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Ion Exchange Chromatography of Nucleoside Polyphosphates

ROLF BERGKVIST and ADAM DEUTSCH

Institute of Chemistry, University of Lund, Sweden

Ion-exchange chromatography of the mono-, di- and triphosphates of adenosine, guanosine, inosine and uridine on Dowex-1 formate columns by elution with formic acid containing successively increased amounts of formate, is described.

Separation of the different adenosine-5′-phosphates, *viz.* adenosine monophosphate (AMP), adenosine diphosphate (ADP) and adenosine triphosphate (ATP) by ion-exchange chromatography, has been achieved by Cohn and Carter[1] by using a column of the strong base resin Dowex-1 in the chloride form and elution by dilute hydrochloric acid, containing successively increased amounts of alkali chloride. Application of the method to the resolution of analogous series of polyphosphates, derived from other nucleosides, gives equally satisfactory results, as shown in the case of the inosine polyphosphates[2]. Resolution of mixtures containing both series of polyphosphates could even be accomplished by slight modification of the original method[2].

The isolation[3] of guanosine triphosphate (GTP) and uridine triphosphate (UTP) from rabbit skeletal muscle made further changes in the method necessary, in order to render it adaptable to the chromatography of mixtures containing polyphosphates of the different nucleosides, as they occur in muscle extracts. As previously reported, GTP and UTP — although clearly separated from ATP — appear in the effluent in the form of a mixture of both triphosphates on chromatography in the chloride system; in the presence of ITP*, all three triphosphates are eluted at the same anion concentration in the eluent. As no effective resolution could be achieved in the chloride system, recourse was made to the use of Dowex-1 formate columns and elution with formic acid solutions, containing progressively increased amounts of formate, an anion system introduced by Cohn and Carter[4] for the chromatography of nucleic

* ITP = inosine triphosphate; IDP = inosine diphosphate; IMP = inosine monophosphate; GDP = guanosine diphosphate; GMP = guanosine monophosphate; UDP = uridine diphosphate; UMP = uridine monophosphate.

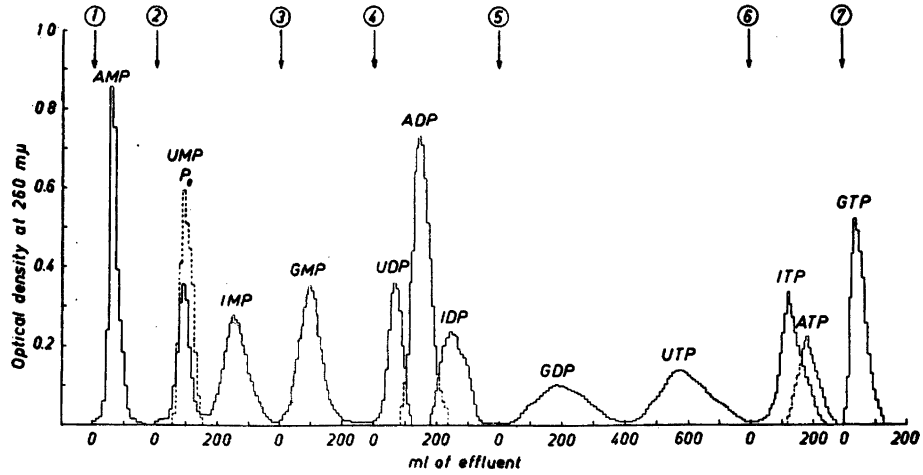

Fig. 1. Ion-exchange chromatography of nucleoside polyphosphates (for details see Experimental part).

acid fragments. Clean separation of GTP and UTP was accomplished in this system as briefly reported at the 2nd meeting of the Swedish Biochemical Society (Uppsala, November 28, 1953). Subsequently, a procedure for the separation of the mono-, di- and triphosphates of adenosine, guanosine, inosine and uridine was worked out, establishing the elution positions of the individual compounds, using Dowex-1 formate columns and formic acid—formate eluents. UMP* and orthophosphate are not separated by this method, but this can easily be achieved by other means. The ineffective separation of ATP and ITP on the other hand, necessitates a second chromatographic procedure in the chloride system for the preparative resolution of mixtures of these two triphosphates.

An ion-exchange procedure by means of a mechanically operated two-column system of anion-exchange with Dowex-1 in the formate form has been developed by Hurlbert, Schmitz, Drumm and Potter[5] for the separation of acid soluble nucleotides from tissue extracts including polyphosphates of cytidine, guanosine and uridine.

EXPERIMENTAL

Materials. The adenosine and inosine polyphosphates were prepared and purified as described previously[2]. GTP and UTP were isolated from rabbit muscle, purified by the present method and precipitated as the barium salts. GDP* and UDP* were obtained by the action[6] of actomyosin gel on the triphosphates and isolated as the barium salts. GMP* was a preparation of the barium salt, obtained by courtesy of Prof. A. R. Todd, Cambridge, and UMP was prepared by acid hydrolysis[7] of UTP. The barium salts were converted to the ammonium salts prior to chromatography, as previously described[2]. All chemicals used were of analytical grade.

Chromatography (Fig. 1). A column of Dowex-1 (250—500 mesh) in the formate form, 0.6 cm^2 × 6 cm, was used, with a flow rate 0.5 ml/min. A mixture of orthophosphate (1.160 mg), AMP (0.780 mg), ADP (1.380 mg), ATP (0.620 mg), GMP (0.970 mg),

GDP (0.780 mg), GTP (1.375 mg), IMP* (1.200 mg), ITP (1.160 mg), IDP* (1.480 mg), UMP (0.600 mg), UDP (0.770 mg) and UTP (1.320 mg) as ammonium salts at pH 11.0 was put on the column and, after washing with water, the following eluting agents were used in succession: (1) 0.1 *M* formic acid, pH 2.5; (2) 0.1 *M* formic acid + 0.05 *M* sodium formate, pH 3.4; (3) 0.1 *M* formic acid + 0.1 *M* sodium formate, pH 3.6; (4) 0.1 *M* formic acid + 0.3 *M* sodium formate, pH 4.1; (5) 0.1 *M* formic acid + 0.4 *M* sodium formate, pH 4.2; (6) 0.1 *M* formic acid + 0.5 *M* sodium formate, pH 4.3; (7) 0.1 *M* formic acid + 1.0 *M* sodium formate, pH 4.5. Recoveries, based on optical density measurements and in addition in some cases on phosphorus analysis, were at least 95 %.

Analytical procedures. Phosphorus determinations were performed as previously described[3] and optical densities were measured in a Beckman Universal Spectrophotometer, Model DU, the molecular extinction coefficients of 14 200 at 257 mμ for adenosine phosphates, 10 850 at 256 mμ for guanosine phosphates, 13 200 at 250 mμ for inosine phosphates and 10 050 at 262 mμ for uridine phosphates being used in the identification of the different fractions in the effluent (purine:total phosphorus:labile phosphorus).

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