On the Possibility of Metabolite Control of Liver Glycogen Synthetase Activity*

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ABSTRACT: A comparison of ATP inhibition of activities of nonactivated and *in vitro* activated liver glycogen synthetase shows that conversion into activated enzyme results in a decrease of synthetase affinity toward the inhibitor. The nucleotide inhibits activities of both enzyme forms by competing with the substrate, UDP-glucose. In the presence of an excess amount of the activator glucose 6-phosphate, a partial reversal of ATP inhibition of activity of nonactivated enzyme is observed. In the presence of magnesium, a limiting amount of glucose 6-phosphate is effective in reversing nucleotide inhibition of synthetase activity. The magnesium ion dependent increase of nonactivated synthetase affinity toward glucose 6-phosphate is observed to be decreased by ATP. These observations suggest that liver glycogen synthetase activity can be regulated by an effect of the nucleotide on enzyme affinity toward the hexosephosphate activator.

The *in vitro* activation of glycogen synthetase (UDP-glucose:α-1,4-glucan α-4-glucosyltransferase, EC 2.4.1.19), observed with extracts of rat liver homogenates (Gold and Segal, 1967), suggests the existence of a mechanism (Mersmann and Segal, 1967) which could explain the increased rate of liver glycogenesis in animals subject to nutritionally or hormonally altered physiological conditions (Hornbrook *et al.*, 1965, 1966; Gold and Segal, 1966; DeWulf and Hers, 1967a,b). A comparison of kinetic properties of nonactivated and *in vitro* activated enzyme show "activation" to result in an enzyme form with a greater affinity toward the substrates, UDP-glucose and glycogen, and the activators, glucose 6-phosphate (G-6-P), P_i, as well as Mg^{2+}, and indicates further the activation is not a change from a G-6-P-dependent to -independent enzyme form (Mersmann and Segal, 1967; Gold, 1968). Although the physiological significance of the *in vitro* activation is obscure at present, evidence has been presented to suggest that induced endocrine alterations effect liver enzyme activity *in vivo* by changing the affinity for UDP-glucose (Hornbrook *et al.*, 1966; Bishop and Larner, 1967). The results of the investigations cited show that an increase in synthetase activity *in vivo*, resulting from treatment of rats with hydrocortisone as well as insulin, can be explained by an increased affinity toward UDP-glucose. The magnitude in change of substrate affinity of the endocrine-induced enzyme activity is similar to that observed upon *in vitro* activation and the analogy serves as presumptive evidence to suggest the *in vitro* activating system, which is not clearly defined in liver, is subject to physiological control.

Recent evidence has suggested the adenine nucleotides to be modulators of the activities of G-6-P-dependent and -independent forms of muscle (Piras *et al.*, 1968) and liver (Gold and Segal, 1967; DeWulf *et al.*, 1968) glycogen synthetases. The studies on muscle and liver enzymes shows that ATP is an inhibitor of activity of each enzyme form and, with respect to muscle enzyme, is kinetically of the substrate-competitive type. The significance of a muscle G-6-P-dependent–independent conversion system has been interpreted in relation to a greater sensitivity of the G-6-P-dependent enzyme to ATP inhibition of activity.

The study presented by this communication is a description of the nature of adenine nucleotide inhibition of the activities of nonactivated and *in vitro* activated forms of liver glycogen synthetase.

Material and Methods

Normal male rats (Sprague–Dawley, 200–250 g) fed a standard laboratory diet were used. All animals were killed before 9:30 AM so as to minimize diurnal changes in liver glycogen. Glucose 6-phosphate, UDP-glucose, ATP, ADP, UTP, UDP, citrate, rabbit liver glycogen, and glycylglycine were obtained from Sigma, St. Louis. UDP-glucose-^{14}C (glucose labeled) was obtained from New England Nuclear Corp., Boston. Other reagents were of the best purity and were obtained from Fisher Scientific Co.

The preparation of enzyme and assay procedure (incorporation of glucose-^{14}C from UDP-glucose-^{14}C into glycogen) has been described (Gold and Segal, 1967; Gold, 1968). The radioactivity of primer glycogen, isolated from the reaction mixture by alcohol precipitation, was determined by suspension liquid scintillation counting. The efficiency of counting was 58% (channels ratio) and 65% (toluene-^{14}C as an internal standard). Enzyme activity is expressed as either counts per minute incorporated into glycogen primer or per cent of activity calculated from counts per minute incorporated. The activity values presented are corrected for small amounts of radioactivity associated with isolated glycogen primer when the reaction is quenched with KOH prior to addition of enzyme to the reaction mixture.

With the synthetase assay conditions as described, maximal activity of noninhibited, nonactivated enzyme, when determined with a saturating amount of G-6-P, was 3800 cpm ± 10–12%. The maximal activity for the *in vitro* activated

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TABLE I: K_i for ATP and ADP of Nonactivated and *in Vitro* Activated Liver Glycogen Synthetase.

	Nonactivated mM Mg^{2+}				Activated mM Mg^{2+}			
	0		2.0		0		2.0	
G-6-P (mM)	ATP	ADP	ATP	ADP	ATP	ADP	ATP	ADP
0			0.3	1.2	0.4	0.4	1.4	1.8
0.05					1.0	0.9	2.3	1.8
0.5	0.2[a]	0.3	1.0	1.6				
1.0	0.2	0.3						
5.0	0.6	0.9	1.7	2.0	4.0	2.9	3.5	

[a] The K_i values are expressed as millimolar concentration.

synthetase, in the presence of an optimal amount of G-6-P, was 5800 cpm ± 10%.

Results

The inability of G-6-P to reverse ATP inhibition of activity is shown in Figure 1 where it is observed that a high concentration of activator is moderately capable of reversing nucleotide inhibition of activity of nonactivated enzyme (Figure 1B). Since an earlier report demonstrated that Mg^{2+} increases the nonactivated enzyme affinity toward G-6-P (Gold, 1968), the effect of a combination of activator and cation on nucleotide inhibition was determined. The results (Figure 1A) show that G-6-P at less than a saturating level partially reverses ATP inhibition of activity as noted by the increased activity as well as increased K_i for ATP (Table I). The observed effect of G-6-P in the presence of Mg^{2+} does not rule out the possibility that the cation, and not the hexose phosphate, is responsible for reversal of ATP inhibition since even in the absence of G-6-P, Mg^{2+} is an effective stimulator of enzyme activity. The activation of enzyme, which occurs upon *in vitro* incubation as described in Figure 1, results in a decreased affinity toward the nucleotide inhibitor (Figure 1D, Table I). The decreased sensitivity of activated enzyme toward ATP inhibition could reflect either a loss of enzyme capability to bind ATP at a site specific for the nucleotide or, since activation results in an increased affinity toward UDP-glucose (Mersmann and Segal, 1967), a decreased binding of the nucleotide at the substrate binding site. Since the ATP inhibition kinetics as described in Figure 1 are of a first-order nature, the possibility that ATP competes with a single binding site does seem likely.

To show changes in activity which occur upon incubation of the enzyme preparation, absolute activities of the enzyme forms assayed with conditions similar to Figure 1 are presented in Table II. The data of Table II show that in the absence of Mg^{2+}, and with a G-6-P concentration which is limiting for nonactivated enzyme (0.5 mM), the conversion into activated form could result in as much as a 20-fold increase in activity at the same level of hexose phosphate activator. The implication from this comparison of activities would be that if *in vitro* conversion into active enzyme does represent *in vivo* changes in activity then conversion into active enzyme appears to be the primary means for regulation of the enzyme. The comparison shows further that the degree of activity increase upon conversion into active enzyme is independent of the concentration of nucleotide inhibitor. At the same G-6-P concentration as stated above, the data of Table II show that in the presence of Mg^{2+}, conversion into active enzyme results in a two- to fivefold increase in activity either in the absence of ATP or presence of ATP at concentrations less than the Mg^{2+}. The implication from this comparison is that the degree of activation upon conver-

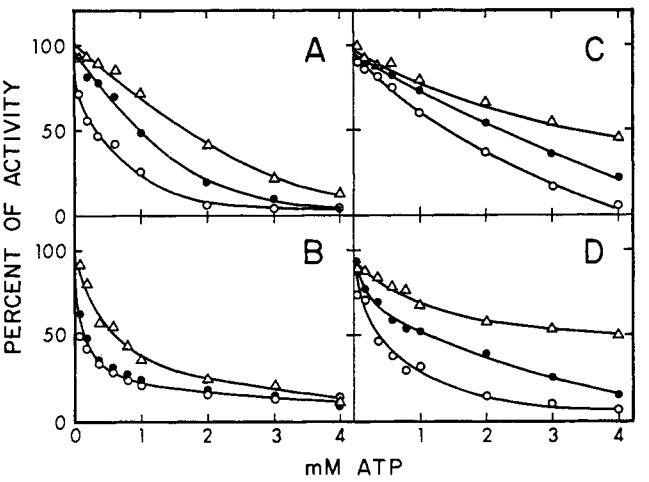

FIGURE 1: The activity of nonactivated (A and B) and *in vitro* activated (C and D) glycogen synthetase *vs.* ATP. Activated enzyme was prepared by incubating an 8000*g* extract of rat liver, blended in three volumes of cold 0.1 M glycylglycine (pH 7.4) at 20° for 50 min. Nonactivated enzyme was maintained at 0°. The assays were initiated by adding 0.05 ml of enzyme to a mixture of 0.25 μmole of UDP-glucose (35,000–40,000 cpm of UDP-glucose-^{14}C, glucose labeled), 60 μmoles of glycylglycine (pH 7.4), 5 mg of rabbit liver glycogen, and additions as indicated. The final volume was 1.0 ml. The reactions were stopped with 2.0 ml of hot 30% KOH, after 2 min at 37°, the glycogen was precipitated with 1.1 volumes of 95% ethanol, and the mixture was centrifuged. The glycogen pellet was redissolved in water and again precipitated with 95% ethanol. After centrifugation, the glycogen was dissolved in 1.2 ml of water and 1.0 ml was counted. Symbols are (A, nonactivated) ○, 0.5; ●, 1.0; △, 5.0 mM G-6-P. (B, nonactivated) ○, 0; ●, 0.5; △, 5.0 mM G-6-P. (C and D, activated) ○, 0; ●, 0.05; △, 5.0 mM G-6-P. In A and C, the assays were done in the presence of 2.0 mM Mg^{2+}. The per cent of activity is calculated from: $v_i/v_0 \times 100$.

TABLE II: Comparison of the Effects of ATP, Glucose 6-Phosphate, and Magnesium on the Activities of Nonactivated and *in Vitro* Activated Liver Glycogen Synthetase.

Compd Tested (mM)		Nonactivated mM G-6-P			Activated mM G-6-P			
Mg^{2+}	ATP	0	0.5	5.0	0	0.05	0.5	5.0
0	0	73[a]	950	3340	1540	4050	6400	6375
0	0.4	56	400	2160	970	3140	5000	5125
0	1.0	62	210	1060	530	2500	4200	4350
0	2.0	50	220	910	200	1540	4000	4175
0	4.0	45	140	480	180	745	3300	3400
2.0	0	1330	4450	4600	5560	6500	6800	6800
2.0	0.4	935	3300	3930	4000	6000	6350	6400
2.0	1.0	280	2200	3300	3140	5100	5700	5650
2.0	2.0	130	980	2010	1935	3550	5450	5460
2.0	4.0	50	180	670	340	1260	4000	3940

[a] Counts per minute incorporated into glycogen during the 2-min enzyme assay period. The preparation of enzyme and assay conditions are the same as Figure 1.

sion into active enzyme is a function of the level of ATP as well as the relative ratio between ATP and Mg^{2+}. The interpretation is equivocal since, at this time, it has not been demonstrated that Mg^{2+} is an obligatory cofactor for glycogen synthetase activity.

Since, as stated above, Mg^{2+} stimulates synthetase activity by increasing the affinity toward G-6-P, a comparison of the effects of ATP on the G-6-P stimulation of activity in the absence and presence of Mg^{2+} was determined (Figure 2). The sigmoid nature of G-6-P stimulation of activity is observed, in the absence of Mg^{2+} (Figure 2B), as reported (Mersmann and Segal, 1967). In the presence of ATP, the sigmoidocity of the G-6-P saturation curves is increased and suggests that the nucleotide decreases enzyme sensitivity to stimulation by the activator. In the presence of Mg^{2+} (Figure 2A), enzyme sensitivity to stimulation by G-6-P is increased as shown by a shift to the left of the G-6-P saturation curve. In the presence of low concentrations of ATP (Figure 2A), the enzyme retains sensitivity to G-6-P stimulation as observed by a lack of shift to the right of the G-6-P saturation curve. A decrease in enzyme sensitivity to stimulation by the activator does occur when the concentration of ATP is either equivalent to or greater than the Mg^{2+} present. The results shown in Figure 2 imply that in contrast to Mg^{2+}, ATP effects enzyme activity by decreasing the affinity toward G-6-P. The nucleotide effect on enzyme affinity toward the hexose phosphate may be apparent since, as will be shown. ATP competes with the substrate, UDP-glucose. It does seem likely, however, that ATP interacts with G-6-P since, in the presence of Mg^{2+}, a nucleotide concentration eightfold greater than the substrate is required before a noticeable effect on enzyme affinity toward the hexose phosphate is observed. Figure 3 shows a comparison of the effect ATP and Mg^{2+} on *in vitro* activated enzyme affinity for G-6-P. The results of the experiment suggest that ATP does not greatly alter the G-6-P sensitivity of this enzyme form. The nucleotide inhibition of the activity, as observed in Figure 3, is most likely a result of an interaction between ATP and UDP-glucose.

An estimate of the nature of ATP effect on Mg^{2+} stimulation of the activity of nonactivated enzyme, in the presence of G-6-P, was determined and the results are presented in Figure 4. At each level of G-6-P tested, the experiment shows that an increase in ATP results in a greater amount of Mg^{2+} required to reverse inhibition of activity as noted by a shift to the right of the Mg^{2+} saturation curve. In the presence of 2.0 mM Mg^{2+}, which causes maximal stimulation of activity in the absence of nucleotide, the experiment suggests that a decrease in ATP concentration from 2.0 to 0.2 mM could at 0.1, 0.5, and 5.0 mM G-6-P, respectively, result in a ten-, three-, and twofold increase in enzyme activity. Although the mechanism of Mg^{2+} stimulation of activity is not known, as yet, the effects as noted in Figure 4 may be a result of nucleotide chelation of the metal cation.

Double-reciprocal plots of the velocity of nonactivated (Figure 5) and activated (Figure 6) enzyme *vs.* UDP-glucose show that ATP is a substrate-competitive inhibitor of activity of the enzyme forms. The K_m' values for noninhibited activities agree with reported values using similar assay conditions (Mersmann and Segal, 1967; Hornbrook *et al.*, 1966). The measurement of effect of ATP on nonactivated enzyme affinity for substrate shows that in the absence of Mg^{2+} (Figure 5A) an ATP concentration near the K_i (1.0 mM used in the experiment) causes a sixfold increase in K_m' for UDP-glucose as compared with noninhibited activity. When activity is determined in the presence of Mg^{2+} (Figure 5B), an ATP concentration near the K_i for this experimental condition (0.2 mM ATP was used) did not change the K_m' for UDP-glucose when estimated in the absence of inhibitor (data not shown on the figure). Although these results imply the effect of nucleotide inhibition is *via* direct competition with the substrate, it can not be ruled out that competition of ATP with G-6-P binding decreases the enzyme affinity toward the substrate.

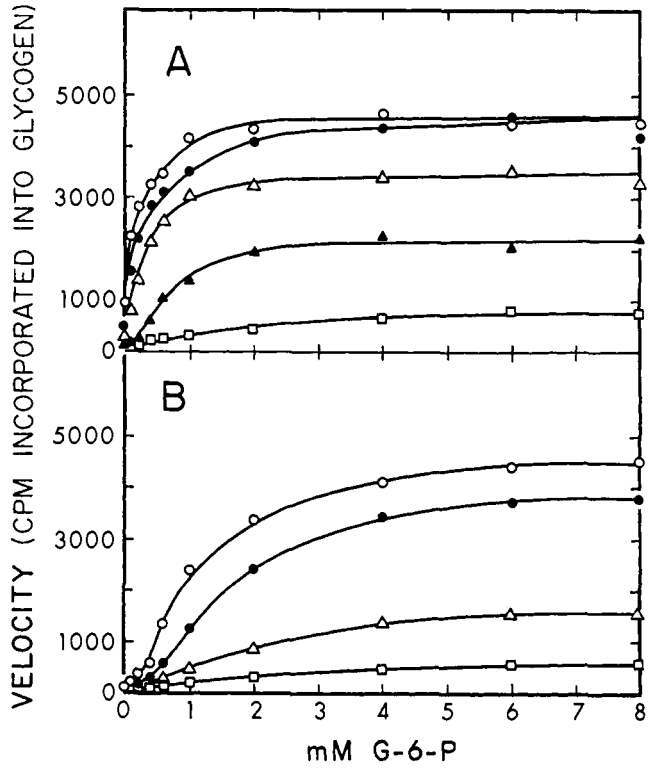

FIGURE 2: The effect of ATP and Mg^{2+} on the activity of nonactivated glycogen synthetase *vs.* G-6-P. The preparation of enzyme and assay conditions are the same as Figure 1. Symbols are ○, 0; ●, 0.2; △, 1.0; ▲, 2.0; □, 4.0 mM ATP. In A, the enzyme assays were done in the presence of 2.0 mM Mg^{2+}.

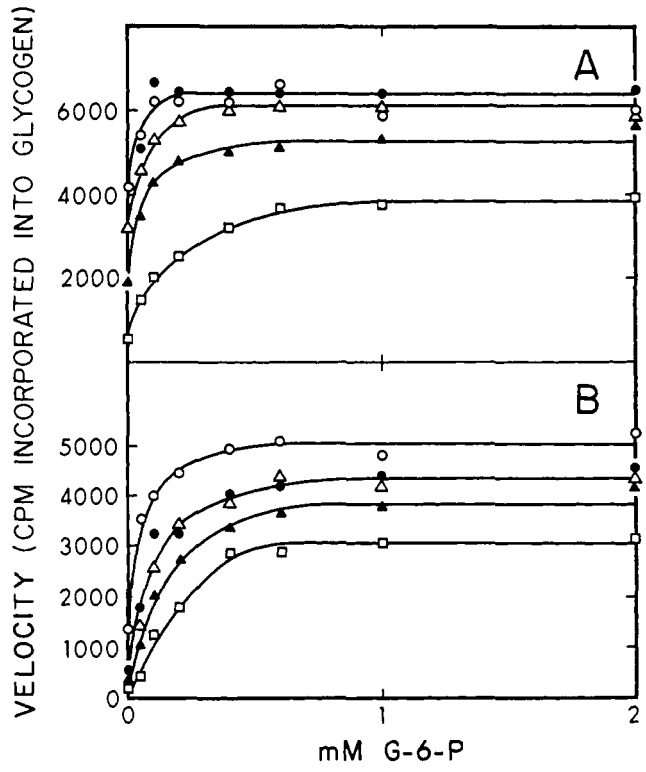

FIGURE 3: The effect of ATP and Mg^{2+} on the activity of *in vitro* activated glycogen synthetase *vs.* G-6-P. The preparation of enzyme and assay conditions are the same as Figure 1. Symbols are ○, 0; ●, 0.2; △, 1.0; ▲, 2.0; □, 4.0 mM ATP. In A, the enzyme assays were done in the presence of 2.0 mM Mg^{2+}.

The nature of Mg^{2+} stimulation of activity of the nonactivated enzyme is an effect on affinity for the substrate. The K_m' values measured in the presence of 0.5 mM G-6-P as well as 0.1, 0.4, and 2.0 mM Mg^{2+} were 0.5 and 0.7, 0.3 and 0.4, 0.2, and 0.18 mM UDP-glucose, respectively, with separate enzyme preparations. In the absence of Mg^{2+}, as well as in the presence of the low Mg^{2+} concentrations, the double-reciprocal plot curved upward and the K_m' values were estimated from the linear portion of the curve obtained with UDP-glucose concentrations greater than 1.0 mM. With the highest Mg^{2+} level used, the double-reciprocal plots of velocity *vs.* substrate were linear.

The ATP inhibition of the *in vitro* activated enzyme, when assayed with a saturating amount of G-6-P, appears to be a simple substrate-competitive type as observed by the linearity of the double-reciprocal plots (Figure 6A). A more complex interaction between enzyme, substrate, activator, and inhibitor is suggested from the curvilinearity of the double-reciprocal plots of the nonactivated enzyme (Figure 5) and with the activated enzyme when assayed with a limiting G-6-P amount (Figure 6B).

A comparison of the effect of ADP on the activities of the synthetase forms was made to determine if ADP and ATP inhibition kinetics were similar (Figure 7). The activity assessments in the presence of a limiting amount of G-6-P shows the shapes of the ADP inhibition curves to resemble those obtained with ATP. When activity is measured in the presence of Mg^{2+} and a saturating amount of G-6-P, ADP inhibition of nonactivated and activated enzyme is not of first-order kinetics, as observed with ATP using similar assay conditions, and suggests that maximal amounts of activators decrease the ADP inhibition sensitivity of the enzyme forms. A comparison of the data of Figure 7 and Table I shows that in the absence of Mg^{2+}, the K_i for ADP of nonactivated enzyme is similar to the value for ATP; however, Mg^{2+} increases the K_i for ADP to a greater extent than observed with ATP. These results may be because of the decreased ability of ADP to bind Mg^{2+} at the pH used in the experiment.

Since there is an apparent competition between the Mg^{2+} stimulation and ATP inhibition of enzyme activity, an estimate of enzyme specificity for divalent cations was made by comparing the velocity of nonactivated and *in vitro* activated forms *vs.* Mg^{2+}, Ca^{2+}, and Mn^{2+} (Figure 8). A difference between Mg^{2+} and Ca^{2+} in effect on the maximal velocity was observed with nonactivated enzyme (Figure 8B) which disappears on activation (Figure 8A). Conversion into active enzyme results in an increase in the enzyme affinity for the cations tested. The greatest change occurs with Ca^{2+} and Mg^{2+} although Mn^{2+} stimulates the activity of both forms more at concentrations less than 1.0 mM. For this experiment, Mg^{2+} was added as the acetate; the effect on the activity is the same as with the chloride salt (Gold, 1968). The effect of combinations of the salts on enzyme activity was additive to a total cation concentration of 2.0–3.0 mM and could reflect a competition between the metal ions.

In view of an earlier report on the inhibition of the synthe-

TABLE III: Comparison of the Effects of UTP, UDP, and Citrate on Glucose 6-Phosphate and Magnesium-Stimulated Activities of Nonactivated and *in Vitro* Activated Liver Glycogen Synthetase.

Compd Tested (1.0 mM)	Nonactivated mM Mg^{2+}				Activated mM Mg^{2+}			
	0		2.0		0		2.0	
	mM G-6-P				mM G-6-P			
	0.5	5.0	0.5	5.0	0.05	5.0	0.05	5.0
None	100	100	100	100	100	100	100	100
UTP	26[a]	14	6	24	16	33	37	42
UDP	10	16	9	25	13	25	30	37
Citrate	100	80	55	88	82	97	75	100

[a] Per cent of original activity calculated as: $v_i/v_0 \times 100$. Values are averages of three experiments. Experimental conditions are the same as Figure 1.

tase activity by UDP, but not UTP or citrate (Steiner *et al.*, 1965), a comparison of the effects of these compounds on the nonactivated and activated enzyme was made and is presented in Table III. The nucleotides were strongly inhibitory at 1.0 mM concentration either in the absence or presence of Mg^{2+}. Of interest is the citrate inhibition of activity of nonactivated enzyme when assayed in the presence of Mg^{2+} and limiting G-6-P. Although the citrate:Mg^{2+} ratio was 0.5, the 50% inhibition of activity of the enzyme under these conditions is presumptive evidence for a role of free Mg^{2+} in altering enzyme affinity for G-6-P.

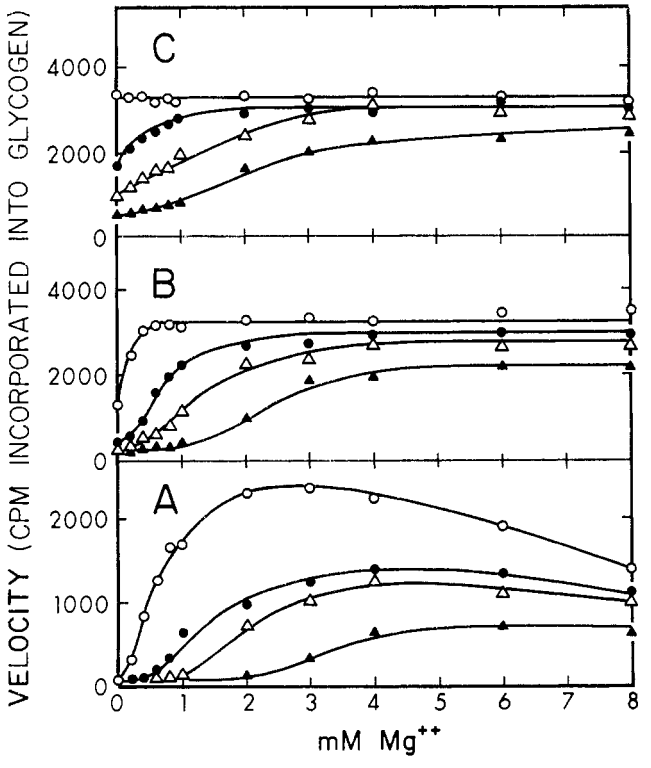

FIGURE 4: The effect of ATP and G-6-P on the activity of nonactivated glycogen synthetase *vs.* Mg^{2+}. The preparation of enzyme and assay conditions are the same as Figure 1. Symbols are ○, 0; ●, 0.2; △, 1.0; ▲, 2.0 mM ATP. G-6-P concentrations were 0.1, 0.5, and 5.0 mM in A, B, and C, respectively.

Discussion

The present study shows the nucleotides ATP, ADP, UTP, as well as UDP to be inhibitors of the activity of nonactivated and *in vitro* activated forms of liver glycogen synthetase. In this investigation, an enzyme preparation was used representing the total liver glycogen synthetase activity. An

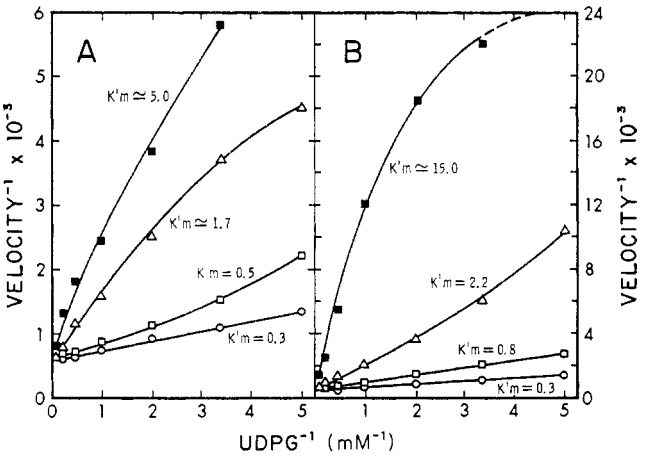

FIGURE 5: Double-reciprocal plot of velocity of nonactivated enzyme *vs.* UDP-glucose. The preparation of enzyme is the same as Figure 1. The specific activity of the substrate was 20,000 cpm (UDP-glucose-^{14}C) per 1.0 μmole of UDP-glucose. In A, the G-6-P concentration was 5.0 mM and the substrate concentration ranged from 0.2 to 10.0 mM. In A, the symbols are ○, 0; □, 0.2; △, 1.0; ■, 2.0 mM ATP. The ordinate on the left corresponds to A. In B, the G-6-P concentration was 0.5 mM and the assays were done in the presence of 2.0 mM Mg^{2+}. The substrate concentration in B ranged from 0.2 to 20.0 mM. The symbols in B are ○, 0; □, 1.0; △, 2.0; ■, 4.0 mM ATP. The ordinate on the right corresponds to B.

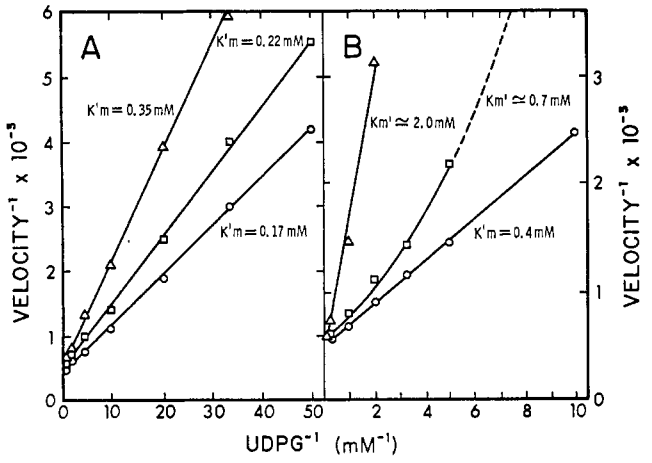

FIGURE 6: Double-reciprocal plot of velocity of *in vitro* activated enzyme *vs.* UDP-glucose. The enzyme preparation is the same as Figure 1. The specific activity of the substrate was 20,000 cpm (UDP-glucose-^{14}C) per 1.0 μmole of UDP-glucose. In A, the G-6-P concentration was 5.0 mM and the substrate concentration ranged from 0.02 to 1.5 mM. The symbols in A are ○, 0; □, 0.4; △, 2.0 mM ATP. The ordinate on the left corresponds to A. In B, the G-6-P concentration was 0.05 mM and the substrate concentration ranged from 0.1 to 8.0 mM. The symbols in B are ○; □, 0.4; △, 2.0 mM ATP. The ordinate on the right corresponds to B.

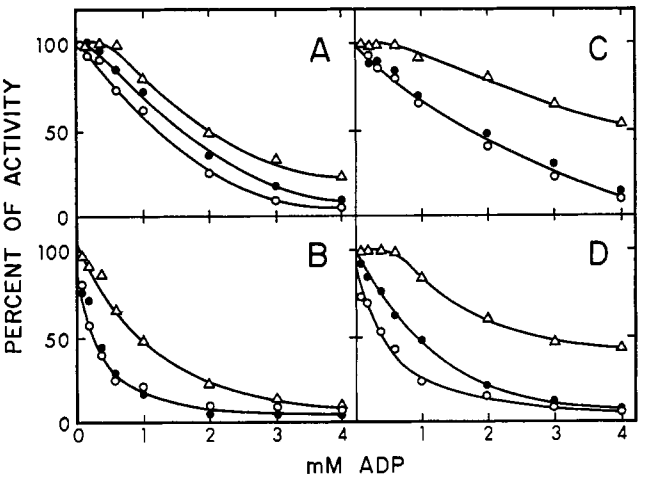

FIGURE 7: The activity of nonactivated, A and B, and *in vitro* activated, C and D, glycogen synthetase *vs.* ADP. The preparation of enzyme and assay conditions are the same as Figure 1. The symbols are (A, nonactivated) ○, 0.5; ●, 1.0; △, 5.0 mM G-6-P. (B, nonactivated) ○, 0; ●, 0.5; △, 5.0 mM G-6-P. (C and D, activated) ○, 0; ●, 0.05; △, 5.0 mM G-6-P. In A and C, the assays were done in the presence of 2.0 mM Mg^{2+}. The per cent of activity is calculated from: $v_i/v_0 \times 100$.

earlier report (Steiner *et al.*, 1965) with a purified liver enzyme preparation, probably representing the activated enzyme of the present study, showed no inhibition by ATP and UTP with similar concentrations of nucleotides as used in this investigation. The lack of nucleotide inhibition of purified, activated enzyme may be a result of the observation that high levels of glycogen in the assay system can reverse ATP inhibition of *in vitro* activated enzyme (Gold and Segal, 1967).

The ATP inhibition of activity of the two enzyme forms is competitive with the substrate, UDP-glucose. The nonlinearity of double-reciprocal plots of velocity *vs.* UDP-glucose suggest the inhibition to be complex and ATP may be competitive with both UDP-glucose and G-6-P.[1] A complex mechanism of inhibition of activity is suggested from the study with ADP as well especially when activity is determined in the presence of a saturating amount of G-6-P where a sigmoid relation of activity *vs.* ADP is observed. With the assay conditions used for these studies, the limiting amount of UDP-glucose (less than saturating) should result in first-order ATP and ADP inhibition kinetics if the nucleotide affinity for UDP-glucose binding site was either greater than or equivalent to the nucleotide affinity for binding of G-6-P. With a limiting G-6-P concentration, nucleotide inhibition is of first-order kinetics and seems to reflect a limitation on enzyme affinity for UDP-glucose in view of the effect of G-6-P on decreasing the K_m' for the substrate. A partially competitive inhibition of the purified glycogen synthetase by UDP has been suggested (Steiner *et al.*, 1965).

Of interest in the present study is the effect of Mg^{2+} on reversal of nucleotide inhibition of activity. In contrast to an earlier report suggesting that Mg^{2+} is required for conversion of nonactive G-6-P-dependent enzyme into G-6-P-independent enzyme (Hizukuri and Larner, 1964), the effects of the cation described in the present study does not appear a result of an enzyme interconversion. The mechanism of Mg^{2+} stimulation of glycogen synthetase activity is not known and it is possible that the reversal of nucleotide inhibition is because of an effect of Mg^{2+} on the increase of synthetase affinity toward G-6-P.[2] ATP chelation of Mg^{2+} does take place at the pH used in this study (Alberty, 1968) and ATP hydrolysis, which occurs in energy utilizing reactions, could result in the release of Mg^{2+}. The *in vitro* conversion into an activated enzyme form, which occurs upon incubation of liver extracts in the absence of added cofactors, results in a decreased affinity toward ATP and ADP. An estimate of the effect of UDP-glucose on nucleotide inhibition of the activity of nonactivated enzyme shows that in the presence of G-6-P and Mg^{2+}, the reversal of inhibition is in part a result of an increase of enzyme affinity for substrate.[3] A comparison of the data of Table II and Figure 5 shows that the twofold change in substrate affinity in the presence of Mg^{2+} (Figure 5) could also account for the tenfold increase in activity at 1.0 mM ATP and 0.5 mM G-6-P (Table II, values of physiological significance).

The results of this investigation show that ADP, like ATP,

[1] A double-reciprocal plot of velocity *vs.* G-6-P from data similar to Figure 2B shows that 0.2 mM ATP increases the K_a' for G-6-P from 1.5 to 5.0 mM without changing V_{max}, as estimated from extrapolated values approaching an infinite G-6-P concentration.

[2] The K_a' for G-6-P, determined in the absence of Mg^{2+}, is 1.0–2.0 mM and is decreased to 0.1 mM in the presence of 2.0 mM Mg^{2+} (Gold, 1968).

[3] In Figure 5, the K_m' for UDP-glucose, at 1.0 mM ATP, is 1.7 mM in the absence of Mg^{2+} and is 0.8 mM in the presence of the cation. The enzyme affinity for substrate, measured in the presence of Mg^{2+}, was determined with a G-6-P concentration one-tenth of that used for the K_m' estimate in the absence of cation since in the presence of Mg^{2+} and absence of ATP, increasing the G-6-P concentration does not greatly increase enzyme activity (Table II).

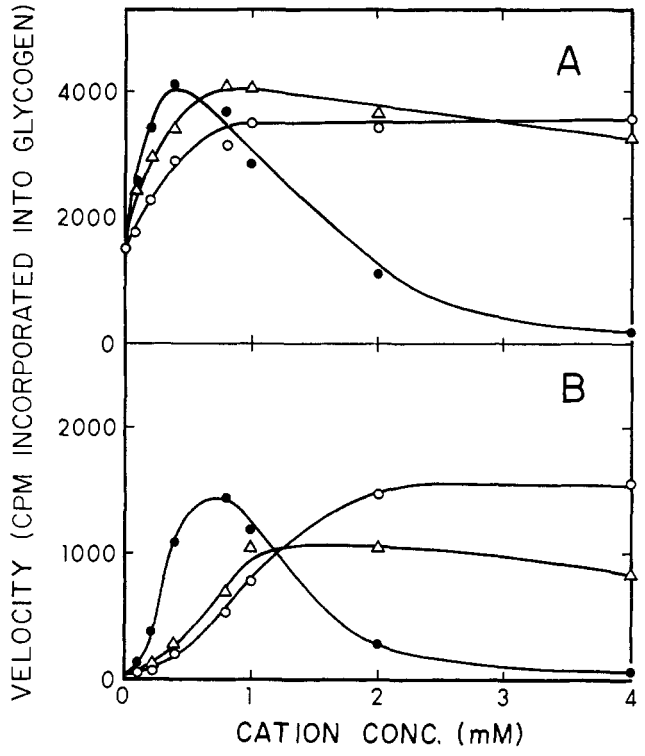

FIGURE 8: The velocity of activated, A, and nonactivated, B, glycogen synthetase *vs.* Mg^{2+}, Mn^{2+}, and Ca^{2+}. The preparation of enzyme and assay conditions are the same as Figure 1. Symbols are ○, $Mg(CH_3COO)_2$; ●, $MnCl_2$; △, $CaCl_2$.

is an effective inhibitor of glycogen synthetase activity. The liver enzyme forms, however, appear less sensitive to ADP inhibition as noted by the higher K_i for ADP as well as higher order of inhibition kinetics when assayed with maximal amounts of activators. Arguments have been presented to suggest that the nonactivated enzyme, as measured, is the form naturally present in liver (Gold and Segal, 1967; Mersmann and Segal, 1967; DeWulf *et al.*, 1968). The analogy between the kinetic changes of the enzyme which occur either by endocrine alteration or *in vitro* activation suggest further that conversion into active enzyme, as observed *in vitro*, accounts for *in vivo* activity changes. In view of the similarity between ATP and ADP inhibition of activity, conversion to active enzyme is most likely the means for the change in glycogen synthetase activity associated with an increased rate of glycogen synthesis. From the results of this investigation, however, it appears possible that the synthetase activity can be regulated by adenine nucleotides either directly by competing with the enzyme for G-6-P or indirectly by limiting the availability of Mg^{2+} which alters enzyme affinity for substrate as well as G-6-P.

The control of glycogen synthetase by adenine nucleotides as inhibitors of activity appears contrary to present views on the relation between cellular energy state and gluconeogenesis or glycolysis (Atkinson, 1965). Glycogen synthetase appears unique, however, in that the enzyme response to insulin as well as hydrocortisone, hormones with opposing actions on liver carbohydrate metabolism, is manifested in both instances by an increased enzyme activity. The insulin sensitivity of the enzyme would suggest that glycogen synthetase, as other liver glucose-utilizing enzymes, is subject to similar metabolite control mechanisms. It is possible that the activity characteristics of liver glycogen synthetase, as suggested from this report, may in part account for observed changes in the *in vivo* rate of glycogen synthesis which occur with various physiologically altered conditions. The increase in the rate of glucose incorporation into liver glycogen, which is independent of a glucose mass action effect (Friedman *et al.*, 1967; DeWulf and Hers, 1967a), could be a result of an increase in glycogen synthetase activity as a result of the utilization of ATP required for rapid phosphorylation of glucose. Similarly, an increase utilization of nucleotides (Hems *et al.*, 1966) due to the activities of energy-requiring gluconeogenic enzymes, pyruvate carboxylase (Scrutton and Utter, 1968) and phosphoenolpyruvate carboxykinase (Foster *et al.*, 1966), could result in an increase in glycogen synthetase activity and subsequent glycogen synthesis *via* the mechanism suggested by this investigation.

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