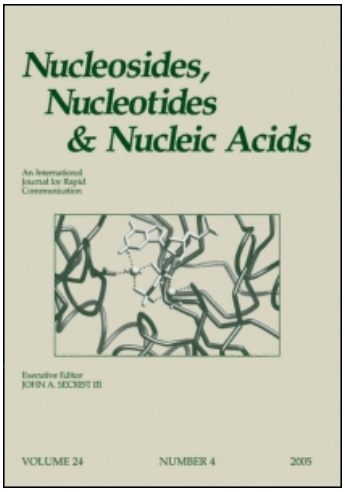

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

A Polymeric Support for Chemical and Enzymatic Nucleic Acid Synthesis (1)

G. Groger[a]; H. Seliger[a]
[a] Universität Ulm, Sektion Polymere, Oberer Eselsberg, Ulm, Fed. Rep. of Germany

To cite this Article Groger, G. and Seliger, H.(1988) 'A Polymeric Support for Chemical and Enzymatic Nucleic Acid Synthesis (1)', Nucleosides, Nucleotides and Nucleic Acids, 7: 5, 773 — 778
To link to this Article: DOI: 10.1080/07328318808056328
URL: http://dx.doi.org/10.1080/07328318808056328

NUCLEOSIDES & NUCLEOTIDES, 7(5&6), 773-778 (1988)

A POLYMERIC SUPPORT FOR CHEMICAL AND ENZYMATIC NUCLEIC ACID SYNTHESIS (1)

G. Gröger and H. Seliger*

Universität Ulm, Sektion Polymere, Oberer Eselsberg, D-7900 Ulm, Fed. Rep. of Germany

Abstract. A non-swellable, highly porous support material - CPG 3000 - was used in building up covalently bound nucleic acids by combined chemical and enzymatic methods. Bases are optimal accessible for hybridization and enzymatic reactions because they are not involved in the linking procedure.

INTRODUCTION

The synthesis of nucleic acid fragments is usually done by a combination of chemical and enzymatic methods. While chemical methods are generally in use for the preparation of oligonucleotides, the joining of these two polynucleotides requires enzymatic ligation. The two parts of the synthesis are normally "decoupled", i.e. the chemical steps are done on a solid support, the enzymatic ligations in solution. Although first reports on the immobilization of ready-made oligonucleotides for hybridization[2] and solid-phase enzymatic joining[3] reactions have been published nearly 20 years ago, it was only very recently that an attempt was reported by Hostomsky et al.[4] to use the same polymer support for the chemical build-up of an oligodeoxynucleotide chain and its enzymatic lengthening with DNA ligase.

Hydrophilic swellable materials, like cellulose, Sepharose or Sephacryl, were the carriers applied in all these experiments. However, such supports are not very suitable for chemical oligonucleotide synthesis, since the inclusion of reagents leads to prolonged washing times. Also hydroxyl groups exposed by mechanical rupture of the matrix may lead

to additional irreversible fixation of oligonucleotide chains. Porous non-swellable silicagel- or CPG-based supports, such as Fractosil 1000 or CPG 500, on the other hand, were reported to be of limited applicability for the enzymatic elongation[4] or degradation[5] of immobilized oligonucleotides, although they are materials of choice for the chemical synthesis. Experiments done earlier in our laboratory[6] suggested that enzyme reactions on such supports were hindered mainly by a too low average pore size and a too broad pore size distribution. Consequently, we have developed support systems based on CPG 3000 and wish to describe their use for both the chemical solid-phase oligonucleotide synthesis and conversions of the grafted chains with a variety of enzymes used in the construction of double-stranded nucleic acids.

RESULTS

Chemical oligonucleotide synthesis:

Aminopropylated CPG 3000 was loaded with 10.4 µmol/g 5'-(di-p-anisyl-)phenylmethyl-thymidine-3'-(p-nitrophenyl-) succinate according to literature[7] and subjected to 19 elongation cycles using 5'-(di-p-anisyl-)phenyl-methylthymidine-3'-(methoxy-)diisopropylamino-phosphane as monomers. The immobilized icosathymidylate was stripped of protecting groups[7] without removal from the carrier. A sample was ammonolyzed for analysis and the icosathymidylate characterized by gel electrophoresis (yield 7.8 µmol/g; average yield per condensation 98.5%).

Enzymatic reactions of immobilized oligonucleotide:

The immobilized icosathymidylate was used as a test substrate for a number of enzymatic reactions, which are summarized in Chart 1.

A) 100 µg (dT_{20})-CPG 3000 were incubated in 10 µl solution (Tris.HCl pH 7.6, 40 mM; magnesium chloride 10 mM; DTT 1 mM; glycerol 5%) with 0.1 µmol γ-^{32}P-ATP + 1 µmol ATP and 4 u T4-polynucleotide kinase at 37°C for 15 min. The yield of phosphorylated oligonucleotide was nearly quantitative.

TABLE 1. Yields of ligation product using $d(T_7A_2)rA$ as acceptor fragment.

Amount of acceptor fragment	Yield of ligation product
1 nmol	55 %
2 nmol	70 %
4 nmol	66 %
5 nmol	82 %

B) As a test for DNA ligation sample (A) was mixed with icosaadenylate and $dCTAGGT_{10}$ (1 nmol each) in 15 µl water, heated to 100°C and slowly cooled for hybridization. 2 µl ligation buffer (760 mM Tris.HCl pH 7.6; 10 mM magnesium chloride; 1 mM ATP), 1 µl 1 mM ATP and 2 µl DNA ligase solution (6 u/µl) were added and the mixture incubated 24 h at 20°. After removal of the splint and washing a sample was ammonolyzed and the yield of ligation product determined after gel electrophoresis to be 95%.

C) Single-stranded ligation of sample (A) to riboadenylate-terminated deoxyoligonucleotides[8] was effected by the following procedure: 100 µg of immobilized (A) are co-lyophilized with 1 - 5 nmol acceptor fragment. The compounds were dissolved in 20 µl solution containing 75 µM ATP, 2 mM spermine, 20 mM magnesium chloride, 20 mM DTT, 50 mM HEPES pH 7.5, 15% DMSO, 20% polyethylene glycol (M=6000) and 28 µg/µl RNA ligase and incubated for 48 h at room temperature. The support was washed thrice with 500 µl each of buffer containing 10 mM Tris.HCl, pH 7.6 and 2.5 mM EDTA. A sample was ammonolyzed and the yield of ligation product determined electrophoretically. The yields were generally between 60 and 80%, as shown in TABLE 1.

D) Replication of an immobilized DNA-RNA hybrid strand with DNA polymerase was the next reaction tested. For this purpose the primer $(dA)_{16}$ (200 pmol) was dissolved in 16 µl water and 3 µl buffer (500 mM Tris.HCl, pH 7.2; 100 mM magnesium sulfate; 1 mM DTT; 500 µg/ml BSA). In this solution we suspended 100 µg support from reaction (C) containing $dT_7dA_2rAdT_{20}$-CPG 3000. Hybridization was effected by heating

3 min. to 100°C and slow cooling. 5 µl deoxynucleoside triphosphate mixture (each 2.5 mM), 3 µl α-^{32}P-dATP (2 µM) and 3 µl containing 21 u DNA-polymerase (Klenow-fragment) were added and the mixture incubated 2 h at 37°C. The assay for replication was done by workup a) of a sample of the double-stranded product after ammonolysis and b) of the replication product after denaturation. In both cases the replication product was shown to be a homologous series of bands corresponding to oligonucleotides with 14 to 30 bases.

DISCUSSION

Our results clearly demonstrate the applicability of CPG 3000 as a support material for chemical as well as enzymatic oligonucleotide chain elongations in solid phase. Although for the experiments described above we could do, for simplicity, with only an oligo-(dT)-CPG 3000, wide-pore CPG is an optimal material for the preparation of oligonucleotides containing any sequence of all four bases[9], and a support anchor can be chosen so that it is not cleaved during deprotection[5].

Using the immobilized icosathymidylate we have followed two routes for the construction of double-stranded nucleic acid. An excellent yield was obtained in the conventional combination of sequentially overlapping oligomers with T4-DNA ligase. Alternatively, we have successfully applied the new strategy of single-stranded joining of DNA fragments terminating in a 3'-ribonucleoside with RNA ligase[8] followed by replication of the immobilized template with DNA polymerase and an oligoadenylate primer.

The release of the products from the solid phase was usually effected by ammonia treatment. While this chemical cleavage may be satisfactory in most cases, it will lead to partial degradation at the deoxy-to-ribo junctions in polynucleotide fragments obtained from experiment (D). Orienting experiments on the incubation of the product from reaction (D) with Eco RI indicate that the nucleic acid load can alternatively be cleaved from the support by restriction endonucleases, if appropriate recognition sequences are inserted into the spacer.

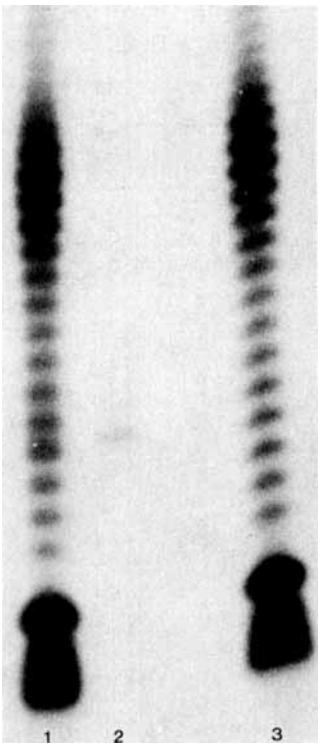

FIG. 1

DNA-Polymerase reaction (Klenow fragment)
on CPG 3000 support

100 µg support, 0.2 µM α-^{32}P-ATP, 1.6 µM dNTP,
50 mM Tris.Cl, pH 7.2, 10 mM $MgSO_4$, 100 µM DTT,
50 µg/ml BSA, 21 u DNA-polymerase
lane 1: duplex DNA removed from the support
by ammonia treatment
lane 2: ref. dT
lane 3: DNA-strand removed by heat denaturing
of the support

Experiments designed to further widen the scope of enzymatic reactions of oligonucleotides bound to CPG 3000 are under way. Applications of this system e.g. in gene synthesis[10] as well as for hybridization and affinity purification purposes are being studied.

ACKNOWLEDGEMENTS

Financial support from the Deutsche Forschungsgemeinschaft is gratefully acknowledged.

REFERENCES

1. Polymer supports synthesis XIV. (Part XIII, H. Seliger, R. Rösch, in prep.)
2.a) P.T. Gilham, Biochemistry 7, 2809-2813 (1968)
b) D.J. Arndt-Jovin, T.M. Jovin, W. Bähr, A. Frischauf, M. Marquardt, Eur. J. Biochem. 54, 411-418 (1975)
c) S.G. Siddel, Eur. J. Biochem. 92, 621-629 (1978)
d) B.E. Noyes, G.R. Stark, Cell 5, 301-310 (1975)
3.a) N.R. Cozzarelli, N.E. Melechen, T.M. Jovin, A. Kornberg, Biochem. Biophys. Res. Comm. 28, 578-586 (1967)
b) A. Panet, H.G. Khorana, J. Biol. Chem. 249, 5213-5221 (1974)
4.a) Z. Hostomsky, J. Smrt, Nucl. Acids Res. Symp. Series 18, 241-247 (1987)
b) Z. Hostomsky, J. Smrt, L. Arnold, Z. Tocik, V. Paces, Nucl. Acids Res. 15, 4849-4856 (1987)
5. M.S. Urdea, T. Horn, Tetr. Lett. 27, 2933-2936 (1986)
6. G. Gröger, Diplom Thesis
7. M.D. Matteucci, M.H. Caruthers, Tetr. Lett. 21, 3243-3246 (1980)
8. D. Zeh, H. Seliger, Nucl. Nucl. 6, 485-488 (1987)
9. H. Seliger, A. Herold, U. Kotschi, J. Lyons, G. Schmidt, Proceedings of the 2nd Int. Symp. on Phosphorus Chemistry Directed Towards Biology, Lodz, Poland, Sept. 8-12, 1986 (Elsevier 1987)
10. H. Seliger, H. Fancelli, G. Gröger, S. Klein, U. Kotschi, R. Schlenk, G. Schmidt, E. Schwichtenberg, D. Zeh, W. Stegmaier, K.-H. Voigt, Nucl. Nucl., in prep.