A high-capacity column for affinity purification of sequence-specific DNA-binding proteins

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Christopher J.Larson and Gregory L.Verdine
Department of Chemistry, Harvard University, Cambridge, MA 02138, USA

Submitted April 27, 1992

Studies on the isolation, cloning and biochemical characterization of DNA-binding proteins have commonly been hindered by difficulties in purification that arise from the low abundance of regulatory molecules. Although this problem has been lessened by the advent of DNA affinity chromatography (1–5), current methods remain less than ideal, having unstable attachment of DNA to the resin (5), modifications of the 5′- or 3′-positions that preclude enzymatic multimerization (3), low capacity (2, 4), or non site-specific coupling (2, 5). Here we report an improved method for synthesis of a DNA affinity column, involving site-specific linkage of a monomeric or concatenated oligonucleotide to an activated chromatographic support. In the present method, efficient and site-specific coupling is accomplished between DNA bearing a reactive alkylamine-tethered nucleoside and a carboxyl-activated support; attachment of the tether to a DNA base (6–8) and co-synthetic 5′-phosphorylation allow for facile generation of concatenated DNA.

An example of this approach is illustrated in Figure 1. A 29-mer oligonucleotide was synthesized (10 μmol scale) using PAC phosphoramidites (Milligen), in addition to the phosphoramidite of O^6-phenyl-2′-deoxyinosine [d(φI), Ref. 8]. A 5′-phosphoryl substituent was added during synthesis using the Phosphate-ON reagent (Clontech). The resin-bound oligonucleotide was treated with concentrated aqueous ammonia (7 ml, 4 h, rt) to remove the protecting groups, then lyophilized. It was redissolved in 1 M aqueous 1,4-diaminobutane (800 μl) and heated at 55°C for 12 h, to convert the φI moiety to N^6-(4-aminobutyl)-A. After cooling to rt, the solution was neutralized with 8 ml 5 M ammonium acetate and lyophilized. The complementary strand was synthesized (10 μmol) using standard phosphoramidites (Milligen) in addition to Phosphate-ON. The crude oligonucleotides were purified on a 20% denaturing polyacrylamide gel and recovered by the crush-and-soak technique, to yield ~1.4 μmol of each.

The two strands were annealed in 1×TE containing 100 mM NaCl, dialyzed (Centricon) into deionized distilled H_2O (ddw), lyophilized, and redissolved into ddw at a concentration of 1 nmol per μl. Ligations were typically carried out on a 500 nmol scale in a solution containing duplex oligonucleotide (500 μl), 10× One-Phor-All buffer (200 μl, Pharmacia), 100 mM ATP (50 μl), 10 mg/ml BSA (25 μl), 1 M DTT (25 μl), ddw (1200 μl), and T4 DNA ligase (100 μl at 20 units/μl), for 16 h at 16°C. The reaction mixture was extracted with phenol and phenol-chloroform, then precipitated with ethanol. The ligated DNA was then coupled to NHS-activated Sepharose (Pharmacia HiTrap, 1 ml bed volume) according to the manufacturer's protocol. Quantification of free DNA revealed that ~250 nmol (50%) had coupled, regardless of whether it was ligated or unligated. The DNA that failed to couple could be used in a subsequent coupling reaction, again with ~50% yield. DNA lacking an alkylamine tether did not couple. The 50% coupling yield and resulting column capacity of 250 nmol/ml observed here are significantly higher than the 10% yield and 2–3 nmol/ml capacity gained with the most widely used method (2).

In work to be published elsewhere, the columns reported here have been used to purify the transcription factor H2TF1 (9), which had previously eluded attempts using existing methodologies (D.Potter, P.Eckes, C.J.L., P.Sharp and G.L.V., manuscript in preparation). Routes other than the convertible nucleoside approach have been reported for attaching an alkylamine function to a DNA base (10); these should also be suitable for use in synthesis of DNA affinity columns by the present method.

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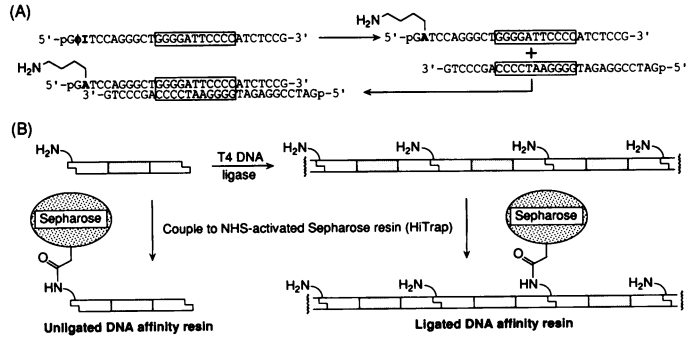

Figure 1. (A) Synthesis of a duplex oligonucleotide containing a single alkylamine-tethered dA residue (A); (B) Synthesis of an unligated and ligated affinity resin.