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New and Efficient Solid Support for the Synthesis of Nucleic Acids

M. P. Reddy*, M. A. Michael, Firdous Farooqui and N. S. Girgis

Advanced Technology Center, Beckman Instruments Inc., 2500 Harbor Blvd., Fullerton, CA 92634

Abstract: Controlled pore glass (CPG) is presently the most widely used solid support for the solid phase synthesis of nucleic acids. We have in our study explored the use of several organic solid supports as alternatives to CPG and found Fractogel (Toyopearl) solid support which is a methacrylate - vinylidene copolymer as an efficient one. This support was derivatized with the nucleosides through the optimized spacer arm to furnish nucleoside loadings of up to 125 μmole/gm. Oligonucleotides of various lengths have been successfully synthesized and analyzed. The integrity of the synthesized oligonucleotides has been established.

It is well established that oligonucleotide synthesis proceeds efficiently when it is assembled on a solid support, and the solid support plays an important role in this process. Although various organic solid supports[1-5] were employed for the synthesis of oligonucleotides, they were not adequate for reasons such as slow diffusion rates of activated nucleotides into the support, excessive swelling of the low cross-linked polymer supports and irreversible adsorption of reagents onto the polymer.[6] Matteucci and Caruthers[6-7] have appropriately modified and efficiently used silica gel solid supports for the synthesis of oligonucleotides; controlled pore glass (CPG) solid support which has been very widely used in the automated DNA synthesizers belongs to this class.[7-9] The CPG solid support however has certain limitations such as its instability towards the DNA synthesis conditions which results in the generation of undesirable sequences of various lengths.[10-11] Also, it is unstable under the basic deprotection conditions.[12] Some polystyrene based solid supports have recently been developed to address this need.[11,13-15]

We have in our study[16] explored several new organic solid supports for the synthesis of nucleic acid molecules and have succeeded in developing Fractogel (Toyopearl)[17] resin as an efficient solid support. Fractogel (Toyopearl) is a methacrylate based support copolymerized with vinylalcohol. These spherical particles have the following properties; a) Stable at pH 1 to 14 and upto the pressure of 100 psi. b) Compatible with most organic solvents with minimal volume change. c) Stable upto 100^{0}C. d) Flow properties are compatible with the commercial DNA synthesizers. e) Well defined pores suitable for the synthesis of oligonucleotides.

The Fractogel solid support has been derivatized using various spacers differing in the length and the hydrophilic nature. Among them the chemistry depicted in Figure 1 gave optimal performance in the oligonucleotide synthesis. The hydroxyl groups of dry Fractogel beads (I) were activated with 1,1'-carbonyldiimidazole, followed by reaction with 1,12-diaminododecane. The remaining unreacted groups of the support II were capped by treatment with propylamine. The level of amino functionality in the support III could be controlled in the range of 50 to 300 μmole/gm as measured by the picric acid test.[18] The support III was loaded with the desired nucleoside by treatment with p-nitrophenyl nucleoside succinate ester. After capping the remaining unreacted amino groups with acetic anhydride/pyridine/N,N-dimethylaminopyridine, the Fractogel support furnished nucleoside loadings in

the range of 10-125 μmoles per gram as measured by the DMT cation assay.[19]

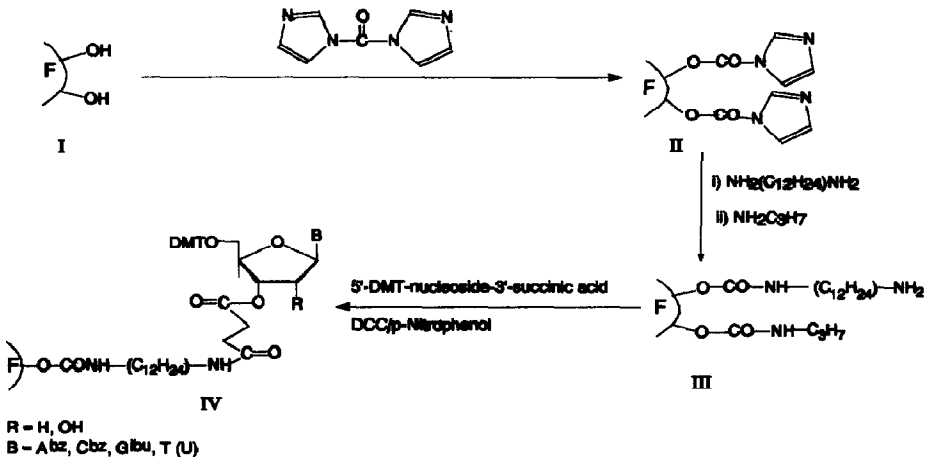

Figure 1

The nucleoside loaded Fractogel suport could be successfully used to synthesize oligonucleotides of various lengths in the range of 17 mer to 120 mer on various commercially available synthesizers: ABI PCR mate, Biosearch 8750, Pharmacia Gene assembler and Beckman Oligo-1000. This support with one pore size can synthesize both short and long oligonucleotides. The step wise coupling efficiency as monitored by the DMT assay was typically > 98.5%.

The oligonucleotides were analyzed by reverse phase HPLC, capillary electrophoresis and slab gel electrophoresis. Figure 2 shows the capillary electrophoresis pattern of a 101 mer synthesized on the Fractogel solid support using the Beckman Oligo-1000. The required peak was integrated to be about 34% of the total mixture. The nucleoside composition analysis gave very similar results compared to the same oligomer synthesized on the CPG support.[20]

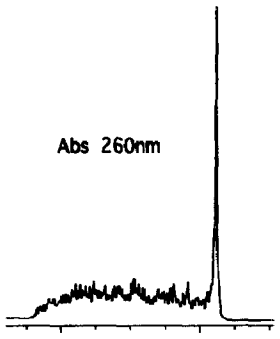

Figure 2: Capillary electrophoretic scan of a 101 mer run on the Beckman P/ACE 2000 using a gel filled capillary. Sequence:5'AACGTCGGTAACGTACACGG TAGCTACGGACACCGTGGCAATACGGGT AACCTGTGGAACGTACACGGAAGAGACT AGGGATGGGAGTACGGATGGGT3'

To evaluate the effect of nucleoside loading on the synthesis efficiency, we have synthesized oligonucleotides on the Fractogel solid supports with different thymidine loadings: 11, 30 and 82 μmole/gm and they all performed equivalent to each other. Figure 3 shows the comparison of capillary electrophoretic scans of a 35 mer.

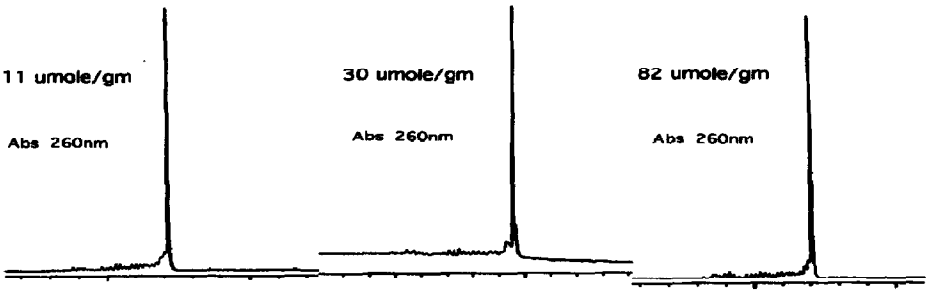

Figure 3: Capillary electrophoretic scans of a 35 mer synthesized on 11, 30 or 82μmole/gm supports Sequence : $^{5'}$GATGCCAGTTCGGTCATACACGTAGTACTACGACT$^{3'}$

Oligoribonucleotides were also successfully synthesized on Fractogel support. For this purpose, the amino Fractogel support III was reacted with the 5'-dimethoxytrityl-2'-tert-butyldimethylsilyl-3'-succinic acid to obtain ribonucleoside loadings of 25-50 μmole/gm of the support. Figure 4 shows the capillary electropherogram of a 21mer oligoribonucleotide.

Encouraged by the successful synthesis of oligonucleotides and oligoribonucleotides, we have explored the synthesis of oligonucleoside phosphorothioates, one of the leading classes of antisense nucleic acids. Using Beaucage reagent,[21] a 25 mer phosphorothioate oligomer was successfully synthesized and analyzed by reverse phase HPLC and capillary electrophoresis. Figure 5 shows the capillary gel electrophoretic scan.

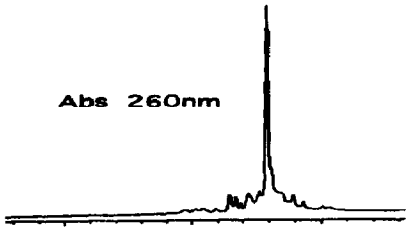

Figure 4: Capillary electrophoretic scan of a 21 mer RNA: $^{5'}$CTGGACACTAGTCCGACTGCT$^{3'}$

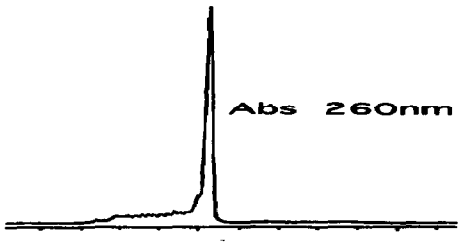

Figure 5: Capillary electrophoretic scan of a 25 mer oligonucleoside phosphorothioates: $^{5'}$AGTCAGTCAGTCAGTCAGTCAGTCT$^{3'}$

The integrity of the oligonucleotides was established by the nucleoside composition analysis and by using them in the following applications: PCR amplification, DNA sequencing by dideoxy termination method, 5'-Kinasing, 3'-terminal transferase extension, hybridization probes and Tm analysis. There was no discernible difference in the performance as compared to the oligonucleotides synthesized using CPG as a solid support. Also, the Fractogel solid support was found to be compatible with methylamine/ammonia, a fast cleavage and deprotection reagent developed in our laboratory.[22]

In conclusion, we have developed a suitably derivatized Fractogel (Toyopearl) as an efficient solid support for the synthesis of oligodeoxyribonucleotides, oligoribonucleotides and oligonucleoside phosphorothioates. Work is in progress to synthesize the other classes of nucleic acid molecules.

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