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Short communication

Determination of N-nitroso-L-arginine in rat brain extracts by capillary electrophoresis using photodiode-array detection

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Abstract

N-Nitroso-L-arginine was described as one of the products of L-arginine metabolism in biological media. A simple and rapid method to determine its concentration in rat brain was developed. Capillary electrophoresis with a photodiode-array detector was used at 254 nm, permitting the quantification of N-nitroso-L-arginine. The detection limit in biological solution was 1 μg/ml.

Keywords: N-Nitroso-L-arginine

1. Introduction

N-Nitroso-L-arginine was recently discovered by voltammetry in rat brain, but at present there is no other analytical technique permitting its determination [1]. Numerous methods have been used for amino acid determination and are used to detect L-citrulline, N-hydroxy-L-arginine and L-arginine in biological media [2]. Analysis by capillary isotachophoresis of S-nitroso compounds was successful [3] and capillary zone electrophoretic detection of biological S-nitro derivatives was realised without derivatization [4]. Here, a simple and sensitive method for the determination of N-nitroso-L-arginine by capillary electrophoresis in biological solutions is presented.

2. Experimental

2.1. Capillary electrophoresis

Determinations were performed with a P/ACE system 5510 with a photodiode-array detector using dual-wavelength detection at 205 and 254 nm (Beckman, Gagny, France). The voltage was set at 30 kV (positive mode) and a fused-silica capillary was used (37 cm×50 μm I.D.). The electrolyte was phosphate buffer (25 m*M*, pH 2) with 2.5 m*M* lauryl sulfate. The temperature was set at 25°C. Pressure injection (5 s) was realised. L-Arginine, L-citrulline, N-hydroxy-L-arginine and N-nitroso-L-arginine standards were prepared in the electrolyte by serial dilution (Interchim, Paris, France).

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2.2. Sample preparation

Tissue determinations were carried out after incubation with L-arginine. Briefly, 200–300 mg of fresh rat brain cortex was crushed in acetate buffer (500 μl, 0.1 *M*, pH 4) with L-arginine (5 mg). The sample was incubated at 20°C for 4 h. Then the extract was filtered using a small ultrafiltration device (Ultrafree-MC, Millipore, St-Quentin, France) for 10 min and the ultrafiltrates were used for capillary electrophoresis.

3. Results and discussion

Electropherograms of standards are shown in Fig. 1. The migration times of L-arginine and N-hydroxy-L-arginine were 1.85±0.15 min and those of L-citrulline and N-nitroso-L-arginine were 3.47±0.2 and 3.6±0.18 min, respectively. N-Nitroso-L-arginine is detectable at 254 nm due to its maximum absorbance at 260 nm in the electrolyte. The detection limit, at a signal-to-noise ratio of 2, was 1 μg/ml for N-nitroso-L-arginine at 254 nm, determined by using serial dilution of brain ultrafiltrate with acetate buffer. At 205 nm, the detection limits were 100, 5 and 5 μg/ml for L-arginine, N-hydroxy-L-arginine and L-citrulline, respectively. These different limits were determined with serial dilution in acetate buffer according to a signal-to-noise ratio of 2. These compounds are undetectable at 254 nm. The regression lines of the peak area vs. standard concentrations were linear with correlation coefficients of $r=0.998$ over the range studied (500 μg/ml to 1 μg/ml). The ultrafiltrates were stable at ambient temperature for one working day without degradation of N-nitroso-L-arginine. Standards must be prepared on the day of analysis. The intra- and inter-assay reproducibility of the N-nitroso-L-arginine assay was below 6% in the concentration range. The analytical recovery in the concentration range studied (5, 100 and 350 μg/ml) was 97–103%. Storage of the brain ultrafiltrates at −80°C showed no detectable change after three months. A typical brain extract is shown in Fig. 2. The production rate of N-nitroso-L-arginine is 74±17 ng/g/min in rat brain cortex. This enzyme

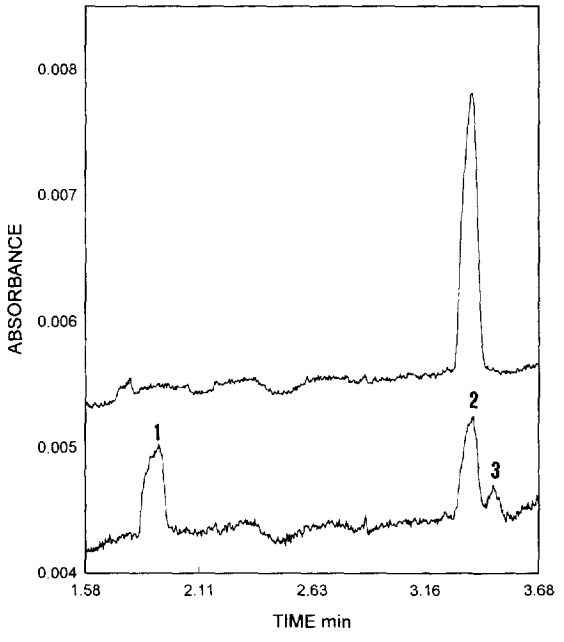

Fig. 1. Electropherogram of N-hydroxy-L-arginine, N-nitroso-L-arginine and citrulline. The concentration of compounds at 1.85 (1), 3.28 (2) and 3.45 min (3) were 8, 9 and 10 μg/ml, respectively. Upper trace is 254 nm and lower trace is 205 nm.

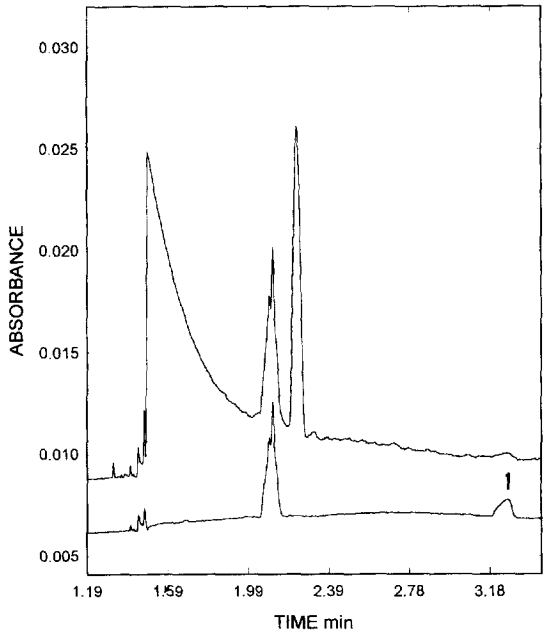

Fig. 2. Electropherogram of brain ultrafiltrate after a 4-h incubation. The upper trace is 205 nm and the lower trace is 254 nm. The concentration of N-nitroso-L-arginine was 6 μg/ml (1).

activity is similar to values which were obtained by others [1,5,6] in rat brain, measuring either citrulline or nitrite production. A high concentration of L-arginine was added to rat brain extract and this gave rise to maximum velocity for NO-synthase [5,6]. Direct injections from the rat cortex did not permit N-nitroso-L-arginine determination. N-Nitroso-L-arginine is rapidly degraded in neutral solutions (life span of 2 s). When N-nitroso-L-arginine was dissolved in phosphate buffer, pH 7.4, the L-citrulline peak appeared at 205 nm. The dual-wavelength detection permitted the degradation of the standards to be followed. The photodiode-array detector was necessary to develop this assay and to control the migration time with compounds that are detected at 205 nm in biological media. A simple filter photometric detector was used at 254 nm, but it improved the limit of sensitivity only slightly. The fact that direct brain ultrafiltrate without incubation or with incubation at pH 7.4 did not permit the detection of N-nitroso-L-arginine is due to its immediate degradation (2 s). Acidification stopped this phenomenon, but the stabilized amount is below the detection limit of the described procedure. The concentration of nitrite, a NO-synthase metabolite, is in the nanomolar range in rat brain and that of N-nitroso-L-arginine, using a classical analytical method, must be in the same range. The detection limit could be lowered using concentrating procedures such as solvent extraction or cation-exchange resins, but the nanomolar range will be attained with difficulty. Another approach could be to work with cell extracts where nitrite is present in the millimolar range. An acidic electrolyte (pH 2) was chosen in order to stabilise this compound, otherwise more basic electrolytes, up to pH 4, were used and gave slower migration times than this assay. The lauryl sulfate concentration permitted the separation of other compounds at pH 2 from 1.25 m*M* to 10 m*M*; the chosen concentration avoided an excessively long migration time. Using pH 2 without additive, the electrophoretic mobility of N-nitroso-L-arginine was equal to the value of citrulline. The electrophoretic mobilities of L-arginine and N-hydroxy-L-arginine were also the same.

In conclusion, this preliminary study showed that capillary electrophoresis of N-nitroso-L-arginine permitted NO-synthase activity to be followed. This assay of N-nitroso-L-arginine could permit comparison with voltammetry, as microelectrodes are difficult to build for non-specialist laboratories. It is necessary to improve the limit of detection for direct determination of N-nitroso-L-arginine in brain extracts and different possibilities are being explored in our laboratory.

References

[1] A. Meulemans, Neurosci. Lett., 157 (1993) 7.
[2] H.J. Issaq and K.C. Chan, Electrophoresis, 16 (1995) 467.
[3] D. Tsikas, R.H. Boger, S.M. Bode-Boger, G. Brunner and J.C. Frolich, J. Chromatogr. A, 699 (1995) 363.
[4] J.S. Stamler and J. Loscalzo, Anal. Chem., 64 (1992) 779.
[5] M.J. Barjavel and H.N. Bhargava, Pharmacology, 50 (1995) 168.
[6] K. Ohta, N. Araki, M. Shibata, J. Hamada, S. Komatsumoto, K. Shimazu and Y. Fukuuchi, Neurosci. Lett., 176 (1994) 165.