Volume 302, number 3, 197–200 FEBS 11020 May 1992

Interaction of mannose-6-phosphate with the hysteretic transition in glucose-6-phosphate hydrolysis in intact liver microsomes

Hubert Vidal[a,*], Alfred Berteloot[c,d], Marie-Josée Larue[a], Jean-François St-Denis[b] and Gérald van de Werve[a,b,c]

Laboratoire d'Endocrinologie Métabolique, Departments of [a]Nutrition and [b]Biochemistry, [c]Groupe de Recherche en Transport Membranaire and [d]Department of Physiology, University of Montreal, Montreal, Que. H3C 3J7, Canada

Received 16 March 1992

We showed previously that glucose-6-phosphatase activity was characterised in intact liver microsomes by a hysteretic transition between a rapid and a slower catalytic form of the enzyme. We have now further investigated the substrate specificity of these two kinetic forms. It was found that the pre-incubation of intact microsomes with mannose-6-phosphate or glucose-6-phosphate (50 μM for 30 s) suppressed the burst in glucose-6-phosphatase activity, that the hysteretic transition was reversible and that mannose-6-phosphate inhibited glucose-6-phosphate hydrolysis during the first seconds of incubation, but not anymore after the burst. Our results indicate (i) that mannose-6-phosphate is recognised by the enzyme and can promote the hysteretic transition and (ii) that the transient phase is part of the catalytic mechanism itself.

Liver; Glucose-6-phosphatase; Microsome; Hysteresis; Mannose-6-phosphate

1. INTRODUCTION

Since its discovery in 1949 [1] the kinetics, regulation and molecular organisation of microsomal glucose-6-phosphatase (G6Pase) have been widely studied (see [2] for a review). Permeabilisation of the microsomal membrane by detergent treatment results in a loss of specifity of G6Pase for glucose-6-phosphate (G6P) associated with a marked increase in the catalytic rate of the enzyme [3,4]. This apparent latency of G6Pase has been taken as evidence for the concept of a specific, rate-limiting transport of G6P across the microsomal membrane [4–6]. However, several authors have also considered that the difference in G6Pase activity between intact and permeabilised microsomes might result from detergent-induced modifications in the conformation and thus in the properties of the enzyme protein [2,7–10]. The conformational model implies that G6Pase binds its substrate at the outer face of the microsomal membrane, hydrolyses it and releases the products in the cisternae. In a recent study [11] we have described the rapid kinetics of G6P hydrolysis in fasted rat liver microsomes and shown that both glucose and phosphate productions are characterised by a burst of activity in intact vesicles. Solubilisation of the microsomal membrane by detergent resulted in a loss of this property. Moreover, the initial rates of G6P hydrolysis were equal when measured in intact microsomes (initial rate of the burst) or in deoxycholate-treated vesicles, indicating that the substrate has similar access to G6Pase either in intact or in permeabilised microsomes during the first seconds of incubation. However, over the first 5–10 s of incubation the rate of G6Pase decreased in intact microsomes to achieve a lower steady-state rate [11]. From these results we have concluded (i) that there is no separate and rate-limiting transport of G6P in intact microsomes, (ii) that the latency of G6Pase in intact microsomes is compatible with the concept of a slow hysteretic transition and (iii) that solubilisation of the microsomal membrane by detergent treatment precludes the hysteretic relaxation process. In the present study we investigated the substrate specificity of the two kinetic forms of G6Pase involved in the hysteretic transition observed during the catalytic process of the enzyme in intact microsomes of fasted rat liver.

Present address: INSERM U197, Faculté de Médecine Alexis Carrel, 69008 Lyon, France.

Correspondence address: G. van de Werve, Laboratoire d'Endocrinologie Métabolique, Department of Nutrition, University of Montreal, CP 6128, succursale A, Montreal, Que. H3C 3J7, Canada. Fax: (1) (514) 343-7395.

2. MATERIALS AND METHODS

2.1. *Microsomes and reagents*

Procedures for preparing and permeabilising rat liver microsomes, rapid kinetics, analytical methods and sources of chemicals were fully described in our previous report [11]. Mannose-6-phosphate (M6P), free of G6P contamination (#M 6876), was from Sigma.

2.2. *Curve fitting*

In intact microsomes, the time-dependent production of [U-^{14}C]glucose from [U-^{14}C]G6P was fitted to equation 1 by non-linear regression analysis of the data points, as previously justified [11].

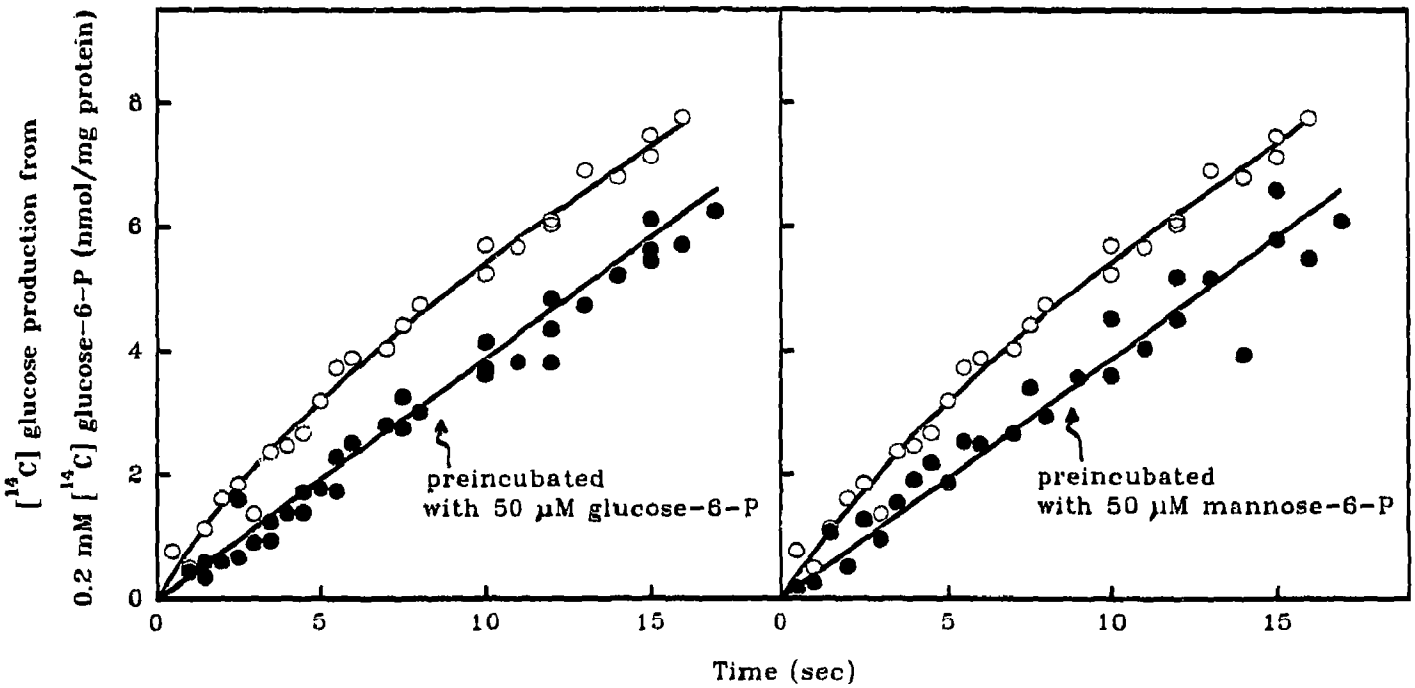

Fig. 1. Effects of the pre-incubation of microsomes with G6P or M6P on the kinetic behavior of G6Pase. Microsomes were pre-incubated for 30 s at 30°C with either 0.05 mM G6P (left panel) or 0.05 mM M6P (right panel) before measuring the production of [U-^{14}C]glucose from [U-^{14}C]G6P (0.2 mM) using a fast-sampling, rapid-filtration apparatus [11]. (○) Control microsomes; (●) microsomes pre-incubated with the indicated phosphorylated sugar.

$$P = Bt + A(1-e^{-\alpha t}) \quad (1)$$

Equation 1 allows the simultaneous determination of the kinetic parameters, A (amplitude of the burst), α (inverse of the time constant of the burst) and B (steady state rate of glucose production), which characterise the time-dependent production of glucose from G6P.

The initial rate of glucose production (nmol/s·mg of protein) can then be estimated by calculating the slope of the zero-time tangent to the fitted curve using the zero-time derivative of equation 1, given by equation 2.

$$V_i = B+(A\cdot\alpha) \quad (2)$$

Alternatively, the time-dependent hydrolysis of G6P was fitted by non-linear regression to equation 3 which applies to any mechanism of hysteresis as shown by Neet and Ainslie [12].

$$P = V_{ss}\,t - \left[\frac{V_{ss}-V_i}{\alpha}\right](1-e^{-\alpha t}) \quad (3)$$

In this equation, V_{ss} stands for the steady-state rate of hydrolysis (and is thus equivalent to B in equation 1), V_i is the initial rate of enzyme activity as described above and α has the same meaning as in equation 1 (inverse of the time constant of the transient phase). Equations 1 and 3 are equivalent but equation 3 allows V_i to be directly determined with its corresponding standard error for regression. The amplitude term can then be calculated according to equation 4.

$$A = \frac{V_{ss}-V_i}{\alpha} \quad (4)$$

When equation 3 was used, V_{ss} was first determined by linear regression of the linear part of the time curves of glucose production and fixed thereafter to this value during the fit to equation 3 in order to get a better estimate of the two other parameters (V_i and α).

Computer aided linear and non-linear regression analyses were performed with commercially available software (Enzfitter, Elsevier Biosoft, Cambridge, UK).

3. RESULTS AND DISCUSSION

3.1. *Effect of M6P and G6P on the burst of G6Pase activity in intact microsomes*

From the left panel of Fig. 1 it can be seen that the burst in glucose production observed before [11] and measured with 0.2 mM [U-^{14}C]G6P (open symbols) was completely suppressed (closed symbols) when the microsomes had been pre-incubated for 30 s at 30°C with 50 μM G6P prior to the addition of 0.2 mM [U-^{14}C]G6P. Most interestingly, if the pre-incubation with 50 μM G6P was prolonged up to 30 min at 20°C in order to completely hydrolyse the available substrate, and then 0.2 mM [U-^{14}C]G6P was added, although the rate of glucose production was decreased, a burst with a similar time constant was still recovered (Fig. 2). These observations thus indicate that the hysteretic transition is related to the catalytic process itself, that it is closely linked to substrate hydrolysis and that it is reversible.

Since our results suggest that G6Pase exists under the same conformational state in intact microsomes as in permeabilised vesicles at the start of the catalysis [11], one may expect that the enzyme would be able to recog-

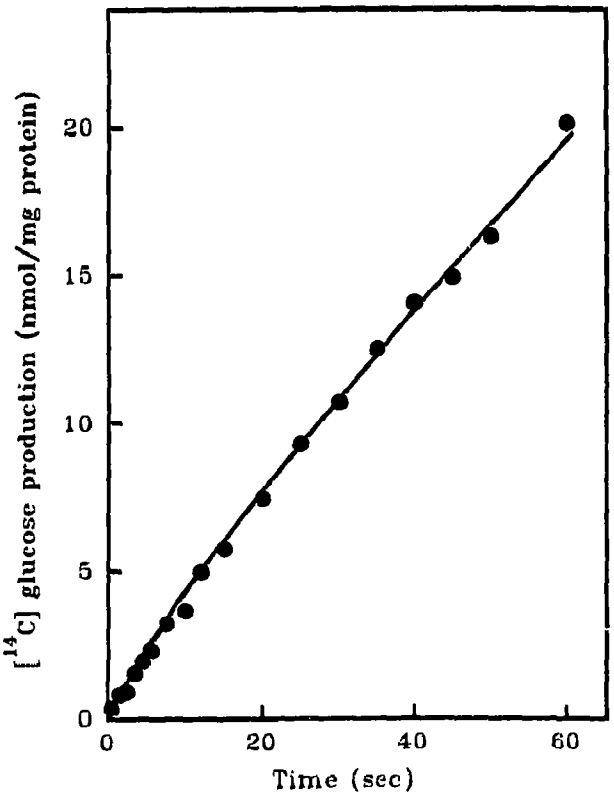

Fig. 2. Reversibility of the hysteretic transition in G6Pase activity. Microsomes were pre-incubated for 30 min at 20°C with 0.05 mM G6P before measuring the production of [U-^{14}C]glucose from [U-^{14}C]G6P (0.2 mM) using a fast-sampling, rapid-filtration apparatus [11].

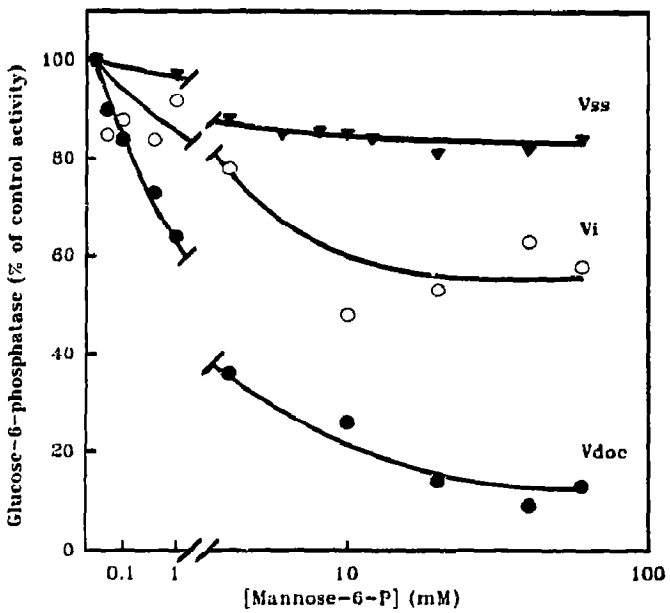

Fig. 3. Inhibition by M6P of the production of [U-^{14}C]glucose from [U-^{14}C]G6P in intact and permeabilised microsomes. [U-^{14}C]G6P (0.2 mM) hydrolysis was measured at 25°C in the presence of the indicated concentrations of unlabelled M6P, using a fast-sampling, rapid-filtration apparatus [11]. Steady-state rates (V_{ss}) (▼) were determined by non-linear regression analysis as described in section 2. Initial velocities (V_i) (○) of glucose production in intact microsomes were calculated from the kinetic parameters according to equation 2 and rates of hydrolysis in detergent-treated microsomes (V_{doc}) (●) were determined by linear regression of the data points. The results are expressed as the percentage of the enzymatic velocities measured in the absence of M6P. The experiment shown is one out of a series of experiments giving similar results (V_{ss} intact microsomes = 0.097, V_i intact microsomes = 0.181, and V_{doc} detergent-treated microsomes = 0.181 nmol/s·mg).

nise M6P over the first few seconds of incubation in intact vesicles. This seems indeed to be the case as pre-incubation with M6P (free of G6P contamination) (Fig. 1, right panel), as with G6P (left panel), suppressed the burst in [^{14}C]glucose production.

3.2. *Effect of M6P on [U-^{14}C]G6P hydrolysis in intact and detergent-treated microsomes*

A corollary to the recognition of M6P by G6Pase in intact microsomes is that G6P hydrolysis should be inhibited by M6P during the first seconds of incubation. We therefore investigated the competitive effect of increasing concentrations of M6P (0.05–60 mM) on the two phases, initial velocity of the burst (V_i) and steady-state rate (V_{ss}) of 0.2 mM [U-^{14}C]G6P hydrolysis in intact microsomes and on G6Pase activity (V_{doc}) in deoxycholate-permeabilised vesicles. Fig. 3 shows that M6P addition had virtually no effect on V_{ss} but almost completely inhibited V_{doc}, as reported before [3,4]. The salient observation, however, was that the initial velocity of the burst (V_i) in intact microsomes was also inhibited by M6P. The inhibition of G6Pase by M6P corresponds to the disappearance of the burst, and the rate of glucose production is then linear with time and similar to V_{ss}. M6P is thus accessible to the enzyme in tightly sealed microsomes, supporting our previous conclusion that a kinetic form of the enzyme similar to that in deoxycholate-treated vesicles is present in intact microsomes upon mixing with substrate [11]. Some isomerisation step must exist between the M6P- and G6P-generated forms of the enzyme, or alternatively, two different enzyme configurations, both accessible to G6P, are produced upon incubation with either M6P or G6P.

Taken together, our previous report [11] and the present results demonstrate: (i) that the apparent latency of G6Pase in intact liver microsomes cannot be explained by a rate-limiting transport of substrate into the intravesicular compartment [11]; (ii) that this latency actually depends on a transiet phase in the molecular mechanism of hydrolysis itself and that the transition is reversible; (iii) that binding of substrate in intact microsomes initially involves a free enzyme form with kinetic properties indistinguishable from those of the enzyme in detergent-treated membranes, including a lack of specificity for G6P; (iv) that further ligand binding must occur on (an)other conformational state(s) of the pro-

tein which is (are) induced upon ligand binding and/or the formation of the protein–phosphate complex during the first enzyme turnovers; and (v) that this second conformational state retains some 'memory' of the first bound substrate since the conformational change can be induced by M6P which is not further hydrolysed.

Acknowledgements: This work was supported by Grants ME-10783 and MT-10804 (to G.v.d.W.) from the Medical Research Council of Canada. H.V. was a recipient of a fellowship of the Association Jacques Cartier (Université des Frères Lumière, Lyon, France).

REFERENCES

[1] de Duve, C., Berthet, J., Hers, H.G. and Dupret, L. (1949) Bull. Ste. Chim. Biol. 31, 1242–1253.
[2] Nordlie, R.C. and Sukalski, K.A. (1985) in: The Enzymes of Biological Membranes, vol. 2 (Martonosi, A.N., ed.) pp. 349–398, Plenum, New York.
[3] Arion, W.J., Wallin, B.K., Carlson, P.W. and Lange, A.J. (1972) J. Biol. Chem. 247, 2558–2565.
[4] Arion, W.J., Ballas, L.M., Lange, A.J. and Wallin, B.K. (1976) J. Biol. Chem. 251, 4901–4907.
[5] Arion, W.J., Wallin, B.K., Lange, A.J. and Ballas, L.M. (1975) Mol. Cell. Biochem. 6, 75–83.
[6] Waddell, I.D. and Burchell, A. (1991) Biochem. J. 275, 133–137.
[7] Stetten, M.R. and Burnett, F.F. (1967) Biochim. Biophys. Acta 139, 138–147.
[8] Zakim, D. and Edmondson, D.E. (1982) J. Biol. Chem. 257, 1145–1148.
[9] Schulze, H.-U., Nolte, B. and Kannler, R. (1986) J. Biol. Chem. 261, 16571–16578.
[10] Ness, G.C., Sukalski, K.A., Sample, C.E., Pendleton, L.C., McCreery, M.J. and Nordlie, R.C. (1989) J. Biol. Chem. 264, 7111–7114.
[11] Berteloot, A., Vidal, H. and van de Werve, G. (1991) J. Biol. Chem. 266, 5497–5507.
[12] Neet, K.E. and Ainslie Jr., R.A. (1980) Methods Enzymol. 64, 192–226.