HYPOTHESIS

DUAL FUNCTION AND COMMON IDENTITY OF PROTEINS IN GLYCOGEN METABOLISM: A HYPOTHESIS

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Hers, De Wulf and Stalmans have recently reviewed the control of liver glycogen metabolism in the pages of this journal [1] and have offered a scheme for the regulation of the activities of various of the enzymes involved. We have elsewhere proposed an alternative regulatory scheme [2] and present some features of it here as a comment on the hypothesis of Hers et al.

As our understanding of the control of glycogen metabolism, first in muscle, and, more recently, in liver has increased, so has the apparent complexity of the process. We feel that the point has been reached where possible simplifications are emerging which remove at least some of the problems that have been posed. There are ten key enzymes involved in the metabolism of the chain-forming $1 \rightarrow 4$-bonds and the regulation of this metabolism, namely: phosphorylase, phosphorylase *b* kinase, phosphorylase *b* kinase-kinase, phosphorylase *b* kinase-phosphatase, phosphorylase *a* phosphatase, synthetase, synthetase *a* kinase, synthetase *b* phosphatase, synthetase *b* phosphatase-kinase and synthetase *b* phosphatase-phosphatase. In addition, five of these enzymes are reported to exist in two forms. This, in turn, implies the existence of further regulatory enzymes and suggests linear regulatory systems extending indefinitely in either direction. Indeed, the activation of phosphorylase ($b \rightarrow a$) by the production of active phosphorylase *b* kinase, catalyzed in turn by phosphorylase *b* kinase-kinase, has been referred to as a cascade process [1] and likened to the blood clotting mechanism [3]. It is our contention [2] that the system may not be linear but cyclic, and the total number of proteins involved may not be so numerous as is suggested by the number of different names that have been employed. Two recent experimental observations have added weight to this contention. These are: first, that in muscle, synthetase *a* kinase and phosphorylase *b* kinase-kinase are one and the same [4, 5], and secondly, that in liver the process of inactivation of phosphorylase by dephosphorylation is closely linked to the activation of synthetase, also by dephosphorylation [1]. Fig. 1 depicts the various enzymes involved and their modes of interconversion; it does not, however, include the notion that phosphorylase *a* phosphatase also exists in two forms [6–8].

The idea that the regulation of synthetase and phosphorylase activities are linked by more than a similarity of mechanism was postulated some years ago by Larner and his colleagues who tested for the identity of phosphorylase *b* kinase with (i) glycogen synthetase and (ii) synthetase *a* kinase, but concluded that no identity existed [9, 10]. An identity has, however, now been discovered, as already mentioned, between synthetase *a* kinase and phosphorylase *b* kinase-kinase [4, 5]. On the basis of our assumptions [2] that (a) the system must be cyclic rather than linear, and (b) the regulatory mechanisms for glycogen anabolism and catabolism must be inextricably inter-

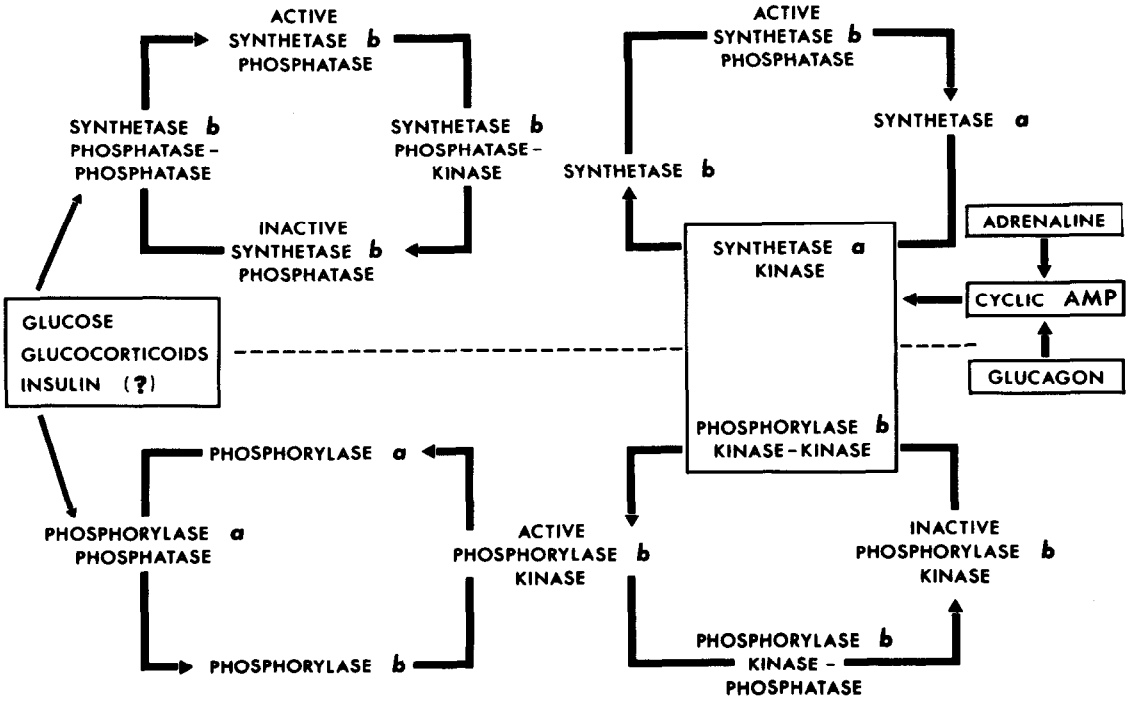

Fig. 1. Regulation and inter-relation of glycogen synthetase, glycogen phosphorylase and their regulatory enzymes. The kinases within the box are identical [4, 5]. Other enzymes reflected across the dotted line may be similar or identical (see text).

linked, we propose that the common identity has to extend beyond an identity of regulatory enzymes and must include an identity of protein substrates. The hypothesis we will outline states first that each of the two proteins (synthetase and phosphorylase) within the cyclic system exists in two catalytic forms, one form of which operates on glycogen, while the other operates on another enzyme within the system. Secondly, there are only two points of external control, both of which are under hormonal regulation.

Fig. 1 has been drawn with the deliberate intention of emphasizing possible identities within the group of nine supposedly different proteins. If one assumes that proteins that are reflected across the dotted line are the same protein, then the number is reduced to four, of which two exist in phosphorylated and dephosphorylated forms, these being the proteins within the cycle. This idea is expressed in fig. 2 where the names of enzymes and substrates, which may in fact be the same protein, are collected together at the same points. Implicit in this scheme is the notion that glycogenesis and glycogenolysis cannot be fully operative at the same time. Segal and his collaborators [11, 12] showed that in a liver homogenate, synthetase is inactive, but is gradually activated with time, and Hers et al. [13] have shown that the onset of synthetase activation is preceded by phosphorylase inactivation. We interpret this sequence of events as the action of phosphorylase *a* phosphatase on phosphorylase *a*, converting it into phosphorylase *b*, which is the active form of synthetase *b* phosphatase. While Hers et al. [14] had been the first to propose two forms of synthetase *b* phosphatase, they have now withdrawn this idea [1] in favour of a single form which is inhibited by phosphorylase *a*. We see no reason to withdraw the original idea and suggest that the so-called inhibition of synthetase *b* phosphatase is the result of a competition between the inactive (phosphorylase *a*) and the active (phosphorylase *b*) forms of the synthetase *b* phosphatase for the syn-

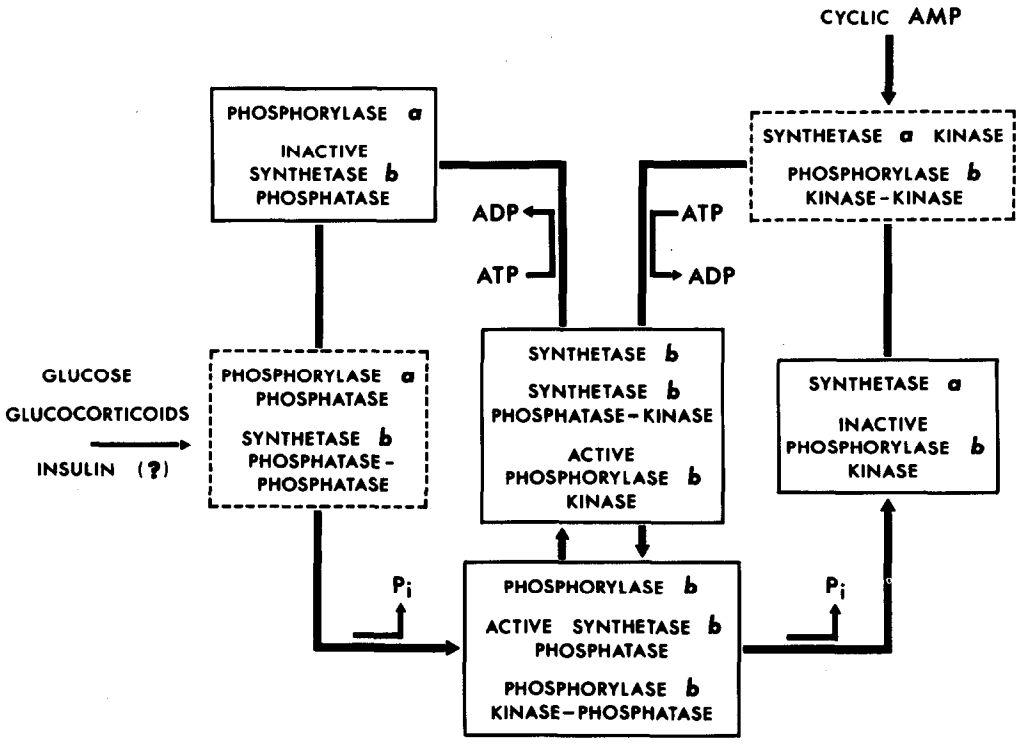

Fig. 2. Regulation of activity of glycogen synthetase and phosphorylase, drawn according to the hypothesis outlined in the text. Names appearing in the same box represent different functions of the same protein. Boxes surrounded by dotted lines represent the points of hormonal control, and these proteins behave only as protein-modifying enzymes. Proteins whose names appear within solid boxes can behave as a glycogen-modifying enzyme, as a protein-modifying enzyme, or as a substrate for a protein-modifying enzyme.

thetase *b* substrate. Hers et al. [14] noted that muscle phosphorylase *b* was less inhibitory than muscle phosphorylase *a* in the liver synthetase activation system. This serves to reinforce the fact that muscle and liver phosphorylase *b* are not identical, otherwise muscle phosphorylase *b* should have accelerated liver synthetase activation. That they are different is already known in that muscle phosphorylase *b* becomes active in glycogenolysis in the presence of AMP, while the liver enzyme does not [15].

A feature of this system is that, in the presence of ATP, the relation of phosphorylase *b* to synthetase *b* (active phosphorylase *b* kinase) is that of substrate to enzyme. In the absence of ATP the relationship is reversed, and synthetase *b* becomes the substrate for active synthetase *b* phosphatase (phosphorylase *b*). This reversibility of function implies that the amounts of phosphorylase and its kinase ought to be comparable, and this situation indeed exists in rabbit muscle [16]; the relative amounts are not known in liver. Our hypothesis may perhaps explain this hitherto puzzling situation in muscle. Also implicit in the hypothesis is that the inactivation of phosphorylase *a* by its conversion into phosphorylase *b* results in turn in the inactivation of phosphorylase *b* kinase by its being dephosphorylated by phosphorylase *b* (phosphorylase *b* kinase-phosphatase). This idea has the advantage that when phosphorylase *a* is converted into *b*, the $b \rightarrow a$ activation mechanism is automatically switched off, otherwise futile recycling could occur.

The external influences on this system occur at the points of action of the protein phosphatase and pro-

tein kinase. Adrenaline and glucagon promote cyclic AMP formation, which induces simultaneous curtailment of glycogenesis and activation of glycogenolysis. In liver, glucose and glucocorticoids, and more doubtfully insulin (perhaps through an as yet unidentified second messenger comparable to cyclic AMP), activate the protein phosphatase.

It should also be noted that much of the evidence on which this scheme is based is gathered from experiments employing crude homogenates or partly purified preparations. Indeed, it is somewhat surprising to realize that in the case of liver only phosphorylase *b* has been purified to near homogeneity, while the list of purified muscle enzymes, though more substantial, is far from complete. We hope that this hypothesis will stimulate further efforts to purify the various enzymes and to test for the identities we have suggested.

Acknowledgements

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