METABOLISM OF HUMAN ERYTHROCYTE GLUCOSE-6-PHOSPHATE DEHYDROGENASE. III - ISOLATION OF A PROTEIN INVOLVED IN THE 6-PHOSPHOGLUCONATE-INDUCED INACTIVATION *

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Received 8 July 1968

1. Introduction

Glucose-6-phosphate dehydrogenase (G6PD) from human erythrocytes is inactivated when partially purified preparations of the enzyme are incubated at 37° with either glucose 6-P (G-6-P), 6-phosphogluconate (6-Pga) or NADPH [1].

Data reported in this paper are consistent with the involvement of a protein factor in the 6-Pga-induced inactivation of G6PD: this protein has been isolated and demonstrated to specifically convert erythrocyte G6PD into an inactive form in the presence of 6-Pga, according to the following equation:

$$\text{active G6PD} \xrightarrow{\text{protein + 6-Pga}} \text{inactive G6PD}$$

2. Materials and methods

G6PD activity was assayed according to Kornberg and Horecker [2] and one unit of activity was defined as the amount determining an absorbancy increase of 1.0/min at 340 mμ. Protein was determined by the method of Lowry et al. [3] and by the turbidimetric procedure of Bücher [4].

G6PD and the inactivating protein were purified from human erythrocytes by modifying the method of De Flora et al. [5] as follows: hemolysate (one volume of packed erythrocytes + four volumes of distilled water) was fractionated with solid ammonium sulfate, according to Marks et al. [6]: the precipitate between 35% and 55% saturation was collected with 10 ml of 0.25M glycyl-glycine buffer, pH 7.5 (AS fraction I) and treated with DEAE-cellulose in 5×10^{-3} M phosphate buffer, pH 7.0, containing 2×10^{-6} M NADP, 5×10^{-4} M EDTA and 1×10^{-3} M ε-aminocaproic acid (dry weight cellulose/mg of protein = 70). After 60 min of gentle stirring at 2°, the mixture was poured into a Buchner funnel and washed extensively with the same buffer (A), to obtain an almost complete removal of hemoglobin; cellulose was washed again with 500 ml of 5×10^{-2} M KCl in buffer A and then treated with 200 ml of 1.1×10^{-1} M KCl in buffer A: the pale yellow filtrate was treated with solid ammonium sulfate at 70% saturation and the precipitate collected with 2.0 ml of 0.25M glycyl-glycine buffer, pH 7.5 (AS fraction II). DEAE-cellulose was then treated with 500 ml of 3×10^{-1} M KCl in buffer A and the filtrate, containing about 20% of the original G6PD activity, discarded. Elution of G6PD was accomplished with 200 ml of 6×10^{-1} M KCl in buffer A: the eluate was treated with solid ammonium sulfate at 65% saturation and the precipitate dissolved in 2.0 ml of 0.25 M glycyl-glycine, pH 7.5, containing 1×10^{-4} M NADP (AS fraction III).

No G6PD activity could be detected in the AS fraction II; on the contrary, the G6PD specific activity of AS fraction III is from 1.000- to 1.500-fold

* This work was supported by the Consiglio Nazionale delle Ricerche, Rome, Italy.

higher than that of starting hemolysates.

Rat liver G6PD was partially purified according to Mangiarotti and Acquarone [7]; highly purified yeast G6PD was a commercial preparation (Zwischenferment, from Boehringer).

Before inactivation experiments all fractions were dialyzed three hours at 2° against 1000 volumes of 0.015 M KCl adjusted to pH 7.5 and containing 2×10^{-6} M NADP.

3. Results and discussion

3.1. *Inactivation of purified erythrocyte G6PD*

G6PD purified from human erythrocytes as described in section 2 is not inactivated by 6-Pga (fig. 1); addition of AS fraction II alone without 6-Pga does not produce any inactivation. On the contrary, a progressive loss of G6PD activity is observed incubating the purified enzyme with AS fraction II, in the presence of 6-Pga. After the first minutes, the inactivation is clearly a first-order reaction and the kinetic constant, as calculated under the conditions of fig. 1, is 5.5×10^{-5} sec^{-1}. As it was observed with less purified preparations [2], the inactivation is not complete and an equilibrium is attained between active and inactive G6PD: this equilibrium has been found to change with different preparations of G6PD and of AS fraction II.

Replacing 6-Pga with G-6-P or with NADPH, G6PD is also inactivated without AS fraction II, suggesting the occurrence in AS fraction III of another or two other inactivating proteins specifically requiring these metabolites. This result was rather unexpected, since no differences of both extent and rate of G6PD inactivation could be shown, incubating AS fraction I with either 6-Pga, or G-6-P or NADPH [2].

3.2. *Features of the inactivating factor*

Heat exposure (10 min at 100°) results in a complete loss of the G6PD-inactivating ability of AS fraction II. The inactivating compound does not diffuse through the dialysis membrane; moreover it is precipitated by ammonium sulfate. These properties indicate that the inactivating component of AS fraction II is a protein.

Although the same equilibrium between active and inactive G6PD is reached at longer time intervals, the initial rate of inactivation is nearly proportional to the amount of the inactivating protein. Raising the pH from 7.0 to 10.0 the rate of inactivation becomes higher, as it was observed for G6PD inactivation in less purified preparations [2].

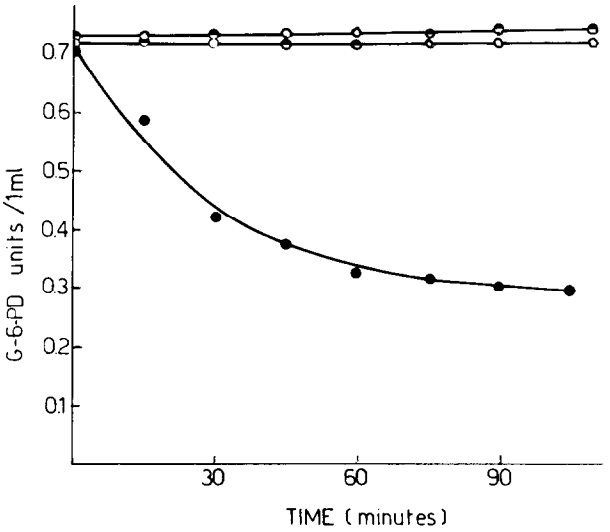

Fig. 1. *G6PD inactivation by the erythrocyte factor requiring 6-Pga.* Each ml of the incubation mixture contained (•——•): 5×10^{-2} M Tris-Hcl, pH 8.5, 1.0 unit of partially purified erythrocyte G6PD (AS fraction III, having a specific activity of 7.2), 7.0 mg of bovine serum albumin, 1×10^{-3} M 6-Pga and 60 μg (expressed as protein) of AS fraction II; o——o, the same without 6-Pga; •——•, the same without AS fraction II. Incubation was at 37° in a water bath; at the times indicated, 0.05 ml samples were assayed for G6PD activity.

3.3. *Specificity towards G6PD from different sources*

The inactivating protein appears not to be specific since a certain loss of activity is detected also with liver G6PD (table 1). The small inactivation of gel eluate in the presence of 6-Pga alone may be explained by the presence in this fraction of another G6PD-inactivating protein requiring 6-Pga, as the one extracted from human erythrocytes: this view is supported by disappearance of susceptibility to 6-Pga inactivation with further fractionation (liver B and C). The inactivation of both erythrocyte and liver G6PD by the same erythrocyte protein, followed by reactivation as for AS fraction I [2], might provide a first approach to hybridization of the dehydrogenases extracted from the two sources.

Table 1
G6PD specificity of the inactivating protein.

Source of G6PD	% inactivation			
	no 6-Pga no AS fraction II	10^{-3}M 6-Pga no AS fraction II	no 6-Pga AS fraction II	10^{-3}M 6-Pga + AS fraction II
Erythrocytes	0	0	0	81
Yeast	2	1	3	1
Liver A	0	12	1	9
Liver B	3	0	0	34
Liver C	0	0	1	33

Experimental conditions as in fig. 1. Liver A was the calcium phosphate gel eluate (specific activity = 4.2), obtained according to Mangiarotti and Acquarone [7]. Livers B and C were prepared by fractionating the gel eluate with solid ammonium sulfate; B was the precipitate at 48% saturation (specific activity = 2.1) and C the precipitate at 57% saturation (specific activity = 10.7); both precipitates were collected with 1.0 ml of 0.25M glycyl-glycine, pH 7.5. Values, corrected for variations of control experiments, refer to residual G6PD activity after 60 minutes of incubation at 37°.

The G6PD-inactivating protein is highly reminiscent of the glutamine synthetase-inactivating enzyme, that Mecke et al. [8] isolated from *E. coli* and Wulff et al. [9] and Kingdon et al. [10] then characterized at a mechanistic level: the GS-inactivating enzyme was demonstrated to be an ATP: glutamine synthetase adenylyl transferase. The similarity between the GS-inactivating enzyme and the G6PD-inactivating protein is stressed by the requirement for both systems of the metabolite formed by the inactivated enzyme, i.e. glutamine and 6-phosphogluconate respectively.

The results previously obtained with less purified preparations indicate that the metabolite-induced inactivation of G6PD is determined by dissociation of the dehydrogenase into subactive subunits [11]; therefore it is conceivable that such a change of the quaternary structure of G6PD occurs also during inactivation by the isolated protein in the reconstructed system.

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