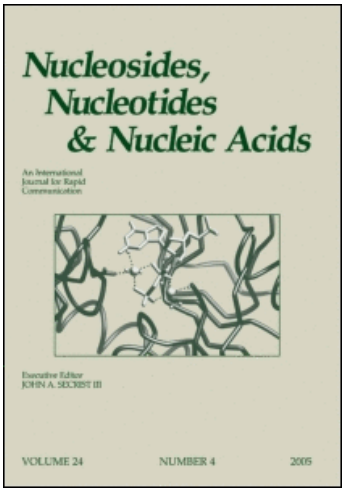

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A New Support For Automated Oligonucleotide Synthesis

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A NEW SUPPORT FOR AUTOMATED OLIGONUCLEOTIDE SYNTHESIS

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Abstract: A particular form of polystyrene has been developed as a solid support for automated oligonucleotide synthesis. Extraneous chain growth is minimized due to the lack of reactive functionality on the new support surface. Therefore, it is well suited to small scale (50 nanomole) synthesis.

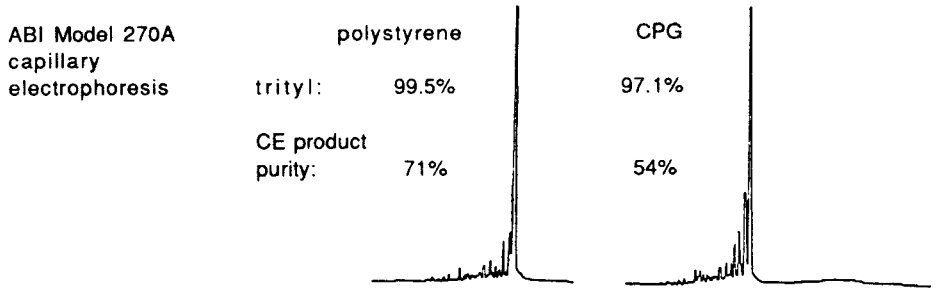
The solid support currently in use with most automated DNA synthesizers is controlled-pore-glass (CPG), an inorganic, rigid silicate matrix of uniform size beads[1] . Compared to other inorganic silica supports, CPG has the positive features of easy covalent derivatization, an efficiently and rapidly washed surface, and rapid reaction kinetics.

Phosphoramidite DNA synthesis on CPG can attain 98-99% repetitive yields. However, as oligonucleotide synthesis is reduced in scale and phosphoramidite excess, to achieve more economical cycles, the deficiencies of CPG become more apparent. Although the silanol matrix of CPG is exhaustively capped prior to synthesis, during detritylation with trichloroacetic acid a small amount of trimethylsilyl capping groups are lost, allowing phosphoramidite to react with the nucleophilic silanols[2]. Extraneous oligonucleotides can be initiated and propagated each synthesis cycle, resulting in 3' phosphate terminus failure sequences. These failures become more problematic at smaller scales.

For a lower scale synthesis, 50 nanomole, we have developed a form of polystyrene which preserves the positive aspects of CPG, and prevents the unwanted participation of the support during synthesis[3]. Polystyrene beads (50-100 micron diameter) of 1000Å pore size and high divinylbenzene cross-link content were derivatized with N-hydroxymethylphthalimide and methanesulfonic acid, followed by hydrazinolysis of the phthalimide group. Synthesis supports (A^{bz}, G^{ibu}, C^{bz}, T) were prepared by coupling 3' p-nitrophenylsuccinate nucleoside esters to the amine[4]. Nucleoside loading can be precisely controlled within a broad range.

Since the physical properties of the polystyrene supports are very similar to CPG, no reagent or cycle changes are necessary. The resulting oligonucleotides are of consistently good quality and yield. With other variables controlled, the polystyrene support gave equal or superior results compared to the CPG support.

A 21-mer oligonucleotide primer was synthesized on 50 nanomole and 0.2 μmole polystyrene and 0.2 μmole CPG columns concurrently on a Model 380B DNA synthesizer. Four



mg. of phosphoramidites were delivered each cycle (6.2 minutes, 3 columns) in order to stringently test the supports. The crude and OPC purified[5] oligonucleotides were analyzed by capillary electrophoresis[6] (ABI Model 270A) using a new gel-filled matrix optimized for oligonucleotide analysis. Trityl yields for polystyrene are excellent (99.0-99.5%), but the CPG trityl yield is fair (97.1%). The trityl data is corroborated by the quantitation of product on capillary electrophoresis. Examination of the CE electropherograms suggest undesired growth off the support may contribute to the comparatively high n-1 mer contamination seen in the CPG samples. Trityl selective OPC purification, and subsequent CE analysis, shows that these contaminants are trityl bearing. Electropherograms of OPC pure samples also demonstrate the superior trityl homogeneity of the polystyrene support derived products. Syntheses conducted with aged, partially degraded reagents also give better results with polystyrene support.

In addition to decreased growth off the support, polystyrene support improves coupling efficiency over that attained with CPG. The advantage is especially evident under more stringent conditions, such as low phosphoramidite excess and impaired synthesis conditions. The difference may reflect a more anhydrous environment of the hydrophobic polystyrene.

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