Separation and Quantitation of Hemoglobin H and Hemoglobin Bart’s by Electrophoresis on Gelatinized Cellulose Acetate

SUPIS CHINDAVANIG, M.S., AND RACHNEEwon GUMNARDPETCH, B.S.

Division of Clinical Chemistry, Department of Pathology, Faculty of Medicine, Ramathibodi Hospital, Bangkok, Thailand

ABSTRACT


STARCH-BLOCK ELECTROPHORESIS is considered a method of choice for separation of fast-migrating hemoglobins, H and Bart’s. This procedure is not simple, and quantitation is not possible by direct scanning with a densitometer. Electrophoresis of hemoglobins on cellulose acetate membrane is rapid and relatively easy. This method allows good separation of the hemoglobin fractions but cannot distinguish the fast migrating hemoglobins H and Bart’s. Recently, a gelatinized cellulose acetate membrane, cellulose diacetate combined with a gel, has been used as a high-resolution electrophoretic medium for the separation of hemoglobin fractions.® 7, 9 Pabis and associates® made a brief mention that this could be used for the preliminary identification of abnormal hemoglobins such as S, C, E, H, and Bart’s.

The present report describes a method for separation of the unstable hemoglobin H and Bart’s by electrophoresis on cellulose acetate gel membrane. Quantitation of these unstable hemoglobins has also been accomplished by scanning with densitometer.

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Materials and Methods

Equipment

1. Model R-101 Microzone Electrophoresis system (Beckman Instruments, Inc., Fullerton, Calif.).
2. Model R-110 Microzone Densitometer (Beckman Instruments, Inc.).
3. Cellogel strips, 5.7 by 14.5 cm. with perforation (Chemetron, Milan, Italy).

Reagents

Buffer. Tris-glycine buffer, Tris 14.1 Gm., glycine 22.6 Gm., distilled water to 1.5 l.
Staining solution. 1 bottle Beckman B 4 dye (0.2% Ponceau S, 3% trichloroacetic acid and 3% sulfosalicylic acid) in 250 ml. distilled water.
Destaining solution. 5% acetic acid.
Mounting solution. Glycerine.

Procedure

Hemolysates were prepared from heparinized venous blood by the method of Chernoff.® The erythrocytes were washed three times with physiologic saline solution and then lysed by addition of 1 ml. of distilled water per ml. of cells. To each
2.5-ml aliquot of hemolyzed cells was added 0.5 ml. of toluene. The cell stroma was separated by low-speed centrifugation. The toluene layer was discarded and the hemolysate was then filtered through wet Whatman filter paper. The electrophoresis was carried out in the Beckman-Spinco Microzone electrophoresis cell. Gelatinized cellulose acetate (Cellogel) was used as a supporting medium. Tris-glycine buffer, pH 8.6, was substituted for the barbital buffer, pH 8.6. The hemolysates were placed on the membrane adjacent to the cathode end. 250-v. direct current was applied for 45 min. The strip was stained with Ponceau S and the excess dye was...
Table 1. Concentrations of Hemoglobin H before and at Intervals after Freezing of the Hemolysates*

<table>
<thead>
<tr>
<th>Sample</th>
<th>Day of Storage</th>
<th>Before Freezing</th>
<th>After Freezing</th>
<th>% Hb H Decreased</th>
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<td>1</td>
<td>2</td>
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<td>13</td>
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<td>9</td>
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* Electrophoresis on gelatinized cellulose acetate, Tris-glycine buffer, 250 volts for 45 min.

washed off with 5% acetic acid. The strip was washed in running water mounted in glycerine and put in plastic envelope. The wet strip was scanned in a Beckman densitometer.

Results and Discussion

The hemoglobins are separated on gelatinized cellulose acetate membranes in a Tris-glycine buffer, pH 8.6, for 45 min. at 250 v. Distinct separation of hemoglobins E, F, A, A₂, H, and Bart’s was achieved (Fig. 1). A₂ and E travel at the same speed and hence cannot be separated. The following factors have been considered.

Supporting Medium

With the use of cellulose acetate gel, the hemoglobin H appeared as a sharply defined narrow band. On cellulose acetate membrane the hemoglobin H appeared as a broad diffused band.

Buffer

Both Tris-glycine and Tris-EDTA borate (TEB) buffer were recommended as good buffers for hemoglobin separation. In our experiment, both Tris-glycine and TEB buffer gave distinct bands of hemoglobin H.

Stability of Hemoglobin H

Effect of Chloroform. When chloroform was used for the extraction of lipid material, the H band did not show up on the electrophoretic pattern. When toluene was used in place of chloroform, the H band appeared prominently (Fig. 2).

Effect of Storage. Hemoglobin H was shown to be denatured after storage of the hemolysates at freezing temperature or at 4 C. It may disappear partially or even completely on the cellulose electrophoresis pattern after being frozen for 24 hr.

We have tested the stability of hemoglobin H hemolysates by our electrophoretic procedure at low voltage. Table 1 shows that hemoglobin H could be identified and the concentration did not change for as long as 5 days.

Effect of Voltage. The unstable hemoglobins cannot be demonstrated by cellulose acetate electrophoresis at 400 v., but satisfactory demonstration was made at 250 v. on gelatinized cellulose acetate membrane. Besides the physical difference between the two supporting media, the higher temperature resulting from the application of 400 v. may lead to more denaturation of the unstable hemoglobins, as suggested by Perutz and Lehman and Grimes, who showed rapid denaturation and precipitation of hemoglobin in solution upon heating to 50 C.

Conclusion

Electrophoresis of hemoglobin solutions on gelatinized cellulose acetate at 250 v. for 45 min. appears to be a simple, reliable, and rapid procedure for the diagnosis of rapidly moving hemoglobins (H and Bart’s) and alpha thalassemia in countries where alpha thalassemic genes are common among the population.

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References


