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Aromatic aldehydes as selective fluorogenic derivatizing agents for α-dicarbonyl compounds. Application to HPLC analysis of some advanced glycation end products and oxidative stress biomarkers in human serum

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ABSTRACT

α-Dicarbonyl compounds (α-DCs) are very clinically important as they are considered as advanced glycation end products (AGEs) precursors and biomarkers for many chronic diseases such as diabetes and vascular diseases, in addition to their major role in progression of complications of such diseases. Aromatic aldehydes and ammonium acetate were productively used as a one-pot co-reagents for fluorogenic derivatization of α-DCs yielding fluorescent imidazole derivatives. Among the tried aromatic aldehydes, 4-carbomethoxybenzaldehyde yielded the products with best fluorescent characters. This approach for fluorogenic derivatization of α-DCs overcome the selectivity problem of the most commonly used derivatization reagent for α-DCs, α-diamino compounds, that can react unselectively with α-DCs and aldehydes. Separation of the formed imidazole derivatives of five α-DCs including glucosone, 3-deoxyglucosone, glyoxal, methyl glyoxal and dimethyl glyoxal together with ethylmethylglyoxal as an internal standard was carried out on an octyl column using a mobile phase consisted of methanol-water (15:85, v/v%) containing 0.2% formic acid with time programed flow, followed by fluorescence detection at excitation/emission wavelengths of 310/410 nm. The method showed excellent sensitivity for the targeted α-DCs with limits of detections ranging from 0.4 to 5.0 nM in human serum. Simple protein precipitation procedure was used for human serum treatment yielding very good recovery (91–105%) for the targeted α-DCs. The developed method was fully validated, then applied to the analysis of the five above mentioned clinically important α-DCs in serum samples of healthy, diabetic, rheumatic and cardiac disorders human volunteers. Due to the excellent analytical features of the developed method, including high selectivity and sensitivity, it was able to detect the pattern of the targeted α-DCs serum levels under the investigated different clinical conditions.

1. Introduction

Among keto compounds, aliphatic α-dicarbonyl compounds (α-DCs) such as α-dialdehydes, α-diketones and α-oxoaldehydes, have been receiving a great consideration by virtue of their high clinical importance. α-DCs have a significant role in the progress of complications associated with many chronic diseases such as diabetes, Alzheimer's disease, nephropathy, macrovascular disease and cataract. In this perspective, reactive α-dialdehydes such as glyoxal (GO) and α-oxoaldehydes including: methylglyoxal (MGO), glucosone (GS) and 3-deoxyglucosone (DG) are reactive intermediates in protein glycation. Thus, glycation occurs via the Maillard reaction between the aldehyde group of reducing sugar and the primary amino group of protein, nucleic acid or lipid giving a Schiff base which produces Amadori products by rearrangement. Amadori products then degrade through oxidation and dehydration reactions to yield mainly GO, MGO, GS and DG. Since these α-oxoaldehydes are more reactive than the reducing sugar from which they are formed, they propagate the reaction through reacting with the amino group of protein and they act as precursors for advanced glycation end products (AGEs) [1,2]. AGEs cause damage to tissues by three main ways: formation of cross-links, interaction

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with cellular receptors, and intracellular glycation [1]. In addition, GO, MGO, GS and DG are produced by non-enzymatic degradation of glucose, so their levels are increased in diabetes patients [2]. Also, it was reported that the level of these α-DCs in biological fluids could be used as an indicator for the risk of diabetic complications progression [3].

GO is also a main product of lipid peroxidation as a result of oxidative stress and increased level of intracellular oxidants. It originates from ω-6 polyunsaturated fatty acids [4,5]. It is also a key product of carbohydrate autoxidation. For example, it is produced by DNA oxidation at deoxyribose $C_{4'}/C_{5'}$ carbons leading to breakage of DNA strands, as well, it is produced by autoxidation of ene-diol tautomer of glycolaldehyde [6]. MGO, which is also considered as a lipid peroxidation product but to much less extent than GO [4], is produced enzymatically from glycolic metabolism of glucose or ketone bodies metabolism [7]. Also, MGO is produced from aminoacetone through the enzymatic activity of serum semicarbazide sensitive amine oxidase (SSAO) [5]. Regarding GS and DG endogenous sources other than Amadori products, they could be produced from glucose autoxidation and hydrolysis of fructose 3-phosphate, respectively [5].

Also, it is noteworthy that another α-DC, dimethylglyoxal (DMGO), was also detected in biological materials and it was considered as a lipid peroxidation product [4]. DMGO was also detected in students after alcohol ingestion and was considered as a minor metabolite for the acetaldehyde produced from metabolic oxidation of ethanol [8]. It was considered as one of the causes of the alcohol toxicity via its favorable redox potential which is able to induce electron transfer in-vivo. Also, mutations and cancer initiation are among the possible toxic responses to DMGO [9].

The determination of the above mentioned α-DCs in human serum has a great significance due to their close correlation with AGEs, tissue modifications, lipid peroxidation and other possible serious toxic responses. In 2006, a summary for the reported analytical methods for the determination of α-DCs in different matrices was reported by Shibamoto [4]. In the last ten years, many methods were reported for determination of α-DCs in biological fluids including: Gas chromatographic methods with flame ionization detection (GC-FID) [10,11] or with mass spectrometric detection (GC–MS) [12,13] and liquid chromatography with tandem MS (LC–MS/MS) [14–16]. However, these methods require the use of expensive and sophisticated instruments and need experienced analyst to perform the analysis. On the other hand, simpler methods such as capillary electrophoretic methods with UV (CE-UV) [17] or amperometric detection (CE-AD) [18], and high-performance LC methods with fluorescence detection (HPLC-FL) were also reported [19–21]. However, these methods suffered from several drawbacks including long derivatization reaction time, need for relatively large sample volume, and need for time consuming and multistep sample preparation. Moreover, most of these methods depended on using α-diamino compounds as derivatizing reagents [10–17,19–21] which are not selective for α-DCs as they can react with any compound having aldehyde group in their structure forming imidazole derivatives [22] instead of the pyrazine derivative formed with the α-DCs. Also, CE-AD method depends on using the non-selective reagent, thiobarbituric acid, which is reactive towards many other compounds including malondialdehyde (MDA), reducing sugars, and pyrimidine [23].

Now it is clear that there is a strong need for a new approach for the determination of α-DCs to overcome the previously mentioned problems of the reported literature. Our research group has previously utilized benzaldehyde or its derivatives for the derivatization of *ortho*-quinones in the presence of ammonium acetate [24–26]. In this paper we aim to use this reaction for the first time for the fluorogenic derivatization of clinically important aliphatic α-DCs including: GS, DG, GO, MGO and DMGO using ethylmethylglyoxal (EMGO) as internal standard an (IS) (Fig. 1) followed by their HPCL-FL analysis in human serum. The most important advantage of using this approach for determination of α-DCs is the expected selectivity. Benzaldehyde or its derivatives in the presence of ammonium acetate can react only with α-DCs to yield fluorescent imidazole derivative and the reagents cannot react with aldehydes or any compound does not have the α-dicarbonyl group.

2. Materials and methods

2.1. Materials and reagents

The utilized reagents were of analytical grade and used without any prior treatment. GO, MGO, DMGO, EMGO were obtained from Sigma-Aldrich (St. Louis, MO, USA). GS and DG were purchased from Carbosynth Ltd (Berkshire, UK). Acetic acid and methanol were from Wako Pure Chemical Industries (Osaka, Japan). The tested aromatic aldehydes are shown in Fig. S1 (Supplementary materials). Most of the aromatic aldehydes including 4-carbomethoxybenzaldehyde (CMBAL) were obtained from Tokyo Chemical Industry (Tokyo). Sigma-Aldrich was the supplier for ammonium acetate, 4-diethylaminobenzaldehyde, 4-formylphenylboronic acid and 4-acetoxybenzaldehyde, while benzaldehyde, 1-naphthaldehyde and 4-methoxybenzaldehyde were from Wako Pure Chemical Industries. 4-Dimehthylaminobenzaldehyde was obtained from Kishida Chemicals (Tokyo). Purified water was obtained using Autostill WG 203 (Yamato Scientific Co., Ltd., Tokyo).

Stock solutions of GS, DG, GO, MGO, DMGO, EMGO (5.0 mM) were prepared in acetonitrile. A mixed standard solution containing the six analytes was prepared by diluting the stock solutions with acetonitrile to obtain the required concentrations. CMBAL (0.2 M) and ammonium acetate (1.0 M) were prepared in methanol and glacial acetic acid, respectively. The analytes solution were kept in −30 °C while other reagents kept in the refrigerator at 4 °C.

2.2. Apparatus and chromatographic condition

A Shimadzu LC-20AT pump (Kyoto, Japan), a Rheodyne injector (Cotati, CA, USA) with a 20-μL sample loop combined with a Shimadzu RF-20AXS fluorescence detector and an EZ Chrom Elite chromatography data acquisition system (Scientific software, Pleasanton, CA, USA) were used to construct the HPLC system. The separation was performed on a Cosmosil 5C8-MS column (150 mm × 4.6 mm i.d., 5 μm particle size) from Nacalai Tesque INC. The mobile phase consisted of methanol-water (15:85, v/v%) containing 0.2% formic acid. The flow rate was time programed as follow; in the first 30 min, the flow rate was kept at 0.6 mL/min then increased to 2.0 mL/min in one minute then was kept constant till 60 min. The $\lambda_{Ex}/\lambda_{Em}$ wavelengths were set at 310/410 nm, respectively.

Shimadzu RF-1500 spectrofluorophotometer was used for recording fluorescence spectra. Himac CR 15 refrigerated centrifuge (Hitachi Koki Co., Ltd., Tokyo) was used during serum samples extraction. Quattro micro TM triple-quadruple mass spectrometer (Waters Co., Milford, MA,USA) was used for recording the mass spectrum (MS) of GO and MGO derivatives of CMBAL after positive electro spray ionization (ESI^+). For the derivatization of the target analytes, a Yamato HF-41 heating block (Tokyo) was used.

2.3. Study of different aromatic aldehydes as fluorogenic reagent for α-DCs

One hundred μL of GO or MGO (40 μM each), as representatives for the DCs, was transferred to a screw-capped vial followed by addition of 50 μL of 1.0 M ammonium acetate and 50 μL of