Blood Cells, Molecules and Diseases 55 (2015) 62–67

Contents lists available at ScienceDirect

Blood Cells, Molecules and Diseases

journal homepage: www.elsevier.com/locate/bcmd

Development of a capillary zone electrophoresis method for rapid determination of human globin chains in α and β-thalassemia subjects

Li Lin [a], Dan-Na Chen [a], Jing Guo [b], Wan-Jun Zhou [a], Xiang-Min Xu [a,*]

[a] Department of Medical Genetics, School of Basic Medical Sciences, Southern Medical University, Guangzhou 510515, Guangdong, PR China
[b] Department of Birth Health and Heredity, Guigang Women and Children Care Hospital, Guigang 537000, Guangxi, PR China

ARTICLE INFO

Article history:
Submitted 20 January 2015
Accepted 22 March 2015
Available online 26 March 2015

Keywords:
Capillary zone electrophoresis
Human globin chain
Hemoglobinopathies

ABSTRACT

Thalassemia is an inherited autosomal recessive blood disorder characterized by the underproduction of globin chains as a consequence of globin gene defects, resulting in malfunctioning red blood cells and oxygen transport. Analysis of globin chains is an important aspect of thalassemia research. In this study we developed a capillary zone electrophoresis (CZE) method for human globin determination in the diagnosis of thalassemia and hemoglobin variants. To demonstrate the utility of this approach, α/β area ratios were determined for samples from 310 thalassemia patients and healthy controls. The separation was performed on uncoated capillary with simple preparation. Distinct globin peaks were resolved in 17 min, and coefficients of variation (CV) for migration time and areas ranged from 0.37%–1.69% and 0.46%–6.71%, respectively. Receiver operating characteristic (ROC) curve analysis of the α/β area ratios gave 100% sensitivity and specificity for indicating β-TI/TM, and 100% sensitivity and 97.4% specificity for Hb H disease. Hemoglobin G-Honolulu (Hb G-Honolulu) and Hb Westmead (Hb WS) were successfully detected using this CZE method. This automated methodology is simple, rapid and cost-effective for the fast determination of human globin chains, which could be an important diagnostic tool in the field of hemoglobinopathies.

1. Introduction

Hemoglobin is a globular protein comprised of two α-like (ζ or α) and two β-like (ε, γ, δ or β) globin chains that form a non-covalent tetrameric complex, which mainly exists in the erythrocytes as a transporter of oxygen and carbon dioxide. Thalassemias are hereditary recessive anemia characterized by insufficient production of α or β globin chains, which lead to the imbalance of human globin chains [1]. For a better understanding of thalassemia, the analysis and identification of globin chains is particularly important in the study of structural modifications, revealing the degree of α/non-α chain imbalance, identifying unknown Hb variants and monitoring globin gene therapies in animal experiment and related hematologic studies [2–4].

To date, a variety of analytical methods have been reported for separation of globin chains, including immunoassay, gel electrophoresis, HPLC and RP-HPLC [5–7]. However, the main drawbacks of these methods are labor intensive, time-consuming sample preparation and limited resolution. Recently, capillary electrophoresis (CE) is becoming an increasingly attractive separation technique for human globin chains due to its ability to separate charged compounds with high efficiency, low sample consumption, fast analysis times, and low solvent consumption. Some researchers have focused on the use of CZE with coated capillary for the separation and quantification of globin chains [8,9]. Nevertheless, expensive cost of commercial permanently coated capillaries limits its application in clinical laboratories. CZE with dynamic modifiers in fused-silica capillary at acid pH is an alternative first line technique that is not, as yet, so widely used.

Here, we have developed a rapid, automated electrophoresis method for separation of α, β, and γ globin chains from both newborn and adult samples without the need for complex sample pre-preparation. This CZE method plays a substantial role in the analysis of thalassemia and hemoglobinopathies.

2. Materials and methods

2.1. Clinical samples

In this study, 24 samples from normal adult (4 cases), α-thalassemia silent (4 cases), α-thalassemia trait (4 cases), Hb H disease (4 cases), β-thalassemia carrier (4 cases) and β-TI/TM (4 cases) samples were used to develop the method. Additionally, one for each of four samples (normal adult, normal newborn, Hb H disease, β-TI/TM) was used to assess assay reproducibility. These samples were pre-typed by molecular genetic assay.

For assay evaluation, a total of 310 clinical samples consisting of normal (n = 58), α-thalassemia silent/trait (n = 107, including 6 Hb WS and 1 Hb G-Honolulu), Hb H disease (n = 39, including 1 Hb G-

* Corresponding author.
E-mail address: xixm@smu.edu.cn (X.-M. Xu).

http://dx.doi.org/10.1016/j.bcmd.2015.03.003
1079-9796/

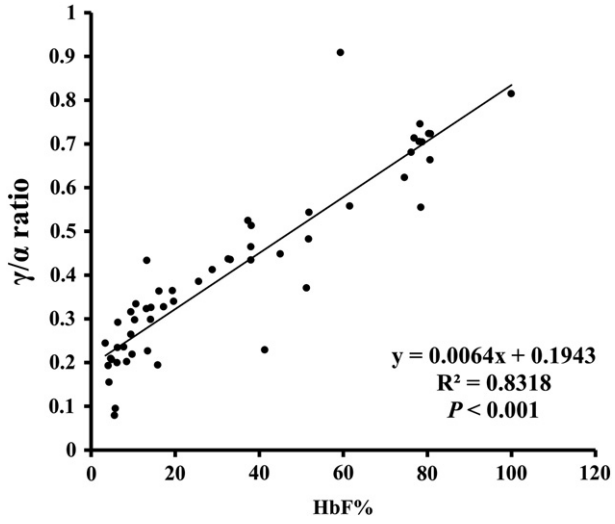

Fig. 1. Correlation analysis between the area ratio of γ/α and HbF via bivariate correlations.

Honolulu), β-thalassemia carriers (n = 43, including 2 Hb E and 1 co-inheritance of α-thalassemia samples with Hb WS) and β- TI/TM (n = 63) were obtained from EDTA-anticoagulated whole blood. Hemoglobin analysis was carried out using the high-performance liquid chromatography (Variant II, Bio-Rad Laboratories, Hercules, CA, USA). Point mutations and deletions in the α-, β-, and γ-globin genes were detected using previously described methods [10]. The study protocol was reviewed and approved by the local medical ethics committee, and written consent was obtained from parents and/or patients prior to commencing the study.

2.2. CZE

CZE was performed using a P/ACE MDQ Capillary Electrophoresis System (Beckman Coulter Inc., USA) equipped with a 70 cm × 50 μm uncoated fused-silica capillary (Ruifeng chromatographic device Co. Ltd., Hebei, China) at 25 °C. The separation voltage was 16 kV and the UV detector was set at 214 nm. The electrophoresis buffer (100 mM sodium phosphate) was adjusted to pH 2.14 with TFA (HPLC grade, Darmstadt, Germany), and contained 0.25% PEG-6000 (purity ≥ 99.5%; Jinhuada Chemical Reagent Co. Ltd., Guangzhou, China). Samples were pressure-injected for 3 s. Prior to separation, 25 μl of whole blood was washed one time with saline to remove plasma (3000 r/min for 3 min), and red cells were hemolyzed in 1 ml deionized water. The hemolysate was centrifuged for 10 min at 4000 r/min to remove cell debris [11].

2.3. Statistical analysis

Mean values of the α/β area ratios between controls and patient groups were compared by ANOVA and ROC curves were plotted to evaluate the sensitivity and specificity and to determine the appropriate cut-off values for predicting thalassemia patients. The area under the curve (AUC) was calculated to further investigate the ability of this method to discriminate thalassemia patients. Bivariate correlation analysis was conducted to determine the association between the area ratio of γ/α and HbF levels in the samples from β-TI/TM by using the spearman correlation coefficient. *P*-values < 0.05 were considered significant, and analysis was carried out with SPSS 13.0 statistical analysis software (IBM, Armonk, NY, USA).

3. Results

3.1. Determination of the α, β and γ peaks

For the initial separation of α, β and γ chains, 8 samples from normal adult (n = 4) and Hb H disease (n = 4) were analyzed. Compared with the normal samples, the peak eluting at 14.69 min was confirmed as α-globin as it was significantly reduced during separation of Hb H samples. Since γ chains contribute to HbF, 52 Hb samples from β-TI/TM patients with different HbF levels (10%–95%) were used to verify the α, β and γ

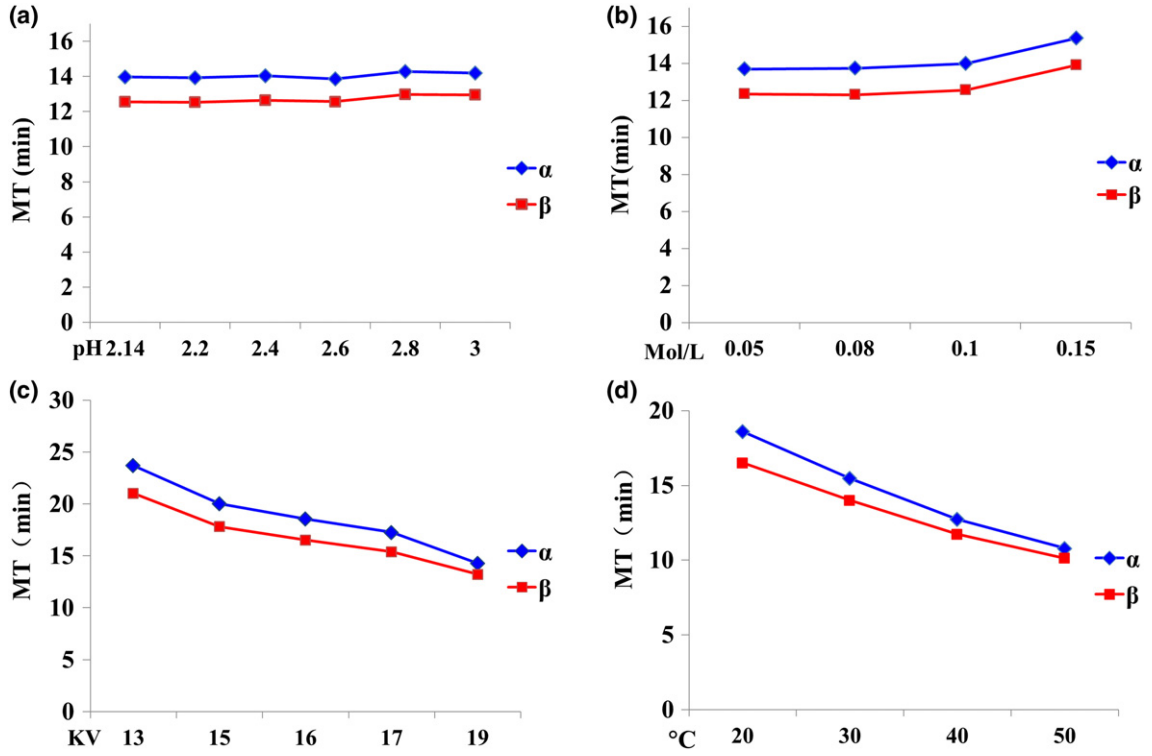

Fig. 2. The optimization of initial conditions of free zone capillary electrophoresis: (a) PH value; (b) the concentration of phosphate buffer; (c) voltage; (d) temperature.