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Short communication

Photodecomposition of o-phthaldialdehyde-derivatized amino acids by the photodiode array detector during their high-performance liquid chromatographic analysis

Sándor Kéki [a], János Török [a], Bernadett Biri [a], Judit Zsuga [b,d], Rudolf Gesztelyi [c], Dániel Bereczki [d], Miklós Zsuga [a,*]

[a] Department of Applied Chemistry, University of Debrecen, Egyetem tér, H-4012 Debrecen, Hungary
[b] Department of Pharmacology and Pharmacotherapy, University of Debrecen, H-4012 Debrecen, Hungary
[c] Departments of Pharmacology and Pharmacodynamics, University of Debrecen, H-4012 Debrecen, Hungary
[d] Department of Neurology, University of Debrecen, Nagyerdei krt 98, H-4012 Debrecen, Hungary

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Abstract

During the high-performance liquid chromatographic (HPLC) analysis of *o*-phthaldialdehyde (OPA)-derivatized amino acids such as arginine and homoarginine, we observed that the response of the fluorescence detection (FLD) system is decreased when the photodiode array detection (DAD) system (located before the FLD system) was simultaneously switched on. The decrease in the FLD response, i.e., the difference in the FLD peak area (ΔA) obtained with DAD modes off (A_{off}) and on (A_{on}) was dependent upon the flow rate, but the relative FLD response decrease ($\Delta A/A_{off}$) was practically independent of the amount of analyte injected. For example, decreasing the flow rate from 1 to 0.5 mL/min resulted in the relative decrease of FLD response from ~5% to 11%. It was concluded that DAD caused a photoinduced partial decomposition of the OPA-derivatized amino acids flowing through the cell. The photoinduced dissociation of OPA derivatives was independently supported by using off-line photodiode array spectrometric measurements with long and short irradiation pulses. Based on the experimental results, for description of the variation of FLD responses with the flow rate upon the irradiation by DAD a simple mathematical model is proposed and reported.

Keywords: OPA-derivatized arginine and homoarginine; High-performance liquid chromatography; Photodecomposition; UV and fluorescence detection

1. Introduction

Determinations of the amino acid levels in biological samples, e.g., from human organisms may provide clues for understanding and prediction of the evolution of several disorders. Unusual variation in these concentrations may reflect to the presence and/or onset of serious disorders. For example, changes in the asymmetric dimethylarginine (ADMA), symmetric dimethylarginine (SDMA) and L-Arginin (Arg) levels in the serum or in the plasma are indicative of the increased risk for coronary heart diseases [1–3] and simultaneous evolution of atherosclerosis and insulin resistance [4–6]. In this respect, due to the possible connection between the corresponding amino acid levels in biological liquids and the disorders several methods have been developed for the qualitative and quantitative analysis of these compounds. Most of these methods include reversed-phase high-performance liquid chromatographic (HPLC) separations of the pre-column-derivatized amino acids using UV and/or fluorescence and/or MS detections [7–12]. Because of the simplicity and reproducibility of the method and due to the UV and high fluorescent sensitivity of the derivatized amino acids, pre-column modification of these compounds with *o*-phthaldialdehyde (OPA) [13] in the presence thiol derivatives such as 2-mercaptoethanol (2-ME), 3-mercaptopropionic acid (3-MPA) or *N*-acetyl-L-cysteine (NAC) have become the most popular and widely accepted methods for the determination of amino acids originating from biological samples [7–12]. The effect of the reaction conditions such as pH, temperature and the ratio of reactant concentrations on the stability of a wide range of OPA-derivatized amino acids (in the presence of 2-ME

* Corresponding author.
E-mail address: zsugam@tigris.unideb.hu (M. Zsuga).

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and 3-MPA) have been thoroughly studied [11,12]. However, to our best knowledge no report on the stability of such compounds under HPLC conditions has been appeared.

In this article, we report a study on the change of fluorescence detection (FLD) system responses caused by the photodiode array detection (DAD) system as a function of the flow rate and a simple mathematical expression for the description of the above issues is also given.

2. Experimental

2.1. Chemicals

L-Argine (Arg) and L-homoarginine (HArg) hydrochloride, *o*-phthaldialdehyde (OPA), 3-mercaptopropionic acid (3-MPA), boric acid, NaOH, methanol and acetonitrile were purchased from Sigma–Aldrich (Steinheim, Germany) and were used without further purification. Ammonia solution and ammonium acetate were received from Reanal (Budapest, Hungary). All reagents used in this study were of highest purity available.

2.2. Sample preparation, derivatization and chromatograpic separation

The derivatization of Arg and HArg was achieved as described in Ref. [12]. Arg and HArg samples of 1000 μL at a concentration of 6–20 μM were mixed with 315 μL of the ortho-phthaldialdehyde/3-mercaptopropionic acid reagent solution. The samples were incubated at 22 °C for 10 min and then were cooled down to 5 °C. After 10 min incubation time aliquots of 10 μL were injected into the chromatographic system which consisted of a Waters 2695 Separations Module equipped with thermostable autosampler (5 °C) and column module (35 °C) and of a Waters 2475 fluorescent and a Waters 2996 photodiode-array detector. Separations were performed using an Agilent Zorbax SB-C18 (4.6 mm × 75 mm, 3.5 μm) column. Isocratic elution at a flow rate ranging from 0.5 to 1 mL/min was applied using a mobile phase A consisting of 2.5 mM ammonium acetate in water (pH 7.2) and phase B of ammonium acetate solution (2.5 mM) and acetonitrile and methanol in 46/44/10 (v/v), respectively. Mobile phase A and B were mixed in a 4:1 v/v ratio. Arg and HArg were detected at $\lambda_{ex} = 337$ nm; $\lambda_{em} = 520$ nm. The precision of parallel injections was better than 0.5%.

2.3. Off-line spectrophotometric measurements

The mixture of Arg and HArg at a concentration of 10 μM (200 μL) was mixed with OPA/3-MPA solutions of 400 μL and diluted with the borate buffer to 1 mL. After 10 min incubation time at ambient temperature the reaction mixture was placed into a quartz cuvette of optical path length of 1 cm and the absorbance at 230 nm was measured with a HP 8453 diode array spectrometer using cycling times of 30 and 1 s.

3. Results and discussion

3.1. Preliminary experiments

During the HPLC analysis of the mixture of *o*-phthaldialdehyde (OPA)-derivatized Arg and HArg we observed that the response of the FLD system decreased when the DAD system that was located before the FLD system was also turned on. Although the relative decrease in the FLD response was not so high, it appeared to be significant. For instance, we observed a decrease in the FLD response by ∼5% at a flow rate of 1 mL/min. We concluded that the decrease in the FLD response is due to the partial photodecomposition of these compounds caused by the light beam emitted from the DAD system. To support this conclusion, independent, off-line photometric experiments were conducted. In these experiments, we used diode array photometers and a reaction mixture similar to that applied for the HPLC experiments but the excess of the OPA/3-mercaptopropionic acid (3-MPA) reagents to Arg and HArg was lowered in order to mimic the conditions occurring after LC separation of the OPA derivatives from the unreacted OPA/3-MPA reagents. As an example, the results of these experiments are presented in Fig. 1.

Fig. 1. shows that by applying a cycling time of 30 s, a relatively slow decrease in the absorbance at 230 nm can be observed. However, when lowering the cycling time from 30 to 1 s, the absorbance decreases rapidly. This observation indicates and supports that photodecomposition of the OPA-derivatized Arg and HArg takes place upon the effect of light emanating, most probably, from the UV lamp of the photometer. On the other hand, under HPLC conditions it was found that the relative change in the FLD response caused by the DAD system

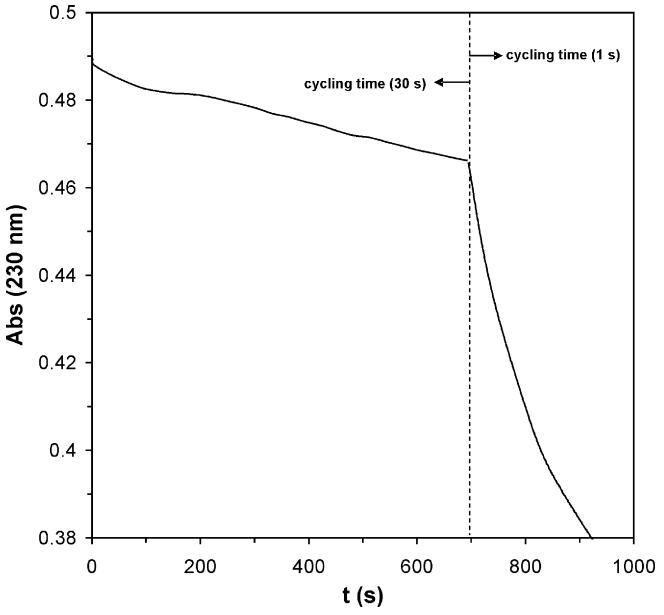

Fig. 1. Absorbance (at 230 nm) *vs*. time plot for the reaction of Arg and HArg with OPA/3-MPA recorded by photodiode array photometer at ambient temperature. Experimental conditions: to the aqueous solution of Arg (10 μM) and HArg (10 μM) of 2 mL 36 μL OPA/3-MPA solution was added and incubated for 15 min at ambient temperature.