CHROMATOGRAPHY OF TRI- AND TETRANUCLEOTIDES FROM PANCREATIC RIBONUCLEASE DIGESTS OF RIBONUCLEIC ACID. *

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Tomlinson and Tener (1962) found that 7 M urea at pH 7.5 greatly facilitated DEAE cellulose chromatography of pancreatic ribonuclease (RNase) digests of ribonucleic acid (RNA). The elution profiles showed a number of well-separated peaks each containing compounds of identical charge, emerging in the order of mono-, di-, tri- and higher oligonucleotides. This chromatography in 7 M urea is also useful for the resolution of individual members of a homologous series (Ap, ApAp, ApApAp, etc.). The subsequent characterization of the material in the peaks usually involves paper chromatography and/or paper electrophoresis. However, these methods are hampered by the decreasing solubility of tetra- and higher oligonucleotides (Lipsett and Heppel, 1963). Furthermore, the identification of such compounds requires quantities which may be handled on columns but which are too large for paper chromatography and paper electrophoresis. The results reported here show that mixtures of oligonucleotides

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of like charge may be further separated by column chromatography on DEAE-cellulose in 7 M urea and 0.1 M formic acid.

High-molecular weight RNA from yeast (Crestfield et al., 1955) was digested with RNase (Worthington) and fractionated by chromatography on DEAE-cellulose in 7 M urea at pH 7.5 with a linear gradient of 0-0.3 M NaCl (Tomlinson and Tener, 1963; Bartos et al., 1963). The material in the trimer and tetramer peak, characterized after desalting (Rushizky and Sober, 1962) and mapping (Rushizky and Knight, 1960) was separately applied to another DEAE-cellulose column equilibrated with 7 M urea but in 0.1 M formic acid. This second fractionation, carried out in the formic acid-7 M urea solution by a linear gradient to 0.1 M NaCl, further separated the tri- and tetranucleotides (Fig. 1). The order of emergence was consistent with their increasing negative charge at pH 3.1-3.3, (values measured with glass electrodes (Leeds and Northrup) for solutions of 7 M urea - 0.1 M formic acid with and without 0.1 M NaCl, respectively). The negative charges calculated were not corrected for the elevation of pK values reported for mononucleotides in 7 M urea (Stockx and Vandendriessche, 1963). When DEAE-cellulose columns were developed with 0.1 M formic acid and linear gradients of NaCl but without urea, quantitative recovery and the same order of emergence as in Fig. 1A were obtained with trinucleotides, but the peaks were broader. When tetranucleotides were used the same procedure (without urea) yielded poor recoveries, overlapping peaks and a different order of emergence than in Fig. 1B.

Chromatography on DEAE-cellulose in urea at pH 7 and 3 should facilitate the isolation of tetra- and larger oligonucleotides

in quantities sufficient to permit characterization by further enzymatic hydrolysis of the isolated fragments.

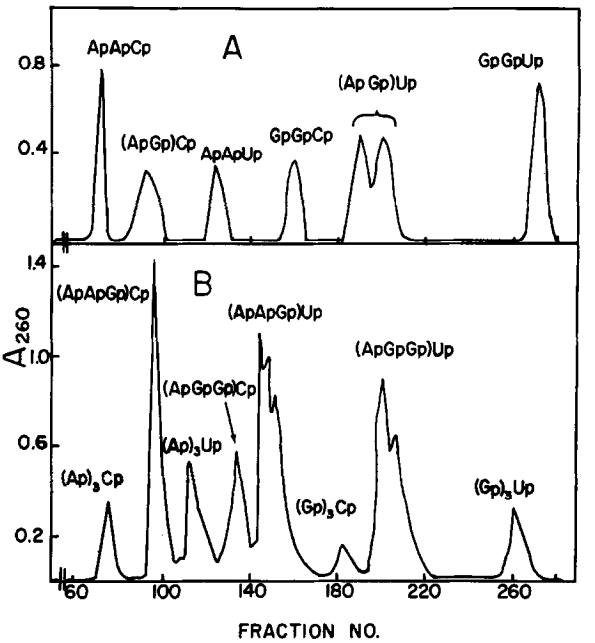

Fig. 1

A - Trinucleotide standards (30-60 absorbancy units at 260 mμ (A_{260}) each in a total volume of 2 ml. of water) were applied to a 2.2 x 75 cm. column packed with 30 g. of DEAE-cellulose (Whatman Floc DE-50, lot No. 76-81, 1.0 meq. N per g. dry adsorbent, 100-230 mesh) and a linear pressure gradient to 15 lbs. The column was equilibrated in 7 M urea and 0.1 M formic acid and developed in the same solution with a 4500 ml. linear gradient from 0.0 - 0.1 M NaCl at a flow rate of 60 ml./hour, 15 ml./fraction.

B - An aliquot (A_{260} = 1000) of a tetranucleotide peak was fractionated as in (A) but using a 6000 ml. linear gradient from 0.0 - 0.1 M NaCl at a flow rate of 100 ml./hour, 20 ml./fraction.

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