THE REGULATION OF LIVER GLYCOGEN SYNTHETASE D PHOSPHATASE BY ATP AND GLUCOSE

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SUMMARY: Synthetase D phosphatase activity in a liver glycogen pellet preparation is inhibited by ATP (physiological concentration). The inhibition can be reversed by glucose concentrations within the usual physiological range. Phosphophosphorylase activity decreases concomitantly with increasing synthetase I activity during the phosphatase incubation but the decrease is modest even in the presence of glucose. The glucose reversal of ATP inhibition is not the result of ATPase or glucokinase activities, which would reduce the ATP concentration. ATP, glucose and glucose 6-phosphate concentrations remain stable during the phosphatase assay.

In liver, as in other animal tissues, glycogen synthesis is catalyzed by glycogen synthetase (UDP-glucose:α-1,4-glucan α-4-glucosyltransferase, E.C. 2.4.1.11). There are two forms of the enzyme, synthetase D and synthetase I.

$$\text{Synthetase I} + n\text{ATP} \longrightarrow \text{Synthetase D-}(PO_4)_n + n\text{ADP} \quad [1]$$

$$\text{Synthetase D-}(PO_4)_n + H_2O \longrightarrow \text{Synthetase I} + n\text{Pi} \quad [2]$$

Synthetase I, the physiologically active form (1) is converted to synthetase D, the inactive form, by a phosphorylation reaction catalyzed by synthetase I kinase (cAMP-stimulatable protein kinase) (Reaction 1) (2). Synthetase D is converted to synthetase I by synthetase D phosphatase (Reaction 2) (3).

Glucose administered orally or intravenously causes an increase in liver synthetase I (4,5,6) but the mechanism of this effect is somewhat unclear. A similar effect of glucose has been demonstrated in the *in vitro* perfused rat liver (7,8) and in intact rats following insulin antibody administration (6); thus, this appears to be a direct effect of glucose on synthetase activation. However, activation of synthetase has also been reported following insulin administration (9,10). But, neither the role of insulin nor the relationship of insulin and glucose in causing synthetase activation is understood.

Recently, Stalmans and associates (11) reported that mouse liver synthetase phosphatase was inhibited by phosphophosphorylase, the active form of phosphorylase in liver. Glucose has been shown to promote the inactivation of phosphophosphorylase by stimulating phosphorylase phosphatase (12,13). They have, therefore, proposed that the glucose effect on synthetase phosphatase is an indirect effect mediated through a decrease in phosphophosphorylase concentration and that this system constitutes a regulatory mechanism for glycogen synthesis.

A critical aspect of this mechanism is that a decrease in phosphophosphorylase must precede an increase in synthetase I activity. Stalmans and associates (11) observed such a delay both *in vivo* and *in vitro* which was measured in minutes.

Recent *in vivo* studies reported from our laboratory showed that glucose administered to a fasted rat either by oral gavage (5) or intravenously (6) produced a prompt, significant conversion of synthetase D to synthetase I. However, contrary to the results of Stalmans and associates (11), no prior change in phosphophosphorylase activity was observed.

We have recently reported that ATP added to crude liver extracts treated with the anion exchange resin, BioRad AG-1, was inhibitory to the phosphatase reaction (14). Similar results were shown with crude extracts of skeletal muscle (15) but unlike muscle no agent tested reversed the inhibition in liver.

It was subsequently determined that ATP was unstable in crude liver extracts and that kinetic studies were impossible. In the present experiments a glycogen pellet preparation has been used in which ATP has been shown to be very stable during the phosphatase incubation. Using this preparation, glucose has been found to overcome ATP inhibition of the phosphatase reaction.

METHODS

The glycogen pellet was prepared from fresh liver taken from fed, male Holtzman rats (180-250 g), anesthetized with seconal (40 mg/kg). A 1:3 (wt/vol) homogenate was prepared in 250 mM sucrose, 50 mM imidazole, pH 7.0. Debris was

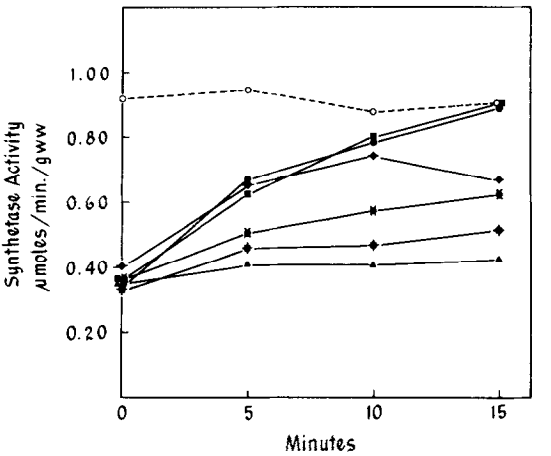

Figure 1. Synthetase D phosphatase activity in the presence of glucose and ATP. A glycogen pellet was prepared as described in the text. The pellet was suspended in 50 mM imidazole buffer, pH 7.0, diluted 4:5 with various additives and the mixtures incubated at 25°. Synthetase I activity was determined at appropriate intervals: no additive (●); 2.5 mM ATP (▲); 7.6 mM glucose (■); 2.5 mM ATP and 4.1 mM glucose (✚); 2.5 mM ATP and 8.8 mM glucose (✖); and 2.5 mM ATP and 13.6 mM glucose (◆). Total synthetase was comparable for all samples and a typical example is shown (○).

removed by centrifugation at 8200 x g for 10 minutes, and the supernatant was centrifuged at 42,000 x g for 45 minutes. The resulting glycogen pellet was washed with buffer and resuspended in 50 mM imidazole, pH 7.0 (one-half volume).

The glycogen content of the pellet was such that the phosphatase incubation mixture usually contained about 2% glycogen. The phosphatase assay was conducted at 25° and synthetase D and total synthetase activity were measured at intervals by the method of Thomas et al (16) in a pH 8.8 test mixture. Results are expressed as μmoles of glucose incorporated into glycogen per gram wet weight of liver per minute. Phosphorylase assays were conducted in the presence and absence of 3 mM AMP by a method developed in this laboratory (17). Results of this assay are expressed in the same fashion as for synthetase activity. Glucose 6-phosphate and ATP were determined by the method of Lowry et al (18). Glucose was determined by the Somogyi-Nelson method (19). Protein was determined by the method of Zak and Cohen (20).

RESULTS

Phosphatase activity measured in the pellet preparation proceeded without

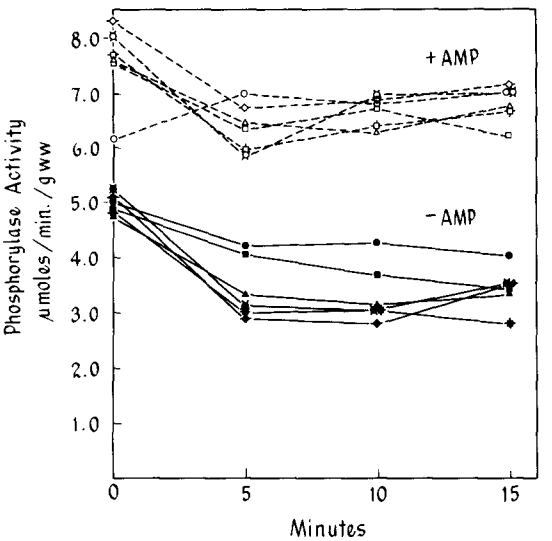

Figure 2. Phosphorylase phosphatase activity in the liver glycogen pellet. During the synthetase phosphatase assays shown in Fig. 1, aliquots were also taken for the assay of phosphophosphorylase activity in the presence (open symbols) and absence (filled symbols) of AMP. Phosphophosphorylase activity was determined at the intervals indicated: no additive (● , ○); 2.5 mM ATP (▲ , △); 7.6 mM glucose (■ , □); 2.5 mM ATP and 4.1 mM glucose (◆ , ◇); 2.5 mM ATP and 8.8 mM glucose (,); and 2.5 mM ATP and 13.6 mM glucose (,).

lag (Fig. 1). When ATP was added to the preparation to about 2.5 mM final concentration the phosphatase reaction was markedly inhibited. Increasing levels of glucose from 4 to 14 mM reversed the ATP inhibition with about 50% reversal achieved at a glucose concentration of 9 mM. In other experiments in the absence of ATP, it was shown that the synthetase phosphatase reaction was stimulated only slightly by increasing glucose levels up to about 8 mM final concentration with no additional effect when the concentration was raised to 20 mM (unpublished results).

Concurrent with the synthetase phosphatase assay phosphophosphorylase activity was also determined (Fig. 2). Phosphophosphorylase activity measured in the presence or absence of AMP was about one-fourth that usually found in crude extracts and decreased only modestly and inconstantly during incubation. The change occurred simultaneously with the activation of synthetase. There was no correlation between the extent of the decrease in phosphophosphorylase and the increase in synthetase D phosphatase activity.

TABLE I

ATP and Glucose Concentrations During Synthetase D Phosphatase Assay

Sample	Additions (mM, final conc.) ATP	Glucose	Incubation Time (min)	ATP Concentration* (mM, final conc.) 0	15	Glucose Conc.** (mM, final conc.) 0	15
1	---	---		0.07	0.15	0.9	3.0
2	2.5	---		2.33	2.61	1.6	2.6
3	---	7.6		0.15	0.15	7.6	9.6
4	2.5	4.1		2.30	2.81	4.1	5.0
5	2.5	8.8		2.41	2.54	8.8	10.1
6	2.5	13.6		2.50	2.52	13.6	13.9

* 100 µl aliquots were removed from the phosphatase incubation mixture and assayed according to the fluorometric method of Lowry et al. (18).

** 100 µl aliquots were removed during the phosphatase assay, deproteinized and assayed for glucose (19).

In order to assure that the inhibitory effect was due to ATP and not to some breakdown product, concentrations of ATP were determined during the assay. The ATP concentration remained essentially unchanged (Table I). Thus, there appears to be no ATPase or nonspecific phosphatase activities in the preparation. The ATP concentration also did not change when incubated in the presence of glucose indicating a lack of glucokinase activity in the preparation. This was supported by the fact that glucose (Table I) and glucose 6-phosphate concentrations (< 0.04 µM/ml) (data not shown) were stable during the course of the phosphatase assay.

DISCUSSION

From the present studies it is clear that the effect of glucose on synthetase activation need not be an indirect effect. Our results indicate that glucose directly deinhibits ATP inhibition of the synthetase phosphatase reaction. Glucose alone had little influence on the reaction.

A physiological concentration of ATP in liver is approximately 2.8 mM (18)

and it probably does not vary greatly. In the glycogen pellet preparation used here a similar concentration of ATP resulted in nearly complete inhibition of the synthetase D phosphatase reaction. The inhibition was completely reversed by 14 mM glucose and was approximately 50% reversed by 9 mM glucose. These are concentrations likely to be attained in liver in vivo. Whether the present observations represent a physiological mechanism for regulating glycogen synthesis is unknown. However, the fact that usual intracellular concentrations of glucose can regulate synthetase phosphatase activity by modulating the inhibitory effect of a physiological concentration of ATP in vitro suggests that this may be important in vivo. This mechanism could explain the effect of glucose on the synthetase system noted both in intact rats and in liver perfused in vitro (4,5,6,7,9).

Recently, Sanada and Segal (21) have reported conformational changes in which synthetase D forms a dimer in the presence of 5 mM ATP. We have previously speculated (14) on the suitability of the dimer as a substrate for synthetase D phosphatase and suggested that only the monomer of synthetase D may serve as a substrate. This could account for the ATP inhibition observed. Furthermore, it is possible that glucose either serves to reverse the dimerization process or modifies the dimer in a manner which would make it an acceptable substrate for synthetase D phosphatase. These interconversions of synthetase D conformers may represent a sensitive and specific means by which synthetase D phosphatase activity is regulated. Bailey and Whelan (13) have previously suggested a control mechanism in which glucose induces conformational changes of liver phosphophosphorylase and stimulates phosphorylase phosphatase activity.

It is becoming more apparent that a number of enzymic functions are accomplished by a single enzyme. Protein kinase is now recognized as catalyzing the conversion of synthetase I to synthetase D (22) and also the conversion of inactive phosphorylase kinase to the active form (23). More recently, a purified synthetase phosphatase preparation from rabbit skeletal muscle has been shown to have histone phosphatase activity (24). Purified rabbit muscle

synthetase D phosphatase also inactivates phosphorylase kinase (25). These activities exhibited by synthetase D phosphatase suggest that the enzyme may have the same kind of broad specificity as found earlier for protein kinase. If this is true, since it is necessary to insure that a specific reaction be regulated, control sites may exist at loci other than on the phosphatase enzyme itself. This would avoid the need for a plethora of unrelated regulatory binding sites on a single enzyme species. Regulation could occur as a result of an interaction of a modifier with the protein substrate. These modifiers would function to control the relative suitability of each potential substrate for binding with the enzymes. Interaction of ATP and glucose with synthetase D may be an example of such a mechanism. Studies to further investigate this possibility are planned.

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