Characterization of Leukocyte Glucose 6-Phosphate Dehydrogenase in Sardinian Mutants *

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Previous studies characterizing the hereditary deficiency of glucose 6-phosphate dehydrogenase (G-6-PD) have been performed for the most part with the erythrocyte enzyme (1–27). It is, however, known that although in the Negro the G-6-PD deficiency is mostly localized in the erythrocytes,[1] in Caucasian mutants the defect involves many other tissues, including leukocytes (30–38). We have recently demonstrated in Sardinian mutants that the leukocyte defect is only detectable in male and female subjects with a complete erythrocyte G-6-PD deficiency (38). This defect is characterized by both a decreased enzyme activity and a reduced electrophoretic mobility on starch gel.

This report deals with studies on crude and purified preparations of G-6-PD obtained from the leukocytes of sensitive Sardinian subjects. The propositi included a large number of subjects belonging to several family groups from different parts of Sardinia. Data will be presented which indicate that the mutant enzyme differs from the normal enzyme both in kinetic and physical properties. The two can be distinguished by differences in electrophoretic mobility, affinity for glucose 6-phosphate and nicotinamide adenine dinucleotide phosphate (NADP), optimal pH activity, stability to inactivation by heat and by nicotinamide adenine dinucleotide phosphate glycohydrolase (NADPase), and protection by NADP. The different enzymes appear similar with regard to the sedimentation pattern on sucrose density gradient ultracentrifugation.

[1] A decrease in the enzyme activity has also been reported for the lens and the liver of primaquine sensitive Negroes (28, 29).

Methods

Subjects

Studies were carried out on 54 subjects belonging to 28 families from different parts of Sardinia; 11 of these propositi have previously experienced acute hemolytic episodes after fava bean ingestion. The controls were normal subjects with no clinical evidence of hematological disease.

Preparation of lysates

For every experiment amounts of blood varying from 30 to 50 ml were collected with siliconized syringes, and 2 mg per ml of EDTA was added. To separate the leukocytes from the erythrocytes, we added blood to an equal volume of a mixture containing 3% dextran, 3% glucose, and 0.9% NaCl and sedimented it for an hour at + 2° C. The supernatant containing leukocytes was collected by suction and centrifuged for 10 minutes at 800 rpm. An equal volume of cold water was added to the precipitated bulk of cells; leukocytes were then lysed by three successive freezings in a dry ice and acetone mixture. The leukocyte stroma were then removed by centrifuging the lysate for 15 minutes at 25,000 × g at + 2° C. We then added 2×10^{-5} M NADP to the part of the lysate which was used for the determination of optimal pH activity, of Michaelis constant (Km) for G-6-P, and of electrophoretic mobility.

Purification of the enzymes

The G-6-PD was purified from leukocytes by the following procedure (39). For each purification 30 to 50 ml of leukocyte lysate from different subjects was utilized; the initial specific activity of the lysate ranged between 0.05 and 0.15 U per mg.

1) First ammonium sulfate step. The leukocyte lysate was saturated to 35% with ammonium sulfate, and after 20 minutes at 0° C it was centrifuged for 10 minutes at 25,000 × g at + 2° C. The collected precipitate was suspended in 0.25 M glycylglycine buffer, pH 7.6, containing 5×10^{-4} M NADP.

2) Calcium phosphate gel absorption. The ammonium sulfate fraction was added to freshly prepared gel [13.8 ml of gel containing 8 mg per ml of calcium phosphate per milliliter of $(NH_4)_2SO_4$ fraction], kept at 0° C for 15 minutes, and then centrifuged at 25,000 × g for 10 minutes at + 2° C. The precipitate was eluted with 0.1 M phosphate buffer, pH 7.6, containing 5×10^{-4} M NADP. The eluate was then separated from the gel by centrifugation at 35,000 rpm for 10 minutes at + 2° C.

3) Second ammonium sulfate step. The eluate from calcium phosphate gel was saturated to 30% with $(NH_4)_2SO_4$, kept at 0° C for 10 minutes, and then centrifuged at 18,000 × g for 10 minutes at + 2° C. The precipitate was discarded, and the supernatant was saturated to 50% with ammonium sulfate, kept at 0° C for 10 minutes, and then centrifuged at 20,000 × g for 10 minutes at + 2° C. The precipitate was collected and resuspended with 0.25 M glycylglycine buffer, pH 7.6, containing 5 × 10⁻⁴ M NADP.

4) Third ammonium sulfate step. The above fraction was saturated to 35% with $(NH_4)_2SO_4$, kept at 0° C for 10 minutes, and then centrifuged at 25,000 × g for 10 minutes at + 2° C. The precipitate was discarded; the supernatant was brought to 47% saturation with ammonium sulfate, and after 10 minutes at 0° C it was centrifuged at 25,000 × g for 10 minutes at + 2° C. The precipitate obtained was then suspended in 0.25 M glycylglycine, pH 7.6, containing 5 × 10⁻⁴ M NADP.

Eight different enzyme purifications were performed, four of normal and four of mutant enzymes. Of these purifications, four (two of normal and two of mutant enzyme) were performed as described in the presence of NADP, and four (two of normal and two of mutant enzyme) in the absence of NADP. In the presence of NADP, the two preparations from control leukocytes gave 346- and 312-fold purifications yielding 11.4 and 12.8% of the original total activity, whereas the two from mutant leukocytes gave 306- and 318-fold purifications with a final yield of 12.2 and 10.8%. In the absence of NADP, the two preparations from control subjects gave 270- and 302-fold purifications yielding 4.5% and 5.2% of the original total activity; the two from mutant subjects gave 246- and 280-fold purifications with a final yield of 3.8% and 4.7%.

NADPase was purified 32-fold from rat spleen according to the previously described method (40).

TABLE I

Glucose 6-phosphate dehydrogenase level in normal and mutant erythrocytes and leukocytes

Subjects	Erythrocytes		Leukocytes	
	No. of subjects	Specific activity*	No. of subjects	Specific activity*
Normal (male and female)†	356	15.2 (11.8–18.6)	26	132.4 (116.5–153.8)
Mutant homozygous and hemizygous†	232	0– 0.8	54	68.3 (51.5– 87.9)
Mutant heterozygous (female)	7	7.3 (5.2– 8.9)	7	130.8 (110.6–155.2)

* One U of glucose 6-phosphate dehydrogenase (G-6-PD) activity is that amount of enzyme which determines an optical density variation of 1.0 at 340 mμ per minute at +20° C; the specific activity is in reference to grams of hemoglobin for the erythrocyte enzyme and to grams of protein for the leukocyte enzyme.

† The activity values for the erythrocyte G-6-PD of the normal subjects, the mutant homozygous subjects, and the mutant hemizygous subjects were obtained from protocols of this laboratory for the period 1958 to 1965.

Starch gel electrophoresis

Starch gel electrophoresis was performed according to the procedure of Smithies (41). Starch gel was prepared in 0.01 M Tris-HCl buffer, pH 8.2, containing 2.7 × 10⁻³ M EDTA and 2 × 10⁻⁵ M NADP. Enzymatic preparations contained 2 × 10⁻⁵ M NADP, and the amounts placed in the slits were 0.05 to 0.1 U. The buffer in the vessels was 0.05 M Tris-HCl, pH 8.4, containing 2.7 × 10⁻³ M EDTA and 2 × 10⁻⁵ M NADP. The run was vertical toward the anode, and it lasted for 4 hours at + 2° C with a voltage output of 8 v per cm applied to the gel column. The G-6-PD activity was directly evidenced on the starch by the use of the following mixture: 5 × 10⁻² M Tris-HCl, pH 8.2; 5 × 10⁻⁵ M G-6-P; 2 × 10⁻⁴ M NADP; 5 × 10⁻⁴ M MgCl₂; 0.015% tetrazole blue nitrite; and 0.008% phenazine metasulfate. The dark blue stain, which indicates the enzyme band, was obtained after 2 hours at 37° C.

Determinations

The sedimentation coefficient on sucrose density gradient ultracentrifugation was determined according to the method of Martin and Ames (42) as previously described (39). G-6-PD activity was determined according to the method of Kornberg and Horecker (43). If not otherwise specified, the assay mixture contained 30 mM glycylglycine, pH 7.5, 2 mM G-6-P, 0.5 mM NADP, and a suitable amount of enzyme to obtain a 0.010 to 0.015 OD increase per minute at 340 mμ. The determinations were performed in cuvettes with an optical trait of 1.0 cm. The carboxyhemoglobin (COHb) was determined by direct spectrophotometric reading at 413 mμ, the proteins were determined according to Lowry, Rosebrough, Farr, and Randall (44), and the hemoglobin was determined according to Drabkin's method (45).

Calculations

One U of G-6-PD was defined as the amount of enzyme that determines an optical density variation of 1.0 at 340 mμ per minute at +20° C; the specific activity was in reference to grams of protein for the leukocyte G-6-PD, and to grams of hemoglobin for the erythrocyte G-6-PD. One U of NADPase was defined as the amount that splits 0.1 μmole of NADP per hour at 37° C.

Reagents and apparatus

The high speed centrifugations were performed in an LRA Lourdes refrigerated centrifuge and a Spinco L2 centrifuge with an SW 39 rotor. The Optica CF4 spectrophotometer was used. Substrates,[2] dextran,[3] starch,[4] and other chemicals[5] were obtained commercially.

Results

Table I shows the G-6-PD activity in erythrocytes and leukocytes from normal and mutant sub-

[2] Sigma Chemical Co., St. Louis, Mo.
[3] Henley Co., New York, N. Y.
[4] Connaught, Toronto.
[5] E. Merck, A. G., Darmstadt, Germany.

TABLE II

*Heat stability of crude leukocyte glucose 6-phosphate dehydrogenase**

Preparation[†]	Amount	No. of patients	% activity at the following times[‡]			
			15 min	30 min	60 min	90 min
	U					
Normal enzyme	2	16	116 ± 9.4	103 ± 9.2	100 ± 8.5	100 ± 8.7
Normal enzyme	1	10	107 ± 9.8	100 ± 10.4	96 ± 7.6	88 ± 11.3
Normal enzyme	0.5	22	98 ± 10.3	95 ± 10.3	84 ± 8.7	72 ± 9.1
Mutant enzyme	2	32	100 ± 11.5	98 ± 10.6	93 ± 12.2	81 ± 10.8
Mutant enzyme	1	32	96 ± 10.8	83 ± 9.9	71 ± 9.1	54 ± 7.5
Mutant enzyme	0.5	54	74 ± 9.7	65 ± 6.3	51 ± 6.8	38 ± 5.3

* The incubation was performed in a thermostated bath at 37° C. The incubation mixture contained, per ml, 30 μmoles of pH 7.5 glycylglycine and enzyme at the indicated concentrations. At the chosen times suitable amounts of the mixture were cooled, diluted with 0.15 M KCl, and assayed for enzyme activity.

† The preparations utilized were those free of added nicotinamide adenine dinucleotide phosphate (NADP).

‡ Per cent of activity related to zero time, mean ± standard error of the reported cases.

jects. The present results, obtained from a large number of subjects, confirm our previous observations in a few cases (38). The leukocyte defect is found only in the homozygous and hemizygous subjects with no detectable erythrocyte enzyme activity (i.e., 2% or less of controls). In the heterozygous subjects with an intermediate level of the erythrocyte G-6-PD, the leukocyte enzyme activity was always normal.

The stability of the leukocyte enzyme to heat (37° C) was tested in normal and mutant subjects with crude (Table II) and purified (Table III) preparations of the enzyme both as a function of time and enzyme concentration. Under all experimental conditions the mutant enzyme is markedly less stable to inactivation by heat.

The crude normal enzyme is activated during the first minutes when incubated at high enzyme concentrations (2 and 1 U per ml). This activation

depends on the amount of EDTA added to the blood as the anticoagulant. When EDTA is present in concentrations higher than those normally used (2 mg per ml of blood) the G-6-PD is activated at lower enzyme concentrations (0.5 U per ml). This activation occurs over a longer period of time. On the other hand, the crude mutant enzyme shows no activation. It is inactivated by heat irrespective of either enzyme concentration or time of incubation and even in the presence of high concentrations of EDTA (4 to 8 mg per ml).

The differences in thermostability between the mutant and normal enzymes are more striking with purified preparations. The purified undialyzed enzyme contains a large amount of ammonium sulfate, which induces a notable activation, as was also observed with the erythrocyte purified G-6-PD (10). The $(NH_4)_2SO_4$-induced activation is present only in the undialyzed control enzyme but

TABLE III

Heat stability of purified leukocyte glucose 6-phosphate dehydrogenase

Preparation*	Amount	% activity at the following times[†]			
		15 min	30 min	60 min	90 min
	U				
Normal undialyzed[‡]	1	175 ± 15.8	170 ± 16.3	181 ± 16.8	168 ± 15.2
Normal undialyzed	0.5	160 ± 17.4	148 ± 15.3	156 ± 14.7	152 ± 16.4
Normal dialyzed§	1	90 ± 10.9	83 ± 10.2	75 ± 8.1	63 ± 6.9
Normal dialyzed	0.5	81 ± 8.8	74 ± 8.5	56 ± 6.0	42 ± 5.7
Mutant undialyzed[‡]	1	65 ± 5.2	51 ± 5.9	40 ± 3.5	31 ± 4.1
Mutant undialyzed	0.5	58 ± 6.0	47 ± 5.5	39 ± 3.1	27 ± 3.3
Mutant dialyzed§	1	46 ± 5.3	41 ± 5.1	23 ± 3.4	10 ± 2.2
Mutant dialyzed	0.5	41 ± 5.5	30 ± 3.9	19 ± 2.7	9 ± 1.8

* The preparations utilized were those free of added NADP.

† All values are the mean ± standard error of six determinations (three for each enzyme preparation).

‡ $(NH_4)_2SO_4$ concentration was 15% in all undialyzed enzyme preparations.

§ Dialysis was carried out against 0.15 M KCl buffered at pH 7.5 for 4 hours at +2° C. For further details see Table II.
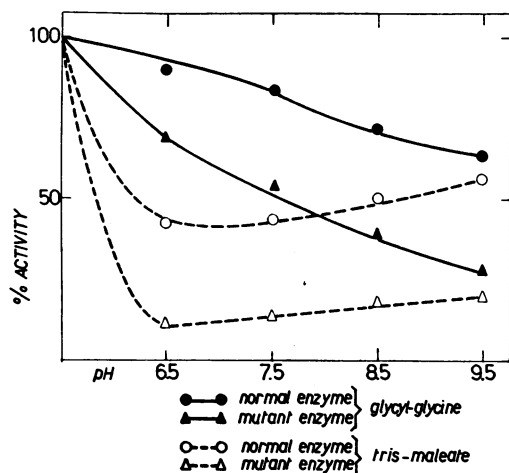
FIG. 1. EFFECTS OF pH AND BUFFER ON HEAT STABIL-ITY OF LEUKOCYTE GLUCOSE 6-PHOSPHATE DEHYDROGENASE. The incubation mixture contained, per milliliter, 30 μmoles glycylglycine or Tris-maleate at the mentioned pH, and 0.5 U crude enzyme. The incubation was performed at 37° C for 60 minutes. Results are expressed as per cent of residual activity and are the mean value of 7 experiments with normal subjects and 16 with mutant subjects. For further details see Table II.
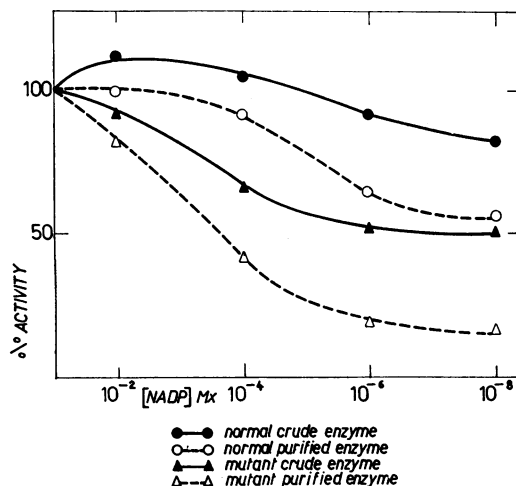

FIG. 2. EFFECT OF NADP ON HEAT STABILITY OF LEU-KOCYTE GLUCOSE 6-PHOSPHATE DEHYDROGENASE. The incubation mixture contained, per milliliter, 30 μmoles gly-cylglycine, pH 7.5; 0.5 U enzymes; and nicotinamide ade-nine dinucleotide phosphate (NADP) at the indicated concentrations. The mixture was incubated at 37° C for 60 minutes. The results are expressed as per cent of residual activity and are the mean value for 2 experiments for the purified enzyme from normal and mutant subjects, 5 experiments for the crude enzyme from normal subjects, and 12 experiments for the crude enzyme from mutant subjects. For further details see Table I. Mx = molar concentration.

is not present in either the dialyzed or undialyzed preparations of the purified mutant G-6-PD.

Ramot and associates (23) have recently ex-cluded a thermostability difference between par-tially purified erythrocyte enzymes from normal and mutant Jewish subjects. On the other hand, they have observed that the early heat activation is absent in nondialyzed preparations of the mutant enzyme.

Kirkman, Schettini, and Pickard (22) have ob-served an increased thermolability of the erythro-cyte enzyme in subjects of Mediterranean origin.

As illustrated in Figure 1, the effect of the buf-fering system and of the pH on the thermostability

TABLE IV

Effect of NADPase and NADP on leukocyte glucose 6-phosphate dehydrogenase*

Compounds added		Crude enzyme†		Purified enzyme†	
NADPase	NADP	Normal	Mutant	Normal	Mutant
U	mole/L				
		100	83	83	41
0.5		88	35	70	19
1.0		65	24	51	3
2.0		52	11	34	4
2.0	10^{-6}	68	16	42	7
2.0	10^{-4}	86	28	65	10
2.0	10^{-2}	94	45	86	28

* The incubation mixture contained, per milliliter, 30 μmoles of pH 7.5 glycylglycine; 1 U of enzyme; and NADP glycohydrolase (NADPase) and NADP at the indicated concentrations. The samples were incu-bated at 37° C for 30 minutes, cooled, and suitably diluted with 0.15 M KCl, and the residual G-6-PD activity was assayed. The values are expressed as per cent of residual activity; they are the mean values of eight experiments for the crude enzyme and of two for the purified enzyme. For futher details see Table II.

† The enzyme preparations utilized were those free of added NADP.

of leukocyte G-6-PD has been studied in crude preparations of the normal and mutant enzymes. As has been reported for other enzymes (46–52) the thermostability of G-6-PD is greatly affected by the buffering system. We observed a greater inactivation when the enzyme was incubated with Tris-maleate buffer than when it was incubated with glycylglycine at the same pH. The enzyme is more stable at high than at low pH for Tris-maleate buffer. Opposite effects are observed with glycylglycine buffer. In all instances the mutant enzyme was more labile than the control enzyme.

As shown in Figure 2, NADP protects both the crude G-6-PD and the purified dialyzed enzymes of normal and mutant subjects from heat inactiva-tion. The protection by NADP is much less ef-
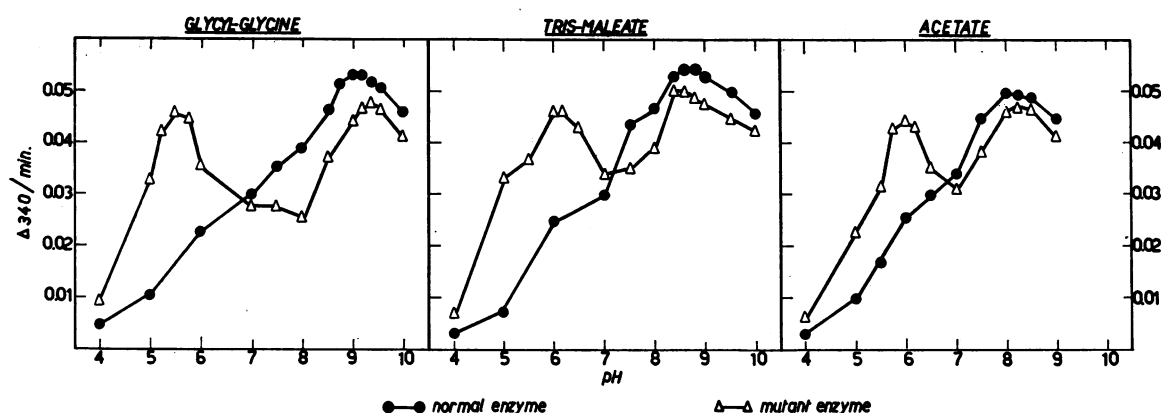
FIG. 3. OPTIMAL pH CURVES OF LEUKOCYTE GLUCOSE 6-PHOSPHATE DEHYDROGENASE. Enzyme assays have been performed in the presence of 30 mM glycylglycine, Tris-maleate, or acetate. The curves were obtained by the mean activity values from 6 normal crude enzyme preparations and 16 mutant crude enzyme preparations.

fective for the mutant than for the control enzyme and less effective for the purified enzyme than for crude preparations of G-6-PD.

The mutant enzyme in both crude and purified preparations is more sensitive to inactivation by NADPase than is the normal enzyme; the protection by NADP is also less effective for the mutant enzyme (see Table IV).

The influence of pH on enzyme activity has been investigated with the three different buffering systems, namely glycylglycine, Tris-maleate, and acetate buffers. Under all experimental conditions the behavior of the mutant enzyme is markedly different from that of the control. Figure 3 shows the pH curves of crude G-6-PD preparations from normal and mutant subjects. The normal enzyme has a single peak of activity that ranges between

8.2 and 9.4 according to the buffer used. The mutant enzyme has two distinct peaks. The first lies between pH 5.5 and 6.5, according to the buffer used; the second ranges between pH 8.0 and 9.5. These striking differences in pH activity have also been obtained with two purified preparations of the normal and mutant enzymes. Similar pH curves showing a biphasic pattern have been described for the erythrocyte enzyme by Kirkman and associates for sensitive subjects of Mediterranean origin and for Seattle and Chinese variants (22, 26, 27) and by Ramot and co-workers (23) in Jewish subjects.

Table V shows the affinity constants for G-6-P and NADP of normal and mutant enzymes at various pH. The estimations for G-6-P have been carried out on crude and purified preparations and

TABLE V

Affinity constants of leukocyte glucose 6-phosphate dehydrogenase*

	Normal subjects		Mutant subjects	
pH	Km† G-6-P	Km NADP	Km G-6-P	Km NADP
5.0	5.7 (4.3–6.9) × 10⁻⁴‡	2.3 (1.1– 5.2) × 10⁻⁵	1.1 (0.5–2.3) × 10⁻⁵	8.0 (5.7–11.2) × 10⁻⁵
6.0	8.0 (6.2–9.4) × 10⁻⁵	5.3 (3.8– 7.6) × 10⁻⁵	1.6 (1.0–3.1) × 10⁻⁵	8.2 (4.5– 9.8) × 10⁻⁵
7.5	1.7 (0.9–2.8) × 10⁻⁵	7.7 (3.4– 9.0) × 10⁻⁶	3.1 (2.2–4.0) × 10⁻⁵	8.1 (3.9–10.4) × 10⁻⁵
8.5	1.6 (0.6–2.9) × 10⁻⁵	8.1 (5.5– 9.4) × 10⁻⁶	3.4 (2.6–4.9) × 10⁻⁵	8.0 (3.7– 9.2) × 10⁻⁵
9.5	2.5 (1.8–3.9) × 10⁻⁵	9.0 (5.2–10.6) × 10⁻⁶	3.9 (3.0–5.3) × 10⁻⁵	8.3 (4.2– 9.7) × 10⁻⁵

* The affinity constants for G-6-P were determined in the presence of 0.5 mM NADP. For normal subjects they are the mean of 6 determinations with crude enzyme and 2 with purified enzyme; for mutant subjects they are the mean of 18 determinations with crude enzyme and 2 with purified enzyme. The affinity constants for NADP were determined with crude enzyme in the presence of 2 mM G-6-P, and they are the mean value of 7 experiments with normal subjects and 16 with mutant subjects.

† Km = Michaelis constant.

‡ Numbers in parentheses are the maximal and minimal values for each kind of experiment.
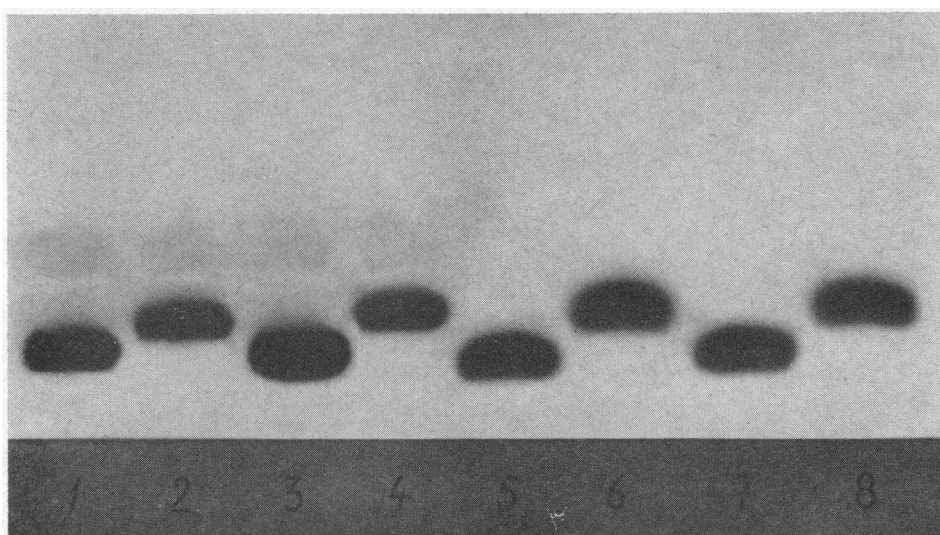
FIG. 4. STARCH GEL ELECTROPHORESIS OF LEUKOCYTE GLUCOSE 6-PHOSPHATE DEHYDROGENASE. Origin appears at the top of the Figure. Migration is vertical toward the anode. Channels 1 and 3 contain normal crude enzyme; channels 2 and 4 contain mutant crude enzyme; channels 5 and 7 contain normal purified enzyme; and channels 6 and 8 contain mutant purified enzyme. For further details see text and (39).

those for NADP on crude preparations only. Both with crude and purified preparations the affinity for G-6-P in the mutant enzyme is markedly higher than that of the normal enzyme at pH 5 and 6, but much lower at pH 7.5, 8.5, and 9.5. On the other hand, the affinity of the mutant enzyme for NADP is much lower than that of the normal enzyme.

Differences in the affinity for substrate have also been reported for the erythrocyte mutant by Marks, Banks, and Gross (12), Kirkman and associates (22, 26, 27), and Ramot and co-workers (23).

The starch gel electrophoretic pattern of G-6-PD was studied with crude enzyme preparations from all the subjects (54 mutant and 22 control subjects) and with four purified preparations of the enzymes. All enzymes, from both normal and mu-
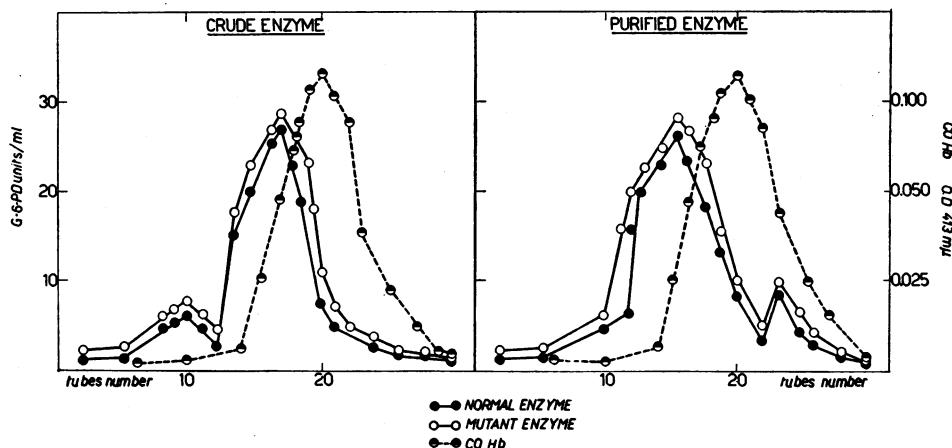

FIG. 5. SEDIMENTATION PATTERN OF LEUKOCYTE GLUCOSE 6-PHOSPHATE DEHYDROGENASE (G-6-PD) ON LINEAR DENSITY SUCROSE GRADIENT. Four experiments with crude enzyme and three with purified enzyme were performed. For details see text and (39). COHb = carboxyhemoglobin.

tant subjects, showed a single electrophoretic band that could be related to G-6-PD activity. With crude preparations a slower band is stained less intensely on gel slices, this band being attributed to 6-phosphogluconic dehydrogenase activity (38). The mutant enzyme from all subjects showed a slower electrophoretic mobility, ranging between 82% and 93% of the normal enzyme. Figure 4 shows the electrophoretic pattern of crude and purified preparations of both normal and mutant enzymes.

Using the same technique, we have recently observed (38) a slower electrophoretic mobility for erythrocyte enzyme of five Sardinian heterozygous women belonging to three unrelated families. In these subjects the enzyme level was about 50% of normal.

The sedimentation coefficients of the crude and purified enzymes, as determined on a linear sucrose density gradient, are similar for the normal and mutant enzymes; both have S values of 6.25 for the main G-6-PD activity peak. As illustrated in Figure 5, there is a second peak of G-6-PD activity from both mutant and control enzymes. This minor peak has a slower sedimentation rate for the purified enzyme (S = 4.4) and a faster sedimentation rate for the crude enzyme (S = 8.8). The molecular weights of these two minor active peaks can be assumed to be half and double the main peak, respectively. They can be attributed to different aggregational states of the enzyme (53–56), that is, to the monomeric subactive state and to a possible tetrameric state. The different aggregational states of the active protein in crude and purified preparations are most probably due to the purification procedure. Additional studies in this laboratory have pointed out that a tetrameric aggregation for the purified leukocyte (57) and also erythrocyte and liver G-6-PD (58) can be demonstrated when the purification is carried on in the presence of high concentrations of Mg^{++}, mercaptoethanol, and NADP.

Discussion

As pointed out by several previous studies (4, 6–9, 11–18, 20–23, 38) G-6-PD deficiency is highly heterogeneous for its genetic, biochemical, and clinical features.

The erythrocyte G-6-PD of different ethnic groups has been widely studied both with crude and purified enzyme preparations. Whereas the Negro subjects appear to constitute a homogeneous group, the Caucasian population is markedly heterogeneous, and several variants have been described that account for the heat stability, kinetic properties, and electrophoretic behavior of the mutant enzyme (12, 15, 17, 18, 20–23, 26, 38). On the other hand, the leukocyte mutant enzyme from sensitive Caucasian subjects has not been widely studied. An altered heat stability has been demonstrated by Ramot and associates (32) and Marks (17). The electrophoretic mobility has been found to be modified by Kirkman, Simon, and Pickard (26) in the Seattle variant and found to be normal by Ramot and co-workers (23) in Jewish subjects.

The present investigation is the first extensive study of the mutant leukocyte enzyme performed on a large number of subjects. These results demonstrate that the stability, kinetic properties, and electrophoretic mobility of the leukocyte G-6-PD of all Sardinian mutant propositi are modified. The alterations are observed both with crude and purified preparations of the enzyme. This would indicate that the differences are not a result of the enzyme purification.

On the basis of the present data, it seems most probable that the primary structure of the mutant leukocyte G-6-PD is altered.

The altered (17, 26, 32) as well as the normal properties (23) of the leukocyte G-6-PD are consistent with a great variability of the observed leukocyte G-6-PD mutation, as has already been found for the erythrocyte enzyme. As the studies done on the leukocyte enzyme are still in progress, there are insufficient data to justify any definitive statement regarding the nature of the relationship between the erythrocyte and the leukocyte G-6-PD mutation. In this regard we have recently demonstrated (39) that the G-6-PD of erythrocytes and leukocytes from normal humans, both crude and purified preparations, have different properties (optimal pH activity, affinity for G-6-P and NADP, stability, inactivation, and electrophoretic mobility). This behavior could perhaps also indicate a different stucture and consequently a different genetic control for the enzyme in the two kinds of cells. This has been shown for pyruvate kinase (59). Only after the structure of the two enzymes is determined can this hypothesis be proved for leukocyte G-6-PD.

Summary

The properties of the mutant glucose 6-phosphate dehydrogenase (G-6-PD) from the leukocytes of 54 subjects belonging to 28 families from different parts of Sardinia have been studied.

We have carried out studies with both crude and purified preparations of the enzyme. The results show that the mutant enzyme differs from the normal on the basis of the optimal pH activity, affinity for G-6-P and nicotinamide adenine dinucleotide phosphate (NADP), electrophoretic mobility, heat stability, NADP glycohydrolase (NADPase)-induced inactivation, and protection afforded by NADP. On the other hand, the sedimentation in the linear sucrose density gradient is similar to normal.

The present findings are discussed with respect to the mutation characteristics of the erythrocyte and leukocyte G-6-PD.

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