Ethnic Variation in Red Cell Glutathione Peroxidase Activity

By E. Beutler and F. Matsumoto

Glutathione peroxidase activity was measured in blood and cultured fibroblasts from healthy persons of several different population groups. Individuals of Jewish ancestry and others of Mediterranean origin were found to manifest a decrease of red cell but not of leukocyte or fibroblast enzyme activity. Oriental populations differed in that the scatter in red cell enzyme activity was significantly lower than in Occidental populations. The erythrocyte enzyme of individuals with low activity was found to be less stable to heating than was the enzyme from persons with high activity. As a possible explanation for these data, a provisional genetic model is presented: a low GSH Px allele with a frequency of 0.556 in the Jewish population and of only 0.181 in the United States-Northern European population. Our results suggest that an association between GSH Px deficiency and hemolytic anemia need not represent a cause-and-effect relationship.

GLUTATHIONE PEROXIDASE (GSH Px) is an enzyme which catalyzes the oxidation of GSH by hydrogen peroxide and certain organic peroxides:

\[
R-O-O-H + 2GSH \rightarrow R-OH + H_2O + GSSG
\]

Hemolytic anemia associated with a moderately severe degree of deficiency of this enzyme was first reported in a Puerto Rican patient after he was transfused with his own stored blood.\(^1\)\(^2\) Subsequently, lowered GSH Px levels were noted in Japanese,\(^3\) French,\(^4\)\(^7\) and United States white\(^4\) patients with mild hemolysis. GSH Px is a selenium-containing enzyme,\(^9\)\(^10\) and it is known that, in experimental animals, selenium deprivation decreases GSH Px activity.\(^11\) Lowered GSH Px activity has also been reported to occur in iron deficiency.\(^12\)\(^14\)

In the course of standardizing an improved technique of GSH Px assay, we noted that one normal Jewish male had red cell GSH Px activity which was consistently only one-half of normal. His family members were found to share the enzyme deficiency, and it was then found that many Jewish donors and other individuals of Mediterranean origin also had much lower GSH Px activities than were found in the United States-Northern European population or in the United States-African population.

MATERIALS AND METHODS

Venous blood samples were collected into tubes containing approximately 1 mg of ethylenediaminetetraacetic acid (EDTA) per ml of blood. All samples, except those obtained from Israel, were refrigerated immediately after they were drawn and were assayed within 1 or 2 days. Samples from Israel were held at room temperature for 2 days prior to assay. Previous studies\(^15\)
have shown that if sterility is maintained there is no change in red cell GSH Px activity even after 5 days at room temperature. Identification of donors classified as Ashkenazi and Sephardic was based on family names. All blood samples were filtered through a SE-cellulose-Sephadex G-25 mixture or through a mixture of α-cellulose and microcrystalline cellulose. Both of these treatments remove over 99% of the leukocytes and most of the platelets. The erythrocytes were washed in saline solution, hemolyzed by freezing and thawing, and assayed for GSH Px activity using t-butylhydroperoxide as a substrate. Leukocytes were separated by sedimentation in PVP citrate and centrifugation over Ficoll-diazotrate. Skin fibroblasts were grown in Eagle’s minimal essential medium containing 20% fetal calf serum, trypsinized, and washed in saline. Leukocytes and fibroblast suspensions were disrupted by freezing and thawing, were centrifuged at 12,000 g for 10 min, and the supernatant was assayed for GSH Px activity. Kinetic and thermal stability studies were carried out on hemoglobin-free GSH Px prepared by ammonium sulfate precipitation and DEAE chromatography, as described elsewhere.

Thermal stability of the enzyme was measured at 68°C at pH 6.8 with 10 mM added GSH and at 57°C at pH 7.5 after dialysis to remove GSH: GSH markedly stabilized the enzyme to heating. The thermal stability was calculated from the regression of the log of enzyme activity against time, and expressed as the T½, the time required for loss of one-half of the enzyme activity. The energy of activation was calculated from an Arrhenius plot at assay temperatures ranging from 15°C to 45°C. Antibody titrations were carried out using antihuman GSH Px rabbit serum prepared by immunizing the rabbit against homogeneous erythrocyte GSH Px. Dilutions of the antiserum were mixed with hemolysate, allowed to stand for 1 hr at 25°C, 1 hr at 4°C, and centrifuged at 36,000 g for 1 hr. GSH Px activity was assayed in the supernatant. Starch-gel electrophoresis of GSH Px was carried out as previously described, with the pH of the buffer system ranging from

![Fig. 1. Distribution of glutathione peroxidase activities of red cells from 145 unrelated individuals of different ethnic origins. Each point represents the value obtained on the red cells of one person.](image-url)
from 6.5 to 8.0 at 0.5 pH unit intervals. Selenium determinations were carried out fluorometrically with 2,3-diaminonaphthalene on plasma and red cells after digestion with concentrated HNO₃ and 70% perchloric acid. Statistical analysis for significance of the differences between means was carried out using Student’s t test.

RESULTS

The results of GSH Px assays on the red cells of 145 normal donors from different ethnic groups are shown in Fig. 1 and in Table 1. It is apparent that both American and Israeli Jews have significantly lower red cell enzyme activity than the non-Jewish Americans of Northern European origin or Afro-Americans. The enzyme activity of Orientals, both of Japanese and Chinese origin, was intermediate and showed much less scatter than did that of the other population groups. There was no difference in the enzyme activity of males and females. The pedigrees of a three-generation Jewish family and two two-generation Jewish families with individuals with low GSH Px activity are shown in Fig. 2.

The partially purified erythrocyte enzyme from deficient, Jewish subjects with enzyme activity of less than 18 U/g Hb was compared with that of Northern European subjects with greater than 30 U enzyme activity per g Hb. As shown in Table 2, no difference was found in the following parameters: $K_m$ GSH, $K_m$ t-butylhydroperoxide, and temperature of activation. Using a hemolysate, no difference was found in the slope of the antibody titration curve or the electrophoretic mobility in starch gel at pH 6.5–8.0. The whole-blood selenium level of one Jewish subject with low activity was 0.146 μg/ml; a level in a United States-Northern European subject was 0.135 μg/ml. The mean of plasma selenium levels on three persons with low activity was 0.146 ± 0.005 μg/ml and in three individuals with high GSH Px activity, 0.122 ± 0.019 μg/ml. The partially purified enzyme from persons with low enzyme activity was significantly less stable to heating than that from those with activity greater than 30 U/g Hb.

The results of enzyme assays carried out on leukocytes and fibroblasts are summarized in Table 3. No difference was observed between the cells from a

| Table 1. Red Cell GSH Px Activity (Mean ± SD [n]) in Various Ethnic Groups |
|-----------------------------|-----------------------------|
|                            | Male                       | Female                     | Male and Female |
| U.S.—N. European           | 27.82 ± 3.78 (13)          | 30.20 ± 4.04 (23)          | 29.37 ± 4.12 (36)†|
| U.S.—African               | 20.53, 30.80               | 29.82 ± 5.37 (6)           | 28.78 ± 5.64 (8) |
| All Jewish                 | *                          | *                          | 21.88 ± 6.19 (54)†|
| U.S. Jewish                | 23.00 ± 6.55 (14)          | 34.51 (1)                  | 23.77 ± 6.97 (15) |
| Israeli Jewish             | *                          | *                          | 21.05 ± 5.81 (39) |
| Sephardic Jews             | *                          | *                          | 20.79 ± 4.93 (16) |
| Ashkenazi Jews             | *                          | *                          | 22.25 ± 6.69 (38) |
| U.S.—Japanese, Chinese     | 24.39 ± 3.13 (11)          | 24.73 ± 2.21 (7)           | 24.52 ± 2.74 (18)†|
| U.S.—Asian Indians         | 27.25 ± 7.80 (5)           | 24.07 (1)                  | 26.72 ± 7.10 (6) |
| U.S.—Mexicans              | 37.66 (1)                  | 27.47 ± 5.73 (8)           | 28.59 ± 6.35 (9) |
| U.S.—Mediterranean         | 25.09 ± 8.65 (4)           | 21.98, 31.33 (2)           | 25.61 ± 7.37 (6) |

*The Israeli-Jewish samples were not identified by sex.
†Means differ with $p < 0.001$.
‡Snedecor’s $F = 2.26 p < 0.01$ for differences of variances.
Fig. 2. Pedigrees of three families with an individual with low GSH Px activity. The numbers inside the symbol indicate enzyme activity in U/g Hb. The familial nature of low GSH Px activities is clearly demonstrated, but the genetic pattern is not clear. Most of the persons in these families are of Ashkenazi Jewish origin. II-K.N. (B family) is of Japanese origin.

Table 2. Comparison of Partially Purified GSH Px From U.S. Jewish Subjects With < 18 U GSH Px per g Hb With U.S.-Northern European Subjects With > 30 U GSH Px per g Hb

<table>
<thead>
<tr>
<th></th>
<th>&lt; 18 U GSH Px/g Hb (U.S.-Jewish)</th>
<th>&gt; 30 U GSH Px/g Hb (U.S.-N. European)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_m$ GSH (mM)</td>
<td>0.729 ± 0.077 (4)</td>
<td>0.650 ± 0.092 (3)</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>$K_m$ TBH (mM)</td>
<td>0.194 ± 0.027 (4)</td>
<td>0.199 ± 0.055 (3)</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>Temperature of activation (cal)</td>
<td>2420 ± 28 (4)</td>
<td>2429 ± 161 (3)</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>Thermostability ($t_{1/2}$ 65°)</td>
<td>122.6 ± 8.95 (4)</td>
<td>150.9 ± 7.04 (3)</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>70°</td>
<td>34.3 ± 5.87 (3)</td>
<td>62.18 ± 9.80 (3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>40° (no GSH)</td>
<td>6.95 ± 1.29 (3)</td>
<td>10.74 ± 1.08 (3)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>
Table 3. GSH Px Activity of Other Blood Cells and Fibroblasts From Individuals With Low and High RBC Enzyme Activity ± SD

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Low RBC Activity (U.S.-Jewish)</th>
<th>High RBC Activity (U.S.-N. European)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocytes</td>
<td>&lt;18</td>
<td>&gt;30</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>73.93 ± 11.02 (4)</td>
<td>77.96 ± 15.1 (3)</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>17.38 ± 7.54 (4)</td>
<td>13.03 ± 2.51 (3)</td>
</tr>
<tr>
<td>Platelets</td>
<td>149.9 (1)</td>
<td>129.8, 139.9 (2)</td>
</tr>
<tr>
<td>Fibroblast</td>
<td>84.83 ± 3.58 (4)</td>
<td>76.6 (1)</td>
</tr>
</tbody>
</table>

Jewish subject with low enzyme activity and those from a subject of Northern European ancestry with normal enzyme activity.

**DISCUSSION**

Glutathione peroxidase activity is known to be subject to environmental influences. The enzyme contains selenium as an integral part of its structure,9,10 and selenium deficiency results in a decrease in enzyme activity.11 The possibility that iron lack also influences glutathione peroxidase activity has been suggested.12-14 However, the different levels of GSH Px found in various population groups are certainly based upon genetic and not environmental or dietary differences. The population samples of Americans from different ethnic and racial backgrounds was drawn from hospital employees, most of them physicians, technicians, and clerical personnel. The United States-Jewish and Israeli-Jewish samples were found to have a virtually identical GSH Px activity. The familial aggregation of lowered GSH Px activity was unmistakable. Additional ethnic variability in the enzyme is suggested by the intermediate levels found in Oriental subjects, with a marked decrease in scatter of values obtained in samples from Japanese and Chinese subjects. A significant feature of the lowering of GSH Px activity which was observed in some population groups was that the deficiency was limited to the erythrocytes. Such a discrepancy between findings in red cells and in other tissues has also been observed in a number of other enzyme deficiencies. In some cases, such divergence can be explained by the existence of two genetically distinct enzymes with similar functions in different tissues. However, since a common electrophoretic mutant of GSH Px, the Thomas variant,21,23 affects red cells, white cells, and fibroblasts, at least a major genetic determinant of GSH Px is common to all of these tissues. Divergence between deficiency in red cells and white cells can sometimes also be explained on the basis of inheritance of an enzyme with reduced stability in vivo: the erythrocytes are more likely to manifest such a deficiency than are shorter-lived nucleated cells. Such a state of affairs has been found with other unstable mutant enzymes, including G-6-PD A-24,25 and pyridoxine kinase.26 It is likely that a similar stability mutation is involved in the case of GSH Px.

Although a consistent difference in stability of the red cell enzyme to heating could be found, the difference was not sufficiently great to permit unequivocal classification of each individual with respect to glutathione peroxidase phenotype. The necessity of relying on quantitative differences in enzyme activity for classification makes analysis of the formal genetics of the deficiency difficult,
since nongenetic factors also produce quantitative differences in enzyme activity. Our previous studies\textsuperscript{23} of electrophoretic variation of GSH P\textsubscript{x} suggested that the structural gene for this enzyme was autosomal, but sex-linked inheritance could not be ruled out. The family studies presented in Fig. 2, however, seemed to rule out sex-linked inheritance of the enzyme deficiency. The findings in the T family are particularly germane in this respect: there appears to be clear-cut father-to-son transmission of low GSH P\textsubscript{x} activity. This also seems to be true in the M family, although here the borderline value of GSH P\textsubscript{x} of subject I I.M. makes the interpretation less certain. The genetic interpretation which is most consistent with the population distribution data shown in Fig. 1 is that the “wild-type” allele, in the homozygous state, (GSH P\textsubscript{x\textsuperscript{H}}/GSH P\textsubscript{x\textsuperscript{L}}) codes for enzyme activity of higher than 28 U of enzyme per g Hb, and that the red cells of individuals homozygous for another allele (GSH P\textsubscript{x\textsuperscript{L}}/GSH P\textsubscript{x\textsuperscript{L}}) have an enzyme activity of below 18 U per g Hb. Heterozygotes for the two alleles (GSH P\textsubscript{x\textsuperscript{L}}/GSH P\textsubscript{x\textsuperscript{H}}) have intermediate enzyme activity. Examination of data from the pooled (United States and Israel) Jewish populations with these assumptions yields gene frequency estimates of 0.556 for GSH P\textsubscript{x\textsuperscript{L}} and of 0.444 for GSH P\textsubscript{x\textsuperscript{H}}. It can be seen in Table 4 that these gene frequencies provide an excellent fit according to the Hardy-Weinberg equation. Similarly, gene frequencies of 0.181 and 0.819 for the GSH P\textsubscript{x\textsuperscript{L}} and GSH P\textsubscript{x\textsuperscript{H}} genes, respectively, were found in the United States-Northern European population. As shown in Table 4, these gene frequencies, too, give an excellent fit for that population. The pedigrees shown in Fig. 2 also give a fair fit to these assumptions, but there are some exceptions. For example, in the B family, subject I K.B. would be classified as being of the GSH P\textsubscript{x\textsuperscript{H}}/GSH P\textsubscript{x\textsuperscript{L}} genotype on the basis of her enzyme activity, which is higher than the arbitrary limit of 28 U/g Hb. All three of her children, on the other hand, would be classified as GSH P\textsubscript{x\textsuperscript{L}}/GSH P\textsubscript{x\textsuperscript{L}}, since their enzyme activity is below 18 U. If the genetic model we propose is correct, one would have to assume that I K.B. is, in point of fact, actually of the GSH P\textsubscript{x\textsuperscript{L}}/GSH P\textsubscript{x\textsuperscript{H}} genotype. Similarly, in the M family, the classification of subject I I.M. as being of the GSH P\textsubscript{x\textsuperscript{H}}/GSH P\textsubscript{x\textsuperscript{L}} genotype

\begin{table}[h]
\centering
\begin{tabular}{llll}
\hline
& GSH P\textsubscript{x} (U/g Hb) & <18 & 18–28 & 28+ \\
Assumed genotype & GSH P\textsubscript{x\textsuperscript{L}}/GSH P\textsubscript{x\textsuperscript{L}} & GSH P\textsubscript{x\textsuperscript{H}}/GSH P\textsubscript{x\textsuperscript{L}} & GSH P\textsubscript{x\textsuperscript{L}}/GSH P\textsubscript{x\textsuperscript{H}} \\
\hline
Jewish population (n = 54) & (GSH P\textsubscript{x\textsuperscript{L}} = 0.556; GSH P\textsubscript{x\textsuperscript{H}} = 0.444)\textsuperscript{*} & 16 & 28 & 10 \\
Observed & 16.67 & 26.67 & 10.67 \\
Expected & & & \chi^2 = 0.02, \ p = 0.99 \\
\hline
U.S.–Northern European population (n = 36) & (GSH P\textsubscript{x\textsuperscript{L}} = 0.181; GSH P\textsubscript{x\textsuperscript{H}} = 0.819)\textsuperscript{*} & 0 & 13 & 23 \\
GSH P\textsubscript{x} (U/g Hb) & <18 & 18–28 & 28+ \\
Assumed genotype & GSH P\textsubscript{x\textsuperscript{L}}/GSH P\textsubscript{x\textsuperscript{L}} & GSH P\textsubscript{x\textsuperscript{H}}/GSH P\textsubscript{x\textsuperscript{L}} & GSH P\textsubscript{x\textsuperscript{L}}/GSH P\textsubscript{x\textsuperscript{H}} \\
Observed & 1.16 & 10.67 & 24.15 \\
Expected & & & \chi^2 = 0.2, \ p > 0.9 \\
\hline
\end{tabular}
\caption{Observed and Expected (Hardy-Weinberg) Frequency of GSH P\textsubscript{x} Phenotypes}
\end{table}

\textsuperscript{*}Gene frequencies are calculated from the assumed genotype.
GLUTATHIONE PEROXIDASE ACTIVITY

(greater than 28 U/g Hb) is inconsistent with the classification of her son, II J.M., as GSH Px^+/GSH Px^- genotype (less than 18 U/g Hb). Such exceptions must be expected when identification of phenotypes depends upon arbitrary quantitative values of the enzymes. We recognize that the genetic model we propose is only the most simple among several possibilities.

The fact that low GSH Px activities occur commonly in healthy persons in some population groups indicates that extreme caution must be used in interpreting the significance of lowered GSH Px activities in persons with anemia. In spite of the fact that lowered GSH Px activities have been observed in a number of individuals with hemolytic anemia, the evidence for a cause-and-effect relationship between the enzyme deficiency and the anemia has not been compelling. In most cases the decrease of activity was modest, often not exceeding that observed in normal persons in this study. It will be important to carry out family studies of other such cases which may be encountered to determine whether, indeed, a causal relationship between GSH Px deficiency and hemolytic anemia exists.

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REFERENCES

14. Hopkins J, Tudhope GR: Glutathione
27. Chern CJ, Beutler E: Biochemical and electrophoretic studies of erythrocyte pyridoxine kinase in caucasian Americans and Afro-Americans. (submitted for publication)
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