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Letter to the Editor

Determination of purine compounds by ion-pair microbore high-performance liquid chromatography: application to ischemic rat kidney mitochondria

Sir,

The main advantage of ion-pair reversed-phase high-performance liquid chromatography (IP-HPLC) consists in the simultaneous measurement of nucleotides, nucleosides and nucleobases [1,2] and it has been applied to various biological materials, e.g. erythrocytes [3], bone marrow cells [4] and heart [5], to determine purine compounds.

We have optimized a method previously applied to isolated mitochondria [6] by using a microbore column. The developed IP-HPLC method allows the simultaneous quantification of nucleotides and their catabolites in mitochondria with high efficiency at a flow-rate of 0.1 ml/min.

EXPERIMENTAL

Chemicals

Nucleotides, nucleosides and nucleobases used as standards were purchased from Sigma (Deisenhofen, F.R.G.) and Boehringer (Mannheim, F.R.G). UV spectrograde acetonitrile and tetrabutylammonium bromide were obtained from VEB Petrolchemisches Kombinat (Schwedt, G.D.R.) and Zentralinstitut für Ernahrung (Potsdam, G.D.R.), respectively.

Equipment

A 1090 M HPLC system from Hewlett Packard (Vienna, Austria) was used, consisting of a ternary DR 5 solvent-delivery system, a diode array detector, an autosampler, an autoinjector and a 79 994 A LC workstation. The column

0378-4347/90/$03.50

was a vertex microbore column (100 mm×2.0 mm I.D.) with an integrated precolumn (7 mm×2.0 mm I.D.) from Knauer (Bad Homburg, F.R.G). Both columns were prepacked with 3-μm ODS Hypersil (Shandon).

Preparation of mitochondria

Mitochondria from non-ischemic and ischemic rat kidney were prepared as described elsewhere [7].

Extraction

A 700-μl aliquot of mitochondrial suspension (ca. 10 mg protein per ml) was treated with 70 μl of 0.33 *M* ice-cold perchloric acid and centrifuged (11 000 *g*; 2 min); 500 μl of the supernatant were neutralized with 75 μl of a solution containing 2 *M* K_2CO_3 and 0.5 *M* triethanolamine. After centrifugation, the neutral extract was cleaned by passing through a cellulose nitrate filter from Sartorius (0.45 μm).

HPLC method

Mobile phase A consisted of 10 m*M* $NH_4H_2PO_4$ (pH 5) and 2 m*M* tetrabutylammonium bromide, and mobile phase B of 10 m*M* $NH_4H_2PO_4$ (pH 6.5), 0.5 m*M* tetrabutylammonium bromide and 25% (v/v) acetonitrile. The gradient elution started with 7% of mobile phase B, increased linearly to 30% B during 12 min and continued for 2 min to 80% B. The column was equilibrated under the initial conditions for 10 min before the next injection. The flow-rate was 0.1 ml/min, and 5 μl of the filtered extract were injected.

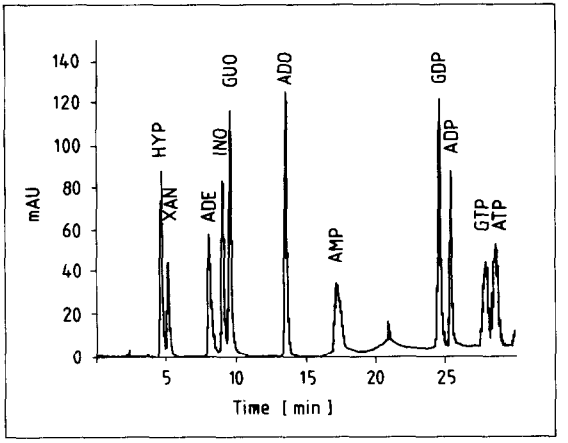

Fig. 1. Separation of purine standards by ion-pair microbore HPLC. Chromatographic conditions were as outlined in *HPLC method*. Peaks: HYP = hypoxanthine; XAN = xanthine; ADE = adenine; INO = inosine; GUO = guanosine; ADO = adenosine; AMP = adenosine 5′-monophosphate; GDP = guanosine 5′-diphosphate; ADP = adenosine 5′-diphosphate; GTP = guanosine 5′-triphosphate; ATP = adenosine 5′-triphosphate.

Purines of interest were routinely identified and checked for purity by the software facilities of the LC workstation. The library software compares spectra acquired during the run by the diode array detector with those of standards stored in the library, and the peak purity function uses spectra corresponding to the upslope, apex and downslope of the peak of interest.

RESULTS AND DISCUSSION

The main advantage of the developed method is the reduction of the flow-rate to 0.1 ml/min. This extremely low flow-rate reduces the consumption of mobile phases 10–30 fold compared with that of standard analytical columns

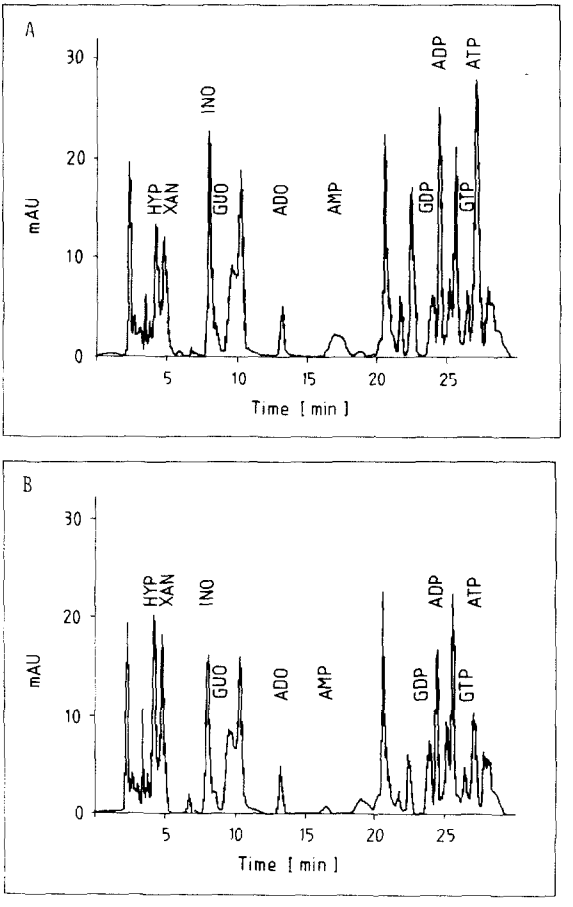

Fig. 2. Chromatograms of neutralized acid extracts obtained from non-ischemic (A) and ischemic (B) rat kidney mitochondria. Experimental conditions as described in Experimental. For peak identification see Fig. 1.

[1–6]. Fig. 1 illustrates the separation of a mixture of standards. The separation time of ca. 30 min corresponds to that achieved with a standard column [3–6].

It is known that the application of IP-HPLC methods to different biological materials requires special adaptations [1,6]. The extraction procedure and the chromatographic conditions described here for the microbore technique are suitable for the determination of purine compounds of kidney mitochondria. Fig. 2 shows chromatograms obtained from extracts of mitochondria from non-ischemic and ischemic rat kidney.

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