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TEMPLATE CHROMATOGRAPHY: APPLICATION OF THE PRINCIPLES OF MOLECULAR BIOLOGY FOR THE SELECTIVE SEPARATION OF NUCLEOTIDES AND PEPTIDES

A PROGRESS REPORT

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SUMMARY

A chromatographic procedure is described for the selective separation of oligonucleotides according to the base-pairing mechanism. Defined oligonucleotides, covalently linked to soluble polymers, have been fixed to DEAE-cellulose in a non-covalent manner.

Complementary oligonucleotides present in the mobile phase undergo selective adsorption on the immobilized template when chromatographed under the conditions necessary for base pairing and are desorbed in a temperature gradient.

Template chromatography enables the base-pairing specificity of oligonucleotides and also oligonucleotide–peptide interactions to be studied.

Affinity chromatography was developed by taking advantage of the relatively specific enzyme–substrate interactions and has now achieved general application in the separation of natural products[1]. The specificity of the base pairing in DNA occupies a predominant position in the field of biological interactions. However, the extent of this specificity is not known for oligomers. In order to investigate this problem in more depth, indirect spectroscopic methods[2] and also hybridization techniques[3] have been employed. The study of base pairing under chromatographic conditions, which we call template chromatography, is still in its early stages. Such investigations should yield information on the specificity of base pairing and would at the same time be of considerable importance in the development of methods for separating selectively oligo- and polynucleotides.

Basically, there are two possible methods of approach: the template can be an immobilized RNA or DNA as reported by Saxinger *et al.*[4], or oligonucleotides of a defined sequence can be attached to the stationary phase. The application of oligonucleotide homologues exhibiting a broad molecular-weight range[5] is useless for the exact investigation of the base-pairing mechanism. Consequently, only strictly defined oligonucleotides or a very narrow range of homologues should be immobilized on the stationary phase. In addition, undesirable side-reactions that occur during the immobilization procedure should be eliminated as far as possible.

Consequently, we decided against the procedure of Astell *et al.*[6], which consists of the covalent fixation of unprotected nucleotides to an insoluble carrier (cellulose), as the exact binding site in unknown and also cleavage of the nucleotide chain could occur. In addition to these points, steric factors inherent in the cellulose matrix could affect the accessibility of the oligonucleotides. A new method was used to immobilize the oligonucleotides[7,8], the latter being synthesized in a defined manner on a soluble polymer, *i.e.* polyvinyl alcohol (PVA), according to the liquid phase method[9–11].

Oligomers of 5′-deoxythymidylic acid with a narrow molecular-weight range, *i.e.* $(pT)_n$ ($n = 7$–10), can also be bound to PVA, and this technique is discussed in more detail below.

We have shown that nucleotides bound to soluble polymers undergo irreversible adsorption on DEAE-cellulose under the chromatographic conditions necessary for base pairing[7,8], and thus represent an ideal support for chromatographic investigations. The fixation proceeds via secondary forces and thus leaves the nucleotide groups free for interaction with nucleotides in the mobile phase. These supports for template chromatography can be synthesized in large amounts and can be used for preparative-scale separations, which is in contrast to the existing methods of fixation on cellulose filters described in the literature[6]. This is of considerable importance for the planned preparative-scale recovery of defined oligonucleotides from RNA and DNA hydrolyzates. This aspect also necessitates a study of the specificity of base pairing for oligonucleotides.[12,13]

Using the same techniques, oligonucleotide–peptide interactions could also be investigated, which play an important role in the molecular biological regualtion mechanisms and which until now have mainly been studied using physico-chemical methods.

DEAE-cellulose coated with polymer-bound oligomers of 5′-deoxythymydylic acid [$PVA(pT)_n$–DEAE-cellulose] adsorbs specifically complementary oligomers of 5′-deoxyadenylic acid[7]. The degree of retardation which the complementary oligonucleotides of the mobile phase experience increases, under otherwise standard

TABLE I

CONDITIONS FOR THE SEPARATION OF OLIGONUCLEOTIDES USING COLUMN CHROMATOGRAPHY ON $PVA(pT)_n$–DEAE-CELLULOSE ($n = 7$–10) AND THE CHARACTERIZATION OF THE PEAK FRACTIONS IN FIGS. 1a–1d

Fig. No.	*Peak No.*	*Main product*	*Oligonucleotide (A_{260} units)*			*R_F value rel. to pA*	*Molarity of NaCl buffer*
			Applied	*Recovered*	*Eluted*		
1a	a	d(A–C–C–A–T–T–C–A)	120	120	120	0.34	1
1b	b_1	X*	1000	996	600		0.5
	b_2	d(A–G–A–A)			396	0.75	
1c	c_1	d(A–G–A–A–A–T)	300	240	108	0.41, 0.82**	0.5
	c_2	X*			20	0.82, 0.41**	
1d	d_1	d(A–G–A–A–A–T–A–A–A–A)	113	91	36	0.18	0.5
	d_2	50% main product, X*			19		

* Product not identified.

** Traces only.

conditions, with the chain length and is strongly dependent on temperature and concentration. Mixtures of low-molecular-weight synthetic oligomers of 5'-deoxyadenylic acid were separated on PVA(pT)$_n$–DEAE-cellulose under preparative-scale conditions into molecularly homogeneous fractions.

However, higher-molecular-weight mixtures of these compounds cannot be unequivocally separated according to chain-length. Well-defined separations of high-molecular-weight oligonucleotides therefore require a support which can bind and immobilize oligonucleotides with uniform molecular weight. These supports can be synthesized using the liquid-phase method and their synthesis and application will be reported elsewhere.

The base pairing between oligonucleotides shows rigorous specificity, as we have been able to show using synthetic oligonucleotides[8]. Thus the following oligomers of 5'-deoxyadenylic acid which exhibit one or two failure sequences (*i.e.*, G, T instead of A), d(A–G–A–A), d(A–G–A–A–A–T) and d(A–G–A–A–A–T–A–A–A–A) are not adsorbed on PVA(pT)$_n$–DEAE-cellulose. From these results, we conclude that the base pairing between oligonucleotides proceeds according to the "all or nothing" principle and complementary segments of an oligonucleotide alone cannot result in the formation of stable base pairing.

The chromatographic conditions and results are summarized in Table I and Fig. 1.

In order to investigate peptide–oligonucleotide interactions, we chromatographed common amino acids on PVA(pT)$_n$–DEAE-cellulose under the conditions

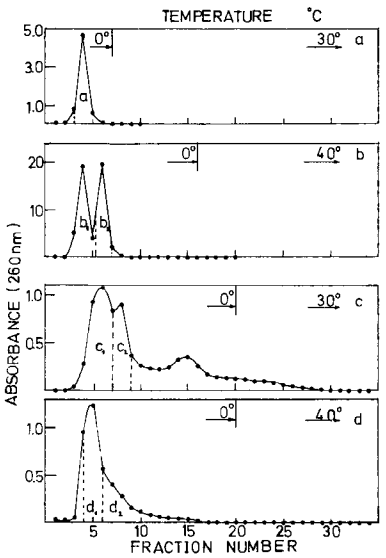

Fig. 1. Template chromatography of phage-specific DNA fragments on PVA(pT)$_n$–DEAE-cellulose in NaCl buffer in the range 0–40°. Column size: 23 × 2 cm. Fractions of 20 ml/h were collected, those within the vertical dotted lines being combined and characterized using paper chromatography. (a) 120 A_{260} units, d(A–C–C–A–T–T–C–A). (b) 1000 A_{260} units, d(A–G–A–A). (c) 300 A_{260} units, d(A–G–A–A–A–T). (d) 113 A_{260} units, d(A–G–A–A–A–T–A–A–A–A). The substances corresponding to the peaks are listed in Table I.

for base pairing, whereby tyrosine and tryptophan undergo significant retardation. This was confirmed by chromatographing tryptophan-containing peptides and it was shown that such peptides can be separated from those which contain no tryptophan[14].

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