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Note

Separation of naturally occurring adenine nucleosides and nucleotides by anion-exchange chromatography

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Since the discovery of poly(A) sequences at the 3′ ends in mRNA[1], an intensive study of poly(A) metabolism has been made both at the analytic and enzymic levels. The poly(A) sequence is usually obtained by digestion of the purified mRNA with RNase A and T_1 (ref. 2). Recently, there have been some investigations on the enzymes which hydrolyze poly(A); the determination of the substrate specificity of these enzymes both for exoribonucleases[3] and for endoribonucleases[4–7] depends on methods available for the characterization of the products. In the present study we describe a chromatographic technique which could be used for the separation and identification of adenine nucleosides and nucleotides formed after enzymatic hydrolysis of poly(A). The basic principles of this method were described some time ago[8,9].

EXPERIMENTAL

Anion-exchange chromatography of synthetic mixtures of adenine nucleosides and nucleotides was carried out on DEAE-Sephadex A-25 (particle size, 40–120 μm; Pharmacia, Uppsala, Sweden). Adenosine, 2′,3′-AMP, 5′-AMP, ADP and ATP were obtained from Boehringer (Mannheim, G.F.R.), 2′-AMP and 3′-AMP from Serva (Heidelberg, G.F.R.) and 1-methyladenosine (1-m-Ado) and N^6-m-Ado from P-L Biochemicals (Milwaukee, Wisc., U.S.A.). A column (27 × 0.8 cm) with a cooling jacket was used for the separation. The DEAE-Sephadex gel was equilibrated with 0.05 *M* ammonium bicarbonate (pH 8.0). An amount of 1.0 ml of a mixture containing 100 μg of each of the nucleosides and nucleotides was applied to the column. After washing with the equilibration buffer, the column was eluted with a linear salt-and-pH gradient (200 ml) of 0.05 *M* NH_4HCO_3 (pH 8.0) to 0.4 *M* NH_4HCO_3 (pH 9.0, adjusted with ammonia). Fractions of 3 ml were collected; the flow-rate was 6 drops/min. The column was monitored with an Uvicord II at 254 nm.

RESULTS AND DISCUSSION

A typical elution profile of the chromatographic separation at 20° on DEAE-Sephadex A-25 with a linear salt-and-pH gradient is shown in Fig. 1. The eluted nucleosides and nucleotides could be identified on the basis of their previously deter-

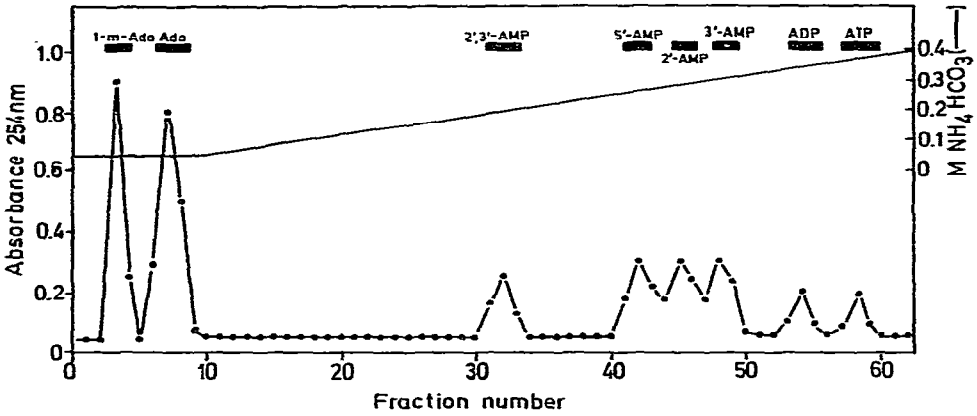

Fig. 1. Separation of adenine nucleosides and nucleotides on DEAE-Sephadex A-25 in an NH_4HCO gradient. The solid bars indicate the positions of the authentic compounds.

mined elution positions. In the presence of 0.05 *M* NH_4HCO_3 the nucleotides are bound to the anion exchanger, while the nucleosides are eluted. The first peak contains 1-m-Ado, whereas N^6-m-Ado is always eluted at the same position as Ado. The nucleosides can be recovered by increasing the concentration of NH_4HCO_3. For the separation of the monophosphates it was necessary to combine the use of the salt with a pH gradient. Under these conditions a resolution of 2',3'-AMP, 5'-AMP, 3'-AMP and 2'-AMP was obtained. The separation of these monophosphates allows identification of each suspected product of a poly(A)-degrading exoribonuclease. The advantage of the volatile bicarbonate buffer system used is the possibility of converting the separated nucleic acid component into a salt-free state[8].

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