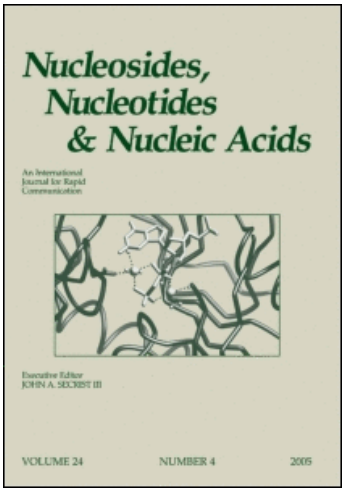

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Cleavage Of Oligodeoxyribonucleotides From Polymer Supports And Their Rapid Deprotection Under Microwaves

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CLEAVAGE OF OLIGODEOXYRIBONUCLEOTIDES FROM POLYMER SUPPORTS AND THEIR RAPID DEPROTECTION UNDER MICROWAVES

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ABSTRACT

Novel conditions for the cleavage of oligodeoxynucleotides from polymer supports and their complete deprotection under microwaves have been developed. The oligonucleotides synthesized using phosphoramidite synthons carrying base labile (Pac, Dmf and t-Bpac) and conventional (Bz for A and C and Pac for G) protecing groups for nucleic bases were deprotected using 0.2M sodium hydroxide (MeOH : H_2O :: 1:1, v/v) = Reagent A and 1M sodium hydroxide (MeOH : H_2O :: 1:1, v/v) = Reagent B, respectively under microwaves. The deprotected oligonucleotides were found to be comparable with the corresponding oligonucleotides deprotected under standard conditions (aq. ammonia at 55°C).

INTRODUCTION

Synthetic oligonucleotides are now valuable tools in modern biological studies. Chemical methods are now well established for their synthesis. However, the high demands of these molecules for various biomedical applications including their recent use as antisense oligonucleotides have provided impetus to DNA chemists to develop rapid and clean post synthesis work-up methods for these molecules.[1-3] Recently, some attempts have been made in this direction by employing base labile protecting groups (Pac, Dmf and t-Bpac)[4] or using special deprotection conditions, viz., aqueous ammonia and methyl amine[5] and gaseous amines.[6] In case of special deprotection conditions, the first one requires cytosine synthon protected exclusively with acetyl group, while gaseous amines treatment needs special deprotection device. We wish to describe here the use of methanolic sodium hydroxide as safe and rapid deptotecting agent under microwaves[7,8].

Though the use of methanolic sodium hydroxide has been described by Koster et al. as deprotecting reagent for N-acyl nucleosides, the $t_{1/2}$ (deprotection) reported for various N-acyl nucleosides were considerably too high to be adapted for DNA synthesis. We have reinvestigated the use of methanolic sodium hydroxide for the deprotection of oligodeoxynucleotides. A large number of oligodeoxynucleotides synthesized using phosphoramidite synthons carrying conventional (except G) and base labile protecting groups for nucleic bases were deprotected under the proposed conditions and compared with the corresponding standard oligonucleotides. These were found to be identical in all respect. No deamination of cytosine was observed at nucleoside and oligonucleotide level under the proposed conditions.

RESULTS AND DISCUSSION

Two reagents, viz., 0.2M sodium hydroxide solution (methanol : water :: 1 : 1, v/v), Reagent A and 1M sodium hydroxide solution (methanol : water :: 1 : 1, v/v), Reagent B, have been employed in the present investigation for the deprotection of oligonucleotides under microwave irradiation. The number of oligodeoxynucleotides were synthesized (Table-1) using phosphoramidite synthons carrying base labile and conventional protecting groups for nucleic bases. The oligonucleotides with base labile protecing groups were deprotected in 4 min with Reagent A and 2 min with Reagent B under microwaves. While in case of oligonucleotides with conventional protecting groups, it was not possible to deprotect them completely when isobutyryl (Ibu) group was employed at guanine. Oligonucleotides devoid of G could be deprotected with Reagent B in just 4 min under microwaves. It was, therefore, decided to synthesize oligonucleotides with conventional protecting groups except G where Pac was employed for the protection of guanine.

Since deamination is well known in cytidine in strong alkaline conditions, it was, therefore, considered necessary to find out if deamination occured during the course of deprotection under the proposed conditions. Deamination studies were performed at nucleoside and oligonucleotide level. N-Acyl cytidine was subjected to Reagent A and Reagent B treatment under microwaves followed by analysis on HPLC and UV-spectroscopy. There was no change in HPLC pattern and UV spectrum was noticed on

Table-1 : Deprotection of N-acyl 2'-deoxynucleosides and oligodeoxynucleotides using Reagent A and Reagent B under microwaves

N-acyl nucleosides /Oligonucleotide	Reagent A (time in sec)	Reagent B (time in sec)
N-Benzoyl-2'-deoxyadenosine	--	90
N-Benzoyl-2'-deoxycytidine	--	120
N-Phenoxyacetyl-2'-deoxyguanosine	72	30
N- Phenoxyacetyl -2'-deoxyadenosine	120	90
N-Isobutyryl-2'-deoxycytidine	108	72
d(TAG CTG ATA GTC TTT), I	240	--
d(TCT CTC TCT C), II	--	120
d(GAA AAG ACC ATA TGA GCA CAG AAA GC), III	--	240
d(CCC CCC CCC C), IV	--	240
d(TTT GAT GGT GTA TTC CCC CCA TC), V	240	--
d(GGA GCC TCA TTG TTA AAA ATA), VI	240	--

[#] *Synthesized using nucleosidephosphoramidites having conventional protecting group*

comparison with the standard cytidine. Likewise oligonucleotides deprotected with either Reagent A or Reagent B showed any deamination products.

Figure 1 shows the HPLC profile of I, the corresponding oligomer deprotected under standard conditions and a co-injection of I and its corresponding standard oligomer. The HPLC profiles of oligomer II, its corresponding standard oligomer and co-injection of II with the corresponding standard oligomer are shown in Fig. 2. Figure 3 shows the HPLC pattern of a mixture of nucleosides obtained after the treatment of III first with snake venom phosphodiesterase (SVPD) and then alkaline phosphatase (AP). Figure 4 shows the HPLC pattern of individual nucleosides obtained after SVPD and AP treatment of III on co-injection with the corresponding standard nucleosides.

EXPERIMENTAL

Oligodeoxyribonucleotides were assembled on a Pharmacia.LKB Gene Assembler Plus.[10] Two types of nucleosidephosphoramidite synthons were used, viz., labile and conventional protecting groups. Syntheses were carried out at 0.2 μmol scale. Desalting was performed on Sephadex G-25 column using 0.1M triethylammonium acetate, pH 7.5. Purification of desalted oligonucleotides was accomplished on reverse phase - high pressure liquid chromatography (RP-HPLC) using Lichrosphere RP-18 column (Merck).

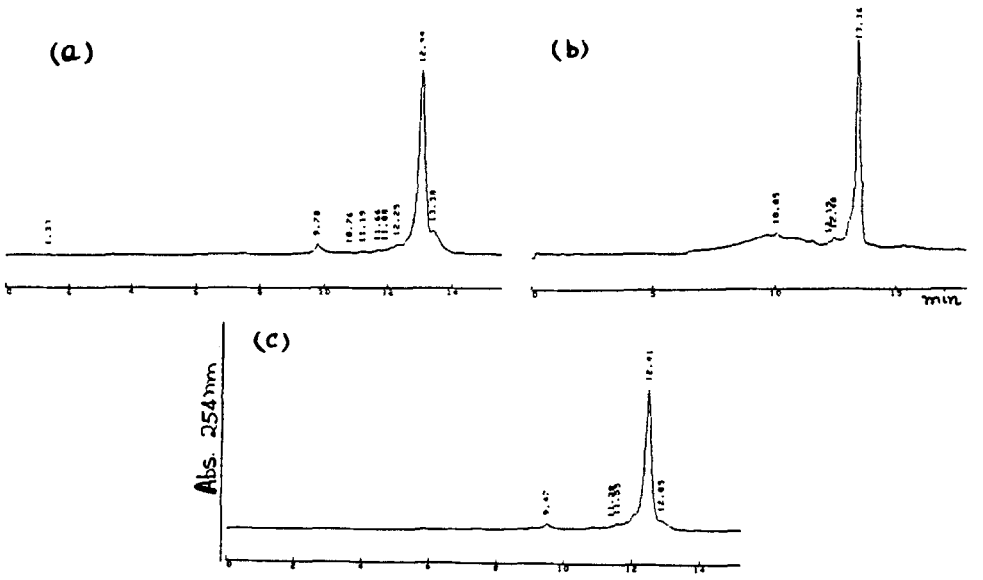

Figure 1. Reverse phase-HPLC profiles of d(TAG CTG ATA GTC TTT), I (a) deprotected with aq. ammonia, 16h, 55°C, (b) deprotected under MW using Reagent A and (c) Co-injection of oligomers obtained from (a) and (b). HPLC conditions : column, Lichrosphere RP 18; Buffer A, 0.1M ammonium acetate, pH 7.1; Solvent B, acetonitrile; Gradient, 0-20% B in 25 min. Auf. 0.08.

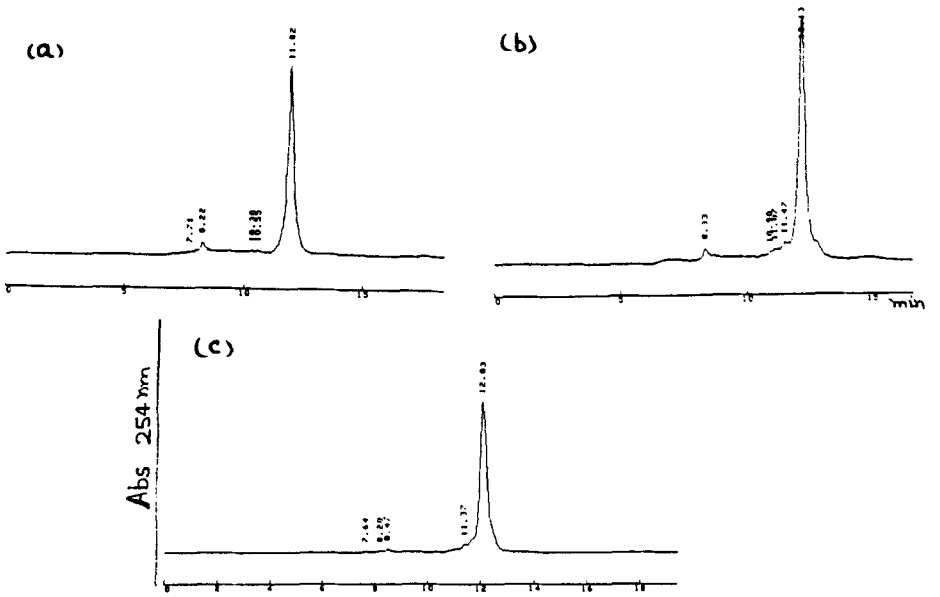

Figure 2. Reverse phase-HPLC profiles of d(TCT CTC TCT C) (II) (a) deprotected with aq. ammonia, 16h, 55°C, (b) deprotected under MW using Reagent B and (c) Co-injection of oligomers obtained from (a) and (b). HPLC conditions : column, Lichrosphere RP 18; Buffer A, 0.1M ammonium acetate, pH 7.1; Solvent B, acetonitrile; Gradient, 0-20% B in 25 min. Auf. 0.08.

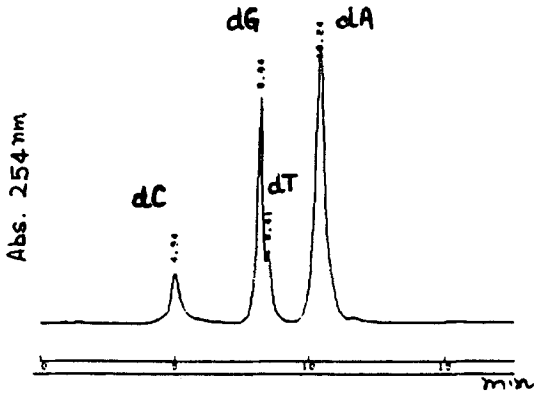

Figure 3. Reverse phase-HPLC profile of d(GAA AAG ACC ATA TGA GCA CAG AAA GC) III, digested first with snake venom phosphodiesterase followed by alkaline phosphatase. Column: Lichrosphere C18; Buffer A , 0.1M Ammonium acetate, pH 7.1; Buffer B, 100% Acetonitrile. Gradient: 0-20% B in 25 min. Auf: 0.08

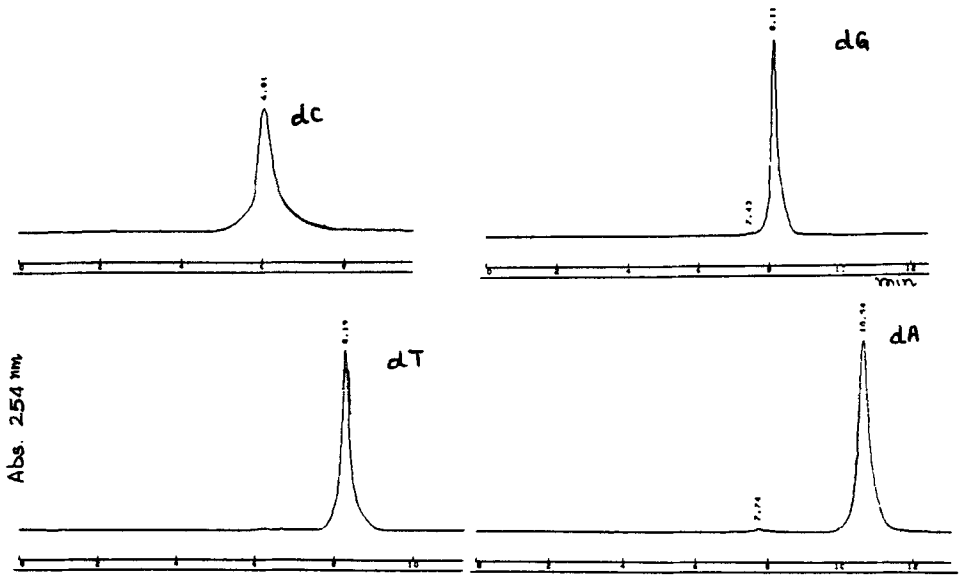

Figure 4. HPLC analysis of nucleosides dC, dG, dT and dA obtained after the snake venom phosphodiesterase and alkaline phosphatase treatment of d(GAA AAG ACC ATA TGA GCA CAG AAA GC) III co-injected with the corresponding standard dC, dG, dT and dA.

Cleavage from support and removal of protecting groups

A. Oligonucleotide chain, assembled using labile protecting groups carrying phosphoramidite synthons, bound to polymer support was suspended in 0.2M sodium hydroxide solution (methanol : water :: 1 : 1, v/v, 4 ml) and the vial was kept inside a domestic microwave oven operating at 2750Hz. The suspension was irradiated for 4 min. Each exposure was of the order of 6sec followed by cooling step (1s). After 4 min exposure, the vial was removed, cooled and the solution neutralized with acetic acid. The neutralized solution was transfered in another vial and concentrated in a speed vac. The solid residue was dissolved in distilled water (100 µl) and loaded on to a desalting column. The eluted solution was again concentrated in speed vac. The resulting residue was reconstituted in distilled water and analysed on RP-HPLC.

B. Oligonucleotide synthesized using conventional protecting groups carrying phosphoramidite synthons were deprotected under identical conditions as described above except that the 1.0M sodium hydroxide solution (methanol : water :: 1 : 1, v/v, 4 ml) was employed as deprotecting agent.

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