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SYNTHESIS OF OLIGORIBONUCLEOTIDES BY THE PHOSPHORAMIDITE APPROACH USING 5'-LEVULINYL AND 2'-TETRAHYDROFURANYL PROTECTION

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Abstract---Four decaribonucleotides (10mers) and one heneicosaribonucleotide (21mer) were synthesized by the phosphoramidite approach using the levulinyl group for protection of the 5'-hydroxyl group, which was compatible with the 2'-tetrahydrofuranyl group during chain elongation.

Recent developments in the chemical synthesis of oligonucleotides have made it possible to synthesize relatively long-chain RNA fragments. Syntheses of a 43mer and a 34mer have been achieved using the tert-butyldimethylsilyl[1] and o-nitrobenzyl[2] groups respectively as the 2'-protecting group. On the other hand, the synthesis of oligoribonucleotides using an acid-labile 2'-acetal or ketal protecting group has been difficult because of incompatibility with the 5'-dimethoxytrityl group during chain elongation on a polymer support. Rao et al.[3] have reported the synthesis of a 19mer by the phosphoramidite approach using the 1-[(2-chloro-4-methyl)phenyl]-4-methoxypiperidin-4-yl(Ctmp) group for protection of the 2'-hydroxyl group, and Tanimura et al.[4] have recently reported the synthesis of four 10mers and a 13mer using the 5'-9-phenylxanthen-9-yl (Pix) or (4-methoxy)phenylxanthen-9-yl (Mox) group in combination with the 2'-tetrahydropyranyl group. We have previously reported the synthesis of a 18mer in the 3'-direction using p-anisidate protection of the phosphate residue and 2'-tetrahydrofuranyl protection[5]. This method is, however, limited to the phosphotriester approach. In this paper we describe the synthesis of oligoribonucleotides by the phosphoramidite approach using the levulinyl and tetrahydrofuranyl groups for protection of the 5'- and 2'-hydroxyl groups respectively.

The starting materials, N-acyl-5'-O-levulinyl-2'-O-tetrahydrofuranylnucleosides, were prepared from N-acyl-2'-O-tetrahydrofuranylnucleosides with levulinic acid and 2-chloro-1-methylpyridinium iodide as described[6], although yields were low (30-60%) due to the occurrence of 3'-acylation. The 5'-O-levulinyl derivatives were phosphitylated with 2-cyanoethyl N,N-diisopropylchlorophosphoramidite[7] and purified by chromatography on silica gel. The ^{31}P-NMR spectral data ($CDCl_3$/trimethyl phosphate) were as follows: B=bzA, δ =148.62, 147.74 ppm; B=ibG, δ =148.62, 148.01 ppm; B=bzC, δ =148.15, 147.47 ppm; B=U δ =148.01, 147.74 ppm.

To test the stability of the protecting groups against hydrazine, 5'-O-dimethoxytritylthymidyl-3'-[O^P-(2-cyanoethyl)]-5'-(4-N,3'-O-dibenzoyldeoxycytidine), d(DMTr)T$_{p(CE)}$bzC(Bz), was treated with 0.5M

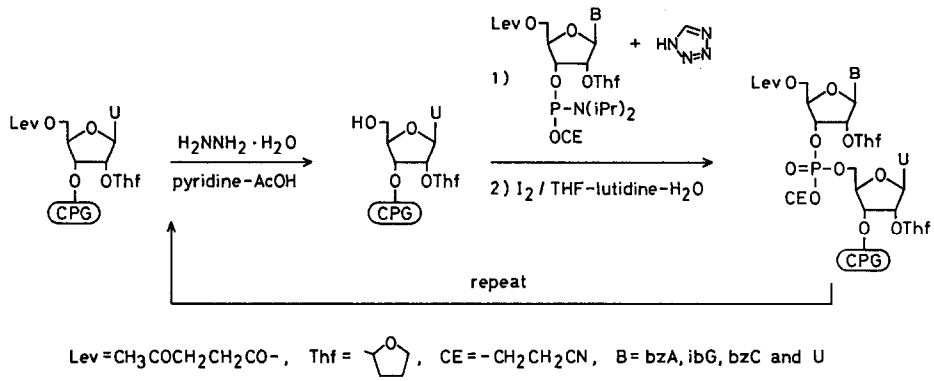

Fig. 1 Chain elongation on controlled pore glass.

hydrazine monohydrate in pyridine-acetic acid (3:2, v/v). It was found that the cyanoethyl group protecting the phosphate residue was stable, although removal of the benzoyl group protecting adenine and cytosine was detected after treatment for a few hours. As described below, this debenzoylation had no significant influence upon the synthesis of the 21mer.

Four 10mers, AAAAAAAAAU(A_9U), GGGGGGGGGU(G_9U), CCCCCCCCCU(C_9U) and UUUUUUUUUU(U_{10}), were synthesized with an Applied Biosystems 381A synthesizer using 1 μmol (33 mg) of uridine-CPG as illustrated in Fig. 1. The procedure for chain elongation was as follows : 1) deprotection of the 5'-levulinyl group with 0.5M hydrazine monohydrate in pyridine-acetic acid (3:2, v/v) for 10 min, 2) coupling with 70 mM nucleoside 3'-phosphoramidite and 0.25 M tetrazole in acetonitrile for 20 min, 3) capping with acetic anhydride and 4-dimethylaminopyridine for 0.5 min, 4) oxidation with iodine in tetrahydrofuran-2,6-lutidine-water for 1 min.

The 10mers were cleaved from the support with ammonia water and deprotected by heating at 55°C for 5 hr except that the solution of U_{10} was not heated. After evaporation of ammonia water, the tetrahydrofuranyl group was removed by treatment with 0.01 N HCl for 5 hr. The solution of each 10mer was neutralized with 0.1M NH_4OH, followed by HPLC analysis. The elution profiles are shown in Fig. 2, and each main peak was partitioned, ^{32}P-labeled and analyzed by gel electrophoresis (Fig. 4).

A 21mer (GCCUAGCUGAUGAAGGGUGAU), whose sequence was a part of satellite DNA transcripts of the newt[8,9], was synthesized by the same procedure except that the acid treatment was performed for 24 hr. The main peak (Fig. 3) was partitioned and further purified by polyacrylamide gel electrophoresis. One thirtieth of the crude product was purified, and 0.55 A_{260} unit was obtained. The overall yield was ca. 8%. The 5'-

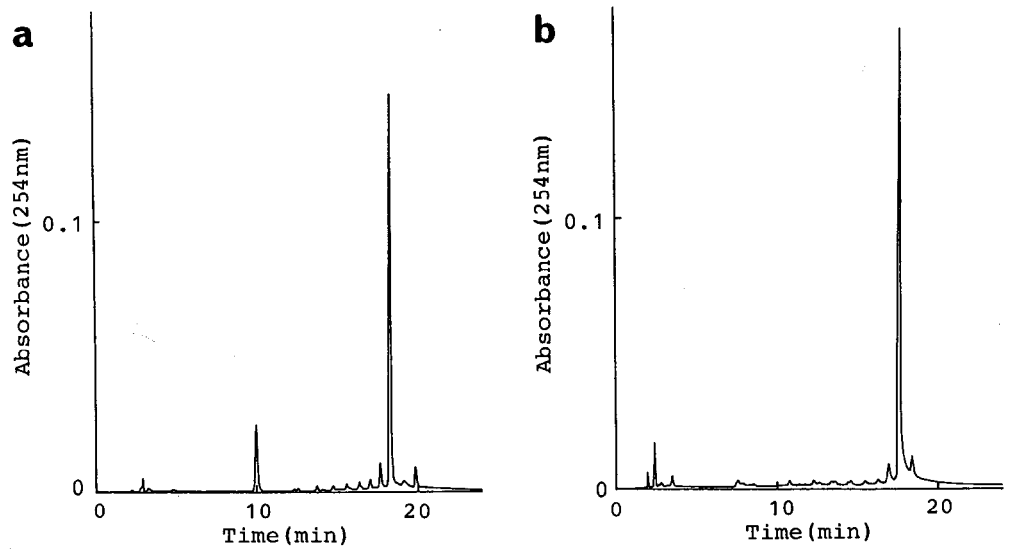

Fig. 2 HPLC analysis of crude 10mers, C_9U (a) and G_9U (b), using a μBONDASPHERE 5μ C18-300Å column (3.9 mmI.D. x 150 mmL.) with a linear gradient of acetonitrile (from 0 to 7.5% during 20 min) in 0.1M triethylammonium acetate (pH 7.0). Column temperature was 40°C (a) and 80°C (b).

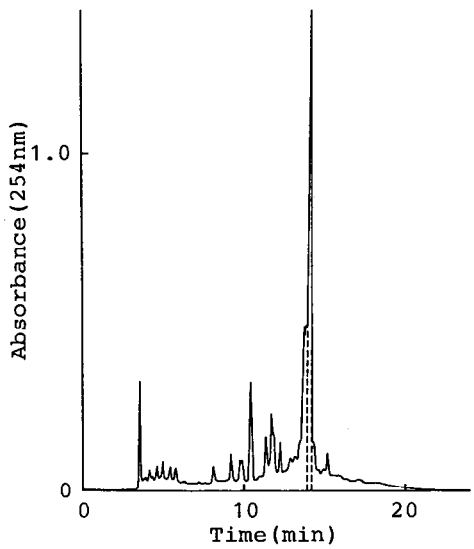

Fig. 3 Purification of the 21mer by reverse-phase HPLC.

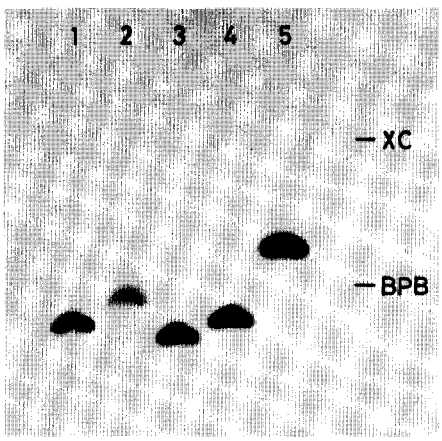

Fig. 4 Autoradiogram of the 5'-^{32}P-labeled oligoribonucleotides after electrophoresis on 10% polyacrylamide gel containing 8M urea. Lane 1, A_9U; lane 2, G_9U; lane 3, C_9U; lane 4, U_{10}; lane 5, 21mer.

terminal guanosine was identified by paper electrophoresis after digestion of the 5'-^{32}P-labeled 21mer with nuclease P1, and the sequence was confirmed by two-dimensional homochromatography[10].

These results demonstrate that this method is facile and effective for the synthesis of oligoribonucleotides with an automatic synthesizer. The synthesis of longer RNA fragments is under investigation.

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