RESOLUTION OF GENETIC VARIANTS OF HUMAN ERYTHROCYTE GLUCOSE 6-PHOSPHATE DEHYDROGENASE BY THIN-LAYER CHROMATOGRAPHY

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Three common genetic variants of red cell glucose 6-phosphate dehydrogenase (D-glucose 6-phosphate:NADP oxidoreductase, E.C.1.1.1.49) are found in Nigerian subjects (Luzzatto, Allan, & De Flora, 1965). Two of them have normal enzyme activity but are electrophoretically different, A and B, the third one is associated with enzyme deficiency but is electrophoretically undistinguishable from A, and is referred to as A^- **. It has previously been shown in this laboratory that, despite electrophoretic identity, A and A^- differ in some physico-chemical properties and can be resolved by chromatography on DEAE-Sephadex (Luzzatto and Allan, 1965). More recently, Yoshida, Stamatoyannopoulos, and Motulsky (1967) have confirmed these results by using a different ion-exchanger and have further shown that the "deficiency" in glucose 6-phosphate dehydrogenase activity in A^- erythrocytes is largely due to a high rate of breakdown of the enzyme during cell ageing. In this report we present a new technique, whereby the A and A^- variants can be resolved very rapidly and revealed directly on thin layer chromatography (TLC) plates.

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**We have followed the nomenclature recommended by W.H.O., Bull. Wld. Hlth. Org. 36, 319 (1967).

MATERIALS AND METHODS

The techniques for the purification of the enzyme variants and their classification by electrophoretic mobility were as described previously (Luzzatto and Allan, 1965). Descending chromatography by salt gradient elution on glass plates coated with DEAE-cellulose powder was essentially an adaptation of the ascending technique of Wieland and Determan (1962). Eight grams of MN 300 DEAE and 2 grams of MN 300 cellulose powder (Macherey, Nagel & Co., 516 Duren, West Germany) were homogenized in 90 ml of 0.1 mM EDTA. The suspension was immediately poured onto a Shandon adjustable spreader and five 20 x 20-cm plates were prepared on a Shandon Unoplan leveler, with a layer thickness of 0.4 mm. Plates were dried at room temperature. Samples of different enzyme variants were applied to the plates and chromatographed and detected as described in the figure legends.

RESULTS AND DISCUSSION

The behaviour, relative to each other, of the three common enzyme variants by this technique is strikingly different from their behaviour in starch-gel electrophoresis. Under the conditions of our experiment the A and B types are undistinguishable (they both remain at the origin), but A^- migrates down the plate as a single band, with slight trailing; it can thus be fully and immediately separated from both the A and B types.

Four other genetic variants of glucose 6-phosphate dehydrogenase were analyzed by this technique. Two among these, obtained from Greek subjects (Rattazzi, Lenzerini, Siniscalco, and Luzzatto, in preparation) which have the same electrophoretic mobility like A, migrate faster than A^- on TLC, and are thus resolved from both A and A^- by this technique. Of the other two, isolated from Nigerian subjects (Luzzatto and Afolayan, submitted for publication)

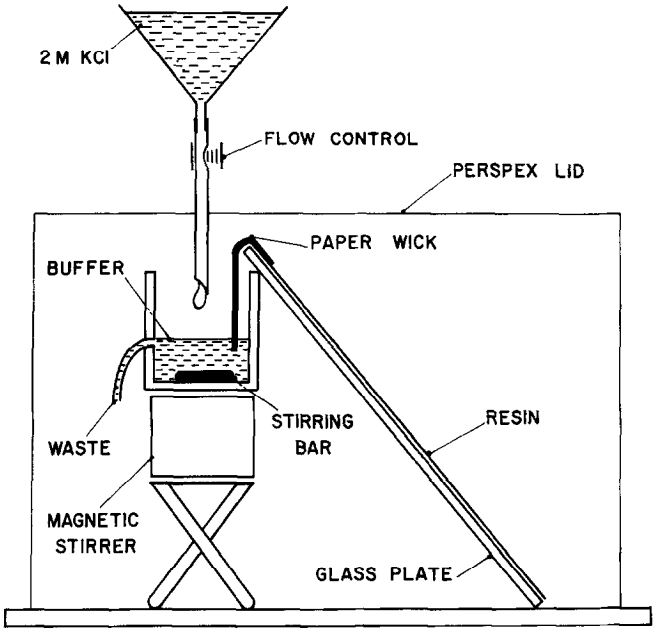

Figure 1. Set-up for descending ion-exchange TLC by gradient elution. The buffer reservoir is a 25 x 5 x 5-cm perspex trough containing 150 ml of 0.45 M KCl, 5 mM potassium phosphate buffer, pH 6.9, 0.1 mM EDTA and 2 μM NADP. Plates were pre-equilibrated by applying a paper wick (2 layers of Whatman 3 MM) and letting buffer flow from the trough until the whole plate was wet (1 hour). The samples (previously dialyzed against 5 mM phosphate buffer, pH 6.9, 50 mM KCl, 0.1 mM EDTA, 2 μM NADP) were then applied in a volume of 0.01-0.03 ml (containing 0.003 to 0.01 international enzyme units) on the origin line (5 cm from the upper edge). The KCl reservoir (containing 150 ml of 2 M KCl) was immediately opened to a flow rate of 40 ml/hour. Linearity of the gradient was checked by testing the chloride concentration in the trough at serial times during the run.

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and having electrophoretic mobility lower than B on starch gel, one remains at the origin in TLC and the other migrates less than A^-

In summary, it is possible to resolve the A and A^- variants of glucose 6-phosphate dehydrogenase in a 4 hour TLC run on DEAE-cellulose by gradient elution. Several other variants can be distinguished under the same conditions. It appears that this simple technique might be usefully adopted in the initial characterization

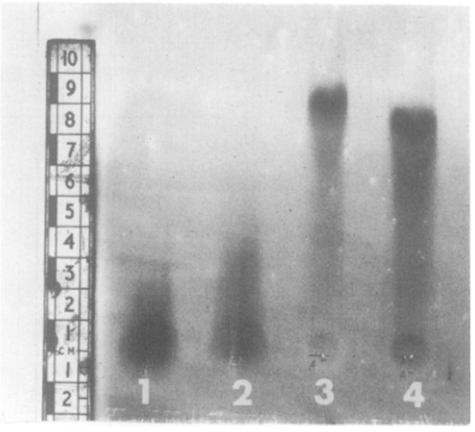

Figure 2. TLC of glucose 6-phosphate dehydrogenase types A, B and A⁻. The plate was processed as described in the legend to Figure 1. After a 4-hour run the plate was removed and rinsed in 100 ml of 0.05 M Tris-HCl, pH 8.6, for 3 minutes, and then overlaid with 60 ml of staining solution, prepared as follows: 0.5 M Tris-HCl, pH 8.6, 0.01 M $MgSO_4$, NADP 5 mg, glucose 6-phosphate 5 mg, nitro blue tetrazolium 2.5 mg, phenazine methosulfate 0.5 mg. After staining for 15 minutes the plate was rinsed in H_2O for 30 minutes and then dried at room temperature. Composition of samples: (1) A; (2) B; (3) A⁻; (4) A + A⁻.

of a glucose 6-phosphate dehydrogenase variant, since the information yielded is different from, and possibly complementary to that obtained from starch-gel electrophoresis.

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