DIFFERENT PROPERTIES OF GLUCOSE 6-PHOSPHATE DEHYDROGENASE FROM HUMAN ERYTHROCYTES WITH NORMAL AND ABNORMAL ENZYME LEVELS*

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Approximately one-fifth of Nigerian males have an erythrocyte glucose 6-phosphate dehydrogenase (G6PD) activity reduced to about 15% of normal (Gilles & Taylor, 1961; Edozien, 1965). This enzyme deficiency is genetically determined and underlies the clinical condition commonly known as primaquine sensitivity (Tarlov, Brewer, Carson & Alving, 1962). There is good evidence that the mutation in Negroes is distinct from those causing G6PD deficiency in various Caucasian groups (Kirkman, McCurdy & Naiman, 1964; Marks, 1965; Motulsky, 1965). It is not yet known whether the low level of activity in the G6PD deficient red cells from Negro subjects results from a structural abnormality of the enzyme, from a decreased rate of production, or from both. No consistent differences have been detected between G6PD purified from normal and deficient erythrocytes in terms of Michaelis constants (Kirkman, Riley & Crowell, 1960), the time course of inactivation at 37° (Marks, Szeinberg & Banks, 1961), and some immunological

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properties (Marks & Tsutsui, 1963). On the basis of electrophoretic mobility the enzyme from deficient cells in American Negroes is indistinguishable from that of the A variant found in normal cells, and the former has therefore been designated as A^- (Boyer, Porter & Weilbacher, 1962; Kirkman & Hendrickson, 1963). Similar electrophoretic data have been obtained in Nigerians (Porter, Boyer, Watson-Williams, Adam, Szeinberg & Siniscalco, 1964; Luzzatto, Allan & De Flora, 1965).

The present communication is concerned with a further attempt at detecting a structural difference, or lack of it, between the enzyme types found in normal and G6PD deficient red cells from Nigerian subjects. It will be shown that the A and A^- enzyme species differ in their thermal inactivation profiles and in chromatographic behaviour.

MATERIALS AND METHODS

G6PD was partially purified from the erythrocytes of expired Blood Bank blood as described by Chung & Langdon (1963a). The experiments to be reported were performed with preparations carried through the first ammonium sulphate fractionation, having a specific activity of about 3 units (μmoles NADPH produced/min)/mg of protein for the A enzyme, and about 0.5 units/mg of protein for the A^- enzyme. Each preparation was from cells from a single individual. Starch-gel electrophoresis was carried out with the discontinuous buffer system of Smithies (as described by Porter et al., 1964) and after 16 hours at 4°C the gel was sliced and stained according to Boyer et al. (1962). G6PD activity was determined by the method of Kornberg & Horecker (1955), proteins by the method of Lowry et al. (1951) and chloride by titration with $AgNO_3$.

RESULTS AND DISCUSSION

1. Heat inactivation. The effect of heat on the partially purified enzyme

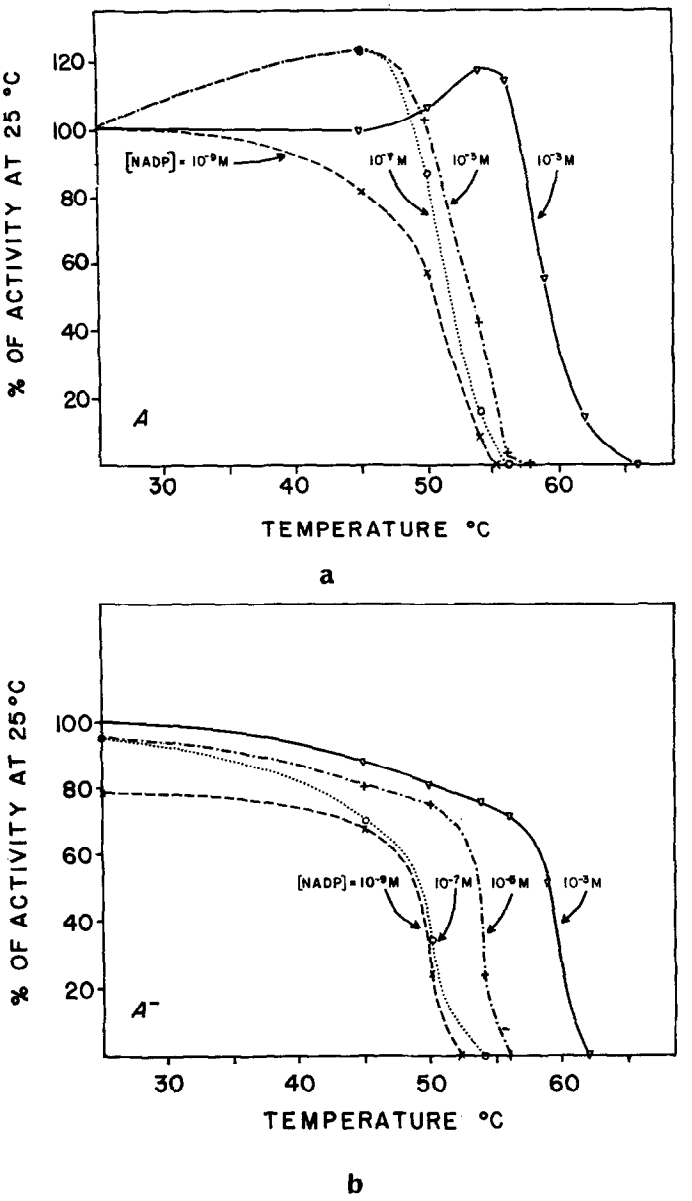

Fig. 1. Heat inactivation of G6PD at various NADP concentrations. Partially purified enzyme preparations of types A and A^- were dialyzed in pairs at 4°C for 3 hours against 3 changes of 100 volumes of 0.02 M potassium phosphate buffer, pH 7.0, containing 0.2 M KCl, 2×10^{-4} M EDTA and 2×10^{-6} M NADP, and then for 30 minutes against 2 changes of 500 volumes of a buffer of otherwise identical composition, but containing only 10^{-9} M NADP. The dialyzed solutions were adjusted to the same enzyme activity concentration. Aliquots of the two solutions were brought to the specified NADP concentrations, heated for 7 minutes at the desired temperatures, chilled on ice and then assayed at room temperature (25°C). (a) Enzyme type A. (b) Enzyme type A^-.

was studied by incubating for 7 minutes at increasing temperatures in the presence of various concentrations of NADP, and then testing the remaining activity at room temperature under standard conditions. The results obtained with the A enzyme are shown in Fig. 1a. It is seen that in excess of NADP the enzyme is unaffected by heat between 25 and 45°C, it undergoes a moderate activation between 50 and 56°C, and is then abruptly inactivated starting at 57°C. The transition temperature, defined as the temperature at which the enzyme retains 50% of the original activity, is 59°C. This sharp inactivation profile is reminiscent of the co-operative breakdown of a highly stabilized structure, such as a helix-random coil transition (Sheraga, 1963). If the concentration of NADP is decreased, we observe a concomitant decrease first in the transition temperature, and then in the sharpness of the transition. This is consistent with the concept that NADP is specifically involved in stabilizing the active conformation of the enzyme and is in agreement with previous findings by Marks et al. (1961), Kirkman & Hendrickson (1962) and Chung & Langdon (1963b). The effect of heat on enzyme type A^-, studied in the same way, is shown in Fig. 1b. In excess of NADP the transition temperature is still 59°C, but it is apparent that:(1) the inactivation profile is less sharp at all NADP concentrations, and there is no activation in the 50-56°C region; (2) decreasing the concentration of NADP has a more pronounced effect on the A^- enzyme. The latter finding is clearly demonstrated in Fig. 2, where the transition temperature is plotted against the NADP concentration for both enzyme types. Thus it seems that the thermal inactivation profiles are more informative than the time course of inactivation at 37°C in the study of the variants of red cell G6PD. Under the conditions of the experiment the transition temperature was independent

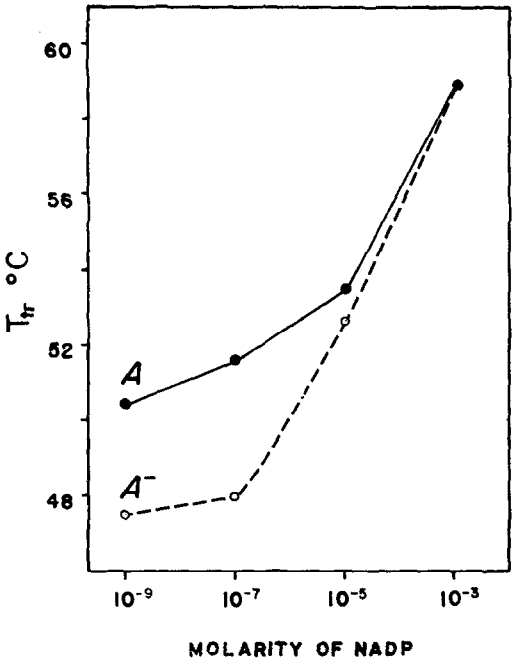

Fig. 2. Transition temperature for heat inactivation of G6PD as a function of NADP concentration. The transition temperature (defined as the temperature at which the enzyme retains 50% of its original activity) is plotted against NADP concentration for the A and A^- enzyme types. The data are from the same experiments shown in Fig. 1.

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of enzyme concentration over an eight-fold range. The activity recovered after incubation at 59°C was somewhat higher if the solution was "slow-cooled" than if it was "quick-cooled", suggesting that the unfolding caused by heat is partially reversible.

2. Chromatography on DEAE-sephadex. The patterns of elution of the enzyme types A and A^- from DEAE-sephadex columns are shown in Fig. 3, in the top and middle sections respectively. It is seen that in both cases the G6PD activity emerges as a single peak, but the salt concentration at which the enzyme is eluted is about 0.22 M KCl for type A and about 0.17 M KCl for type A^-. If a mixture of the two enzymes is chromatographed, two peaks

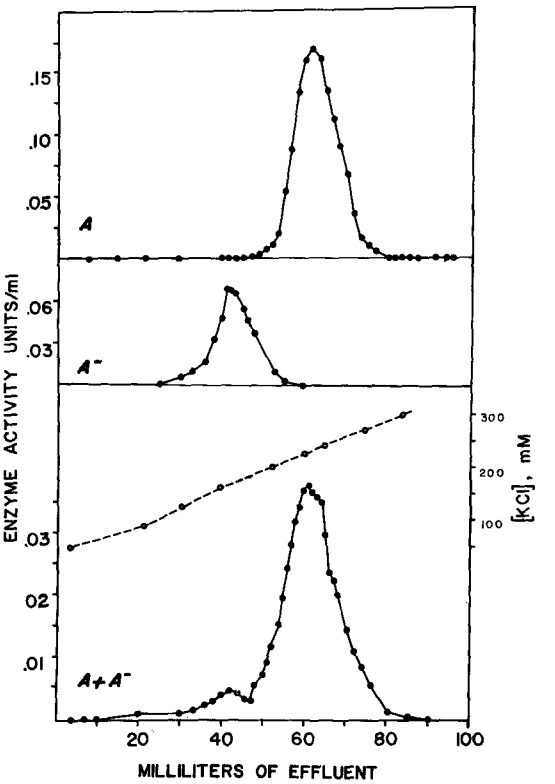

Fig. 3. Chromatography of G6PD types A and A^- on DEAE-Sephadex A-50. For each experiment a 1 x 20 cm column was packed in 0.025 M phosphate buffer, pH 6.9, containing 0.05 M KCl, 10^{-4} M EDTA and 10^{-6} M NADP. One to two units of enzyme (previously dialyzed against the same buffer) was layered on the column, which was then flushed with 20 ml of the same buffer. Elution was then started with a 120 ml linear gradient of KCl (dissolved in the same buffer), from 75 to 400 mM. The chromatograms were run at 4-6°C. One ml fractions were collected and assayed for protein, G6PD activity and chloride concentration. Top: enzyme type A. Recovery of activity 95%. Middle: enzyme type A^-. Recovery of activity 48%. Bottom: mixture of the A and A^- enzymes, in amounts having equal activity. Recovery 61%. Continuous lines, enzyme activity; broken line, KCl concentration. The profile of the chloride gradient is shown for simplicity only in the bottom section: it was similar in the other two chromatograms.

of activity are eluted, which correspond closely in their positions to those found with the two separate enzyme types. When the A^- type is chromatographed the recovery of enzyme activity is considerably lower than when the A type is

chromatographed (48 and 95% respectively). Correspondingly, in the "mixed" chromatogram there is much less activity associated with the first peak than with the second peak. On the basis of the findings with the two enzyme types chromatographed separately it seems most likely that the two peaks in the third "mixed" chromatogram, which have not yet been positively identified, represent indeed the A and A^- fractions of the mixture. It thus appears to be possible to resolve these two enzyme types on a DEAE-sephadex column. The separation is unlikely to originate from a difference in molecular size, since the sedimentation coefficient has been found to be the same (6.9s) for both G6PD types (Luzzatto, unpublished), and since with a molecular weight of approximately 190,000 (Chung & Langdon, 1963a) no "gel filtration" effect can be expected on DEAE-sephadex A-50. More likely, the separation is based on the fact that the A^- enzyme has a different primary structure. This may in turn affect the electrostatic charge of the protein molecule under the conditions of this experiment (but not under those obtaining in starch-gel electrophoresis), or it may affect the non-electrostatic forces involved in the binding interaction between the protein molecules and the resin particles.

In conclusion, the evidence presented suggests that the mutation in G6PD-deficient erythrocytes from Nigerian subjects bears directly on the primary structure of the enzyme molecule. Further characterization of the structural difference between the A and A^- enzymes is in progress. At the present time it is not possible to infer whether the mutation also entails a decrease in the rate of production of glucose 6-phosphate dehydrogenase.

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