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The profile of free amino acids in latent fingerprint of healthy and beta-thalassemic volunteers

Alaa Khedr*

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, King Abdulaziz University, Jeddah 21589, P.O. Box 80260, Saudi Arabia

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ABSTRACT

The aim of the present work is to apply a non-invasive test, using thumb fingerprint residue analysis, for detection of beta-thalassemia (β-Thal). The relative percentages of free amino acids (AA) in the latent fingerprint of β-Thal patients and healthy subjects were compared. The sample included 24 β-Thal patient and 24 healthy subjects, aged 5–10 years old. Twenty-three AA plus ammonia were analyzed by a sensitive high-performance liquid chromatographic method with fluorescence detection. The profile of AA was calculated based on the percentage of relative amount of each AA to serine (Ser) as it found to be the predominant peak. The statistical and chromatographic profiles of β-Thal patients were characterized by significant decrease of ornithine, lysine, and zero tyrosine, with significant increase of ammonia, and proline. Other amino acids that exist in low ratios were estimated statistically for significance changes. The relative percentages of each AA of healthy subjects were approximately constant. For this reason, these mentioned AA were assigned as major fingerprint markers of β-Thal.

1. Introduction

The chemical composition of latent fingerprints has been investigated by several authors [1–3]. Hamilton [1], Oro and Skewes [2], and Hadorn et al. [3], using ultra-micro methods, found the relative concentrations of amino acids (AA) on the surface of the fingers of 10 subjects to be essentially constant. This included aspartic acid, glutamic acid, histidine, serine, threonine, glycine, alanine, valine, isoleucine, leucine, tryptophane, ornithine, lysine, and tyrosine, in addition to ammonia and smaller amounts of citrulline, methionine and arginine. Lee and Gaensslen reported that the total amounts of AA present in fingerprints were in the range of 0.3–2.59 mg L^{-1} [4]. Also, it has been concluded that serine, glycine and alanine were the most abundant amino acids [1–4]. Quantitatively, amino acid concentrations can vary as much as 2–20 times depending on the collection method and the location of the sample collected from (on the body) [4]. The AA content, collectively or separately, has been investigated in plasma or urine as biomarkers for some diseases or as an indication of various metabolic disorder, for example: cases of colon carcinoma [5], Parkinsonism [6], oxidative stress of AA [7], and hemoglobinopathy [8]. β-Thalassemia is a form of blood disorder due to mutations in the β-globin (HBB) gene on chromosome 11, inherited in an autosomal recessive fashion and the severity of the disease depends on the nature of the mutation [8]. This genetic defect results in a reduced rate of synthesis of one of the globin chains that make up hemoglobin. Reduced synthesis of one of the globin chains can cause the formation of abnormal hemoglobin molecules, thus causing anemia, the characteristic presenting symptom of the thalassemia. The genetic defect may be due to substitution of one amino acid for another [9]. Fucharoen and Winichagoon [10] reported that β-Thal is a heterogeneous disorder, caused by various defects in the β-globin gene. Hemoglobin (Hb) E arises from a mutation of the β-globin chain which replaces glutamic acid with lysine. Abdulrazzaq et al. [11] described the relation between amino acids metabolism and thalassemia major. He concluded that lower plasma values of essential amino acids and a decrease in urinary amino acids are the most important characteristic features in thalassemic patients. Growth impairment both in height and weight also occurs in thalassemic patients compared to a control population. Several authors illustrated the correlation between amino acid content, as markers, in plasma or urine and β-Thal [12,13]. A comprehensive review article has been compiled by Molnar-Perl [14] discussing the separation and simultaneous quantitation of amino acids and amines in the same matrix by high-performance liquid chromatography (HPLC). The same review cited the most applied HPLC methods for the analysis of amino acids and some amines using different derivatization reagents and detection methods. The most convenient reagent was dansyl chloride (Dns-Cl), as it gave less or no reaction by-products, in addition to its derivative stability [15]. However, the publications that described the use of Dns-Cl, showed uncompleted separation of some AA due to overlap or bad resolu-

* Tel.: +966 553399718; fax: +966 26951696.
E-mail addresses: akhedr@kau.edu.sa, akhedr@lycos.com.

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tion of certain AA. This was in addition to the extensive sample pretreatment requirements.

In this work we described and validated a new HPLC analytical method of AA using Dns-Cl as derivatizating reagent. The developed method was applied to the analysis of AA in the latent fingerprints of healthy and β-Thal people. The profiles of AA of both healthy and β-Thal subjects were also described.

2. Experimental

2.1. Chemicals and reagents

All solvents were of HPLC grade, Merck (Darmstadt, Germany). All amino acid reference standards were purchased from Permed Scientific Chemicals Ltd. (Bedford, England). Amino acids used were; L-aspartic acid (Asp), L-glutamic acid (Glu), L-asparagine H_2O (Asn), DL-histidine HCl (His), DL-citrulline (Cit), DL-serine (Ser), DL-threonine (Thr), glycine (Gly), L-alanine (Ala), L-arginine (Arg), L-proline (Pro), DL-valine (Val), DL-norvaline (Nva), DL-methionine (Met), DL-isoleucine (Ile), L-leucine (Leu), DL-norleucine (Nle), L-B-phenylalanine (Phe), L-tryptophan (Trp), L-cystine (Cys), DL-ornithine HCl (Orn), DL-lysine HCl (Lys), DL-tyrosine (Tyr). 1-Hexanesulfonic acid sodium salt (HSA-Na, >98%), and dansyl chloride (≥99.0% HPLC for fluorescence BioChemika) were purchased from Sigma–Aldrich (St. Louis, MO, USA). All other materials were of analytical grade.

2.2. Equipment

High-performance liquid chromatographic system used was consisting of an Alliance Waters Separations Module 2695, and Waters 2475 multi wavelength fluorescence detector (Milford, MA, USA). The HPLC system control and data processing were performed by Waters Empower software Build 1154 (Milford, MA, USA) run on an IBM-compatible PC. Screw capped autosampler vials (flat-bottom 1.8 mL and V-shaped 300-μL) were from Alltech GmbH (Unterhaching, Germany). Heraeus heating oven (Kendro, Hanau, Germany) was adjusted at 60 °C. Calibrated digital microtransfer pipettes 5–250 μL, Brand (Wertheim, Germany), were used. Kenwood Microwave model MW450, 17 L capacity—800 W, adjusted at the second power level equivalent to 264 W and 416 MHz (Kenwood Electronics Co. Ltd., Songjiang, Shanghai, China).

2.3. Chromatographic conditions

Analytes were separated on the following two columns in sequence; analytical column 1, Zorbax Eclipse XDB C18, 250 mm × 4.6 mm, 80 Å, 5 μm, connected to analytical column 2, Zorbax Extend C18, 150 mm × 4.6 mm, 80 Å, 5 μm. Pre-column used was, Zorbax Eclipse XDB C18, 12.5 mm × 4.6 mm, 80 Å, 5 μm. All columns and pre-column were from Agilent (Agilent Technologies, Palo Alto, CA, USA). The fluorescence detector was set at 340 and 515 nm, as excitation and emission wavelengths, respectively. The mobile phase was prepared by dissolving 600 mg of HSA-Na in 100 mL water, from this solution a volume of 50 mL was mixed with 950 mL acetonitrile (mobile phase A) and the remaining 50 mL was mixed with 950 mL water containing 1.36 g sodium acetate trihydrate, filtered through a 4.5 Å Nylon membrane filter and adjusted to pH 7.6 with few drops of 0.01 M NaOH (mobile phase B). The mobile phase flow rate was adjusted to 1 mL min^{-1}. The HPLC pump was programmed to deliver the mobile phase as follows; from 0 to 5 min; isocratic elution of 10.0% mobile phase A, 90.0% mobile phase B; from 5 to 100 min; gradient elution of mobile phase A (10.0–43.5%) and mobile phase B (90.0–56.5%); from 100 to 110 min; gradient elution of mobile phase A (43.5–66.0%) and mobile phase B (56.5–34.0%). A sample volume of 10 μL was injected.

2.4. Standard solutions and quality control samples

Separate solutions of each AA and ammonium chloride were prepared in water to get a stock solution of 1.0 mg mL^{-1}. Appropriate dilutions in water were prepared from these stock solutions to obtain calibration standards that contain 23 AA and ammonium chloride (6 concentration levels). The first quality control samples (QC1) were prepared in the concentration range of 25, 50, and 100% of the upper linear limit of each substance. The second quality control sample (QC2) was a real sample (200 μL) of latent fingerprint of healthy subject. (Sample collection and preparation is described in Section 2.7.) The QC samples were divided to small aliquots and stored in borosilicate glass vials at −20 °C until use. The samples were thawed and a volume of 50 μL of each level was derivatized and analyzed at time intervals of; 0, 10 and 30 days. The mass concentrations of each substance were calculated from the corresponding calibration curve using peak area. The calibration curves were constructed using the least-square method for the calculation of slope, intercept and correlation coefficient.

2.5. Derivatization reagent and bicarbonate solution

One hundred milligrams of dansyl chloride was accurately weighed into 10-mL volumetric flask, dissolved in a solvent mixture of acetonitrile: acetone (9:1, v/v), and completed to volume with the same solvent. Sodium bicarbonate (0.1 M) solution was prepared in water and adjusted to pH 9.7 ± 0.1 with 0.1 M sodium hydroxide.

2.6. Derivatization preparation

A volume of 50 μL Dns-Cl solution, 50 μL aqueous standard solutions of AA or sample solution, and 200 μL 0.1 M bicarbonate solution were transferred to autosampler vial (1.8-mL capacity). The vial was capped well, swirled, and left to stand inside the microwave oven over the rotating glass platform. A plastic container (250-mL, microwave-safe) was inverted over the vial just for safety reason, the power was switched on for 5 min (at the 2nd energy level, 264 W), vial allowed to cool at room temperature for 2 min, irradiated again for another 5 min, cooled, and the reaction mixture was transferred to a 300-μL V-shaped autosampler and a volume of 10 μL was injected for HPLC analysis. Alternatively, the derivatization reaction was also conducted at 60 °C for 60 min in hot-air oven.

2.7. Sample collection and preparation

Twenty-four β-Thal volunteers and 24 healthy volunteers, aged 5–10 years old, were enrolled in this study. Volunteers were advised to wash hand by tap water and wait about 2 min to air-dry not to touch anything before sampling. The thumb finger was introduced into a clean 25-mL glass beaker containing 200 μL distilled water and keep touching the wetted inner surface for 2 min. From this solution, a volume of 50 μL was used for derivatization with Dns-Cl and analyzed as described above. Samples were immediately labeled with the corresponding clinical case report number. The collected samples were either derivatized and analyzed immediately or stored in the freezer at −20 °C until derivatization and analysis.