Effect of oxidized glutathione on human red cell acid phosphatases

E. Bottini and G. Modiano

Gruppo di ricerca "Commissione per la Genetica" del C.N.R. e Clinica Pediatrica dell'Università di Roma; Istituto di Genetica dell'Università di Roma.

Received July 29, 1964

Hopkinson, Spencer and Harris (1963) did recently describe the occurrence of a new biochemical polymorphism involving the acid phosphatases of human red blood cells. They were able to identify by starch gel electrophoresis five distinct patterns of fairly common occurrence and proved them to be an additional example of true genetical polymorphism, depending upon an autosomal locus with multiple allelism.

They noted also that beside the usual electrophoretic bands typical for each genotype, additional bands with greater anodic mobility may be found after long storage (Hopkinson et al., 1964).

This situation seemed to us comparable to some extent to the appearance of relatively large amounts of the so called Hb A_3 in old haemolysates. Moreover Huisman and Dozy (1962) were able to obtain the transformation of haemoglobin into a fast moving component similar to Hb A_3 by incubating fresh haemolysates with oxidized glutathione.

Thus the experiments described below were performed in an attempt to clarify the relationship between the presence of oxidized glutathione in the sample and the formation of fast moving bands of acid phosphatases. In addition, the correlation between the formation of these bands and the resulting loss of total enzyme activity was investigated.

Materials and methods.

Haemolysates were prepared by repeatedly freezing and thaw-

ing of fresh human red cells.

Starch gel electrophoresis of the red cell acid phosphatases and of haemoglobin were performed according to the techniques of Hopkinson et al. (1963) and Poulik (1957) respectively.

The quantitative estimation of phosphatases activity was obtained with the method reported by Torriani (1960), slightly modified to make it suitable for haemolysates (Bottini and Modiano,1964a).

Results.

(A) Electrophoretic data. Electrophoretic analysis of fresh haemolysates from a B subject (Hopkinson et al., 1963) incubated with GSSG (8 mg/ml, final concentration) for three hours at 37°C showed the following (Fig. 1):

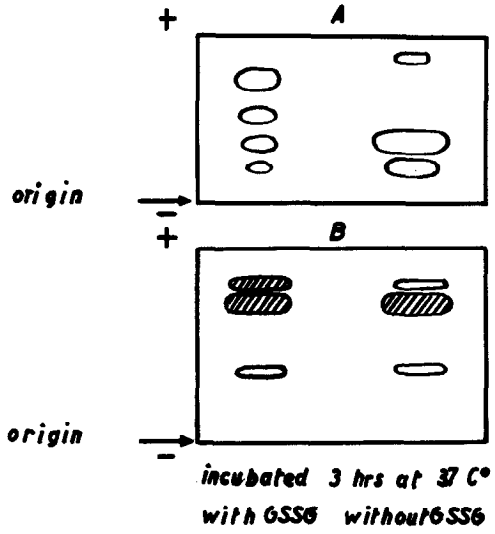

Fig. 1. Schematic representation of acid phosphatases (A) and haemoglobin (B) electrophoteric patterns.

Oxidized glutathione (GSSG) was purchased from Nutritional Biochemical Corporation (USA) and reduced glutathione (GSH) from British Drug House (England).

1) a very well defined fast haemoglobin band.

2) a qualitative as well as quantitative profound modification of electrophoretic pattern of the acid phosphatases. The two normal bands were much less intense. Two new bands were present, the faster one being more intense and both displaced more towards the anode than the normal ones. The faster of the two new bands was definitely, but only slightly, slower than the faint fast band which is usually found in old haemolysates and was always present in the controls of this experimental series.

From the staining intensity of the starch gels it appears that the total enzymatic activity of the treated samples has been drastically reduced. This effect becomes clearer after prolonging the incubation time, but it is already evident with an incubation time of one hour.

Incidentally it may be noted that these GSSG-induced alterations are much more intense and appear faster for acid phosphatases than for haemoglobin.

The electrophoretic patterns were normal in haemolysates incubated with GSH (8 mg/ml) only. When both (GSSG and GSH 8 mg/ml final concentration) are incubated with haemolysates, the previously described modifications still appear but less pronounced than with GSSG alone.

(B). Quantitative data. The acid phosphatases activity of a haemolysate incubated with GSSG decreases progressively with time, as shown in Fig. 2. From this type of experiment as well as from others in which different concentrations of GSSG were used, the kinetics of the decrease of enzymatic activity appeared biphasic. The decrease was positively correlated with GSSG concentration. The GSH alone causes if anything a small decrease in enzyme activity, however if the haemolysate is incubated simultaneously with both GSH and GSSG the latter shows a much less pronounced effect (Table I).

The observed GSSG-induced decrease of enzymatic activity was found to be independent of the p-nitrophenolphosphate

(p-NP-phosphate) concentration used to measure it, in a range between 0,9-14,4 mg/ml (final concentration).

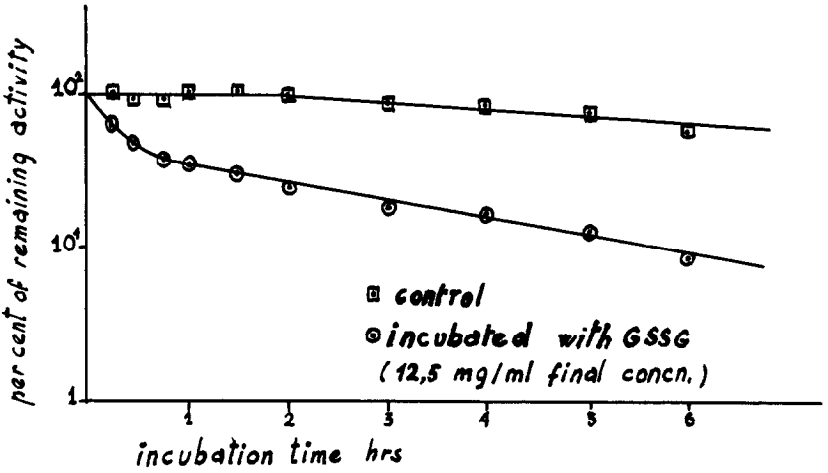

Fig. 2. Rate of change of acid phosphatases activity with and without GSSG.

Table I

Acid phosphatases activity of an haemolysate incubated with GSSG, with GSH and with GSSG and GSH.

Solutions tested	M p-NP produced in 30' by 10 ml of solution
haemolysate + diluent of GSSG and GSH	9.23
haemolysate + GSSG 10 mg/ml (final concn.)	1.46
haemolysate + GSH 10 mg/ml (final concn.)	8.46
haemolysate + GSSG 10 mg/ml and GSH 10 mg/ml (final concn.)	5.11

The haemolysate had the same concentration in all solutions. The incubation time at 37°C was 3 hrs 43' ± 3'.

Conclusions.

The electrophoretic and quantitative findings are best explained assuming that the GSSG could react with acid phosphatases giving rise to molecules of type protein.S—SG (with one or more mixed disulfide bonds). These molecules, which would retain a partial enzymatic activity and exhibit an increased anodic mobility, would then completely loose their activity following a first order reaction.

Whatever may be the interpretation of our findings, they suggest that the lower levels of red cell acid phosphatases reported in carriers of the G6PD-deficiency (Oski, Shahidi and Diamond, 1963) may be due to the excess of GSSG known to be present in the red cells of these individuals. Preliminary experiments on the effect of some oxidative drugs on intact erythrocytes of both normal and G-6-PD deficient individuals seem to confirm this impression (Bottini and Modiano, 1964b).

It would be interesting to find out whether the effect of glutathione on red cell proteins is a fairly general phenomenon since this might have a direct bearing on the mechanism of red cell distruction in the presence of toxic drugs or food stuff. Such studies could moreover contribute critical data for understanding the role of glutathione in the red cell metabolism.

Acknowledgements. We wish to express our gratitude to Prof.D. Cavallini and to Prof.M.Siniscalco for their helpful criticism and for having read the manuscript before publication. The skilfull technical assistance of Mr.C.Santolamazza has been very much appreciated.

References.

1) (a) Bottini,E. and Modiano,G.: (in preparation) 1964.
(b) Bottini,E. and Modiano,G.: proposed to the "Lancet" for publication, 1964.
2) Hopkinson,D.A.; Spencer,N. and Harris,H.: Nature,199,969, 1963.
3) Hopkinson,D.A.; Spencer,N. and Harris,H.: Am.J.Hum.Gen.,16,141, 1964.
4) Huisman,T.H.J. and Dozy,A.M.: J.Lab.Clin.Med.,60,302,1962.
5) Oski,F.A.; Shahidi,N.T. and Diamond,L.K.: Science,139,409,1963.
6) Poulik,M.D.: Nature,180,1477,1957.
7) Torriani,A.: Bioch.Bioph.Acta,38,460,1960.