Tetrahedron Letters, Vol.31, No.17, pp 2471-2474, 1990
Printed in Great Britain

0040-4039/90 $3.00 + .00
Pergamon Press plc

A UNIVERSAL SOLID SUPPORT FOR THE SYNTHESIS OF 3'-THIOL GROUP CONTAINING OLIGONUCLEOTIDES

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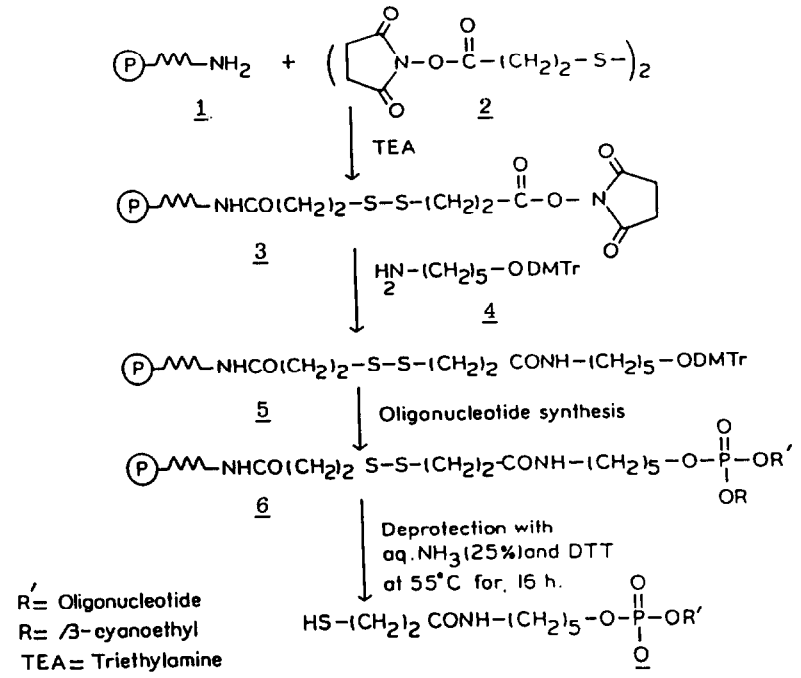
Summary: Use of a derivatised polymer support 5 involving the 4,4'-dimethoxytrityloxypentyl-3-mercaptopropionamide group allows the automated synthesis of oligonucleotides bearing thiol group at their 3'-termini.

The modified oligodeoxynucleotides are of immence use in molecular biological studies (1-6). A large number of methods (7-14) has been described for the selective modification of 3' or 5'-termini of oligonucleotides. For a specific purpose, we were interested to couple a protein to the 3'-terminus of a synthetic oligonucleotide under very mild chemical conditions. The high selectivity and reactivity of sulfhydryl group under mild chemical conditions with a variety of thiol group specific reagents prompted us to use 3'-thiol group containing oligonucleotides to couple with the desired protein.

The method known (15) to date for the selective introduction of a free sulfhydryl group at the 3'-termini of oligonucleotides, in our opinion, suffers from some potential drawbacks:(i) polymer support functionalization is laborious and (ii) an extra nucleotide unit is added to the oligonucleotide or four different polymer supports are required.

In the present communication we wish to describe a general method for the synthesis of 3'-thiol group containing oligonucleotides. The method involves the preparation of a universal Controlled-Pore Glass (CPG)-based support compatible with the established phosphoramidite chemistry of oligonucleotide synthesis to give rise to a 3'-thiol group containing oligonucleotides during final deprotection.

The functionalization of the CPG-based polymer support begins with the reaction of 3-aminopropylated-CPG with a large excess (ten fold) of a commercially available homobifunctional reagent, 3,3'-dithiobis(N-succinimidyl propionate) (DSP) in presence of triethylamine in ethylene dichloride (Scheme-1). Excess DSP was washed off and the polymer support 3 was then reacted with five fold excess of 4,4'-dimethoxytrityloxypentylamine 4 (16) in presence of triethylamine in ethylene dichloride. Excess reagent 4 was removed by washing to obtain the desired polymer support 5. Determination of the amount of 4,4'-dimethoxytrityl cation released by the acidic treat-



SCHEME - 1

ment of a sample of support 5 showed a loading of approximately 25 μM per gram of the 4,4'-dimethoxytrityloxypentyl-3-mercaptopropionamide derivative. The support was dried under vacuum and the remaining active ester functionalities and amino groups on the support were capped following the published procedures (17). In order to ascertain that all the 4,4'-dimethoxytrityloxypentyl-3-mercaptopropionamide groups are linked to the polymer support via disulfide linkage, a small portion of the polymer support 5 was treated with 50 mM DTT solution, pH 8.5 for 6h at 40° C followed by the loading determination by acid treatment method indicating the complete loss of 4,4'-dimethoxytrityloxypentyl-3-mercaptopropionamide groups on polymer support 5 due to the cleavage of disulfide linkage.

To test the utility of the derivatised polymer support 5 for the synthesis of 3'-thiol group containing oligonucleotides, three sequences, viz., d(TTT TT---SH), d(TGC CAG ACA G ---SH) and d(CGG ATC CGC GGA TCC G---SH) were synthesized, using 1 μM scale on derivatised polymer support 5 and 12 μM of 5'-O-dimethoxytrityldeoxynucleoside 3'-N,N-diisopropylamino-2-cyanoethylphosphoramidite per cycle with a cycle time of 7.5 min following the standard protocol (18) on Pharmacia Gene Assembler Plus. The coupling efficiency per cycle based on released 4,4'-dimethoxytrityl cation was found to be > 98%.

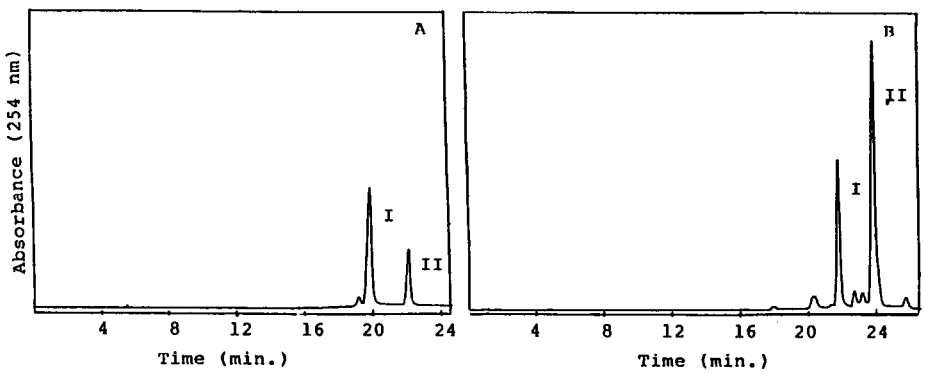


Fig:1: (A) HPLC profile of d(TTT TT SH) (peak II) co-injected with d(TTT TT) (peak I); (B) HPLC elution pattern of 1,5-I-AEDANS labelled d(TTT TT - - SH) (peak II) co-injected with d(TTT TT - - SH) (peak I). Column μ Bondapak (300x2mm); flow rate 0.5 ml/min; gradient 0-20% B in 25 min. A=0.1M ammonium acetate buffer, pH 7.1 containing 5% acetonitrile, B=100% acetonitrile.

Following the last detritylation step in the machine itself, the polymer support was removed and exposed to 50 mM solution of DTT in aq. ammonia (25%) (2 ml) for 16 hours at 55° C. This process allows the removal of 3'-thiol oligomer from the polymer support due to the cleavage of disulfide linkage and deprotection of the classical protecting groups (β-cyanoethyl from phosphates and acyl from nucleic bases). The ammonia solution was concentrated and desalted on Bio-gel P-2 column using 0.1 M triethylammonium acetate buffer, pH 7.5. The sequence was then subjected to HPLC purification on C_{18} reverse phase column.

Fig.1A shows the reverse phase HPLC profile of the purified d(TTT TT--SH) co-injected with a previously synthesized d(TTT TT). The later eluting peak II is of the desired 3'-thiol containing oligomer d(TTT TT--SH). The chromatographic properties of the 3'-thiol containing oligomer was unchanged after exposure to snake venom phosphodiesterase.

d(TTT TT --SH) (2.0 A_{260}units) alongwith 1 eq. of DTT was taken in 200 μl of 5% sodium bicarbonate solution. A thiol group specific fluorescent probe N-(Iodoacetaminoethyl)- 1-naphthylamine-5'-sulphonic acid (1,5-I-AEDANS) (10 eq.) was dissolved in 200 μl of 5% sodium bicarbonate and added to the oligonucleotide containing tube. The reaction was complete in 30 min as monitored by HPLC. A control consisting of the 1,5-I-AEDANS with DTT was always performed to distinguish between peaks due to free fluorophore and its conjugates with DTT and the desired fluorescentally labelled oligonucleotide. Fig 1B shows the HPLC profile of the purified fluorescent labelled oligomer co-injected with the purified 3'-thiol containing oligomer. The later eluting peak II is of the desired fluorescent labelled oligomer.

CPG---SH (100 mg, 22 μM -SH groups/g support, 500 Å pore diameter) was activated with 2,2'-dithiobis (5-nitropyridine) (DTNP) according to the published method (19). Activated support (60 mg) was treated with 3.0 A_{260} units of d(CGG ATC CGC GCA TCC G---SH) in 200 μl of 1.0 M NaCl, 1.0 mM EDTA, 50 mM Tris-HCl, pH 8.0 for 10 min. The amount of the oligonucleotide immobilised on the support was found to be quantitative. The immobilised oligonucleotide eluted quantitatively from the support on treatment with 50 mM DTT solution pH 8.5.

Acknowledgement: We are thankful to the Department of Biotechnology, Govt. of India for financial support. Two of us are thankful to the C.S.I.R. New Delhi for the award of a JRF (PK) and a Res. Associateship(SS).

References and notes

1. Leary, J. J., Brigati, D. J. and Ward, P. C. (1983) Proc. Natl. Acad. Sci. (USA), 80, 4045.
2. Murasugi, A. and Wallace, R. B. (1984) DNA, 3, 269.
3. Ghosh, S. S. and Musso, G. F. (1987) Nucl. Acids Res., 15, 5353.
4. Riley, L. K., Marshall, M. E. and Coleman, M. S. (1986) DNA, 5, 333.
5. Draper, D. E. and Gold, L. (1980) Biochemistry, 19, 1774.
6. Eshaghpour, H., Soll, D. and Crothers, D. M. (1979) Nucl. Acids Res., 6, 1485.
7. Agrawal, S., Christodoulou, C. and Gait, M. J. (1986) Nucl. Acids Res., 14, 6227.
8. Smith, L. M., Fung, S., Hunkapiller, M. W., Hunkapiller, T. J. and Hood, L. E. (1985) Nucl. Acids Res., 13, 2399.
9. Gaur, R. K., Sharma, P. and Gupta, K. C. (1989) Nucl. Acids Res.,17, 4404.
10. Sproat, B. S., Beijer, B., Rider, P. and Neuner, P. (1987) Nucl. Acids Res., 15, 4837.
11. Connolly, B. A. (1985) Nucl. Acids Res., 13, 4484.
12. Horn, T. and Urdea, M.S. (1986) Tetrahedron Lett., 27, 4705.
13. Markiewicz, W. T. and Wyrzykiewicz, T. K. (1989) Nucl. Acids Res., 17, 7149.
14. Sinha, N. D. and Cook, R. M. (1988) Nucl. Acids Res., 16, 2659.
15. Zuckermann, R., Corey, D. and Schultz, P. (1987) Nucl. Acids Res.,15, 5305.
16. 4,4'-dimethoxytrityloxypentylamine was prepared by the reaction of 4,4'-dimethoxytrityl chloride with N-(9-fluorenylmethyloxycarbonyl) aminopentanol(see ref.7) in pyridine followed by deprotection of amino group using morpholin. nmr ($CDCl_3$) δ: 1.5 (m,6H,3x-CH_2), 2.9 (t,2H,-NCH_2), 3.3 (t,2H,-OCH_2), 3.8 (s,6H,2x-OCH_3) and 6.8-7.5 (m,13H,Ar-H).
17. Matteucci, M. D. and Caruthers, M. H. (1981) J. Am. Chem. Soc., 103, 3185.
18. Gene Assembler Plus Manual, Pharmacia Fine Chemicals AB, Sweden.
19. Bischoff, R., Coull, J. M. and Regnier, F. E. (1987) Anal. Biochem., 164, 336.

(Received in UK 15 January 1990)