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Note

High-performance liquid chromatography of hemoglobins

I. Determination of hemoglobin A_2

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The separation of hemoglobin variants has traditionally been carried out by electrophoresis [1, 2] or ion-exchange chromatography on carbohydrate gels [3]. Ion-exchange chromatography gives good resolution and quantitation but is very slow. In an effort to decrease analysis time, microchromatography on small ion-exchange columns was implemented for the determination of specific hemoglobin variants [3]. These columns take only 30—200 min to develop but fractions must be collected manually and the procedure cannot be automated. Over the past few years, support materials for the high-performance liquid chromatography of proteins have been developed and refined [4—6]. The method described herein uses a high-performance anion-exchange support for the rapid determination of hemoglobins A_2 and A_0.

Hemoglobin A_2 levels are elevated in certain clinical states, most notably, in β-thalassemia [7]. A rapid method for the routine determination of Hb A_2 and A_0 is therefore valuable in the clinical recognition of patients with certain hemoglobinopathies.

EXPERIMENTAL

Chemicals

Tris(hydroxymethyl)aminomethane (Tris) was purchased from ICN Pharmaceuticals (Cleveland, Ohio, U.S.A.). Sodium acetate and glacial acetic acid were from Mallinckrodt (Paris, Ky., U.S.A.). The Hb A_2 Quik Column Control (2.47% Hb A_2) was from Helena Laboratories (Beaumont, Texas, U.S.A.). The blood was from anonymous samples which would have been discarded after other determinations had been made.

Apparatus

SynChropak AX 300 columns, 250 × 4.1 mm I.D., particle size 10 μm,

were obtained from SynChrom, Inc. (Linden, Ind., U.S.A.). A Micromeritics 7000 liquid chromatograph and a Model 785 variable wavelength detector (Micromeritics Instrument Corp., Norcross, Ga, U.S.A.) were used for the analyses with a Model CV-6-UHPa-N-60 injection valve (Valco, Houston, Texas, U.S.A.).

Methods

Solvent A (0.02 *M*, pH 8.0) was prepared by adding 2.42 g of Tris to 1 l of distilled water and adjusting the pH with acetic acid. Solvent B was the same as solvent A with the addition of 13.6 g of sodium acetate (0.1 *M*). Hemoglobin standards were dissolved in distilled water at a concentration of 15 mg/ml. Hemolyzed blood was diluted tenfold. No further sample preparation was necessary. Sample size was 10—20 μl. Full-scale absorbance of the detector was set at 0.05 at a wavelength of 410 nm. A 10-min linear gradient from 0 to 30% was used with a step to 100% after A_0 had eluted. The flow-rate was 2.5 ml/min.

RESULTS AND DISCUSSION

Fig. 1 shows the analysis of the hemoglobin standard under the above conditions. Slight variations in operating conditions can result in radical differences in resolution, as is seen in Fig. 2. Here Hb A_2 and A_0 are widely separated but Hb A_1 elutes with Hb A_0. Fig. 3 shows a sample of blood from a person with

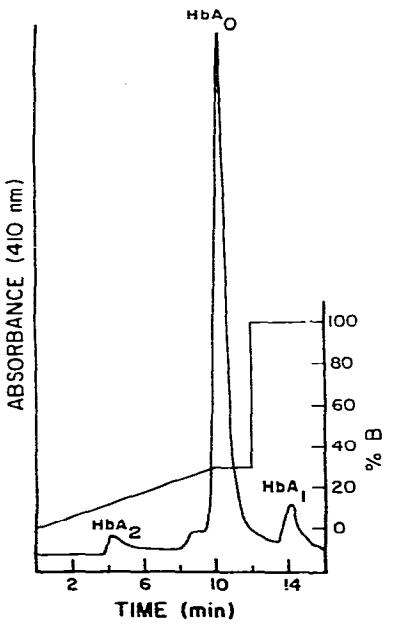
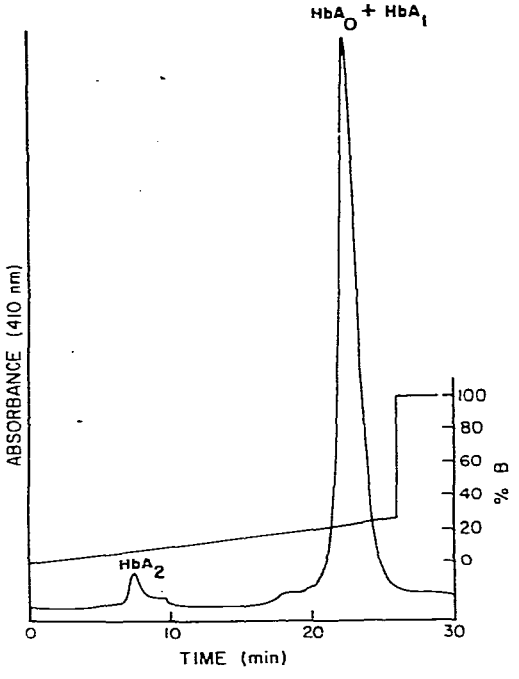


Fig. 1. Analysis of standard Hb A_2 sample. Column: SynChropak AX300, 250 × 4.1 mm I.D. Solvents A and B as in text, with a 10-min linear gradient from 0 to 30% then step to 100%. Flow-rate 2.5 ml/min; pressure 1200 p.s.i. Detection at 410 nm.

β-thalassemia trait. The Hb A_2 peak was approximately twice as large as that of normal blood.

Analysis of hemoglobins by high-performance ion-exchange chromatography allows rapid separations with the capabilities of automated injection and quantitation. With a 5-min recycle time, samples may be analyzed every 20 min with minimal preparation. A drop of blood is more than adequate for the procedure.



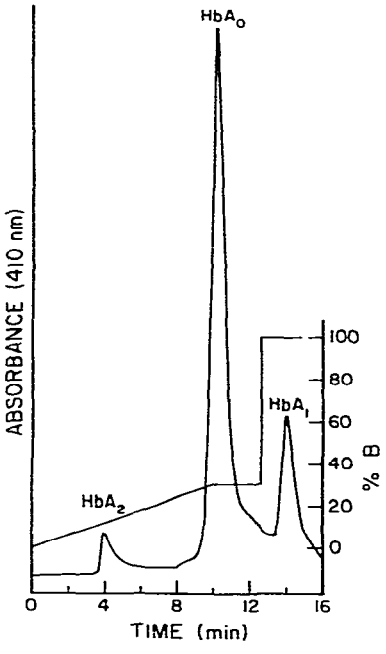


Fig. 2. Analysis of standard Hb A_2 sample. Column: SynChropak AX 300, 250 × 4.1 mm I.D. Solvents A and B as in text, with a 40-min linear gradient from 0 to 40% then step to 100%. Flow-rate 2.0 ml/min; pressure 1020 p.s.i. Dtection at 410 nm.

Fig. 3. Analysis of human blood showing β-thalassemia trait. Conditions as in Fig. 1.

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REFERENCES

1 R.M. Schmidt, D.L. Rucknagel and T.F. Necheles, J. Lab. Clin. Med., 86 (1975) 873.
2 R.N. Wrightstone, Test of the Month, 2 (1976).
3 T.H.J. Huisman and J.H.P. Jonxis, in M.K. Schwartz (Editor), The Hemoglobinopathies (Clinical and Biochemical Analysis, Vol. 6), Marcel Dekker, New York, Basel, 1977, p. 124.
4 S.H. Chang, R. Noel and F.E. Regnier, Anal. Chem., 48 (1976) 1839.
5 S.H. Chang, K.M. Gooding and F.E. Regnier, J. Chromatogr., 125 (1976) 103.
6 T.D. Schlabach, A.J. Alpert and F.E. Regnier, Clin. Chem., 24 (1978) 1351.
7 D.J. Weatherall, in R.M. Hardisty and D.J. Weatherall (Editors), Blood and Its Disorders, Blackwell Scientific Publications, Oxford, London, Edinburgh, Melbourne, 1974, p. 630.