BRIEF REPORT

Rapid Differentiation of Polypeptide Chains of Hemoglobin by Cellulose Acetate Electrophoresis of Hemolysates

By SATOSHI UEDA AND ROSE G. SCHNEIDER

IDENTIFICATION of the abnormal polypeptide chain of a hemoglobin (Hb) variant is often useful in connection with structural analyses and Chernoff and Pettit have recently improved on existing methods for this purpose by using starch gel electrophoresis of globin in urea barbital buffer. Izzo used this same buffer for electrophoresis of globin on cellulose acetate. Ohba et al. have proposed starch gel electrophoresis of whole hemolysates in the presence of p-chloromercuribenzoic acid. We now report a simple, rapid method of differentiating polypeptide chains of hemoglobin by cellulose acetate electrophoresis of whole hemolysates in urea barbital buffer.

MATERIALS AND METHODS

Hemolysates were prepared by addition of water and toluene to saline washed erythrocytes, and hemoglobins were isolated by chromatography on DEAE Sephadex or CM Sephadex C50. Globin was prepared by the acid acetone method. Two types of buffer were used—the pH 8.0 barbital buffer of Chernoff and Pettit and 0.05 M barbital buffer, pH 8.6 (Na barbital 10.3 g/L and diethylbarbituric acid 1.4 g/L); each buffer was made 6 M in respect to urea and 0.05 M to 2-mercaptoethanol. The final pH value of the latter buffer was about 8.9.

A small amount of hemolysate, containing 2 to 10 per cent hemoglobin, is diluted and a diluted sample is added an equal volume of 2-mercaptoethanol. The mixture is refrigerated for 2-8 hours. [The given proportion of mercaptoethanol is arbitrary; in some experiments 1 part mercaptoethanol to 4 parts of diluted hemolysate proved satisfactory.] Isolated hemoglobins are treated in the same way, and they may be analyzed simultaneously with whole hemolysates and with solutions of globin. Electrophoresis is performed on a cellulose acetate strip about 8 cm. long (buffer to buffer) and up to 12 cm. wide. The strip is immersed in the buffer until thoroughly wet; it is then blotted lightly and positioned in the chamber, which is surrounded with crushed ice during electrophoresis.

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*We usually used Titan cellulose acetate, made by the Helena Co. (9786 Lincoln Court, Taylor, Michigan, 48180). A suitable electrophoresis chamber is also made by this company, but any chamber with a short migration path, about 7 to 11 cm. (buffer to buffer), may be used. Most of the analyses described were performed on an apparatus designed to hold glass slides for agar gel electrophoresis.
DIFFERENTIATION OF POLYPEPTIDE CHAINS

Fig. 1.—Cellulose acetate electrophoresis, barbital buffer, pH 8.0 with 6 M urea, of isolated hbs (except AS which is the whole hemolysate) Cathode to right. Duration of electrophoresis: (left) 1 hr. (right) 11/2 hrs.

The prepared samples (about 2 to 5 lambda) are streaked with a micro pipette on previously penciled lines in the center of the cellulose acetate strip, and one hundred to two hundred v, yielding currents of 1 to 4 ma, are applied for 1 to 3 hours; the cellulose acetate is then stained with Ponceau S and it may be cleared for storage according to directions furnished by the manufacturer.

RESULTS AND DISCUSSION

Results are similar with both buffers—pH 8.0 and pH 8.6—but the latter is more stable and easier to prepare. Shortly after application of the current, several heme pigmented fractions move rapidly towards the anode, disappearing in about 20 minutes. A high concentration of mercaptoethanol in the sample is necessary for this to occur. All the globin chains move to the cathode, and they separate satisfactorily in 1 to 3 hours. Representative separations are illustrated in Figure 1 and Figure 2 and the relative mobilities of the polypeptide chains examined are indicated in Figure 3. Each abnormal α or β polypeptide chain was readily identified except for β Sabine,7 which moves like β A. In Hb Sabine, as in many of the other unstable hemoglobin, the substitution is a neutral one (β 91 leu→pro), so no alteration in charge of the
Fig. 2.—Cellulose acetate electrophoresis, barbital buffer, pH 8.6, with 6 M urea, of hemolysates or globin. The A globin contains a small amount of A. Cathode to right. Duration of electrophoresis: (a) 2 hrs. (b) 3 hrs. (c) 2 hrs. (d) 2 hrs.

globin chain would be expected. The diminished anodal mobility of these hemoglobins is probably due to loss of heme, an event to which they are peculiarly prone.
Fig. 3.—Relative mobilities of polypeptide chains of various hemoglobins, cellulose acetate electrophoresis, urea barbital buffer. Cathode to right. The structural alteration of each variant chain, and published references to the samples used are:

- G St-I (same as G Philadelphia), α 68, Asp NH₂→ Lys (11); Sealy (same as Hasharon and Sinai), α 47 Asp→ His; δ 40 Gly→ Arg; C, β 6 Glu→ Lys; S, β 6 Glu→ Val; D Los Angeles, β 121 Glu→ Glu NH₂; G Galveston, β 43 Glu→ Ala; α I, G Coushatta, β 22 Glu→ Ala; P, β 117 His→ Arg; I, α 16 Lys→ Glu; Sabine, β 91 Leu→ Pro.

The abnormal delta chains of Hb A'₂ move only slightly more cathodally than the normal α chains, and these two chains do not separate well. In connection with the analyses of Hb A'₂ we noted that nonheme proteins, which were present in large amounts in the first chromatographic separation of this variant, move electrophoretically like β or γ polypeptide chains; in samples with considerable nonheme protein, such as the Hb F in Figure 1 (left), there is a characteristically fuzzy appearance in the region of the γ chain.

In analyses of whole hemolysates a faint line, moving like normal δ chains, is often apparent, especially in samples with high Hb A₂ values; it is absent in samples freed of Hb A₂. No fetal variants were available during this study, but Hb Barts was analyzed and, as expected, contained only gamma chains (Fig. 1, right).

Our experience with Hb P (α₂β₂δ₂β₁₇α₁₆) illustrates the usefulness of the method. Although this variant was originally described in 1957, a blood sample for structural analysis was not available until recently and only a very
small amount was provided. It was helpful, therefore, to be able to identify
the abnormal chain with less than 0.1 ml hemolysate (Fig. 2 d).

This method, like that of Chernoff and Pettit, utilizes 6 M urea to dis-
sociate the polypeptide chains of hemoglobin and electrophoresis in barbital
buffer to separate them; the high concentration of mercaptoethanol of the
present method permits simultaneous removal of heme from globin, perhaps
by providing the nonpolar solvent required for this process. Prior preparation
of globin is therefore unnecessary.

The use of cellulose acetate as the supporting medium for electrophoresis
eliminates the time consuming task of preparing starch gels, particularly
tedious in the presence of urea. Electrophoretic separations are achieved in
one to three hours, instead of the 22 hours recommended for starch gel and
handling, staining, and storage are considerably simplified.

**SUMMARY**

Separation of the polypeptide chains of hemoglobin and identification of
an abnormal chain may be achieved in a few hours by electrophoresis on
cellulose acetate in barbital buffer pH 8.6, with 6 M urea. The method is
applicable to whole hemolysates to which a large amount of 2-mercaptoethanol
has been added. It requires minimal preparative work.

**SUMMARIO IN INTERLINGUA**

Le separation del catenas polipeptidic de hemoglobina e le identification de un catena
anormal pote esser effectuate in pauc horas per electrophorese super acetato de cellulosa
in tampon de barbital a pH 8,6 con 6 M urea. Le metodo es applicabile a hemolysatos
integre al quales un grande quantitate de 2-mercapto-ethanol ha esite addite. Le metodo
require solo un minimo de travalio preparatori.

**ACKNOWLEDGMENT**

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