ELECTROPHORETIC ABNORMALITY IN TRIOSEPHOSPHATE ISOMERASE DEFICIENCY

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Received May 3, 1968

A deficiency of the enzyme triosephosphate isomerase (TPI) causes nonspherocytic hemolytic disease associated with increased susceptibility to infection and with neurologic disorders (Schneider et al., 1965). This rare disorder is inherited as an autosomal recessive trait. Column chromatographic study of TPI on DEAE sephadex failed to reveal any significant difference between the normal enzyme and residual enzyme from a deficient subject (Schneider et al., 1968). We have devised a method for the detection of TPI on starch gel after electrophoresis, which depends upon the following two reactions:

$$\text{GAP} \xrightleftharpoons{\text{TPI}} \text{DHAP}$$

$$\text{DHAP} + \text{NADH} \xrightleftharpoons{\alpha\text{-glycerophosphate dehydrogenase}} \alpha\text{-glycerophosphate} + \text{NAD}$$

Disappearance of NADH fluorescence under long-wave UV light indi-

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Abbreviations: GAP = glyceraldehyde-3-phosphate
DHAP = dihydroxyacetone phosphate
TPI = triosephosphate isomerase

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cates the areas of the gel to which triosephosphate isomerase activity has moved during electrophoresis. When normal hemolysates are subjected to electrophoresis, three major bands of enzyme activity are found. The hemolysate from a TPI-deficient subject reveals only a moderate decrease in the activity of the slowest moving band, but virtual disappearance of the fastest moving isozymes.

MATERIALS AND METHODS

Electrophoresis was carried out in a horizontal system using the apparatus designed by Smithies (1955). The electrode compartments contained 0.1 M tris and 4.5 mM EDTA adjusted with hydrochloric acid to a pH of 9.3 at 25° C. A 13% starch gel was prepared in a 1:10 dilution of this buffer. Washed red cells were hemolyzed in 4 volumes of distilled water, centrifuged to remove stroma, dialyzed against the gel buffer, diluted in gel buffer, and applied to the gel by saturating and blotting Whatman #3 MM filter paper rectangles. A final dilution of 1:50 to 1:100 of normal red cells was found to give satisfactory enzyme concentration for electrophoresis. Electrophoresis was carried out at a gradient of approximately 10 V/cm for 16 hours in a cold room. After slicing, the gel was overlaid with a few milliliters of a mixture comprised of 3 mM DL-glyceraldehyde-3-phosphoric acid (Sigma), approximately 0.5 mM NADH, .05 M triethanolamine buffer pH 8.0, .005 M EDTA and approximately .008 units of α-glycerophosphate dehydrogenase per ml.

RESULTS

When hemolysates prepared from normal erythrocytes were subjected to electrophoresis in this system three zones of defluorescence appeared on the gel within 15 to 20 minutes. A fourth, more rapidly moving zone appeared just ahead of the main three bands

after prolonged incubation. The fourth zone was difficult to define because of the spreading of defluorescence from the main three bands. Red cell samples from 27 normal Caucasian and 26 normal Negro subjects have been examined electrophoretically without any essential difference in the enzyme pattern observed, except for minor variations in the intensity of the most rapidly moving major band. The relative position of the bands was not altered when electrophoresis was carried out on 10% or 16% starch gel. Rabbit red cells, fat, muscle, liver, kidney, heart, and brain gave a 3-banded pattern very similar to that observed with human red cells, but with only approximately one-half the mobility. Three bands of enzyme activity have also been observed in pig muscle (Scopes, 1964).

The red cells of one subject with TPI deficiency and of her mother were examined. The results of some of these studies are shown in Figure 1. It is apparent that the most rapidly moving band was not seen in the hemolysate prepared from the enzyme deficient subject, even when the hemolysate concentration was adjusted in such a manner that the same amount of enzyme was electrophoresed as in the normal subject. When the same dilution of TPI-deficient and normal red cells was subjected to electrophoresis it was apparent that even the most slowly moving band, although detectable, was decreased in intensity in the red cells of the TPI-deficient subject. Mixtures of normal and TPI-deficient hemolysates resulted in electrophoretic patterns which resembled closely that of the heterozygous sample.

DISCUSSION

The three-banded distribution of most of the TPI activity on electrophoresis could be interpreted as being the result of the aggregation of two separate subunits, A and B, to give

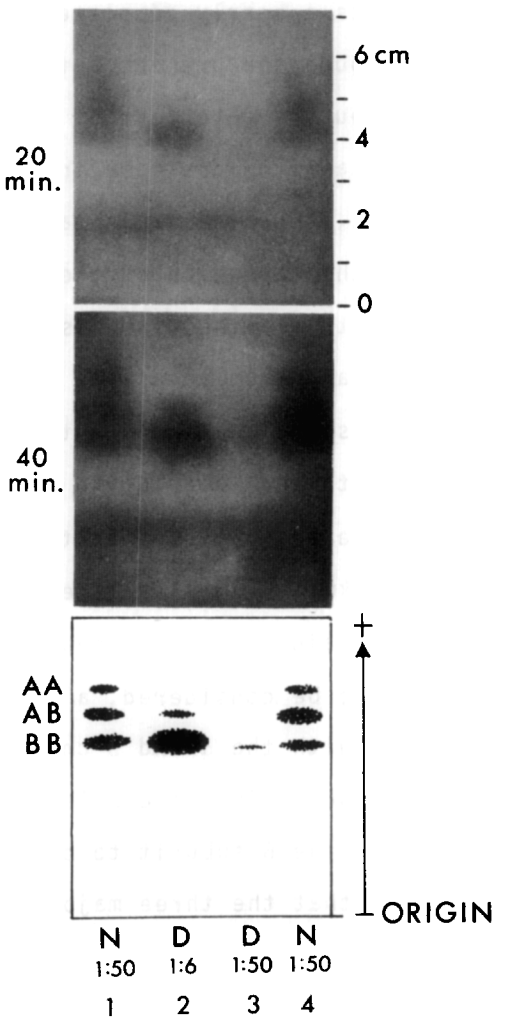

Figure 1: Starch gel electrophoresis of a red cell lysate from a normal subject (N) and a subject with triosephosphosphate isomerase deficiency (D). The 1:50 dilution of normal hemolysate and 1:6 dilution of deficient hemolysate contain approximately the same amount of enzyme activity. Conditions of electrophoresis as given in the text. Photographed with a yellow filter under UV light 20 and 40 minutes after application of the reaction mixture to the gel.

dimers AA, AB, and BB. If this interpretation of the electrophoretic findings is correct then the case of TPI deficiency studied clearly represents a nearly complete failure to synthesize the A subunit, although the presence of a weak second band indicates that some A subunits were formed. However, it would also be necessary to explain the decrease in the quantity of the slower moving (B) subunit, since a simple failure to form A subunits should not affect the formation of the BB dimer. It is possible that both subunits are the products of closely-linked genes having arisen from a common ancestral gene by tandem duplication. They might then share a common controlling mechanism as appears to be the case with the loci in the lac operon. The findings might then be considered analogous to the polar mutants involving these genes (Beckwith, 1967). Alternately, the possibility that the A subunit aids in the removal of the B subunit from the ribosome may also be considered, a situation analogous to that thought to occur with the α and β chains of hemoglobin (Colombo and Baglioni, 1966). This seems less likely, however, because of the ability of the B subunit to complex with itself.

It is also possible that the three major bands do not represent the dimeric forms of two different subunits, but merely polymers of a single polypeptide chain, complexes of such a chain with small molecules, or different configurational forms of the same enzyme. In this case it is possible that a mutation which influences the structure of the polypeptide would also influence its capacity to polymerize, to complex, or to assume certain configurations. The failure of the degree of separation to be influenced by the concentration of starch in the starch gel mitigates against major differences in molecular weight of three bands (Smithies, 1962).

Electrophoretic study of other subjects with TPI deficiency, the discovery of electrophoretically detectable alleles, or study of interspecific hybrids may help to clarify the basic structure of the enzyme and the mechanism of decrease of enzyme activity in TPI deficiency.

ACKNOWLEDGEMENT

This work was supported in part by a fund established in the name of Barry T. Leithead Research Fellowship, and in part by Public Health Service Grant No. HE 07449 from the National Heart Institute.

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