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Note

Rapid separation of tissue nucleotides on conventional anion-exchange resins at low pressures

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In anticipation of investigating the pool size of nucleic acid precursors in human cell lines and the effects on the pool by the uptake of exogenous RNA and DNA[1,2], existing sensitive chromatographic methods[3–10] for the assay of tissue nucleotides were evaluated. A fairly rapid, sensitive method was sought.

Pellicular ion-exchange systems[11–13] seemingly meet most of our requirements; however, such systems employ specialized high-pressure (*e.g.*, up to 5000 p.s.i.) equipment that was not immediately available to us. Anion-exchange chromatography of the nucleoside phosphates on reversed-phase columns[10] was cursorily investigated, and some promising rapid separations of either the mono-, di-, or triphosphate nucleosides were made on the citrate or perchlorate forms of the quaternary ammonium compound used as the packing. However, mixtures of the nucleoside phosphates were not completely separated in short periods of time, and this system has inherent difficulties as to reproducibility in the preparation of the packing and in the employment of stable and reusable columns. Nevertheless, the success of achieving sharp separations among some of the nucleoside phosphates on the reversed-phase columns, particularly when citrate or perchlorate ions served as the eluting ions, suggested that such counter-ions could be used to the same advantage in the existing conventional anion-exchange procedures that are available for the analyses of nucleoside phosphate mixtures. Thus, existing methods[14–16] were reinvestigated using only counter-ions such as citrate or perchlorate which have a very high order of selectivity for the polystyrene-type resins. The compounds of almost all such counter-ions are nonvolatile substances, but this is of no consequence in a strictly analytical system where recovery of material is not necessary.

One exchanger chosen for these studies was Aminex A-27 (Bio-Rad Labs., Richmond, Calif., U.S.A.), a fine-particle (12–15 μ) styrene–divinylbenzene anion exchanger containing quaternary ammonium exchange groups. As seen in Fig. 1, the twelve common nucleoside phosphates can be separated on a short column (0.62 × 10 cm) in about 1 h at relatively low pressures with alkaline sodium citrate solution as the eluent. The chromatogram in Fig. 1 compares very favorably to similar analyses obtained with pellicular ion exchangers[11–13], particularly in regard to time of analysis and the degree of resolution. As noted in Fig. 1, the separation is carried out at a relatively fast flow-rate (about 2 ml/min cm^2). At half this rate, the analysis takes about

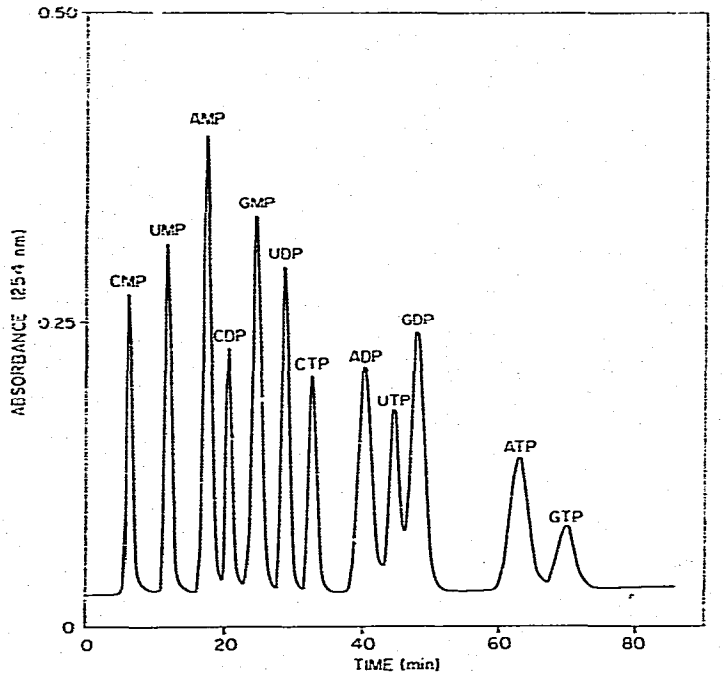

Fig. 1. Separation of the ribonucleoside 5′-mono-, -di-, and -triphosphates. Conditions: column, 0.62 × 10 cm of Aminex A-27 (citrate form); elution, convex gradient formed with a closed constant volume mixing container and an open reservoir vessel. The former contained initially 25 ml of 0.025 *M* sodium citrate solution, and the latter 0.5 *M* citrate solution, both at pH 8.3; flow-rate, 0.6 ml/min; column temperature, 70°; operating pressure, *ca.* 40 p.s.i. Sorbed material was *ca.* 30 nmoles each of the 5′-mono-, -di-, and -triphosphates of cytidine, uridine, adenosine, and guanosine contained in about 30 µl of 0.025 *M* sodium citrate solution; the sample was applied to the column by an off-column septum-type injector with a Hamilton syringe without interrupting the flow of the eluent; recovery was quantitative. The column was monitored with a model UA-2 ISCO ultraviolet analyzer (10-mm path-length flow cell of 50 µl capacity). Peaks were quantitated by peak height and peak width measurements[17,18] of the tracings from an external recorder attached to the ISCO.

2.5 h to complete, but each peak is completely separated from its neighbor with a resolution[18,19] of 1 or greater, and the operating pressure at this flow-rate is only *ca.* 20 p.s.i.

There are several advantages in using conventional ion-exchange resins instead of pellicular exchangers for tissue nucleotide analysis. One of the main advantages lies in the high capacity of the conventional ion exchangers. With a properly chosen counter-ion, this allows, as shown in Fig. 1, difficult separations to be made on short columns in short periods of time. Also, since the columns are short, they can be operated at low pressures compared to pellicular ion-exchange systems. This, in turn, allows one to use readily available, inexpensive chromatographic equipment. However, one advantage of tissue nucleotide analyses via pellicular ion-exchange systems is that with the newer pellicular ion exchangers, separations can be performed at ambient temperatures[13]. In the experiments reported here, a column temperature of at least 50° was necessary before sharp peaks of nucleoside phosphates were obtained.

The citrate form of Aminex A-27 can also be used for the rapid analysis, at

alkaline pH's, of the bases or nucleosides derived from nucleic acids. For the commonly occurring derivatives, such analyses can be performed in less than 30 min on the column described in Fig. 1 with dilute alkaline sodium citrate as the eluent (see also ref. 19). If present with nucleotide material, the bases or nucleosides would interfere with the nucleoside phosphate analysis. However, I have developed a group separation scheme that allows nucleotides and nucleosides to be separated in narrow elution bands from each other and from the purine and pyrimidine bases on polyacrylamide gel columns[20]. Thus, small aliquots of these components (nucleotides, nucleosides, or the free bases) once separated, can be analyzed according to the conditions of Fig. 1, or slight variations of those presented there.

Various parameters associated with these separations are now under investigation to optimize conditions for the routine analyses of samples containing tissue nucleotides.

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