SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS DETECTION OF NITRATE AND NITRITE

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SPEKTROFOTOMETRIJSKA METODA ZA ISTOVREMENU DETEKCIJU NITRATA I NITRITA

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SAŽETAK

Koncentracija azot monoksida može se meriti brojnim metodama, ali kratak poluživot i niske izmerene vrednosti azot monoksida umanjuju im praktični značaj, te ostaju nepodesne za kliničku primenu. Nedostaci pomenutih metoda mogu se eliminisati merenjem stabilnih metabolita NO, kao što su nitriti i nitrati. U ovom radu opisali smo modifikaciju metode koja se zasniva na redukciji NO_3 i simultanoj detekciji svih krajnjih produkata oksidacije NO. Nivo nitrita i nitrata je određivan u serumu dodrovoljnih, zdravih davalaca. Serum je pre izvođenja testa deproteinizovan.. Redukcija nitrata u nitrite postignuta je vanadijumom(III). Za merenje koncentracije nitrita korišćena je kolorimetrijska detekcija sa Griess-ovim reagensom. Koncentracija nitrata izračunavana je kao razlika koncentracije ukupnih NO_x, određene u prisusvu VCl_3 i Griess-ovog reagensa, i koncentracije NO_2, merene samo u prisustvu Griess-a. Na osnovu dobijenih rezultata, zaključili smo da je metoda osetljiva do 0,5μM NO_3 i može se primenjivati na brojnim tečnostima uključujući serum, plazmu i supernatant kulture ćelija. Merni opseg metode značajno je veći pri upotrebi HCl u Griess-ovom reagensu, u odnosu na H_3PO_4, Sa druge strane, bez obzira na sastav Griess-ovog reagensa (HCl ili H_3PO_4), merni opseg metode je širi kada se reagensi inkubiraju na 37°C.

Ključne reči: azot monoksid, nitrati, nitriti, vanadijum(III), Griess-ov reagens.

ABSTRACT

Concentration of nitric oxide can be measured by variety of methods. Its short half life and values of low detectability decrease clinical importance of these methods. Deficiencies of methods used for NO measurement can be eliminated by measurement of stable NO products such as nitrites and nitrates. In this study we have modified a method for simultaneous evaluation of nitrate and nitrite concentrations.

Human sera were collected from blood donor volunteers. Before testing the samples were deproteinized. Reduction of nitrate was achieved with vanadium(III). Nitrite concentration was measured by Griess reaction. The nitrate concentration was calculated as difference of NOx (nitrites and nitrates), determinated in presence of vanadium(III) and nitrites concentration. This assay has shown sensitivity to 0,5μM NO_3 and is useful in variety of fluids including serum, plasma and cell culture media.

We have examined the influence of various factors on detection of NOX, such as reagent composition, volume, and temperature. The method has shown higher sensitivity when Griess reagent with HCl was used, compared to use of Griess reagent with H_3PO_4. It has been also noticed that regardless of which Griess reagent composition was used, sensitivity of this reaction was higher when samples were incubated at 37°C instead at 25°C. All incubations lasted 30 minutes.

Key words: nitric oxide, nitrate, nitrite, vanadium(III), Griess reagent.

INTRODUCTION

Nitric oxide (NO) is a short-life mediator of numerous physiological processes from neurotransmission, muscle relaxation and vasodilatation to antipathogenic and tumoricidal responses (1–5). Biosynthesis of NO from L-arginine by participation of oxygen is only possible in cells that provides NOS activity (Nitric Oxide Synthase) (6). There are two NO synthase enzymes: constitutive cNOS, in endothelial cells, which produces small quantity of NO without induction and inducible iNOS, which on stimulation synthesizes large amount of NO (7). Overproduction of NO can be a promoter of variety of diseases (8–9). That is the main reason for development of reliable techniques for detecting NO production.

Although NO concentration can be measured by many methods (chromatography, electron paramagnetic resonance, electrochemistry) (10), the short half-life and low concentrations of NO (11) reduce the practical significance of these tests, making these procedures unsuitable for clinical use as well as for scientific purposes. Deficiency of mentioned methods can be eliminated by measuring the stable NO metabolites, in particular nitrite (NO_2) and nitrate (NO_3).

Nitrites (NO_2) are representing the stable, final product of the oxidation of NO in aqueous solution (12). Nitrates (NO_3) are formed by reaction of NO with oxyhemoglobin or superoxide (reaction of oxidation) (13,14). In addition, nitrites are converted to nitrates by oxyhemoglobin (12–15). Consequently, plasma, serum and urine, as mediums with oxyhemoglobin and superoxide, predominately contain nitrates, while significant nitrites can accumulate in non-heme-containing fluids such as cerebrospinal (16).

The simplest and most frequently applied method for detection of nitrite anions employs colorimetric detection with Griess reagent, reagent that makes purple azo-colors with nitrites.

Since the conventional Griess reaction has limitations regarding sensitivity (1–5 mM) (17) and inability to detect NO_3 (which doesn't undergo diazotisation), several modifications have been adapted. The detection limit of the assay can be enhanced (linear to 0.2 μM NO_2) (18) by substitution of dapsone for sulfanilamide. Additionally, the total concentration of oxidative endproduct of NO can be determined by converting (reduction) NO_3 to NO_2.

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