*AN ON-OFF MECHANISM FOR LIVER GLYCOGEN SYNTHETASE ACTIVITY**

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A recent report from this laboratory described a time-dependent activation of rat liver glycogen synthetase (UDP-glucose:glycogen glucosyltransferase, EC 2.4.1.11) upon incubation of liver extracts *in vitro*.[1] This activation appears to be closely related to the so-called "D"-to-"I" conversion of the enzyme reported by Larner and his co-workers.[2] Subsequent experiments, as described here, have revealed the kinetic basis of the activity increases. The results establish that the effect of glucose 6-phosphate (G-6-P) in the case of both the nonactivated and activated forms of the liver enzyme is on the apparent K_m of UDPG. Activation consists in the conversion to a form with a greater affinity for both UDPG and G-6-P. The two forms exhibit the same extrapolated V_{max} at infinite UDPG concentration at all levels of G-6-P.

In the light of these observations a different significance from that proposed by previous workers[2] attach to the activation, or "D"-to-"I" conversion, of the enzyme *in vivo*. An increase in the rate of the glycogen synthetase reaction under *in vivo* conditions consequent to such a conversion is a result not of the *elimination* of a G-6-P requirement for the enzyme, but rather of the appearance of a form of the enzyme *more* sensitive to G-6-P and, significantly, to P_i as well.

The terminology "D" for G-6-P-dependent and "I" for G-6-P-independent forms of the enzyme[3] is thus not only inappropriate, but actually misleading, both for the reason mentioned above and because, as will be shown, under physiological conditions the activity of neither form varies substantially in response to reasonable changes in G-6-P levels. We propose to refer to the form which is active *in vivo* as the *a* form (formerly "I") and the physiological inactive form as the *b* form (formerly "D"), in rough analogy to the phosphorylase system.

Methods and Materials.—Sources of materials, preparation of the enzyme, enzyme assay, and procedure for enzyme activation were as previously described[1] (see also legend, Fig. 1).

Results.—G-6-P saturation curves for both the activated (*a*) and nonactivated (*b*) forms of the enzyme are shown in Figure 1 at a concentration of UDPG approximating that in starved liver.[4] At this UDPG concentration, half-saturation with G-6-P for the *b* form occurs at a concentration of about 2 mM, while with the *a* form this is reduced to about 0.06 mM. The latter value is in the range of G-6-P concentrations *in vivo*.[4] At these concentrations of UDPG and G-6-P (0.25 mM and 0.06 mM, respectively) the ratio of activity of the two forms is about 30 to 1. Also of note are the shapes of the G-6-P saturation curves. While the *a* form is highly dependent on the modulator concentration, the *b* form is virtually insensitive to the G-6-P concentration in the physiological range of concentrations. However, while this is of mechanistic interest, it will be seen below that in the presence of P_i G-6-P is relatively ineffective with the *a* form of the enzyme as well.

Figure 2 shows the effect of P_i on the enzyme. With synthetase *a* a maximum

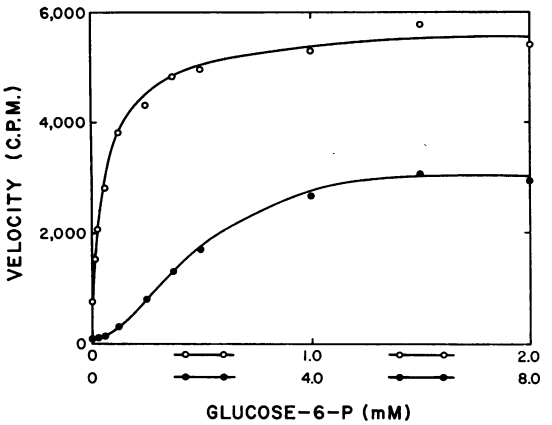

FIG. 1.—Effect of G-6-P concentration on the activity of activated (*a* form) and nonactivated (*b* form) glycogen synthetase. Assays were carried out for 2 min at 37° with 0.2 ml of an 8,500 × *g* extract of liver homogenate in a final volume of 4 ml containing 1 μmole of UDPG (40,000 cpm), 20 mg of rabbit liver glycogen, 280 μmoles of glycyl glycine buffer, pH 7.4, and 4 μmoles EDTA. G-6-P was added as indicated. The reaction was stopped with KOH and the glycogen isolated and counted. The liver was homogenized in 3 vol of 0.01 *M* glycyl glycine buffer, pH 7.4. Activation of the enzyme was effected by incubating the extract at 20° for 50 min. The upper abscissa scale (0 to 2.0 mM) applies to the activated enzyme (*open circles*), and the lower abscissa scale (0 to 8 mM) applies to the nonactivated enzyme (*filled circles*). A Hill plot of the data with the nonactivated enzyme gave a value of *n* of 1.62.

rate about two thirds of that produced by optimal G-6-P was consistently observed from preparation to preparation, as was the inhibition at high P_i levels. The possibility that P_i stimulation was due to the formation of G-6-P via phosphorylase and phosphoglucomutase action was ruled out by G-6-P assays before and after incubation.

Since both G-6-P and P_i were added as K salts, the effect of KCl was tested and found to produce only a small stimulation at a concentration of 50 mM.

The interrelationship of G-6-P and P_i for the *a* form of the enzyme is shown in Figure 3. In the presence of 2.5 mM P_i there was little additional stimulation with G-6-P, and at 10 mM and 25 mM P_i, G-6-P was virtually without effect. It may also be observed that whereas P_i augmented the activity at low G-6-P concentrations, it was somewhat inhibitory at high G-6-P concentrations.

A comparison of the activities of synthetase *a* and *b* at physiological concentrations of UDPG, G-6-P and P_i gave a ratio of activities of about 15 to 1 under these circumstances (Table 1). It is of interest that under these conditions, which simulate those in the liver, essentially the same degree of activation is produced by G-6-P or P_i, or both together. It would appear that very little modulation could

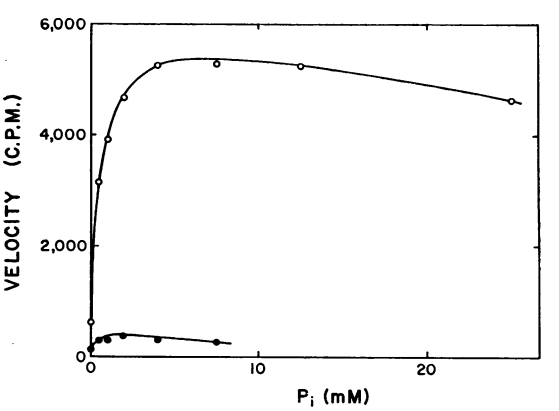

FIG. 2.—Effect of Pi concentration on the activity of glycogen synthetase *a* and *b*. Conditions as in Fig. 1 except Pi was added as indicated. *Open circles, a* form; *filled circles, b* form. The activity of synthetase *a* and *b* in the presence of 1 mM glucose-6-P was 7665 cpm and 2480 cpm, respectively, with this preparation.

TABLE 1

STIMULATION OF GLYCOGEN SYNTHETASE ACTIVITY BY G-6-P AND P_i AT PHYSIOLOGICAL LEVELS OF SUBSTRATE AND MODULATOR

Enzyme preparation	UDPG (mM)	G-6-P (mM)	P_i (mM)	Rate (cpm)
Nonactivated (*b* form)	0.25	0	0	110
	0.25	0.05	0	135
	0.25	0	3.0	300
	0.25	0.05	3.0	357
	0.50	0	0	129
	0.50	0.25	0	387
	0.50	0	3.0	384
	0.50	0.25	3.0	480
Activated (*a* form)	0.25	0	0	1,896
	0.25	0.05	0	4,206
	0.25	0	3.0	4,398
	0.25	0.05	3.0	4,950
	0.50	0	0	3,912
	0.50	0.25	0	8,448
	0.50	0	3.0	6,798
	0.50	0.25	3.0	8,190

Assay and activation conditions were as in Fig. 1; 0.25 mM UDPG and 0.05 mM G-6-P are reasonable approximations of the respective levels in starved liver;[4] 0.5 mM UDPG and 0.25 mM G-6-P are corresponding values in fed liver (H. Burch and O. Lowry, personal communication); 3 mM P_i is a value recently reported by Schulz *et al.*[5]

be effected by reasonable changes in the G-6-P or P_i concentrations. Rather, the system has the characteristics of an on-off switch, with perhaps some leakage in the off position (the leakage through the *b* enzyme appears to be still further reduced by virtue of the strong inhibition of this form by ATP in the presence of G-6-P[1, 6]).

UDPG saturation curves at several G-6-P levels are shown in Figure 4 for the *a* form of the enzyme and in Figure 5 for the *b* form. Apparent K_m values for UDPG are tabulated in Table 2. Several features are to be noted. For both forms of the enzyme the apparent K_m of UDPG decreases with increasing G-6-P to a minimum value of about 0.2 mM for synthetase *a* and about 0.9 mM for synthetase *b* (see also Fig. 6 and *Appendix*). In reflection of the cooperative (sigmoid) kinetics at 0 G-6-P the double reciprocal plots are curved with both forms of the enzyme. Some curvature is still present at 0.063 mM G-6-P with the *a* form at low concentrations of UDPG (not shown), but at G-6-P concentrations of 1 mM and higher the plots are linear at all concentrations of UDPG employed. With the *b* form of the enzyme there is an upward curvature at 1 mM G-6-P, but not at 2 mM or higher,

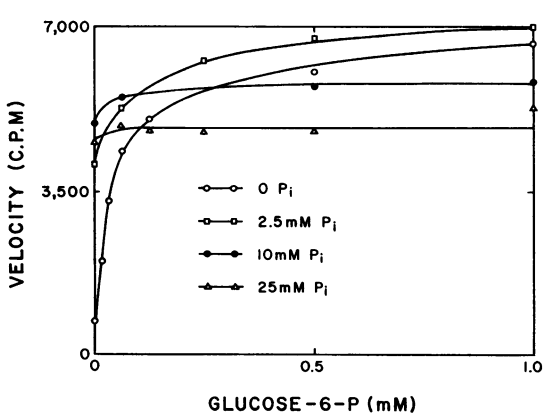

FIG. 3.—Effect of Pi on stimulation by G-6-P. Conditions as in Fig. 1 with G-6-P and Pi added as indicated.

TABLE 2

APPARENT MICHAELIS CONSTANTS (K'_m) OF UDPG AT VARIOUS G-6-P CONCENTRATIONS

G-6-P (mM)	K'_m (mM) Nonactivated enzyme (*b* form)	K'_m (mM) Activated enzyme (*a* form)
0	~16,* ~32*	~2.2,* ~1.5*
0.063		0.83, 0.74
0.5	~4*	
1.0	~2,* ~2*	0.33
2.0	1.1	0.29
4.0	0.79, 1.0, 1.1	0.25, 0.19
8.0	1.1	
12.0	0.69	

Assay and activation conditions were as in Fig. 1.
* Double reciprocal plots were curvilinear, and these values are therefore merely the approximate substrate concentrations for half-maximum velocity.

in the range of UDPG concentrations studied. It should be pointed out that, in cases of cooperative interaction, as the concentration of ligand is reduced sufficiently there is an inflection in the double reciprocal plot, and the slope decreases again (i.e., the double reciprocal plot of a sigmoid curve is itself sigmoid). Thus, if all the velocity measurements are obtained at substrate levels well below the concentra-

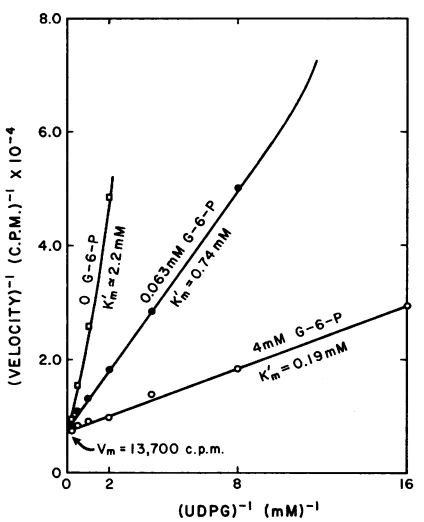

FIG. 4.—Double reciprocal plot of velocity of synthetase *a* vs. UDPG concentration. Assay conditions were as in Fig. 1 except that the UDPG concentration was varied. Separate blanks (KOH before enzyme) were run with each substrate concentration. Concentrations of G-6-P employed and corresponding apparent Michaelis constants (K'_m) are shown. The line at 0 G-6-P is curvilinear and the K'_m value given is merely the approximate UDPG concentration for half-maximum velocity. The line at 0.063 mM G-6-P has an upward curvature at UDPG concentrations below 0.125 mM (points off scale).

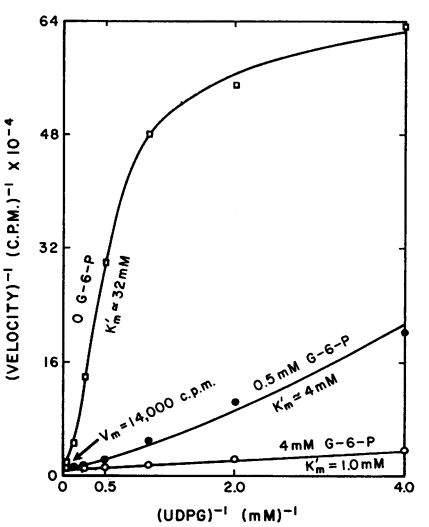

FIG. 5.—Double reciprocal plot of velocity of synthetase *b* vs. UDPG concentration. Conditions as in Fig. 4. The lines at 0 G-6-P and 0.5 mM G-6-P are curvilinear and the corresponding K'_m values given are merely the approximate UDPG concentrations for half-maximum velocity.

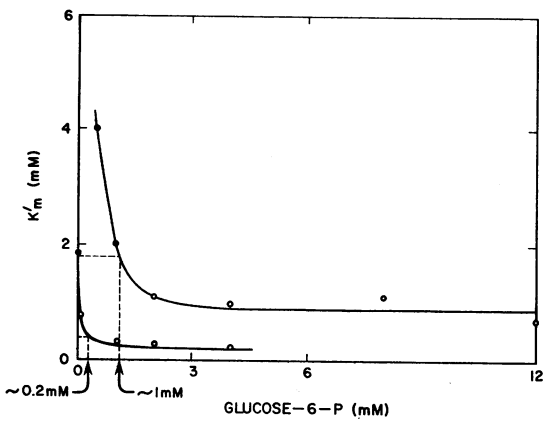

FIG. 6.—Apparent Michaelis constant (K'_m) of UDPG as a function of G-6-P concentration. *Upper curve*, synthetase *b*; *lower curve*, synthetase *a*. Filled circles represent points in which the double reciprocal plots were curvilinear and are therefore merely approximate substrate concentrations for half maximum velocity.

tion for half-maximum velocity, there is a danger of extrapolating the points to a V_{max} value which is far too low.

Discussion.—Metabolic control: From the data presented here and previously,[1] it is a reasonable conclusion that the *b* form of hepatic glycogen synthetase is virtually inactive in the cell. Neither G-6-P nor P_i at levels which may be assumed to be present in the liver elicits significant activity with this form. The activity of synthetase *a* is highly responsive to changes in the G-6-P level around the physiological range in simple cell-free model systems, as is also the case with P_i as demonstrated here.[6a] However, in the presence of both G-6-P and P_i, the enzyme is relatively insensitive to changes in either. Thus the *a* form is a species which functions at one-half to full capacity under all conditions likely to exist *in vivo*. Therefore, the conclusion which has been drawn that synthetase activity in the liver may vary significantly in response to presumed fluctuations in the G-6-P level[8, 9] appears to be incorrect.

The system thus has the characteristics of an on-off process, which on theoretical grounds[10] ought to exist for many, if not all, metabolic pathways. What controls the switch in this case is still not clear, although Larner and his co-workers[2] have made significant contributions to the problem.

As extracted from the liver of normal, fed animals, the enzyme appears to be entirely in the *b*, or physiologically inactive form. On the other hand, Bishop and Larner have reported[11] that a few minutes after insulin administration to normal dogs, a substantial portion of the enzyme has been converted to the *a*, or active, form, and that this is reversed by glucagon. Steiner and King[12] have made similar observations of insulin effects in diabetic rats over a longer time period. Since an elevation in blood glucose brings about an increase in circulating insulin, there appears to be a mechanism for switching on synthetase activity in response to a need to convert precursors to glycogen, and for switching it off again when the influx of nutrients ceases and the net flow of carbohydrate metabolism is in the direction of glycogen utilization.

Kinetics: Larner's method of assay for the two forms of the synthetase involves activity measurements in the presence and absence of G-6-P, and it has been emphasized (e.g., ref. 11) that if meaningful values are to be obtained, relatively high concentrations of UDPG and G-6-P are required. There is a modification which

the present results suggest. Larner refers to the activity obtained in the presence of 5 mM UDPG as the "I" activity, and with 7 mM G-6-P added as the "D"-plus-"I", or total activity. In fact these assays measure neither form uniquely. From the kinetic data at hand (Table 2 and Figs. 4 and 5), the "I" value is seen to be composed of about two thirds of the total *a* activity plus about 20 per cent of the total *b* activity, and the "D"-plus-"I" value consists of almost all the *a* activity plus about 85 per cent of the total *b* activity. Larner's method of assay thus gives only a rough estimate of the two forms. It can be improved by taking the "no G-6-P" assay as representing two thirds of the total *a* form, and the difference between the assays with G-6-P minus the corrected value without G-6-P as representing two thirds of the total *b* form. That is,

total synthetase a = $^3/_2 \times$ "no G-6-P" assay,

total synthetase b = $^3/_2 \times$ ("with G-6-P" assay − total synthetase a).

Without these corrections a conversion of synthetase *b* to synthetase *a* may appear to increase the total activity, since full activity of the *a* form but not the *b* form is obtained in the assay with G-6-P (this is particularly true with the dog[11] enzyme, where the K_m of UDPG for the *b* form is even greater than with the rat enzyme). Thus claims of increased levels of total enzyme under certain conditions[12, 13] are probably incorrect, as also pointed out by Bishop and Larner.[11]

Leaving aside the cooperative interactions, the kinetics of the system suggest a simple mechanism of G-6-P activation peviously described,[14] in which the activator affects only the substrate binding. The rate equation for this system in the double reciprocal form is

$$\frac{1}{v_0} = \frac{1}{V_{\max}} + \frac{K_{ea}}{V_{\max}}\left[\frac{K_a + (A_0)}{K_{es} + (A_0)}\right] \cdot \frac{1}{(S_0)}, \tag{1}$$

where v_0 = initial velocity, $V_{\max}$ = velocity at infinite (S_0), (S_0) = initial substrate concentration, (A_0) = initial activator concentration, K_{ea} = dissociation constant of substrate from ternary complex, K_{es} = dissociation constant of activator from ternary complex, and K_a = dissociation constant of enzyme-activator complex. In addition, there exists a dissociation constant of the enzyme-substrate complex, K_s, and

$$K_s = \frac{K_{ea} \cdot K_a}{K_{es}}. \tag{2}$$

The equations for the limiting cases at $(A_0) = 0$ and $(A_0) = \infty$, respectively, are therefore

$$\frac{1}{v_0} = \frac{1}{V_{\max}} + \frac{K_s}{V_{\max}} \cdot \frac{1}{(S_0)} \tag{3}$$

and

$$\frac{1}{v_0} = \frac{1}{V_{\max}} + \frac{K_{ea}}{V_{\max}} \cdot \frac{1}{(S_0)}. \tag{4}$$

Thus, in principle, the dissociation constant of the enzyme-substrate complex,

K_s, can be obtained from equation (3), and the dissociation constant of the substrate from the ternary complex, K_{ea}, can be obtained from equation (4).

The reciprocals of v_0 and (A_0) at constant (S_0) are not in a linear relationship to one another (Eq. (1)); however, a double reciprocal plot of $(v_0 - v_0')$ versus (A_0) is linear, where v_0' is the velocity at $(A_0) = 0$. This plot could be utilized to obtain K_{es}, once K_s and K_{ea} are known, and K_a could then be obtained from equation (2).

However, the cooperative kinetics at zero or low activator concentration rule out an accurate estimate of K_s with the data at hand. On the other hand, reasonable values for K_{ea} can be obtained from the data at high activator concentration in Table 2. These are 0.9 mM for synthetase *b* and 0.2 mM for synthetase *a*. The K_s values are approximately tenfold higher. An estimate of K_a can be made from the data in Table 2 of about 1 mM for synthetase *b* and 0.2 mM for synthetase *a* (see *Appendix*). From equation (2) the values for K_{es} would be an order of magnitude lower.

What is to be emphasized is the conclusion that the basic distinction between the two forms of glycogen synthetase is the approximate fivefold greater intrinsic affinity of the *a* form for both UDPG and G-6-P (the affinity for glycogen in the presence of G-6-P is also greater[1]). Since the apparent affinity of UDPG depends upon the G-6-P concentration, the decrease in the respective dissociation constants produces a cumulative effect on the activity at the low concentrations of UDPG and G-6-P which prevail in the liver.

Summary.—Two forms of rat liver glycogen synthetase exist which differ in their affinity for UDPG and G-6-P. The form with greater affinity for the ligands is referred to as the *a* form and the other the *b* form. The *a* form is activated by P_i as well as G-6-P. Under physiological conditions it appears that the *b* form is virtually inactive while the *a* form functions at one-half to full capacity at all concentrations of ligands likely to exist in the liver. The system thus has the characteristics of an on-off switch for glycogen synthetase activity.

Appendix.—From equation (1)

$$K_m' = K_{ea}\left[\frac{K_a + (A_0)}{K_{es} + (A_0)}\right],$$

where K_m' is the apparent Michaelis constant for UDPG at corresponding (A_0). The other constants have been defined. Let $(A_0)'$ be the activator concentration at which K_m' is twice K_{ea} (the minimum value of K_m' at infinite (A_0)).

Then,

$$2K_{ea} = K_{ea}\left[\frac{K_a + (A_0)'}{K_{es} + (A_0)'}\right],$$

$$(A_0)' = K_a - 2K_{es}.$$

Since K_{es} is an order of magnitude less than K_a, it can be neglected.

Then, K_a is approximately equal to the activator concentration for double the minimum K_m'.

When the data in Table 2 are plotted as K_m' versus (A_0) (Fig. 6), approximate values of K_a can be read off the graphs.

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[6a] Vardanis has recently reported that P_i is an activator of mouse liver glycogen synthetase.[7] It is not clear whether his preparation consists of the *a* or *b* form of the enzyme, although by analogy with the P_i response of the rat enzyme it is presumably the *a* form. No comparison of the kinetics can be made since all the experiments were performed with enzyme that was substantially or totally inactivated during the assay period.

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