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Note

Oligonucleotide separations by high-pressure liquid chromatography on a weak anion exchanger

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The ion-exchange column chromatographic separation of oligonucleotides has conventionally been carried out using a polysaccharide support bearing alkylammonium ion-exchange groups[1–7]. Such materials are capable of high resolution, but their use requires long elution periods. Typical elution times for longer oligomers range from a day to a week[8].

We wish to report a procedure for conducting such separations in less than 90 min. Oligomers in the deoxyribonucleotide series are eluted at elevated temperature and pressure from Zipax* weak anion exchanger (WAX) in the sulfate form by means of a concentration gradient of sulfate anion at pH 4.4. Mono- and dinucleotides are eluted at or very near the V_0 of the column. Larger oligomers are retained in proportion to their charge and base composition.

MATERIALS AND METHODS

Columns are prepared from 316SS tubing and dry-packed with Zipax WAX. The packing technique and high-pressure liquid chromatographic equipment are as described previously[9]. Gradients are formed in a two-chamber device (Glenco Scientific) using buffers prepared from A.R. grade ammonium, sodium or potassium sulfate of low (0.005 A_{254} ml^{-1} of molar solution) ultraviolet absorption. The standard gradient used consists of 40 ml each of 0.001 and 1 M sulfate buffered to pH 4.4 with 0.001 M ammonium acetate. The linear gradient formed is pumped through the column at 1 ml min^{-1} at a column temperature of 50°.

Samples are prepared by removing the acyl blocking groups from chemically synthesized oligomers[10] with ammonium hydroxide, evaporation to remove ammonia, then solution in an appropriate volume of starting buffer. Normal sample size ranges from 0.01 to 1 optical density unit measured at 254 nm, the monitoring wavelength. The lower limit of detectability ranges from 0.001 to 0.01 A_{254}, depending on the elution volume.

RESULTS AND DISCUSSION

The rapidity of high-pressure liquid chromatography commends itself to application in monitoring the homogeneity of chemically synthesized oligonucleo-

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TABLE I

ELUTION VOLUMES OF OLIGONUCLEOTIDES

Oligomer *	*Chain-length*	*Elution volume (ml)*
d-p(A-A-T-T)	4	8.7
d-p(C-G-G-C)	4	9.9
d-EtSp(T-G-T-C)	4	10.3
d-EtSp(T-T-T-T)	4	11.1
d-EtSp(T-T-A-G)	4	11.9
d-EtSp(T-T-T-C)	4	12.8
d-p(C-A-A-A-T)	5	10.2
d-p(C-T-G-T-C)	5	10.7
d-EtSp(C-T-G-T-C)	5	11.3
d-p(T-G-A-A-T-T)	6	14.4
d-EtSp(T-T-A-G-C-A)	6	15.0
d-EtSp(T-T-A-A-T-C)	6	15.3
d-p(G-C-C-G-C-A-G)	7	15.2
d-p(T-G-T-C-T-T-T)	7	17.9
d-EtSp(T-G-T-C-T-T-T)	7	20.1
d-p(G-G-A-T-T-A-A)	7	19.9
d-EtSp(T-T-A-A-T-C-C-A-T)	9	19.5
d-EtSp(A-T-G-G-A-A-A-C-T-G)	10	26.1
d-p(T-G-C-T-A-A-A-T-T-T-G-A)	12	25.7
d-EtSp(T-G-C-T-A-A-A-T-T-T-G-A)	12	28.2
d-EtSp(A-T-G-G-A-A-A-C-T-G-C-G-G-C)	14	32.1

* For nomenclature, see footnote in ref. 10.

tides. The elution volumes of a number of representative oligomers are listed in Table I.

These data were obtained with a 1.2×500 mm column of Zipax WAX using a concentration gradient as described under MATERIALS AND METHODS. The flow-rate was 1.1 ml min^{-1}, giving a linear velocity of 1.6 cm sec^{-1}. As the elution volumes indicate, oligomers are separated not only according to chain-length, but also to some extent by base composition. This is probably due to the protonation of d-pC and d-pA at pH 4.4. Another factor is the increased retention volume of oligomers bearing an ethylmercapto blocking group on the 5'-phosphate. There is presumably a non-ionic interaction between the sulfur atom and the polymer support which compensates for the loss of a charge on the phosphate group.

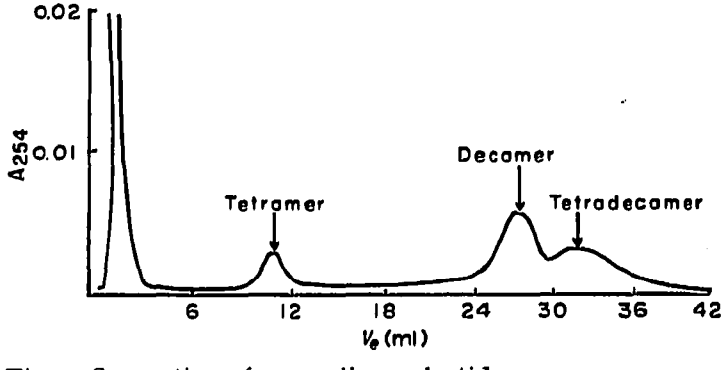

Fig. 1. Separation of some oligonucleotides.

These oligomers are prepared by block condensations. The technique constitutes an analytical method for monitoring condensation reactions. In Fig. 1, the separation of a tetradecamer from its component tetramer and decamer in a reaction mixture is shown.

The yield of a product can be calculated from the chromatogram by integrating the peak areas and normalizing with the calculated extinction coefficients. In cases where a peak is of unknown structure, it is possible to collect the peak, desalt by gel filtration or thin-film dialysis, and carry out a nucleotide composition analysis. Where small amounts of pure materials are necessary, this procedure has been used preparatively; for example, substrates for joining to other oligomers by means of polynucleotide ligase[11] have been purified by this method.

We are continuing to investigate other weak anion-exchange supports and buffer systems for applicability. A much larger column is undergoing evaluation as a possible means of preparative separation on a 10- to 100-μmole scale. A fuller report of this method will be the subject of a future publication.

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REFERENCES

1 H. R. Matthews, *Eur. J. Biochem.*, 7 (1968) 96.
2 R. Černý, E. Černá and J. H. Spencer, *J. Mol. Biol.*, 46 (1969) 145.
3 R. Salmon and A. M. Kaye, *Biochim. Biophys. Acta*, 204 1970) 342.
4 E. Junowicz and J. H. Spencer, *Biochemistry*, 9 (1970) 3640.
5 G. Rushizky and J. H. Mozejko, *Anal. Biochem.*, 42 (1971) 535.
6 G. Haegeman, W. Min Jou and W. Fiers, *J. Mol. Biol.*, 57 (1971) 597.
7 S. D. Ehrlich, J. P. Thiery and G. Bernardi, *J. Mol. Biol.*, 65 (1972) 207.
8 K. L. Agarwal, A. Yamazaki, P. J. Cashion and H. G. Khorana, *Angew. Chem.*, 84 (1972) 489.
9 T. F. Gabriel and J. Michalewsky, *J. Chromatogr.*, 67 (1972) 309.
10 E. P. Heimer, M. Ahmad and A. L. Nussbaum, *Biochem. Biophys. Res. Commun.*, 48 (1972) 348.
11 C. Harvey, to be published.