THE EFFECT OF 6-PHOSPHOGLUCONATE ON THE ACTIVITY OF GLUTATHIONE-REDUCTASE IN MEMBRANE-FREE HEMOLYSATES OF NORMAL HUMAN RED BLOOD CELLS

L. GOTTLIEB, M. BOIS-DELIN, M. SOREL, M. BOULARD and E. BEUTLER*

Groupe de Biochimie de la Cellule Rouge, Service Central de Médecine Nucléaire Hôpital Saint-Louis, Paris, 75475 Paris Cédex 10, France

** Division of Medicine, City of Hope National Medical Center, Duarte, Ca. 91010 USA*

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1. Introduction

In 1970 Carson reported that 'incubation of membrane-free hemolysates with 6-phosphogluconate (6-PGA), (final concentration 10^{-4} M) results in a profound and specific inhibition of glutathione reductase (GR) activity' [1]. Since this phenomenon would provide a metabolic connection between the glycolytic and the pentose-phosphate pathways in the red cell, it seemed important to elucidate the exact nature of the putative inhibitory effect.

2. Methods and results

The presence of 6-phosphogluconate dehydrogenase (G-6-PD) in hemolysates results in regeneration of NADPH, H^+ from NADP formed in the GR reaction. Therefore measurement of GR in the presence of 6-PGA requires either the 6-PGD be inactivated, or that the assays are carried out by measuring the formation of reduced glutathione (GSH). Fig.1 summarizes the pathways which influence NADP levels in a system containig GR, oxidized glutathione (GSSG), 6-PGA, and 6-PGD.

Blood samples were withdrawn from normal donors. Preparation of hemolysates and enzyme assays were carried out according to previously published techniques [2]. Kinetic studies of GR were performed on normal hemolysates with or without the addition of flavine adenine dinucleotide (FAD). In kinetic studies, the concentrations of GSSG were varied from 16.5 to 3 300 μM. In order to eliminate 6-PGD activity from hemolysates, advantage was taken of the well known fact that GR is heat stable, while 6-PGD is readily destroyed at 56°C [3]. A 1:20 water hemolysate was heated to 56°C for 90 min and was then centrifuged to remove the precipitate. As shown in tables 1 and 2, heating destroyed all the activity of 6-PGD without affecting GR activity in the presence or absence of FAD. Furthermore the kinetics of the enzyme were unaffected by heating. Confirming Carson's report, we found that in the unheated hemolysate, there was apparently a total loss of GR activity, when 6-PGA was added. In contrast, the hemolysate which had been heated and was therefore free of 6-PGD activity failed to show any effect of 6-PGA ($0.6\ 10^{-4}$ M to 0.6 M) on the rate of GR activity. The velocity of the GR reaction was also measured by estimating the rate of formation of GSH in a system containing from 16.5 to 3 300 μM of

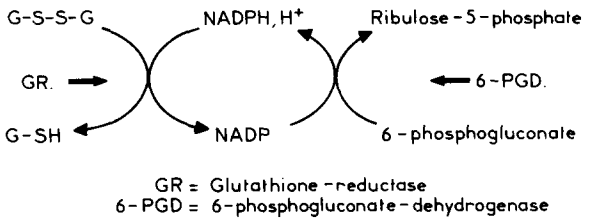

Fig.1. Pathways influencing the level of NADP in the red cell in the presence of 6-PGA (see text).

Table 1
Effect of 6-PGA on $K_{m_{app}}$ and V_{max} of human normal red cell glutathione reductase. Assays were carried out on unheated and heated hemolysates (see text)

	− FAD			+ FAD		
Hemolysate	− 6PGA	+ 6PGA ($6 \cdot 10^{-4}$ M)	+ 6PGA (0.6 M)	− 6PGA	+ 6PGA ($6 \cdot 10^{-4}$ M)	+ 6PGA (0.6 M)
			$K_{m_{app}}$			
Unheated	3.95	no measurable rate		3.80	no measurable rate	
Heated	3.95	4.00	4.00	3.80	3.95	4.00
			V_{max}			
Unheated	9.5	no measurable rate		11	no measurable rate	
Heated	10	10	10	11	10.5	11

GSSG. In this case it was not necessary to use heated hemolysates, since the presence of 6-PGD would not influence the rate of reduction of GSSG to GSH by GR (see fig.1). Fig.2 shows the result of this experiment. It is evident that the presence of 0.6 M 6-phosphogluconic acid had no effect on the rate of formation of GSH in this system.

Although the exact level of 6-PGA in the red blood cell is still unknown because of methodological difficulties, both a very low and a vastly higher concentrations of 6-PGA had no demonstrable effect on the rate of the GR reaction. Our studies indicate therefore that 6-PGA has no inhibitory effect on GR and cannot be considered as an intermediate which connects the metabolism of the glycolytic with the hexose-monophosphate pathways.

Table 2
Activity of GR and 6-PGD before and after heating the hemolysate to 56°C for 90 min.

		Activity (as IU/g of hemoglobin-)	
		GR	6-PGD
Hemolysate	Unheated	5.35	8.02
	Heated	5.40	0.

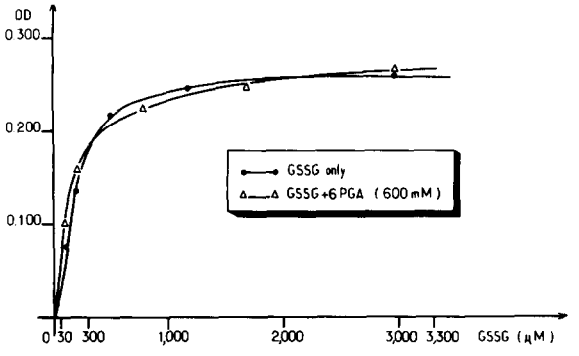

Fig.2. Rate of formation of GSH (O.D.) as a function of the concentration of GSSG, with and without 6-PGA.

Acknowledgement

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References

[1] Carson, P. E. (1970) Proc. Roy. Soc. Med. 2, 63, 175.
[2] Beutler, E. (1971) Red cell metabolism p. 62–66, Grune and Stratton, New York.
[3] Beutler, E., Yeh, M. K. Y. (1963) Blood, 21, 573.