Separation of the Triplexes Poly(dA) · (dT) · (rU) and Poly(dA) · 2(rU) by Affinity Chromatography *

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The presence of (dA)-rich sequences in DNA has been demonstrated through the formation of molecular hybrids with polyuridylic acid. These homopolymeric regions have been found in all eucaryotic DNAs investigated so far and they are 20 – 80 nucleotides long and constitute 0.1 – 0.3 % of the genome.[1] When the level of (dA)-regions is quantitated with denatured DNA immobilized on nitrocellulose filters or in solution, the hybrid poly(dA)·2(rU) is mainly formed.[2] However, the inverted repeated portion of the DNA is recovered as a partly double-stranded structure which will form the hybrid poly(dA)·(dT)·(rU). In order to get a more detailed picture of the function and distribution of these sequences, it is desirable to be able to separate the two triplexes formed.

Bünemann and Müller[3] have developed a cross-linked bisacrylamide gel to which the (A·T)-specific dye *N*-acryloyl-4-amino-malachite green has been attached covalently to polyacrylamide chains that function as spacers within the gel matrix. The gel will separate double-stranded DNAs according to their base composition and molecular weight as well as separating double-stranded from single-stranded DNA.

The source of (^{3}H)poly(rU) was Miles Laboratories Inc. Unlabelled poly(dA), poly(dA)·(dT) and the malachite green gel were obtained from Boehringer Mannheim. The triplexes 2(^{3}H)poly(rU)·(dA) and (^{3}H)poly(rU)·(dA)·(dT) were formed by annealing at 25 °C for 18 h in 2 × SSC (SSC = 0.15 M NaCl, 0.015 M Na_3-citrate), pH 6.0, followed by subsequent treatment of the raw hybrid with ribonuclease A in the same buffer (5 μg/ml for 6 h). After addition of 2½ vol. ethanol the precipitate was collected and dissolved in 2 × SSC, pH 6.0. The single-, double-, or triple-stranded polymers were separately or in different combinations loaded onto the column. After extensive washing with 2 × SSC, pH 6.0 a linear gradient of sodium perchlorate (0 – 0.30 M in 2 × SSC, pH 6.0) was applied and fractions collected. Each fraction was analyzed for A_{260}-absorbing material and radioactivity by liquid scintillation counting.[4]

Table 1. Affinity of nucleic acid homopolymers for the (AT)-specific malachite green gel.

Polymer	Elution properties
poly(dA)	Void vol.
poly(rU)	Void vol.
poly(dA)·(dT)	0.35[a]
poly(dA)·2(rU)	Void vol.
poly(dA)·(dT)·(rU)	0.41[a]

[a] R_F value was calculated as the peak elution fraction divided by total number of fractions generated by a gradient of 0 – 0.30 M $NaClO_4$ in 2 × SSC (SSC = 0.15 M NaCl, 0.015 M Na_3-citrate), pH 6.0. Fractions of 5 ml were collected from a column of 15 ml bed volume.

From Table 1 it can be seen that poly(dA), (^{3}H)poly(rU) and 2(^{3}H)poly(rU)·(dA) will not bind to the gel. The triplex can easily be separated from the single-stranded polymers because of their differential binding properties to hydroxyapatite at different phosphate concentrations (*i.e.*, single strand elution = 0.15 M sodium phosphate, pH 6.8; triplex elution = 0.40 M phosphate buffer). The duplex poly(dA)·(dT) and the triplex (^{3}H)poly(rU)·(dA)·(dT) are both bound to the gel. The sodium perchlorate gradient will elute the duplex and the triplex around 0.20 M $NaClO_4$ (data not shown). They are, however, not completely separated from each other. The final separation is also accomplished here by hydroxyapatite chromatography.

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