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Thin-layer chromatography of oligodeoxyribonucleotides

Recent advances[1–4] and continued interest in the synthesis of oligonucleotides warrant refinements of the existing analytical methods and investigation of new approaches. For analysis of oligomers in the deoxyribo series, paper chromatography in a variety of solvent systems is the most commonly used technique. However, this method requires long development periods (24 h or longer) for reasonable separation of oligomers of four units or higher. There is, therefore, a need for more rapid analytical methods. Previously, quick paper chromatographic systems have been reported[5] but their utility is limited to tetranucleotides or lower homologues.

Separation of mixtures of mononucleotides and nucleosides by thin-layer chromatography (TLC) using various supports is well documented in the literature[6–9]. However, only a few reports dealing with TLC analysis of deoxyribooligonucleotides have appeared[10,11]. Furthermore the application of this technique was limited to selected oligomers in these studies. For instance, LANDO *et al.*[10] separated pyrimidine deoxyribooligomers of seven units or lower by TLC using *n*-butanol, acetic acid and ammonia. We report here results from (a) a new general purpose TLC system and (b) a general application of the modified ammonium acetate system. The technique has been utilized at various stages of a synthetic program using the phosphorothioate blocking group[4] in the deoxyribooligonucleotide series.

Procedure

Silica Gel F-204 plates (substrate thickness 0.25 mm) were used (Brinkmann Instruments Inc., Westbury, N.Y. 11590). Oligomeric materials were spotted in the range of 0.2–0.4 O.D.U. in 1–2 μl solution. The plates were then developed in solvent systems I or II containing sodium dodecylsulfate (SDS) or ammonium acetate as the integral component. The chromatographic development was always in the ascending direction for a period of about 7 h (system I) and 3.5 h (system II). Prior equilibration of the tank was not critical. The solvent system containing

TABLE I

R_F VALUES OF SOME DEOXYRIBOOLIGONUCLEOTIDES

The sequences of the oligomers are: 1 = d-pApApGpApCpApGpCpApTpApT; 2 = d-Et-S-pApApGpApCpApGpCpApTpApT; 3 = d-Et-S-pA-ApGpApCpApGpC; 4 = d-Et-S-pApApG-pApCpA; 5 = d-Et-S-pApApGpA; 6 = d-Et-S-pApA; 7 = d-pApTpApT.

Solvent composition				*R_F of oligonucleotides*						
Ethanol	*methanol*	*0.1 M SDS*	*0.1 M NH_4Ac*	*1*	*2*	*3*	*4*	*5*	*6*	*7*
5	2	3		0.09	0.21	0.30	0.45	0.54	0.74	0.64
5	2		3	0.07	0.13	0.20	0.39	0.57	0.77	0.36
2	5	3				0.52	0.64	0.75		0.74
1	6	3				0.67	0.61			
3.5	3.5	3		0.166	0.32	0.46	0.56	0.58		
7	—	3				0.04	0.17	0.39	0.65	
7	—		3	0.07	0.11	0.14	0.31	0.50	0.77	0.33

TABLE II

R_F VALUES OF SOME DEOXYRIBOOLIGONUCLEOTIDES

The sequence of the oligomers is the same as described in Table I.

Solvent composition				*R_F of oligonucleotides*						
Methanol	*Acetonitrile*	*0.1 M SDS*	*0.1 M NH_4Ac*	*1*	*2*	*3*	*4*	*5*	*6*	*7*
5	2	3				0.52	0.64	0.74		0.7
2	5	3				0.04	0.11	0.26	0.59	
3	4	3		0.07	0.19	0.33	0.52	0.65	0.76	
3	4		3	0.16	0.40	0.43	0.58	0.72	0.90	0.6
	6	4		[a]	[a]	[a]	[a]	[a]	[a]	[a]
	5	5				[b]	[b]	[b]	[b]	

[a] No movement from origin.
[b] Nucleotide moved with the solvent front.

SDS was prepared by the gradual addition of 0.1 *M* SDS to the alcohol solution. At times the addition of detergent resulted in a little cloudiness but it never influenced the results adversely.

Results and discussion

The R_F values presented in Tables I and II are calculated on the basis of the primary front. Most of the oligonucleotides used during this study have their 5'-phosphate terminus substituted with an ethyl thio group, although a fully deprotected tetramer (pApTpApT) and dodecamer are also included. From this investigation there emerge two definite TLC systems. The first system consists of varying compositions of methanol, ethanol and 0.1 *M* SDS or 0.1 *M* ammonium acetate. The corresponding results are presented in Table I. The separations depicted here are quite satisfactory. By varying the proportions of the constituents, tailor-made systems can be prepared to suit the particular problem at hand. For instance, variation in the amount of methanol gives a range of R_F values without affecting the resolution. As regards the concentration of sodium dodecylsulfate or ammonium acetate, best results were obtained with 30% 0.1 *M* aqueous solution of either of these components. By substituting acetonitrile for ethanol, a quick TLC system is obtained which gives comparable results in half the time of development (Table II). The analysis of a dodecadeoxyribonucleotide in 3.5 h is a significant progress in analytical methodology. Currently we are investigating the extension of this technique to preparative scale.

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