmol) were added and the reaction mixture was left with stirring for 3 h. After monitoring the reaction by TLC, the reaction mixture was concentrated on a rotary evaporator under reduced pressure. The syrupy material obtained was taken up in ethyl acetate (50 ml) and washed successively with aqueous sodium bicarbonate (5%, 2 × 25 ml) and saturated sodium chloride solution (1 × 25 ml). The organic layer was collected, dried over anhydrous sodium sulfate and concentrated under reduced pressure to obtain the desired material in 76% yield. The material was subjected to analysis by reverse phase HPLC. Elution was effected with chloroform:methanol (9:4:0.6). The fractions containing pure material were pooled and concentrated under reduced pressure to obtain the desired material in 76% yield. 1H NMR (CDCl3) δ: 3.5–3.65 (m, 10H), 6.9–7.6 (m, 13H).

The mono-tritylated linker molecule was covalently attached to LCAA-CPG using a homobifunctional reagent, succinyl chloride. In a septum-sealed vial, succinyl chloride (6.3 μl, 0.05 mmol) was added dropwise to a stirred solution of triazol (19.8 mg, 0.05 mmol) dissolved in dry acetonitrile (1.0 ml) followed by the addition of 2-(4,4'-dimethoxytrityl)-1,4-anhydroerythritol as an oil which was further purified by silica gel column chromatography. Elution was effected with chloroform:methanol (9:4:0.6). The fractions containing pure material were pooled and concentrated under reduced pressure to obtain the desired material in 76% yield. 1H NMR (CDCl3) δ: 3.5–3.65 (m, 10H), 6.9–7.6 (m, 13H).

RESULTS AND DISCUSSION

We describe here two sets of conditions, namely (i) conventional and (ii) microwave-assisted, for the cleavage of oligodeoxynucleotides from cis-diol group-bearing universal supports and their complete deprotection. Three universal support systems bearing cis-diol groups, as shown in Figure 1, were employed in the present investigation. These conditions have been evolved keeping the following considerations in mind, namely the proposed conditions should be mild, capable of cleaving oligomers from the supports rapidly and without any side product formation and involve the use of commonly available reagents and equipment.

The integrity of the products produced under these conditions was verified by the HPLC and PCR techniques. First, d(TTT) was made on universal support III following the standard phosphoramidite method in an automated DNA synthesizer. The universal support-bound oligomer d(TTT) was divided into different vials and subjected to standard deprotection conditions (Table 1). One of the vials was subjected to depreservation under microwave radiation using reagent A or B. The fully deprotected oligonucleotides were
analyzed by reverse phase HPLC and characterized by co-injecting them with the standard d(TTT). In each case, except microwave-assisted deprotection, oligo d(TTT) eluted in two peaks, namely peaks I and II. The identity of the peaks was established by co-injecting them with the standard d(TTT). The oligomer eluted in peak II was found to be the desired product. The percentage of the desired oligomer (i.e. peak II) in each case is indicated in Table 1. It can be seen that no standard deprotection condition currently being used for oligonucleotide synthesis is sufficient for complete cleavage of oligonucleotide from the universal support and, therefore, each would require an additional deprotection step. Recently, it has been reported that the cleavage of oligomers from cis-diol-based universal supports can be achieved by the use of sodium (14) or lithium (15) ions. Sodium or lithium ion treatment, being mild, has been pursued in detail in this investigation. In order to see the effect of these ions, deprotected oligomers (Table 1, entries 1, 2 and 4) were subjected to varying concentrations (0.25–1.0 M) of sodium chloride at room temperature followed by HPLC analysis. No change in the ratio of the peaks was noticed, clearly indicating that sodium ions alone are not effective in liberating the 3'-hydroxyl function of the oligonucleotides. Then a study of the effect of sodium ions in the presence of the sodium hydroxide was considered. In order to determine the optimum concentrations of sodium chloride and sodium hydroxide, a deprotection kinetic study was carried out using varying concentrations of the same. The results are shown in Figure 2. It became clear that 0.5 M sodium chloride + 0.2 M sodium hydroxide was sufficient for complete cleavage of oligodeoxyribonucleotides with free 3'-hydroxyl function. No significant change in deprotection time was observed on replacing the sodium chloride + sodium hydroxide with a lithium chloride + lithium hydroxide combination. In the case of microwave-assisted deprotection, oligo d(TTT) eluted as a single peak, corresponding to standard d(TTT).

Table 1. Cleavage of oligomer from universal support and deprotection under different conditions

<table>
<thead>
<tr>
<th>Systems</th>
<th>Deprotection conditions</th>
<th>Products*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONVENTIONAL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. dCT dAT dGTT</td>
<td>Aq. NH4, 8h, 60°C</td>
<td>64% I + 36% II</td>
</tr>
<tr>
<td></td>
<td>+ 0.1N NaOH (0.5M NaCl)</td>
<td>100% II</td>
</tr>
<tr>
<td>2. dCT dAT dGTT</td>
<td>Aq. NH4, 24h, 60°C</td>
<td>33% I + 67% II</td>
</tr>
<tr>
<td></td>
<td>+ 0.2N NaOH (0.5M NaCl)</td>
<td>100% II</td>
</tr>
<tr>
<td>3. dCT dAT dGTT</td>
<td>Aq. NH4 + Methyleneimine (1:1)</td>
<td>94% I + 6% II</td>
</tr>
<tr>
<td></td>
<td>5 min, 65°C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+ 0.2N NaOH (0.5M NaCl)</td>
<td>100% II</td>
</tr>
<tr>
<td>4. Labile</td>
<td>dCT dAT dGTT</td>
<td>100% II</td>
</tr>
<tr>
<td></td>
<td>0.1M NaOH, 4min, MW</td>
<td></td>
</tr>
</tbody>
</table>

* I and II denote peaks. Peak I corresponds to oligomer attached to linker and peak II to desired material.
Nucleic Acids Research, 1999, Vol. 27, No. 10

e2

Figure 3. HPLC profiles of oligomer d(CGT CCG CAT AGT TAG TTC GAA GAA TGT AAC) (a) deprotected under two step conditions and (b) its co-injection with the corresponding standard oligomer. HPLC conditions: column, Lichrosphere RP-18; gradient, 0–50% B in 25 min; flow rate, 1 ml/min. Solvent A, 0.1 M ammonium acetate, pH 7.0; solvent B, 100% acetonitrile; Auf 0.08.

Figure 4. HPLC profiles of oligomer d(GAA TTC GAA TTC TCA GTT TCG TAC) cleaved and deprotected under microwave radiation, and its co-injection with the corresponding standard oligomer are shown in Figure 4a and b, respectively.

Figure 5. PCR-amplified product of the protective Antigen gene of anthrax toxin. Lane A, molecular weight standard λ HindIII ladder; lane B, 511 bp amplified PCR product using d(CTT GTG GCA GCT AGC CCG A TT GTA C) (deprotected under standard conditions); lane C, 511 bp amplified PCR product using d(CTT GTG GCA GCT AGC CCG A TT GTA C) (deprotected using aqueous ammonia for 1 h at 60°C followed by salt/alkali treatment for 30 min); lane E, 275 bp amplified PCR product using d(GTA CAA TCG GGC TAG CTG CCA CAA G) (deprotected using aqueous ammonia for 1 h at 60°C followed by salt/alkali treatment for 30 min).

We believe that the proposed conditions for the cleavage of oligomers from cis-diol group-based universal polymer supports and their complete deprotection would be quite useful for laboratories engaged in oligonucleotide synthesis.

ACKNOWLEDGEMENT

Financial support from the Departments of Biotechnology and Science and Technology, New Delhi, India is gratefully acknowledged.
REFERENCES