SYNTHESIS AND PURIFICATION OF MODEL OPERATOR OLIGONUCLEOTIDES

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THE recognition of short sequences of DNA operators and promoters by proteins is of central importance in the control of gene expression. Several bacterial and viral operators have been characterized with respect to the sequence and binding affinity of their corresponding repressors[1,2]. The right operator locus (O_R) of bacteriophage λ is a particularly interesting example which has been studied in detail[3]. It contains three discrete 17bp operators designated O_R1, O_R2 and O_R3 out of which *cro* protein binds preferentially to O_R3 whereas repressor binds preferentially to O_R1 and O_R2.

During our synthetic studies on model peptides and nucleotides of *cro* repressor-operator system, we encountered problems in the purification and recovery of the synthetic nucleotides. In this communication, the synthesis and purification of two model self-complementary oligonucleotides, a tetradecamer sequence (1) from the consensus operator sequence that has been worked out from six binding operator sites of *cro*-protein[4], and another tetradecamer (2), which is based on consensus operator sequence for 434 operator sites[5] are discussed.

A-T-C-A-C-C-G-C-G-G-T-G-A-T (1)
A-C-A-A-T-A-T-A-T-A-T-T-G-T (2)

Synthesis

The protected monomers for the phosphotriester approach were purchased from Vega Biochemicals, USA. Silica resin was functionalized and the first 5-O-protected nucleotide was loaded[6] and measured to be in the range of 60–80 μmol/g. The nucleotides were assembled to a desired chain length by phosphotriester method on a manual bench synthesizer (OMNIFIT, Cambridge, UK). Coupling reactions were usually done in acetonitrile instead of pyridine, without any compromise in yields or in reaction times[7]. Synthesis was performed on 50–60 mg resin each time (~ 3 μmol scale). The protected oligonucleotides were deprotected as usual[7]. The guanine rich sequence (1) was treated with 2-nitrobenzyldoxime 1, 1, 3, 3-tetramethylguanidine prior to deprotection.

Purification

Synthetic oligonucleotides are purified by a combination of ion-exchange and reverse phase HPLC[7] or by gel electrophoresis[7]. Synthetic oligonucleotide (1) was purified by ion-exchange followed by reverse-phase HPLC although the overall recovery was somewhat low (figure 1). However, the same protocol turned out to be unsatisfactory for the A–T-rich sequence (2). Ion-exchange chromatography using potassium hydrogen phosphate or trimethyl ammonium carbonate buffer gave poor resolution. Reverse phase chromatography also did not provide satisfactory results at room temperature or at higher temperature (55°).

Although there are a few reports[8] of seperation of synthetic oligonucleotides using preparative silica plates this method has not been exploited for its potential. This method proved to be of great value in isolating tetradecamer (2). Precoated silica plates (20/20 cm, Kiesel gel 60 F_{254}, E. Merck, W. Germany) were developed in ammonia/*n*-propanol/water (35/65/10) over 4–5 hr. The target sequence (2) showed as the most intense band which moved least and was clearly separated from the deleted sequences. This band was cut off and the oligonucleotide was eluted from the silica gel by triethylammonium bicarbonate buffer (1 M, pH 8.0) and lyophilized. The purity was further checked by

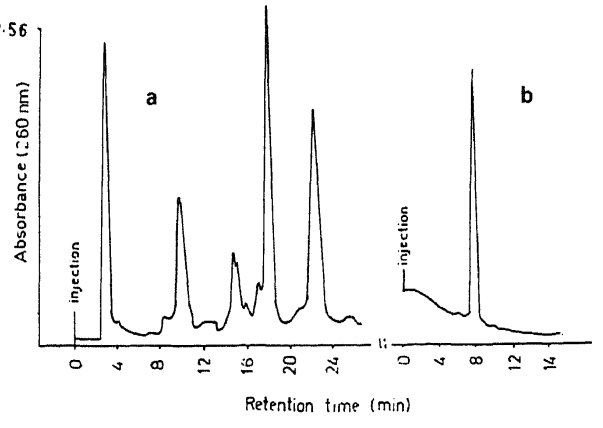

Figure 1a,b. a. Chromatography of synthetic mixture containing the tetradecamer (1) on a 10 SAX radial-PAK-cartridge. Solvent A: 0.008 M KH_2PO_4, pH 6.4 m acetonitrile: water (3: 7 v/v). Solvent B: 0.3 M KH_2PO_4, pH 6.3 in acetonitrile: water (3:7 v/v). Flow: 2 ml/min; temperature: 27°C; detector: 260 nm, 2.56 auf; gradient 0–80% B, 40 min. **b.** Reverse phase chromatography of the tetradecamer, isolated from ion exchange column, on C18 radial-PAK-cartridge. Solvent A: 0.01 M triethylammonium acetate, pH 7.0 solvent B: acetonitrile. Flow 2 ml/min; temperature; 27°C; detector: 260 nm, 2.56 auf: gradient 0–10% B, 20 min.

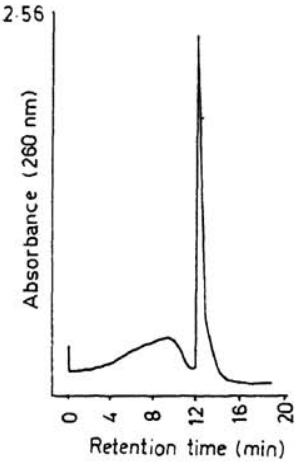

Figure 2. Reverse phase chromatography of the tetradecamer (2), isolated from preparative silica gel plates, on C18 radial-PAK-cartridge, solvent A: 0.01M triethylammonium acetate, pH 7.0, solvent B: acetonitrile, Flow: 2 ml/min; temperature 27°C; detector: 260 nm, 2.56 auf; gradient 0–40% B, 40 min.

reverse phase HPLC (figure 2). The same method of separation was also applied to the purification of tetradecamer sequence (1) satisfactorily. The oligonucleotide could easily be further purified by reverse phase HPLC. We found that after purification, yields were usually 10% higher by preparative TLC method as compared with the corresponding HPLC purification. These results indicate that preparative TLC on precoated silica gel may be routinely used for medium size synthetic oligonucleotides and it offers an economic, faster and higher yielding alternative to the existing methodologies. Of particular interest is that it may be the method of choice when other methods are found unsatisfactory. We are currently developing suitable solvent systems for smaller oligonucleotides (4–6 mer).

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