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The Glucose-induced Switch Between Glycogen Phosphorylase and Glycogen Synthase in the Liver: Outlines of a Theoretical Approach*

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The glucose-induced switch between glycogen phosphorylase and glycogen synthase in the liver is investigated by means of a theoretical approach based on a minimal, bicyclic cascade model involving the reversible phosphorylation of the two enzymes. The aim of the analysis is to evaluate the contribution of different factors to the sequential changes in the activity of glycogen phosphorylase and glycogen synthase observed following the addition of suprathreshold amounts of glucose.

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1. Introduction

Phosphorylation-dephosphorylation cascades represent one of the most exquisite modes of cellular regulation. Examples of such control processes involving multiple cycles of covalent modification are numerous and range from regulation of metabolic pathways to control of the cell cycle (Cohen, 1982, 1985; Boyer & Krebs, 1986). This mode of biochemical regulation illustrates particularly well the notion, repeatedly stressed by Henrik Kacser, to whose memory this volume is dedicated, that metabolic control is a systemic, distributed property (Kacser *et al.*, 1995): in a phosphorylation-dephosphorylation cascade each kinase and phosphatase indeed plays a crucial role in determining the dynamic behavior of the whole system. The organization of the cascade in closely intertwined cycles of covalent modification multiplies the potential for control. Each of the modifying enzymes in every cycle can indeed be activated or inhibited and can thereby significantly modulate the flux (Chock *et al.*, 1980; Goldbeter & Koshland, 1981, 1982; Cárdenas & Cornish-Bowden, 1989; Szedlacsek *et al.*, 1992) or the dynamic behavior through the cascade [for a discussion of the suppression of oscillatory behavior in the phosphorylation-dephosphorylation cascade controlling the onset of mitosis, see Goldbeter (1996) and Goldbeter & Guilmot (1996)].

A prototypic example of phosphorylation-dephosphorylation cascade involved in metabolic regulation is the one controlling the balance between glycogen synthesis and degradation. The enzymes which govern these processes are, respectively, glycogen synthase and glycogen phosphorylase; both enzymes are regulated through reversible phosphorylation (Stalmans *et al.*, 1987; Bollen & Stalmans, 1992). Thus, glycogen synthase is activated from a partially inactive form b to an active form a by glycogen synthase phosphatase, and inactivated by a kinase, whereas phosphorylase is activated from a partially inactive form b to an active form a by phosphorylase kinase, and inactivated by a phosphatase. The coordinated changes in the phosphorylation status of glycogen synthase and glycogen phosphorylase are under hormonal control, through the activation of protein kinases by cyclic AMP and cytosolic Ca^{2+}. In the liver, the switch between glycogen phosphorylase

* This paper is dedicated to the memory of Henrik Kacser.

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and glycogen synthase can also be controlled by glucose. Thus, glucose induces the inhibition of glycogen phosphorylase and the concomitant activation of glycogen synthase (Stalmans *et al*., 1974; Hers, 1976).

In fed animals, the switch between the two enzymes is abrupt: upon addition of sufficient amounts of glucose, the activity of glycogen phosphorylase diminishes, due to the activation of phosphorylase phosphatase. Only when the fraction of active phosphorylase has decreased below some 10% does glycogen synthase begin to be activated (Stalmans *et al*., 1974; Massillon *et al*., 1995). To account for this *hepatic threshold to glucose* and for the time lag in synthase activation, Stalmans and colleagues have proposed a sequential mechanism which rests on the inhibition of glycogen synthase phosphatase by phosphorylase a in the presence of sufficient amounts of glycogen; the latter condition prevails in fed but not in fasted animals (Mvumbi & Stalmans, 1987). The lag in synthase activation following the addition of a suprathreshold amount of glucose is ascribed to the need of first removing the inhibition of the synthase phosphatase by phosphorylase a whose level decreases owing to the activation of phosphorylase phosphatase by glucose (Stalmans *et al*., 1987; Bollen & Stalmans, 1992). These classical studies provide a textbook example of metabolic regulation [see Stryer (1995), p. 597]. The allosteric inhibition of glycogen synthase phosphatase by phosphorylase a has been demonstrated *in vitro* (Alemany & Cohen, 1986).

Observations of Guinovart and co-workers (Carabaza *et al*., 1992), corroborated by more recent studies (Massillon *et al*., 1995), show that removal of the inhibition by phosphorylase a does not suffice to elicit the activation of glycogen synthase. Activation of the latter enzyme, indeed, does not occur with non-metabolizable analogs of glucose, even when such analogs bring about the inhibition of phosphorylase a. These observations point to a role for a phosphorylated derivative of glucose, such as glucose-6-phosphate, in the activation of glycogen synthase. Although the precise mechanism remains to be determined, it appears that glucose-6-P behaves as a positive effector of the synthase phosphatase.

To investigate the origin of the hepatic threshold to glucose and the nature of the switch between glycogen phosphorylase and glycogen synthase, it is useful to

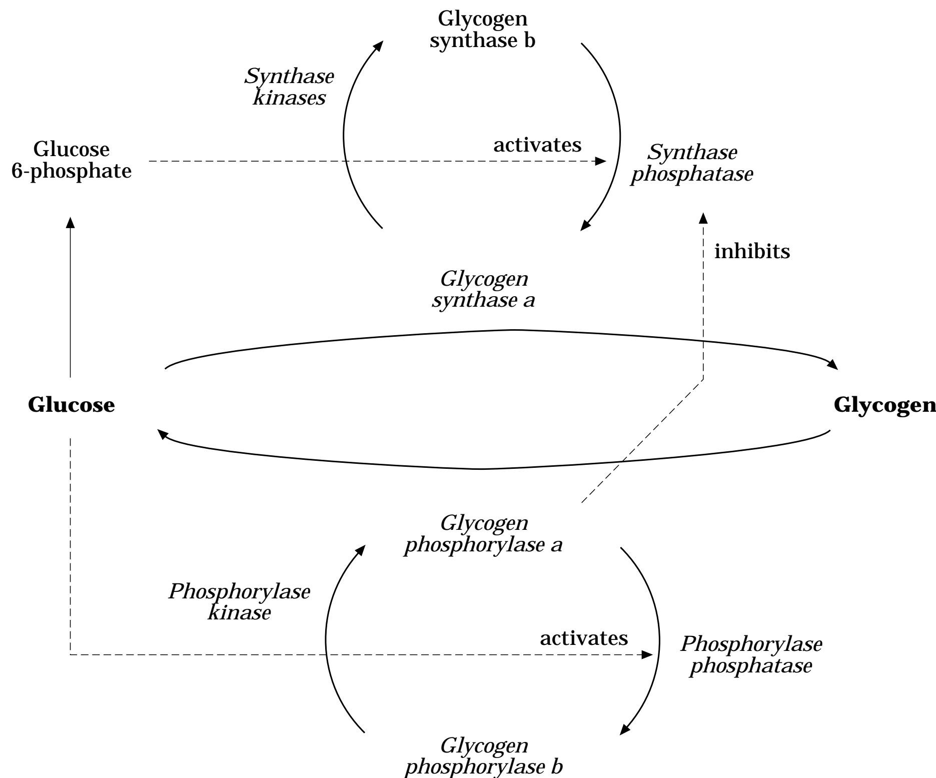

FIG. 1. Scheme of the bicyclic phosphorylation-dephosphorylation cascade considered for the control of glycogen synthesis and degradation. The cascade involves glycogen phosphorylase and glycogen synthase, as well as their converter enzymes. Also taken into account are the activation of phosphorylase phosphatase by glucose, the production of glucose-6-P upon addition of glucose, the activation of the synthase phosphatase by glucose-6-P, and the inhibition of the synthase phosphatase by phosphorylase a.

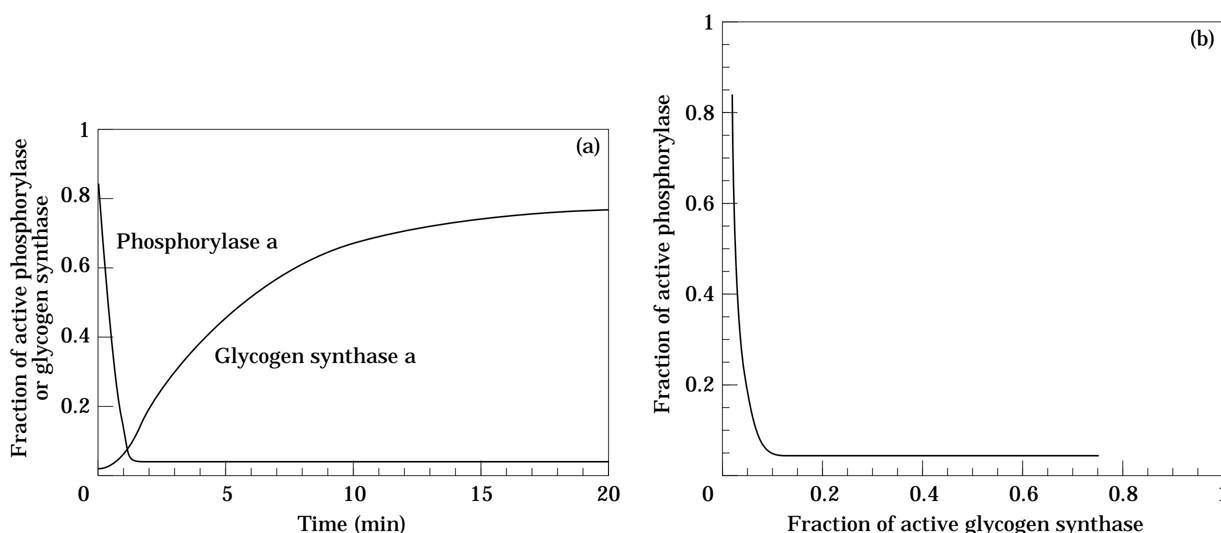

FIG. 2. Sequential inactivation of glycogen phosphorylase and activation of glycogen synthase in response to the addition of glucose. The time evolution of the fractions of active phosphorylase and of active glycogen synthase are plotted as a function of time [part (a)] after the glucose concentration (*Glc*) is raised from 5 mM to 60 mM; the concentration of glucose-6-P is supposed to rise at the same time from 0.1 mM to 0.7 mM. In (b) the fraction of active phosphorylase is plotted as a function of the fraction of active glycogen synthase, to allow comparison with experimental observations which point to the existence of a threshold concentration of phosphorylase a below which glycogen synthase is activated [see fig. 3 in Stalmans *et al*. (1974)]. The curves are obtained by numerical integration of eqns (1) for the following parameter values which appear to be in a physiological range and yield a time course comparable with that observed in the experiments: $K_1 = 0.1$, $K_2 = 0.2$, $K_3 = K_4 = 0.4$, $K_{a1} = K_{a2} = 10$ mM, $K_{a3} = K_{a4} = 0.5$ mM, $\alpha = \beta = 9$, $K_I = 0.1$, $V_1 = 1.25\ \mathrm{min}^{-1}$, $V_4 = 0.2\ \mathrm{min}^{-1}$, $V_{M2} = 0.22\ \mathrm{min}^{-1}$, $V_{M3} = 0.05\ \mathrm{min}^{-1}$. The total amounts of phosphorylase and glycogen synthase, which are used to normalize the Michaelis constants and the maximum rates of the converter enzymes as well as the inhibition constant K_I, are taken equal to 10 μM and 1.5 μM, respectively. The initial conditions are $Pha = 0.845$, $GSa = 0.02$; these values correspond to the steady-state values of the fractions of active phosphorylase and active glycogen synthase obtained when the concentrations of glucose and glucose-6-P are equal to 5 mM and 0.1 mM, respectively. All of these numerical values were chosen so as to match the theoretical curves with experimental observations; orders of magnitude for some of the parameters were kindly provided by Drs M. Bollen and W. Stalmans.

resort to a theoretical analysis which can help in evaluating the contribution of different factors to the threshold phenomenon. The purpose of this note is to present the outlines of such a theoretical approach, based on a minimal, bicyclic cascade model for the sequential changes in the activity of glycogen phosphorylase and glycogen synthase in response to glucose.

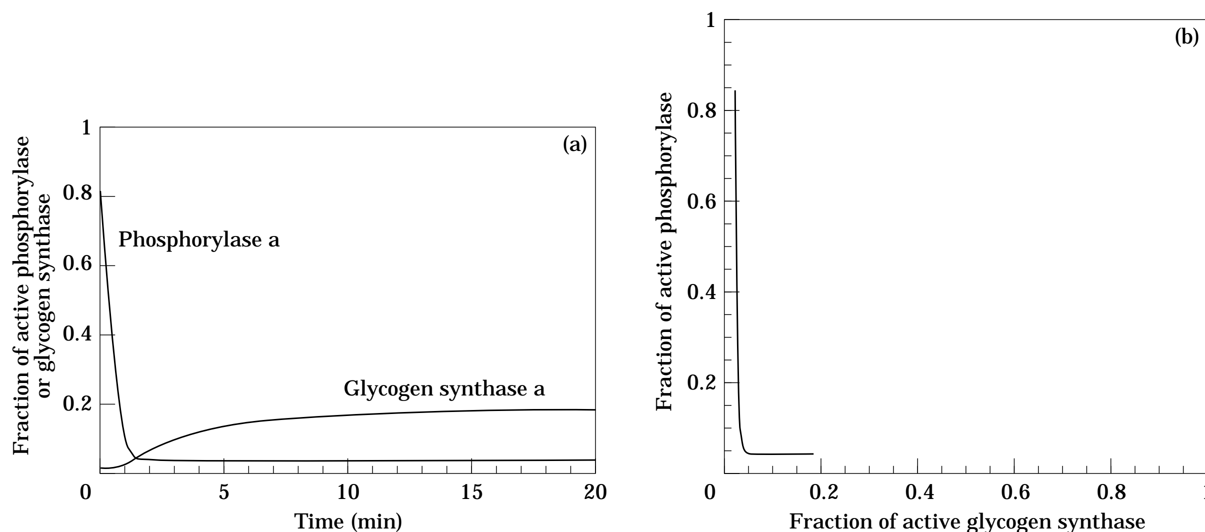

FIG. 3. Failure to significantly activate glycogen synthase upon addition of glucose, in the absence of the formation of glucose-6-P. The curves in parts (a) and (b) are generated as in the corresponding Figs 2(a) and (b), for the same parameter values and initial conditions, except that the concentration of glucose-6-P is held constant at the value of 0.1 mM instead of rising up to 0.7 mM. Here, the inactivation of phosphorylase upon addition of 60 mM glucose is not accompanied by the activation of glycogen synthase up to the level seen in Fig. 2.

2. Minimal Cascade Model Involving Glycogen Phosphorylase and Glycogen Synthase

We focus here on the threshold phenomenon observed as a function of glucose in the liver of fed rats and mice. Assuming that the only effector that changes is glucose, we can describe the dynamics of glycogen phosphorylase and glycogen synthase by the bicyclic phosphorylation-dephosphorylation cascade model schematized in Fig. 1. The level of cAMP is assumed to remain unchanged, so that the rates of phosphorylation of glycogen phosphorylase kinase and of glycogen synthase by the cAMP-dependent protein kinase are taken as constant parameters. Moreover, we assume that the level of Ca^{2+} remains constant, so that its activating effect is simply incorporated into the maximum rate of phosphorylase kinase.

The regulatory interactions retained in this minimal cascade model (see Fig. 1) are thus:

(i) Activation of phosphorylase phosphatase by glucose (*Glc*);
(ii) Inhibition of glycogen synthase phosphatase by phosphorylase a;
(iii) Activation of glycogen synthase phosphatase by glucose-6-P (*G6P*) whose level is taken as increasing with that of glucose [for such a link see, for example, Massillon *et al*. (1995)].

Although multiple phosphorylation of glycogen synthase has been observed *in vitro* (see Roach, 1986), we consider a single phosphorylation site for this enzyme.

The kinetic equations governing the time evolution of the fractions of active glycogen phosphorylase (*Pha*) and active glycogen synthase (*GSa*) are given by the system of eqns. (1):

$$\frac{\mathrm{d}Pha}{\mathrm{d}t} = \frac{V_1(1-Pha)}{K_1+1-Pha} - \frac{V_{M2}\left(1+\dfrac{\alpha Glc}{K_{a1}+Glc}\right)}{\dfrac{K_2}{1+\dfrac{Glc}{K_{a2}}}+Pha} \tag{1a}$$

$$\frac{\mathrm{d}GSa}{\mathrm{d}t} = \frac{V_{M3}\left(1+\dfrac{\beta G6P}{K_{a3}+G6P}\right)\dfrac{K_I}{K_I+Pha}(1-GSa)}{\dfrac{K_3}{1+\dfrac{G6P}{K_{a4}}}+1-GSa} - \frac{V_4 GSa}{K_4+GSa}. \tag{1b}$$

As the question of how glucose affects phosphorylase phosphatase and of how glucose-6-P affects glycogen synthase phosphatase is not yet fully clarified experimentally, we have retained the dual possibility that the effect in each case is both on the Michaelis constant and on the maximum rate. Thus, in the above equations, it is assumed that glucose activates phosphorylase phosphatase (of maximum rate V_{M2} and normalized Michaelis constant K_2) by decreasing the K_m of the enzyme, with an activation constant K_{a2}, and further activates the enzyme by enhancing its maximum rate by a multiplicative factor α, with an activation constant K_{a1}; glucose-6-P similarly decreases the K_m of the synthase phosphatase (of maximum rate V_{M3} and normalized Michaelis constant K_3), with an activation constant K_{a4}, and further activates the enzyme by enhancing its maximum rate by a multiplicative factor β, with an activation constant K_{a3}. Moreover, inhibition of the enzyme by phosphorylase a at the level of the maximum rate is characterized by the inhibition constant K_I, which is normalized by division by the total amount of glycogen phosphorylase. The other parameters, V_1, K_1 and V_4, K_4 refer to the maximum rates and Michaelis constants of phosphorylase kinase and synthase kinase, respectively. All maximum rates and Michaelis constants for the four converting enzymes are normalized by division by the total concentration of target protein, i.e. phosphorylase for the first cycle, and glycogen synthase for the second cycle of the cascade.

3. Dynamics of the Cascade Model as a Function of Glucose

The response of the bicyclic cascade can be determined by numerical integration of eqns (1) in response to step increases in glucose of various magnitudes. A typical response to an increase from 5 up to 60 mM is shown in Fig. 2; the level of glucose-6-P is supposed to follow the level of glucose and to rise instantaneously from 0.1 to 0.7 mM (however, the effect of a delay in the rise of glucose-6-P has also been addressed; see Discussion). As shown in Fig. 2(a), the fraction of active phosphorylase begins to drop immediately, while the level of synthase rises after a lag which can be correlated with the prior inactivation of phosphorylase below a threshold level. The existence of the threshold becomes apparent when plotting the fraction of active synthase as a function of the fraction of active phosphorylase [Fig. 2(b)], as initially done by Stalmans *et al*. (1974) in their treatment of experimental observations.

In the conditions of Fig. 2, however, the decrease in the activity of phosphorylase a brought about by

glucose does not suffice to induce significant activation of the synthase. This is shown in Fig. 3 [parts (a) and (b)] where the level of phosphorylase a is seen to drop after the increase in glucose, without causing an increase in active synthase comparable with that seen in Fig. 2, when the level of glucose-6-P is prevented from rising, as in the experiments performed with non-metabolizable analogs of glucose (Carabaza *et al*., 1992; Massillon *et al*., 1995).

Parameter values can readily be found for which the synthase can be fully activated in the absence of glucose-6-P. For this to be possible the ratio of activities between synthase phosphatase and synthase kinase must be such that the enzyme could be fully activated owing to the decrease in phosphorylase a alone. However, as glucose-6-P has been found experimentally to be required for the activation of glycogen synthase, we have focused on this case in Figs 2 and 3.

Although in this analysis we have only addressed the evolution to stable steady states, it can be argued that stability is not automatically guaranteed as the kinetic equations are highly nonlinear. However, the fact that the present model considers only a sequence of events without any feedback regulation of glycogen phosphorylase by glycogen synthase means that there is no mechanism present which could lead to an instability (for examples of enzyme cascades admitting instabilities leading to oscillatory behavior, see Goldbeter 1996; Goldbeter & Guilmot, 1996). Corroborating this view is the fact that no instability was observed in the numerous conditions investigated numerically.

4. Discussion

The bicyclic cascade model for the control of glycogen phosphorylase and glycogen synthase by glucose provides a minimal theoretical framework for investigating the sequential changes observed for the two enzymes in the liver of fed animals. The model can also be extended to the situation in which inhibition of synthase phosphatase by phosphorylase a is not effective, as in the presence of AMP after addition of fructose, or in the absence of sufficient amounts of glycogen in fasted animals.

The model indicates that the results obtained by Guinovart and co-workers (Carabaza *et al*., 1992) complement rather than contradict those reported by Stalmans and colleagues (Stalmans *et al*., 1987): the theoretical results are consistent with the view that the increase in glucose-6-P and the decrease in phosphorylase a, which follow the rise in glucose are both required for the activation of glycogen synthase up to significant levels. The activity of the latter enzyme is governed by the ratio of synthase phosphatase to synthase kinase; only when the level of phosphorylase a sufficiently decreases and the level of glucose-6-P sufficiently increases can the ratio of these rates be tilted in favor of synthase phosphatase.

When the inhibition of synthase phosphatase by phosphorylase a does not occur, as in fasted animals or in the presence of AMP, the model indicates, in agreement with experimental observations (Stalmans *et al*., 1987), that a rise in glucose-6-P may suffice to induce the rise in glycogen synthase. The activation of the latter enzyme then occurs without any lag, although a time delay could follow from a possible delay in the synthesis of glucose-6-P.

The origin of the sharp threshold observed in the curve showing the activity of phosphorylase vs. that of synthase in fed animals after addition of glucose [see Fig. 3 in Stalmans *et al*. (1974), and also Fig. 2(b) in the present paper] remains to be explored in further detail. One factor which could contribute to the threshold that characterizes the curve of Fig. 2(b) is the rapidity in phosphorylase decline relative to the slower activation kinetics of glycogen synthase. Another contributing factor may arise from the kinetic properties of phosphorylation-dephosphorylation cascades. Previous analyses of phosphorylation-dephosphorylation cycles have shown that sharp thresholds may occur in the curve yielding the steady-state level of phosphorylated protein as a function of the ratio of kinase to phosphatase rates when the converting enzymes operate in the domain of zero-order kinetics, i.e. when they are close to saturation by their protein substrate (Goldbeter & Koshland, 1981, 1982, 1984; Cárdenas & Cornish-Bowden, 1989). Although such a phenomenon of "zero-order ultrasensitivity" has been suggested to occur for muscle glycogen phosphorylase (Meinke *et al*., 1986), kinetic data available for glycogen phosphorylase and glycogen synthase in liver do not suggest that the converting enzymes operate under zero-order kinetics. Nonetheless, they do not exclude this possibility because the fact that glucose and glucose-6-P could act by decreasing the K_m of phosphorylase phosphatase and of synthase phosphatase respectively (as considered here) may well induce these two enzymes to pass from first-order to zero-order kinetics, at least partially. Moreover, the presence of glycogen could also affect the occurrence of zero-order kinetics.

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