Isolation of certain Oligonucleotides Obtained by Degradation of Deoxyribonucleic Acid

By K. BURTON

Medical Research Council Cell Metabolism Research Unit,
Department of Biochemistry, University of Oxford

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1. Pyrimidine oligodeoxynucleotides containing one thymine nucleotide residue and up to five cytosine nucleotide residues were isolated by chromatography on DEAE-Sephadex after degrading calf-thymus DNA with diphenylamine in aqueous formic acid.

In order to test a method for the selective degradation of thymidine in oligonucleotides (Burton & Riley, 1966), it was decided to isolate the series of oligodeoxynucleotides $(C_nT)p_{n+2}$ * containing one thymine nucleotide residue and different numbers

of deoxycytidine nucleotide residues. This short paper describes their isolation from calf-thymus DNA by an extension of chromatographic separations on DEAE-Sephadex that were used to isolate C_nP_{n+1} oligonucleotides (Petersen & Burton, 1964). The amounts of the $(C_nT)p_{n+2}$ nucleotides recovered agree closely with those expected from analyses by other methods (Petersen, 1963).

Table 1. Isolation of $(C_nT)p_{n+2}$ oligonucleotides

Calf-thymus DNA was degraded with 2% (w/v) diphenylamine in 66% (v/v) formic acid and adsorbed on to a column (28 cm. $\times$ 2.3 cm. diam.) of DEAE-Sephadex. P_1 and cytosine nucleotides C_nP_{n+1} were eluted by 2.5N-formic acid. The following fractions (200 ml.) were eluted with a 0.0–0.5M linear gradient of ammonium formate in 3l. of 2.5N-formic acid. E_{280} and E_{260} values were measured after fivefold dilution with 2N-HCl.

Fraction	E_{280}	E_{280}/E_{260}	Provisional identity
1	0.127	0.68	—
2	0.119	0.75	—
3	0.900	1.41	? Mainly $(C_2T)p_4$
4	8.33	1.29	Mainly $(CT)p_3$
5	7.40	0.85	$TP_2 + (CT)p_3$
6	1.82	1.09	? $TP_2 + (C_nT_2)p_{n+2}$
7	3.15	1.19	$(C_nT_2)p_{n+3}$
8	3.82	1.01	? Mainly $(C_2T)p_4$
9	2.95	0.84	$T_2P_3 + \text{other components}$

* Abbreviations: Since all the compounds described in this paper are deoxyribonucleotides, the prefix 'd' is omitted. C and T represent deoxycytidine and thymidine respectively; p represents phosphate esterified with the nucleosides. When p is to the right of the nucleoside symbol it is esterified with the 3'-hydroxyl of the nucleoside; when it is to the left it is a 5'-ester. Thus CpT represents deoxycytidylyl-(3'-5')-thymidine. Sequences that are unknown or expected to consist of mixtures of isomeric nucleotides in unknown proportions are written with the nucleoside symbols in parentheses, e.g. $(CT)p$ represents a mixture of CpT and TpC.

EXPERIMENTAL

Calf-thymus DNA, prepared by the method of Hurst (1958), was degraded with diphenylamine and formic acid and the reagents were removed by ether extraction and

Table 2. Extinction ratios and amounts of $(C_nT)p_{n+2}$ oligonucleotides isolated from calf-thymus DNA

Fractions 1–5 (Table 1) were pooled and the material was chromatographed on a column (32 cm. $\times$ 1 cm. diam.) of DEAE-Sephadex (HCO_3^- form). The eluting solvent was NH_4HCO_3 , pH 8.8, with a 0.0–0.41M linear gradient in 570 ml. followed by 0.41–1.1M in the next 700 ml. E_{280}/E_{260} ratios were measured in 0.1N-HCl on the main portion of each peak. The amounts shown are the totals recovered and include allowances for the small amounts in the regions between the peaks. They are compared with those reported by Petersen (1963), who separated the nucleotides on paper after treatment with phosphomonoesterase.

Peak	E_{280}/E_{260}	Amount recovered (moles of pyrimidine/100 moles of DNA phosphate)	Amounts reported by Petersen (1963)
$(CT)p_3$	1.26	5.15	5.19
$(C_2T)p_4$	1.54	2.70	2.64
$(C_3T)p_5$	1.65	1.19	1.39
$(C_4T)p_6$	1.65	0.50	0.39
? $(C_5T)p_7$	—	0.1	—

evaporation to dryness (Burton & Petersen, 1960; Petersen & Burton, 1964). An amount containing $2.17 \mu\text{moles}$ of total phosphate was adsorbed on to a column ($28 \text{ cm.} \times 2.3 \text{ cm. diam.}$) of DEAE-Sephadex A-25 (formate form). P_i was eluted by 2.5 N -formic acid, appearing at about 0.41. The cytosine nucleotides C_nP_{n+1} were eluted later as a composite peak at about 1.51. When 3.41. had been collected, elution was continued with a gradient of ammonium formate in 2.5 N -formic acid (Table 1). Under these conditions, $(\text{C}_n\text{T})\text{P}_{n+2}$ compounds are eluted first and are followed by the group of compounds $(\text{C}_n\text{T}_2)\text{P}_{n+3}$. Fractions 1–5, comprising the $(\text{C}_n\text{T})\text{P}_{n+2}$ compounds, were pooled, freeze-dried and separated into individual components pTp , $(\text{CT})\text{p}_3$, $(\text{C}_2\text{T})\text{p}_4$ etc. by chromatography on DEAE-Sephadex (HCO_3^- form) with NH_4HCO_3 as the eluting solvent (Table 2). The amounts eluted agree well with those found by Petersen (1963) using other methods. For use in the experiments described in the following paper (Burton & Riley, 1966), the bulk of each peak, excluding the 'head' and 'tail' fractions, was pooled and the salt was removed by rotary evaporation.

For similar separations of the C_nP_{n+1} nucleotides, Petersen & Burton (1964) added urea (7 M) to the eluting solvents as a precaution against losses in chromatography due to secondary binding forces. However, it now appears that the urea is not necessary because it does not improve the recovery of these pyrimidine nucleotides from columns of DEAE-Sephadex. For example, the amounts of C_nP_{n+1} nucleotides recovered without the use of urea are (as moles of cytosine relative to those in C_2p_3): Cp_2 , 2.05; C_3p_4 , 0.41; C_4p_5 , 0.20; C_5p_6 , 0.05; these agree well with the highest recoveries previously obtained in the presence of urea.

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