Transient Hybridization Between Different Hemoglobins Detected by Cross Electrophoresis

Lihong Han [1], Bin Yan [1], Yan Su [2], Liangyi Qin [3], Lijun Gao [2], Lishe Zhou [2], Wenbin Qin [2*]
[1] Department of Basic Medical Science,Putian University,Putian Fujian 351100, China
[2] Laboratory of Hemoglobin, Baotou Medical College, Baotou, China
[3] Clinical laboratory of Nanhui central hospital, Shanghai, China
Email: vhanlihong@163.com

Abstract: In this paper, we aimed to introduce a new method in detecting transient hemoglobin (Hb) hybrids. First, electrophoretic pure HbA, HbA_2, HbF and HbA_1 were prepared by starch–agar mixed gel, and then cross electrophoresis was performed on 2% starch–agar mixed gel. The slow-moving HbA_2 samples were added in the front long slots and the fast-moving HbA, HbF and HbA_1 samples were added in the back short slots of the gel. These two rows of slots are all located on the cathode side of the gel and about 1 cm away from each other. After electrophoresis, which was performed at 5V/cm for about 4 h, the gel was stained with Ponceau Red and Benzidine. The results showed that when fast-moving HbA and HbF crossed slow-moving HbA_2, the band shape of HbA_2 was deformed as a valley, however when fast-moving HbA_1 crossed slow-moving HbA_2, the band shape of HbA_2 was not deformed. This finding demonstrates that HbA and HbF could form transient hybrid with HbA_2 during cross electrophoresis; however HbA_1 could not form any hybrid with HbA_2 because of its chemical modification of Hb subunits.
***Keywords*:** cross electrophoresis, hemoglobin, transient hybrid

1. Introduction

Hemoglobin (Hb) is a well-researched oxygen carrying protein in red blood cells (RBCs). There are HbA_1, HbA, HbF and HbA_2 in adult RBCs. HbA_1 is relatively complicated, it is located in the anode side of HbA during electrophoresis and includes some modified products of Hb, such as glycosylated Hb (HbA_{1c}) and glutathione combined Hb. Glycosylated Hb is formed in a non-enzymatic pathway by Hb's normal exposure to high plasma levels of glucose and glutathione combined Hb is formed during storage [1]. All Hbs are made up of two kinds of similar globular proteins which polymerize together to form a tetramer. The subunit compositions of HbA, HbF and HbA_2 are $\alpha_2\beta_2$, $\alpha_2\gamma_2$ and $\alpha_2\delta_2$, respectively. Four subunits are bound to each other by salt bridges, hydrogen bonds, and hydrophobic interactions. There are two kinds of contacts between α and β subunits: $\alpha_1\beta_1$ and $\alpha_1\beta_2$. Hybridization of Hbs is a more widespread phenomenon and is found in artificial mixtures of different Hbs. This kind of hybridization is generally called asymmetric hybridization. This hybridization happens when two kinds of Hbs are put together, after dissociation and recombination, different Hbs would exchange subunit and generate new hybrid molecule [2-4]. Thus, asymmetric hybridization has nothing to do with electrophoresis; however, electrophoresis technique can be used to identify the hybrid molecules. In our previous study, we had found for the first time that HbA could interact with HbA_2 during cross electrophoresis [5]. We called this interaction as transient hybridization. In this study, we will continue to study the transient hybridization between HbA_2 and other Hbs, and try to understand its molecular mechanism and further application.

2. Materials and methods

2.1 Blood source

This study had been approved by our local ethics committee. Healthy adult blood, umbilical cord blood and diabetes blood were collected from the first affiliated hospital of Baotou medical college. Before the blood samples were collected, all the people who took part in this experiment were asked to sign the consent information. Venous blood samples were anti-coagulated with heparin, stored at 4 °C, and generally analyzed within 24h.

2.2 Preparation of hemolytic fluid of red blood cell

Anti-coagulated venous blood from different individuals was centrifuged at 3000 rpm for 10 min and the upper plasma was aspirated. To 1 ml saline, was added 200 µl of the lower RBCs. The mixture was centrifuged at 3000 rpm for 10 min, after which the supernatant was aspirated. This washing operation was repeated 4-5 times, and then 200µl water

and 100μl CCl_4 were added to the RBCs. After vigorous vortex, the sample was centrifuged at 12000 rpm for 10 min and the upper red hemolysate was piped out carefully for storage at 4℃ .

2.3 Preparation of pure HbA, HbA1, HbA2 and HbF

Electrophoretic pure HbA, HbA_1, HbA_2 and HbF were preparaed by 2% starch-agar mixed gel（starch:agar=4:1）electrophoresis as described earlier [6]. After electrophoresis, the red bands of HbA, HbA_2, HbA_1 and HbF were cut down from the gel and stored in an Eppendroff tube at -80 °C, respectively. Before use, the gels were thawed at room temperature and centrifuged at 10000 rpm for 10 min, and then the red supernatants were piped out for further use.

2.4 Cross electrophoretic technique

Cross electrophoresis was performed on a 2% 10×20 cm starch-agarose mixed gel, which was prepared as earlier [6]. Then two rows of sample slots about 1 cm apart, were made on the cathodic side of the gel which was about 1.5 cm away from the edge as indicated in Figure 1. The front row is made up of long slots, and the back row is made up of short slots. The back short slots were just behind the front long slots and at each side of cross electrophoresis group, control slots were made. 10-15 μL electrophoretic pure Hb samples were subsequently applied on the slots using 3 MM filter paper. Generally, the slow-moving Hbs were added in the front row and the fast-moving Hbs were added in the back row. Electrophoresis was performed in borate buffer at 5 V/cm for 4 h. After electrophoresis, we first observed the red bands on the gel directly, and then stained the gel as usual [6].

3. Results

3.1 Example diagram of cross electrophoretic

In our study, starch-agar mixed gel electrophoresis was used to prepare electrophoretic pure HbA, HbA_2, HbA_1 and HbF. Then, cross electrophoresis was performed to observe the interaction between slow-moving HbA_2 and fast-moving HbA, HbA_1 and HbF, respectively. If there is interaction between each other, the central part of HbA_2 band will bend toward the anode as a valley. Otherwise, the band shape will not be deformed. As shown in Figure 1, when HbA threaded HbA_2, the long HbA_2 band bent toward the anode as a valley. The same phenomenon appeared when HbF threaded HbA_2, showing that HbF may be interacting with HbA_2. However, when HbA_1 threaded HbA_2, the band shape of HbA_2 did not change; indicating that HbA_1 may have no interaction with HbA_2.

3.2 Coarse structures of Hbs

Coarse structures of HbA, HbA_2 and HbF are $\alpha_2\beta_2$, $\alpha_2\delta_2$ and $\alpha_2\gamma_2$, respectively. There has been an 'asymmetric hybridization' with regard to the molecular hybridization of Hb [3,4]. Its reaction formula is assumed as follows in the case of HbA: $\alpha^{A}{}_2\beta^{A}{}_2 + \alpha^{B}{}_2\beta^{B}{}_2 \rightarrow \alpha^{A}{}_2 + \beta^{A}{}_2 + \alpha^{B}{}_2 + \beta^{B}{}_2 \rightarrow \alpha^{A}{}_2\beta^{B}{}_2 + \alpha^{B}{}_2\beta^{A}{}_2$. In this formula, $\alpha^{A}{}_2\beta^{A}{}_2$ represents human normal HbA and $\alpha^{B}{}_2\beta^{B}{}_2$ represents animal HbA variant. After putting two kinds of Hbs together, they will exchange subunit and generate new hybrid molecules. Asymmetric hybridization has nothing to do with electrophoresis; however, electrophoretic technique can be used to identify the hybrid molecules.

4. Discussion

In our study, the hybridization of Hbs is presumed as 'transient hybridization', and the specific reaction formula of HbA and HbA_2 is assumed as follow: $\alpha_2\beta_2 + \alpha_2\delta_2 \longleftrightarrow (\alpha\beta)_2 + (\alpha\delta)_2 \longleftrightarrow 2(\alpha\beta{:}\alpha\delta)$. This progress occurred during electrophoresis when fast-moving Hbs cross slow-moving ones. Thus, transient hybridization belongs to 'dynamic hybridization' of hemoglobin. However, the binding force between the subunits of αβ:αδ was so weak that they gradually dissociated during electrophoresis, causing the middle part of HbA_2 band to move faster and form the special "valley" shape. In contrast with HbA and HbF, the composition of HbA_1 is more complex, which is a mixture of some modification products of Hb, such as glycosylated Hb and glutathione combined Hb, etc. This may have an effect on the interaction between HbA_1 and HbA_2, so we could not see the change of the HbA_2 band after electrophoresis.

As to cross electrophoresis, in 1953, Grassmann and Hubner reported a method to detect the formation of loosely bound addition compound using the technique of continuous paper electrophoresis. They proposed the use of this method in the detection of biologically important reactions, such as the formation of antigen-antibody complexes and of enzyme-substrate complexes. Later, this method was named "cross electrophoresis" by Nakamura [7]. In our study, we initially performed cross electrophoresis on starch-agar gel to detect the interaction between different Hbs. We found that interaction may exist between some Hbs and be absent in other Hbs, the reason was speculated to have a relationship with the homologous and modification of Hbs. We believe that this method can be used to study not only the interaction

between different human Hbs, but also the Hbs molecular evolution of different species.

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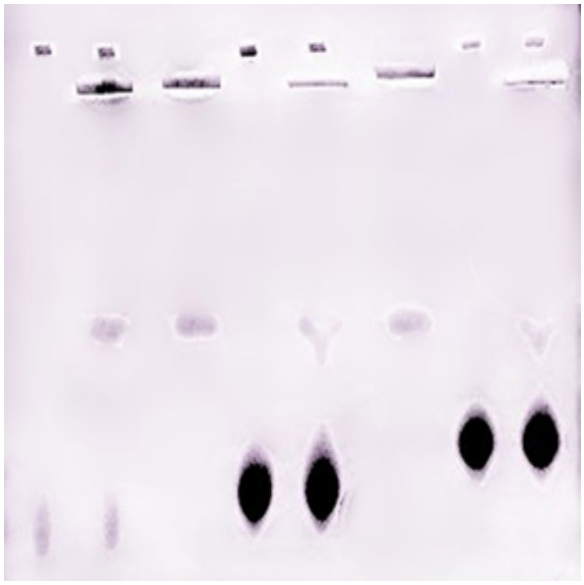

Figure 1. Transient hybrid between different Hbs. Lane 1,3,4,6 and 7 are control samples of HbA_1, HbA_2, HbA, HbA_2 and HbF, respectively. Lane 2, 5 and 8 are hybrid groups between HbA_1 and HbA_2, HbA and HbA_2, HbF and HbA_2, respectively. Electrophoresis was performed in borate buffer at 5 V/cm for 4 h

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