Journal of Chromatography, 135 (1977) 189–192

CHROM. 9887

Note

Purification of oligodeoxynucleotides on Sephadex G-10 columns

P. J. CASHION*, H. J. NOTMAN, T. CADGER and G. M. SATHE
Biology Department, The University of New Brunswick, Fredericton, New Brunswick E3B 5A3 (Canada)
(Received December 20th, 1976)

During the chemical synthesis of DNA, the various protected oligonucleotide intermediates which accumulate must be characterized by a number of analytical methods including spectral measurements, paper (PC) and thin-layer chromatography (TLC) and nuclease digestion[1]. Prior to analysis, the particular oligonucleotide which has protecting groups attached to its bases, sugars and phosphates must be treated sequentially with base and acid to remove these groups. Taken together these analytical methods require mg quantities of the deblocked intermediates for proper analysis. Hitherto the established method for isolating the deprotected oligonucleotide has been PC of the deprotection mixture (requiring *ca.* 16 h) followed by elution and concentration of the desired oligomer (requiring *ca.* 1 h)[2].

A related consideration is the common use of pyridine as a solvent in nucleic acid chemistry and the fact that its strong absorbance in the 250–275 nm range precludes a direct spectral characterization of the nucleotides dissolved therein. By removing excess pyridine under vacuum and exchanging the protonated pyridine, present as a counter-ion to the phosphate groups, with excess aqueous ammonia, the desired spectrum may be recorded[2]. This method, while adequate, results occasionally in loss of sample because of bumping of the aqueous ammonia solution under vacuum.

We report herein a method for the fractionation of the various components of the deprotection mixture based upon Sephadex G-10 or Bio-Gel P-2 column chromatography; we feel this method is simpler, faster and has a higher capacity for isolating mg quantities of oligonucleotides than the currently used methods. This approach results in the isolation, within 45–60 min, of 1–10 mg quantities of pure oligonucleotide (ranging in size from di- to undecanucleotides) free of protecting groups, salts (such as pyridinium acetate) and pyridine.

EXPERIMENTAL AND RESULTS

The protected hexanucleotide[3] [$(MeO)_2$Tr]dbz^{6}A.an^{4}C.an^{4}C.bz^{6}A.ac^{2}G.an^{4}C* was treated with base and acid to remove all protecting groups to give the deprotected

* To whom correspondence should be addressed.

* Abbreviations for the synthetic nucleotides are as suggested by the IUPAC-IUB.[4] A phosphodiester bond is represented by a hyphen (-); a phosphotriester bond by a period (.); each internal triester bond has a *p*-chlorophenyl group attached.

hexanucleotide d(A-C-C-A-G-C) together with acetic acid and the protecting groups: dimethoxytritanol, acetic, benzoic and anisic acids, *p*-chloro phenol, pyridinium acetate salts and free pyridine. The excess pyridinium acetate was removed under high vacuum after *ca.* 30 min; the residue, dissolved in 300 μl ethanol–water–pyridine (1:1:1), was further diluted to 1.5 ml with 80% aqueous ethanol and applied to a Sephadex G-10 column, 2 × 63 cm, equilibrated with aqueous ammonia (4 drops conc. ammonia per liter). Fractions of 3 ml were collected every 2 min. The elution profile is shown in Fig. 1. The first peak contained only hexanucleotide (molecular weight *ca.* $2 \cdot 10^3$ daltons) based upon chromatography in a number of TLC and PC systems; its spectrum with an absorbance maximum at 263 nm was close to the predicted one. The second and smallest peak with an absorbance maximum in the 250–255 nm region contained, among other species, anisic and benzoic acid amides; the final peak, emerging surprisingly far behind the second (given their similar molecular weights) contained pyridine (based on its UV spectrum). Recovery of hexanucleotide added to the column was *ca.* 90%. Similar patterns were obtained with shorter oligomers such as d(A-C), d(C-A), d(G-C), d(A-C-C) and d(A-C-C-A). No separation between the respective oligomers [*e.g.*, between d(A-C) and d(A-C-C-A-G-C)] has been observed. As may be seen from Fig. 2, one does not, in fact, have to remove the large amount of acetic acid and its salts left over from the acid-catalyzed detritylation reaction by lyophilization as was done in Fig. 1; the entire mixture may be applied directly to a column and eluted. The acetic acid emerged as a large trailing peak after fraction 60 (as determined by pH paper).

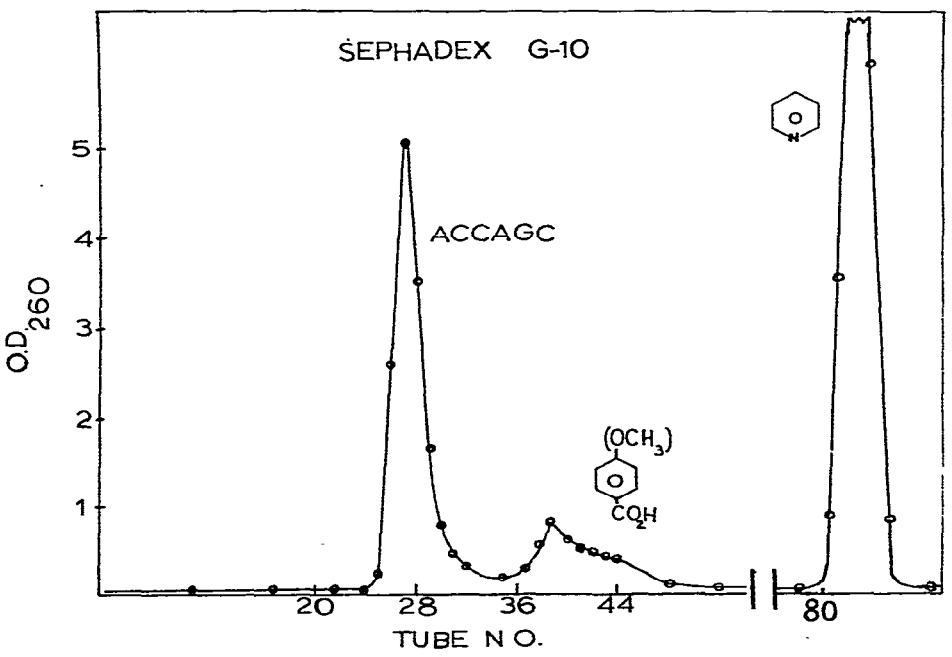

Fig. 1. Sephadex G-10 chromatography of hexanucleotide d(A-C-C-A-G-C) deprotection mixture. See text for experimental details.

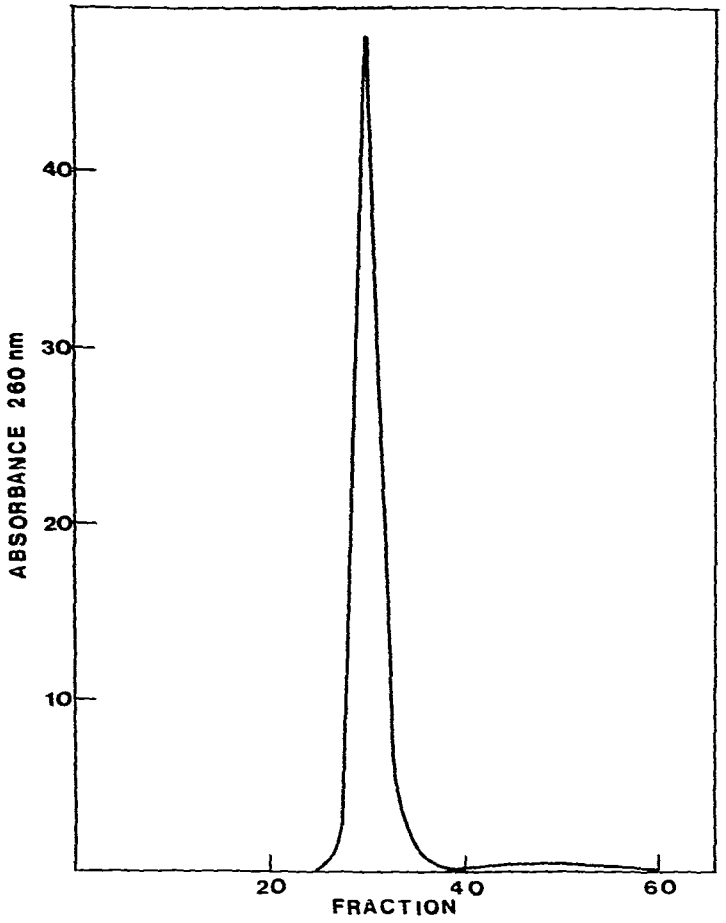

Fig. 2. Bio-Gel chromatography of the undecanucleotide d(A-G-G-T-C-G-A-A-G-G-T) synthesis reaction. The oligonucleotide deprotection solution (2 ml, acetic acid–pyridine–water 8:0.5:1.5) was added directly to a Bio-Gel P-2 column, 2 × 100 cm, equilibrated with aqueous ammonia (pH 8); elution was carried out with the same solution. The major peak represents the undecamer product plus the unreacted starting oligonucleotides; the smaller trailing peak the protecting groups. Fractions of 2 ml were collected every 5 min.

In summary, we have developed a simple method using Sephadex G-10 and Bio Gel P-2 columns for the rapid isolation (45–60 min) of mg quantities of deprotected DNA oligomers which may in turn be characterized directly by conventional spectral, chromatographic and nuclease digestion methods.

ACKNOWLEDGEMENT

Financial support from the National Research Council of Canada is acknowledged gratefully.

REFERENCES

1 K. L. Agarwal, A. Yamazaki, P. J. Cashion and H. G. Khorana, *Angew. Chem., Int. Ed. Engl.*, 11 (1972) 451.
2 H. G. Khorana, K. L. Agarwal, P. Besmer, H. Büchi, M. Caruthers, P. J. Cashion, M. Fridkin, E. Jay, K. Kleppe, R. Kleppe, A. Kumar, P. C. Loewen, R. C. Miller, K. Minamoto, A. Panet, U. L. RajBhandary, B. Ramamoorthy, T. Sekiya, T. Takeya and H. van der Sande, *J. Biol. Chem.*, 251 (1976) 565 and subsequent 10 papers in this issue.
3 P. J. Cashion, K. Porter, T. Cadger, G. Sathe, T. Tranquilla, H. Notman and E. Jay, *Tetrahedron Lett.*, (1976) 3769.
4 IUPAC-IUB, *Biochemistry*, 9 (1970) 4022.