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Short communication

Measurement of nitrite and nitrate levels in biological samples by capillary electrophoresis

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Abstract

Nitrite is one of the products of NO-synthase in biological media. It is slowly oxidized in animals to nitrate. We developed a simple and rapid method to determine simultaneously nitrite and nitrate in biological samples. Capillary ion electrophoresis with direct UV detection at 214 nm was used employing a carrier electrolyte consisting of 10 m*M* sodium sulfate and an electroosmotic flow modifier. The detection limit in ultrafiltrates of plasma, urine and brain tissue extracts was 25 ng/ml for both compounds. Nitrate levels in human plasma and urine were in the μg/ml range. Nitrite could not be detected. Rat brain tissue extracts contained detectable amounts of nitrite and nitrate.

1. Introduction

The biological determination of nitrite is gaining importance since it was demonstrated that an ubiquitous and multiple messenger essential for cellular life [endothelial derived relaxing factor (EDRF)] is degraded to nitrite [1,2]. Numerous methods have been used for nitrite determination; mainly spectrophotometric techniques with Griess coloration [3], enzymatic techniques, and high-performance liquid chromatography with either UV absorbance or electrochemical detection [4,5]. Capillary electrophoresis has been used for anion and cation determination in various media [6].

Here we describe a simple and sensitive method for the determination of nitrite and nitrate in biological samples.

2. Experimental

2.1. Capillary electrophoresis

Nitrite and nitrate determinations were performed with either a CIA analyser or a Quanta 4000 interfaced to the Millennium software which permitted automated processing of the analytical results (Waters, Paris, France). The voltage was set at 15 kV (negative mode) and a fused-silica capillary was used (60 cm × 100 μm I.D.). The electrolyte was sodium sulfate con-

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taining an electroosmotic modifier (OFM-OH, Waters, Paris) in pure bidistilled water (2.5 ml OFM-OH in 100 ml of water, 10 m*M* Na_2SO_4). Nitrite and nitrate standards were commercially available (Analys, Manosque, France) and dilutions were made in pure bidistilled water on each analysis day. The detector was set at 214 nm and 0.02 AUFS with a 0.3-s time constant. The temperature was set at 25°C for both the capillary and the electrolyte. Hydrostatic injection was realised during 20 s (10 cm height).

2.2. Sample preparation

All biological samples were ultrafiltered using a commercial device (Ultrafree-CL, Millipore). A 2-ml sample of human plasma or urine was centrifuged at 1000 *g* for 30 min at 4°C; the ultrafiltrate was diluted with pure bidistilled water before injection. The dilution factor was 1:50 to 1:100 for urine and 1:10 for plasma. For tissue determinations rat brain cortex was used; 40–200 mg wet weight was crushed in 2 ml of pure bidistilled water, then ultrafiltred and injected.

3. Results and discussion

Electropherograms of human urine and rat brain tissue extract are shown in Figs. 1 and 2, respectively. Nitrite and nitrate migration times are respectively 3.1 ± 0.3 min and 3.16 ± 0.3 min; the addition of nitrite or nitrate standards confirmed their identity. The regression lines of the detector response (mV) vs. standard concentrations (ng/ml) were linear with correlation coefficients $r = 0.9978$ for nitrate and $r = 0.9973$ for nitrite over the range studied (10 ng/ml–5 μg/ml). The ultrafiltrates were stable at ambient temperature for a few hours without any conversion of nitrite to nitrate; this permitted multiple injections of the same sample. The detection limit in the ultrafiltrate was 25 ng/ml for nitrite and nitrate at a signal-to-noise ratio of 3. The intra- and inter-assay reproducibility of the nitrite and nitrate assay were below 2.4% and 5%, respectively. The analytical recovery in the range studied was 95.2–104.5%. Storage of the ultrafiltrates at −80°C showed no detectable change after 6 months. Plasma and urine nitrate concentrations in men were 92 ± 18.5 μmol/l (n =

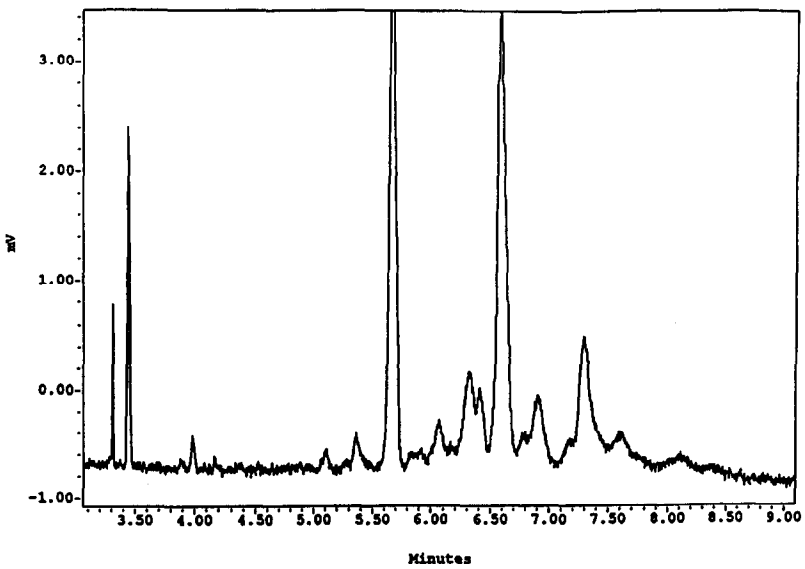

Fig. 1. Electropherogram of human urine diluted 1:100. The nitrate peak is at 3.43 min and the amount is 2.15 μg/ml. Electrolyte was sulfate-OFM.

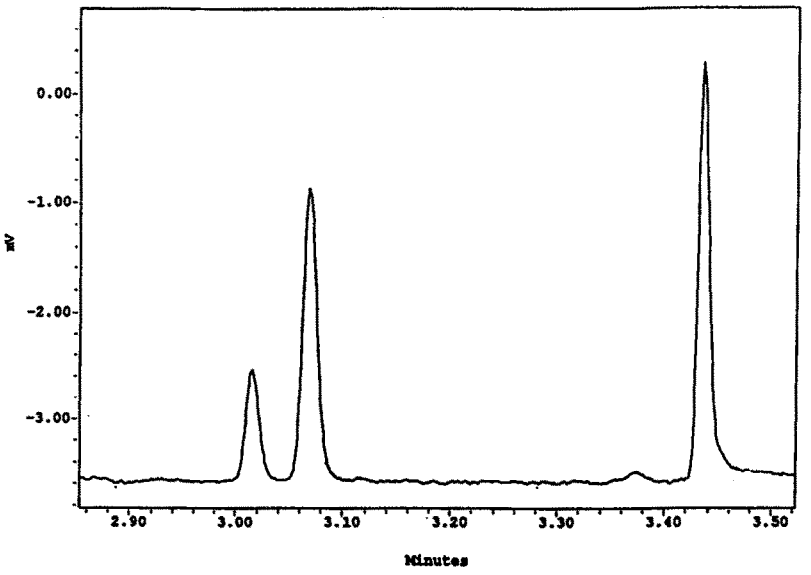

Fig. 2. Electropherogram of rat brain ultrafiltrate. Nitrite and nitrate peaks were at 3.02 and 3.07 min respectively and their concentrations were 0.20 and 0.54 μg/ml, respectively.

10) and 2.2 ± 1.8 mmol/l ($n = 10$), respectively. Nitrite could not be detected in human plasma and urine. Nitrite and nitrate rat brain concentrations were 47.6 ± 34.4 μmol/kg ($n = 10$) and 253.5 ± 111.2 μmol/kg ($n = 10$), respectively.

In conclusion, nitrite and nitrate determinations in biological samples are necessary and useful because nitrite is formed directly by EDRF degradation and subsequently transformed to nitrate by haemoglobin or other molecules. Nitrite could not be detected in plasma and urine, as was also observed by others, due to the presence of multiple oxidants [7]. Nitrate was always found in biological ultrafiltrates. The measured detection limit for nitrite was 800 ng/ml for urine and plasma due to the high chloride concentration in the ultrafiltrates. This detection limit for nitrite could be lowered by using anion-exchange resins. This was not necessary in our hospital laboratory. The nitrate assay gave always a sufficient response for clinical practice. We have also tried to use a chromate-OFM electrolyte with indirect UV detection. This allowed the detection of other anions but nitrite is masked by sulfate and the observed detection limit is identical; the sulfate-OFM electrolyte was also tested at 185 nm but the noise increased more rapidly than the signal; moreover, the chloride peak is also multiplied by a factor of 25. Determination of nitrite and nitrate in tissues was possible due to the high concentrations of the analytes. Gradual conversion of nitrite to nitrate was observed in biological tissues, which is catalyzed by cellular components and ferrohemoproteins. It is important to detect nitrate and nitrite simultaneously. The assay described here is simple and rapid and permitted the simultaneous detection of nitrite and nitrate levels in biological materials.

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