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Lab Highlights

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NOBEL LAUREATE USES SEP-PAK C_{18} CARTRIDGES to PURIFY OLIGONUCLEOTIDES

H. Gobind **Khorana** and colleagues at MIT have recently reported in the Proceedings of the National Academy of Science (USA) a **SEP-PAK** C_{18} Cartridge method used to purify synthetic oligonucleotides and DNA fragments. [1] They describe their procedure as **"extremely rapid, efficient, and general"** and further state that the " **SEP-PAK** [Cartridge] procedure, together with rapid methods available for the synthesis of oligonucleotides, further **expedites the preparation of synthetic DNA."**

Merrifield-type solid-phase synthesis with phosphite triester chemistry was used to prepare the "oligos" of defined sequence by adding specific, fully protected nucleotides to the terminal 5'-hydroxyl group of the bound oligo chain. When the synthesis was completed, the methoxy and N-protecting groups were removed, and the crude dimethoxytrityl [$(MeO)_2Tr$]-oligonucleotide(s) was purified on a **SEP-PAK** C_{18} Cartridge as follows:

> A wash sequence of (a) 10 ml of CH_3CN, (b) 5 ml of 30% CH_3CN in 100 mM triethylammonium bicarbonate [$NHEt_3HCO_3$, TEAB], and (c) 10 ml of 25 mM TEAB, respectively, was used to prepare the **SEP-PAK** C_{18} Cartridge. Then, a solution of the crude $(MeO)_2Tr$-oligo(s) in 10 ml of 25 mM TEAB was loaded onto the bed. **Failed sequences** containing a terminal 5'-OH group were separated next with 10-15 ml of 10% CH_3CN/90% 25mM TEAB. Finally, using 5 ml of 30% CH_3CN/70% 100 mM TEAB, the **desired** $(MeO)_2Tr$-oligo was easily eluted from the bed.

Purification was verified rapidly by a selective color test for the $(MeO)_2Tr$ group. In addition, vivid visual demonstration of the effectiveness of this **SEP-PAK** C_{18} Cartridge purification was provided by polyacrylamide gel electrophoresis [PAGE] (photographs in paper).

Next, all remaining protecting groups were removed with 80% acetic acid, and the oligo was further refined by prep PAGE. The desired gel band was extracted with 1 M TEAB; then, the extract was diluted 1:5 with water, filtered, and loaded onto another prepared **SEP-PAK** C_{18} Cartridge. After washing out urea and salts with 10 ml of 25 mM TEAB, the oligo was removed with 3 ml of 30% CH_3CN/70% 100 mM TEAB, and lyophilized.

Using T4 polynucleotide kinase, 5'-^{32}**P**-phosphorylation of the oligos was accomplished, and the reaction mixture was purified, once again, with a **SEP-PAK** C_{18} Cartridge, prepared as above. P_i and ATP were removed with 10 ml of 5% CH_3CN/95% 25 mM TEAB; the 5'-phosphorylated oligo was then isolated using 2 ml of

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30% CH_3CN/70% 100 mM TEAB. Each ^{32}P-labeled oligo was checked for purity by a combination of gel electrophoresis and two-dimensional homochromatography sequence analysis (photos in paper).

Thus, a simple **SEP-PAK** C_{18} Cartridge method has proven to be an effective way to:

- ☞ separate $(MeO)_2Tr$-protected oligonucleotides from truncated sequences after solid-phase synthesis;
- ☞ remove urea and salts and recover oligos after PAGE; and
- ☞ purify 5'-end-phosphorylated oligos (cold or isotopically labeled) obtained via polynucleotide kinase reactions.

[Khorana and co-workers report that the method is also useful when purifying double-stranded DNA (tested with restriction fragments up to 1600 base-pairs) with "almost quantitative recoveries".] [1]

When combined with other advances in DNA synthesis, recombinant cloning, and gene expression, this **SEP-PAK** C_{18} Cartridge method can contribute significantly to the analysis of structure/function relationships of any particular nucleotide in a gene sequence. [2]

REFERENCES:

1. Lo, K-M, Jones, S S, Hackett, N R, and Khorana, H G, *Specific amino acid substitutions in bacterioopsin: Replacement of a restriction fragment in the structural gene by synthetic DNA fragments containing altered codons,* Proc. Natl. Acad. Sci. USA, **81**: 2285-2289 (1984) [SP 84093*]

2. Craik, C S, *Use of Oligonucleotides for Site-Specific Mutagenesis,* BioTechniques **3**(1): 12-19 (1985) [SP 85007*]

*[**SEP-PAK** Cartridge Bibliography File Access Number--explanation to appear in future LAB HIGHLIGHT]