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# SPECIFICITY OF ACTIVATION OF GLYCOGEN SYNTHASE *I* FROM SKELETAL MUSCLE AND HEART*

J. A. THOMAS, K. K. SCHLENDER AND J. LARNER

*Department of Biochemistry and Biophysics, Iowa State University, Ames, Iowa; Department of Pharmacology and Therapeutics, Medical College of Ohio, Toledo, Ohio; and Department of Pharmacology, University of Virginia School of Medicine, Charlottesville, Va. (U.S.A.)*

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## SUMMARY

The activation specificity for rabbit skeletal muscle and bovine heart glycogen synthase *I* (UDPglucose:glycogen $\alpha$-4-glucosyltransferase, EC 2.4.1.11) has been investigated. It is shown that both enzymes may be fully activated by divalent inorganic anions and several organic phosphate compounds. The activation constant was lowest for glucose 6-phosphate (5 $\mu$M), but millimolar concentrations of several other compounds could also completely activate the enzyme. $Na_2SO_4$ (concentration required to give 50% activation = 0.2 mM) is shown to activate glycogen synthase *I* with no effect on the *D* form of the enzyme. Assays in the presence of millimolar concentrations of $Na_2SO_4$, 5 mM UDPglucose, and pH 7.8 are proposed as a measure of the amount of glycogen synthase *I* in any enzyme preparation.

## INTRODUCTION

In recent years, two forms (*I* and *D*) of UDPglucose:glycogen $\alpha$-4-glucosyltransferase, EC 2.4.1.11 (glycogen synthase) have been described from several mammalian sources. These enzyme forms are interconverted by phosphorylation and dephosphorylation, and each form is activated and inhibited by several metabolites. The specificity of rabbit skeletal muscle glycogen synthase *D* for activators was examined in some detail and it was shown to be very specific for glucose 6-phosphate (ref. 1). The enzyme from other sources has also been shown to be activated by glucose-6-*P*[2–4] and inhibited by ATP[5–8]. However, little information is available on the specificity of glycogen synthase *I*. In some instances, this enzyme appears to be activated by glucose 6-phosphate, sulfate, or inorganic phosphate[8–10].

This paper describes the activation specificity of purified glycogen synthase *I* from both skeletal muscle and heart. This work provides the basis for a comparison of the *I* and *D* forms of the enzyme and permits some conclusions on the effect of

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phosphorylation of glycogen synthase on the enzyme site responsible for binding low molecular weight activators.

## METHODS

### *Enzyme preparations*

Glycogen synthase *I* from bovine heart was prepared as described in the preceding report[11]. The same enzyme was prepared from rabbit skeletal muscle by the method of Schlender and Larner[12]. Heart glycogen synthase *D* was prepared by methods similar to those used for synthase *I* except that the enzyme was not converted to the *I* form during preparation. Since the original heart tissue contained very little synthase *I*, the resulting synthase *D* preparation contained less than 7% glycogen synthase *I*.

A partially purified preparation of heart glycogen synthase was prepared by centrifugation (30 000 rev./min; 3 h) of a crude heart extract that was made as described in the preceding report[11]. The pellets obtained were resuspended in one-tenth the original extract volume with a hand homogenizer (50 mM Tris, 5 mM EDTA, 5 mM dithiothreitol; pH 7.8). The suspension was centrifuged at 12 000 rev./min for 1 h to remove insoluble material and the supernatant was used for enzyme assays.

### *Enzyme assay*

Assays were performed as described in the preceding report[11].

## RESULTS

### *Activation of purified glycogen synthase I*

In the course of purification of glycogen synthase *I*, it was found that the percentage *I* activity in the preparation decreased as much as 2-fold after eluting the enzyme from the DEAE-cellulose column. It seemed possible that some activator was removed from the enzyme preparation by the column chromatography step. Subsequently, it was shown that the activity could be completely restored by assaying the enzyme in the presence of an anion such as $SO_4^{2-}$.

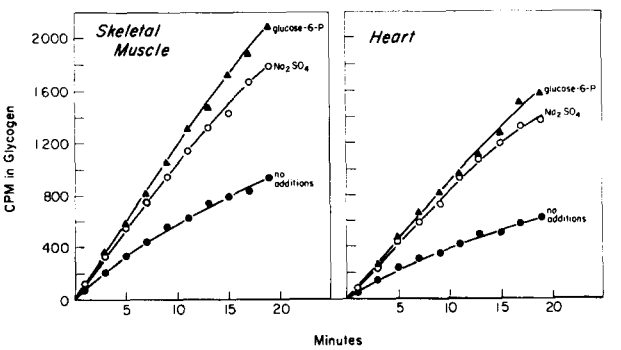


Fig. 1. The effect of activators on the time course of the reaction. Glycogen synthase *I* was assayed at 30 °C in a solution containing 50 mM Tris (pH 7.8) ,5 mM EDTA, 10 mg/ml rabbit liver glycogen, and 5 mM UDP[$^{14}$C]glucose ($4 \cdot 10^5$ cpm/$\mu$mole), and aliquots of 50 $\mu$l were removed at the times indicated. The assay mixtures contained (●—●) no additions, (○—○) 10 mM $Na_2SO_4$ or (▲—▲) 6.7 mM glucose-6-*P*.

Fig. 1 shows the effect of both $Na_2SO_4$ and glucose-6-*P* in an assay of the purified synthase *I*. The activity of both skeletal muscle and heart enzymes is nearly the same with either glucose-6-*P* or $Na_2SO_4$ present, while the reaction rate is reduced to approximately one-half with no addition. Since it was reported that skeletal muscle glycogen synthase *D* was not activated by the concentration of $Na_2SO_4$ used here[1], we concluded that the progress curves shown were produced by "non-activated" glycogen synthase *I* (no additions) and "activated" glycogen synthase *I* (either $Na_2SO_4$ or glucose-6-*P* added).

TABLE I

SPECIFICITY OF INORGANIC ANION ACTIVATION

| *Addition (2 mM)* | *Enzyme activity** | | | |
|---|---|---|---|---|
| | *Skeletal muscle* | | *Heart* | |
| | *+ glucose-6-P*** | *I/total* | *+ glucose-6-P*** | *I/total* |
| Water | 17 | 0.48 | 21 | 0.42 |
| Sodium cacodylate | 18 | 0.54 | 20 | 0.46 |
| $NaNO_3$ | 17 | 0.54 | 22 | 0.41 |
| NaBr | 17 | 0.53 | 20 | 0.44 |
| NaF | 18 | 0.53 | 20 | 0.42 |
| $NaHCO_3$ | 18 | 0.48 | 22 | 0.41 |
| $Na_4P_2O_7$ | 17 | 0.87 | 22 | 0.85 |
| $Na_2SO_4$ | 17 | 0.83 | 21 | 0.84 |
| $Na_2SO_3$ | 18 | 0.81 | 22 | 0.81 |
| $Na_2HPO_4$ | 17 | 0.77 | 20 | 0.80 |
| $Na_2HAsO_4$ | 17 | 0.76 | 21 | 0.72 |

* nmoles glucose incorporated into glycogen in 10 min.
** Enzyme assayed with 6.7 mM glucose-6-*P*.

TABLE II

SPECIFICITY OF ORGANIC ANION ACTIVATION

| *Addition (2 mM)* | *Enzyme activity** | | | |
|---|---|---|---|---|
| | *Skeletal muscle* | | *Heart* | |
| | *+ glucose-6-P*** | *I/total* | *+ glucose-6-P*** | *I/total* |
| Water | 15 | 0.54 | 18 | 0.41 |
| Sodium formate | 16 | 0.56 | 19 | 0.44 |
| Sodium acetate | 18 | 0.55 | 18 | 0.50 |
| Trisodium citrate | 16 | 0.58 | 19 | 0.42 |
| Phosphoenolpyruvate | 16 | 0.71 | 19 | 0.63 |
| Glucose-1-*P* | 16 | 0.68 | 19 | 0.65 |
| α-Glycerophosphate | 17 | 0.72 | 18 | 0.72 |
| Fructose-1,6-$P_2$ | 16 | 0.82 | 18 | 0.81 |
| Cyclic 3′:5′-AMP | 17 | 0.56 | 19 | 0.50 |
| ATP | 15 | 0.61 | 18 | 0.52 |
| ADP | 16 | 0.68 | 18 | 0.60 |
| AMP | 16 | 0.63 | 18 | 0.52 |
| UTP | 14 | 0.44 | 15 | 0.36 |
| UDP | 11 | 0.14 | 11 | 0.10 |
| UMP | 14 | 0.35 | 15 | 0.32 |

* nmoles glucose incorporated into glycogen in 10 min.
** Enzyme assayed with 6.7 mM glucose-6-*P*.

The specificity of activation of the purified glycogen synthase *I* is shown in Tables I and II. Of the inorganic compounds tested (Table I), divalent anions and pyrophosphate activated the enzymes. It has been reported that $P_i$ inhibits at acid pH[5]. Several organic compounds were also effective (Table II) but the specificity was somewhat less obvious. Compounds containing a divalent phosphate moiety (with the exception of uridine nucleotides) were activators. All uridine nucleotides were strongly inhibitory in the absence of glucose-6-*P* and somewhat less inhibitory with glucose-6-*P*. Glucose 6-sulfate (not shown) was completely without effect on these enzymes.

TABLE III

ACTIVATION CONSTANTS FOR GLYCOGEN SYNTHASE *I*

| *Activator* | $A_{\frac{1}{2}}$* *(mM)* | |
|---|---|---|
| | *Heart* | *Skeletal muscle* |
| Glucose-6-*P* | 0.005 | 0.005 |
| Glucosamine-6-*P* | 0.02 | 0.02 |
| Ribose-5-*P* | 0.03 | 0.03 |
| Fructose-1,6-$P_2$ | 0.2 | 0.2 |
| $Na_2SO_4$ | 0.2 | 0.2 |
| Gluconic acid-6-*P* | 0.3 | 0.3 |
| $Na_2HPO_4$ | 0.4 | 0.4 |
| α-Glycerophosphate | 1.5 | 1.5 |
| Glucose-1-*P* | 1.1 | 1.1 |

* Concentration of activator required to give half-maximum activation when assayed at 5 mM UDPglucose.

Table III shows the half-maximum activation constants ($A\frac{1}{2}$) for several of the compounds used in Tables I and II. These data indicate that glycogen synthase *I* may be activated by low concentrations of glucose-6-*P*, and several other organic phosphate compounds. In addition, both sulfate and phosphate were effective at quite low concentrations. Thus, the specificity of both skeletal muscle and heart gly-

TABLE IV

ENZYME ACTIVITY WITH SATURATING ACTIVATORS

α-Glycerophosphate and glucose-1-*P* were used at ten times $A\frac{1}{2}$. All other activators were more than 25 times $A\frac{1}{2}$.

| | *Activity** | |
|---|---|---|
| | *Heart* | *Skeletal muscle* |
| Glucose-6-*P* | 1.38 | 1.10 |
| Glucosamine-6-*P* | 1.28 | 1.16 |
| Ribose-5-*P* | 1.14 | 1.04 |
| Fructose-1,6-$P_2$ | 1.24 | 0.94 |
| $Na_2SO_4$ | 1.23 | 1.03 |
| Gluconic acid-6-*P* | 1.26 | 1.09 |
| $Na_2HPO_4$ | 1.10 | 1.03 |
| α-Glycerophosphate | 1.15 | 1.01 |
| Glucose-1-*P* | 1.12 | 1.08 |

* nmoles glucose incorporated into glycogen in 10 min.

cogen synthase *I* for activators was quite broad when contrasted to that of skeletal muscle glycogen synthase *D*[1]. Table IV shows that the maximum activity of these enzymes was virtually constant in the presence of saturating concentrations of any of these activators. Thus, all compounds tested, containing phosphate or some other divalent anion, were capable of completely activating the enzyme.

The interaction between various activators was then explored. Table V shows the effects of combinations of $Na_2SO_4$, and glucose-6-*P* on the activity of heart glycogen synthase *I*. When the activators were added at non-saturating concentrations, enzyme activity was greater with both activators than with one alone. However, at saturating concentrations, addition of a second activator was slightly inhibitory when compared to the activity with glucose-6-*P*. If the activators were acting independently on the enzyme, saturating concentrations would also produce additive effects.

TABLE V

EFFECT OF ACTIVATOR COMBINATIONS ON ENZYME ACTIVITY

| *Addition* | | | | *Increase in activity** | |
|---|---|---|---|---|---|
| $Na_2SO_4$ | | *Glucose-6-P* | | *5 mM UDPglucose* | *0.2 mM UDPglucose* |
| *0.2 mM* | *10 mM* | *5.0 μM* | *250 μM* | | |
| + | | − | | 1.8 | 0.28 |
| − | | + | | 1.8 | 0.35 |
| + | | + | | 2.8 | 0.55 |
| | + | | − | 4.9 | 1.03 |
| | − | | + | 4.7 | 1.85 |
| | + | | + | 4.0 | 1.51 |

* nmoles glucose incorporated into glycogen in 10 min. In each case the activity obtained with no activator was subtracted from the activity obtained with an activator. Without an activator the activity at 5 mM UDPglucose was 5.8 and at 0.2 mM UDPglucose it was 0.55.

*Effects of anions on the pH optimum*

Since anion activation of an enzyme may produce a pH shift of maximum enzyme activity, the effect of pH on the activity of glycogen synthase *I* was examined in the presence and absence of activators (Fig. 2). Both enzymes have a pH optimum of approximately 7.0 when assayed in the absence of activators. Either $Na_2SO_4$ or glucose-6-*P* produced an alkaline shift of the pH optimum to approximately 7.9. The enzyme was found to be stable under the assay conditions over the pH range from 6.0 to 9.5.

The pH studies in Fig. 2 were carried out in Tris–maleate buffer. To establish that the buffer itself was not inhibitory at a pH greater than 7.0 in the absence of activators, the enzyme was assayed in five other buffers at pH 7.7. Enzyme activity was the same in 50 mM Tris–5 mM EDTA, 50 mM glycylglycine, 50 mM imidazole, 50 mM succinate–50 mM cacodylate, and 50 mM bicine.

It should be noted here that these pH optimum curves are similar to those that were originally observed for preparations of glycogen synthase[13,14] containing both the *I* and *D* forms. Since the present data were obtained from enzyme preparations that were relatively free of glycogen synthase *D*, the observed pH shifts must be attributed to only the *I* form of this enzyme.

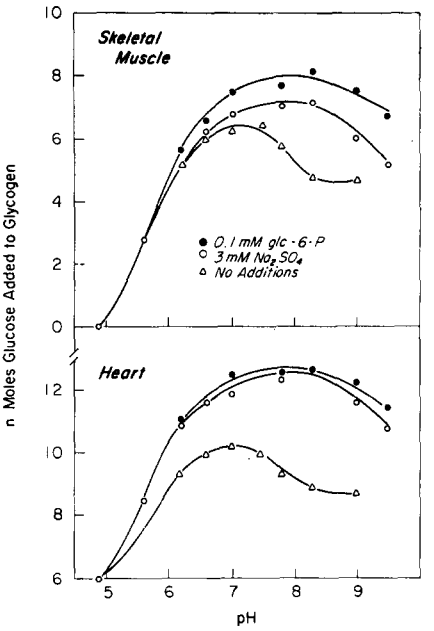


Fig. 2. pH–activity curves for glycogen synthase *I*. The effect of pH was determined in 50 mM Tris–50 mM maleate buffer adjusted to the pH values shown. UDPglucose was present at 5 mM and the activity was measured in the presence of (△—△) no additions, (○—○) 3 mM $Na_2SO_4$, and (●—●) 0.1 mM glucose-6-*P*.

*Comparison of $Na_2SO_4$ activation of glycogen synthase I and D*

Since many enzyme assays are run with crude enzyme preparations, the effects of sulfate and glucose-6-*P* were examined with such an enzyme preparation. Extracts of hearts were prepared, and small molecules were removed by gel filtration on Sephadex G-50. These extracts were diluted to give low glycogen synthase activities and assays were run for 1 h. The glycogen synthase in the extracts was stable to

TABLE VI

ACTIVATION BY $Na_2SO_4$ AND GLUCOSE-6-*P*

The activity of purified enzymes was measured by routine 10-min assays. Crude enzyme fractions were passed over Sephadex G-50 columns, and diluted 6-fold more than the routine procedure (enzyme concentration about 10 munits/ml). Assays were then incubated 60 min.

| *Enzyme source* | *Activity** | | | *Fold-increase by activator* | |
|---|---|---|---|---|---|
| | *No addition* | *28 mM $Na_2SO_4$* | *20 mM Glucose-6-P* | *$Na_2SO_4$* | *Glucose-6-P* |
| Extract** of rat heart | 5.4 | 8.7 | 9.6 | 1.6 | 1.8 |
| | 5.4 | 8.2 | 11.2 | 1.5 | 2.1 |
| | 2.3 | 4.0 | 9.7 | 1.7 | 4.2 |
| | 2.2 | 4.2 | 11.8 | 1.9 | 5.4 |
| Extract** of beef heart | 1.4 | 2.2 | 11.4 | 1.6 | 8.1 |
| Extract** of rabbit muscle | 0.87 | 1.2 | 1.7 | 1.4 | 2.0 |
| Purified beef heart synthase *I* | 12.3 | 18.8 | 19.2 | 1.5 | 1.6 |
| Purified rabbit muscle synthase *I* | 6.0 | 7.8 | 8.0 | 1.3 | 1.3 |

* nmoles glucose incorporated into glycogen in assay.
** Crude extracts were prepared as described in Methods.

these conditions, and any effects of endogenous activators or inhibitors on the assay of glycogen synthase without activators was eliminated.

The results in Table VI show that extracts were all stimulated by both $Na_2SO_4$ and glucose-6-*P*. However, the effect of glucose-6-*P* varied substantially from preparation to preparation, indicating a great variation in the relative amounts of glycogen synthase *I* and *D* in the extracts. The effect of $Na_2SO_4$ was relatively constant, *i.e.* between 1.50- and 1.92-fold. Purified heart glycogen synthase *I* gave a similar response to $Na_2SO_4$. Both crude and purified rabbit muscle enzyme were stimulated approximately 1.4-fold by $Na_2SO_4$.

TABLE VII

EFFECT OF $Na_2SO_4$ ON PURIFIED HEART GLYCOGEN SYNTHASE *D*

| *Addition* | *Activity** | *Fold-increase by activator* |
|---|---|---|
| — | 0.09 | — |
| 25 mM $Na_2SO_4$ | 0.27 | 3.0 |
| 145 mM $Na_2SO_4$ | 0.22 | 2.5 |
| 10 mM glucose-6-*P* | 3.4 | 38 |

* nmoles glucose incorporated into glycogen in 10 min.

The preceding data indicated that $Na_2SO_4$ had little effect on synthase *D* under our assay conditions. Therefore, a purified heart glycogen synthase *D* was prepared and the effect of $Na_2SO_4$ and glucose-6-*P* was examined (Table VII). In this experiment, $Na_2SO_4$ activated the synthase *D* preparation approximately 3-fold and glucose-6-*P* activated 38-fold. A high concentration of $Na_2SO_4$ (145 mM) produced no additional stimulation. The effect of $Na_2SO_4$ may be attributed to contaminating synthase *I*, but since the activity increase with sulfate was higher than previously seen (Table VI) this is not an entirely adequate explanation. This effect of $Na_2SO_4$ is currently being investigated.

*Method for assay of glycogen synthase I*

Since our results indicate that glycogen synthase *I* is activated by $Na_2SO_4$ and the *D* form of the enzyme isn't, it was thought that assays of this enzyme should be done in the presence of $Na_2SO_4$ to determine the amount of the *I* form in an enzyme preparation and in the presence of glucose-6-*P* to determine the total glycogen synthase present. To test this possibility a partially purified enzyme from bovine heart containing approximately equal amounts of both *I* and *D* forms was prepared. Fig. 3 shows the effect of both glucose-6-*P* and $Na_2SO_4$ on assays that were done with both low (0.2 mM) and high (5.0 mM) UDPglucose. Without $Na_2SO_4$ in these assays, the activity of the preparation was highly dependent on the concentration of glucose-6-*P*. Therefore, assays of enzyme preparations that might be contaminated with glucose-6-*P* (crude or partially purified preparations) would be influenced significantly by the amount of glucose-6-*P* contamination. When assayed with $Na_2SO_4$ present, there was a much smaller effect of glucose-6-*P* on the enzyme activity. At low concentrations of glucose-6-*P* (less than 100 $\mu$M) $Na_2SO_4$ is an activator and above that concentration, the enzyme is inhibited by $Na_2SO_4$. Thus, there is much

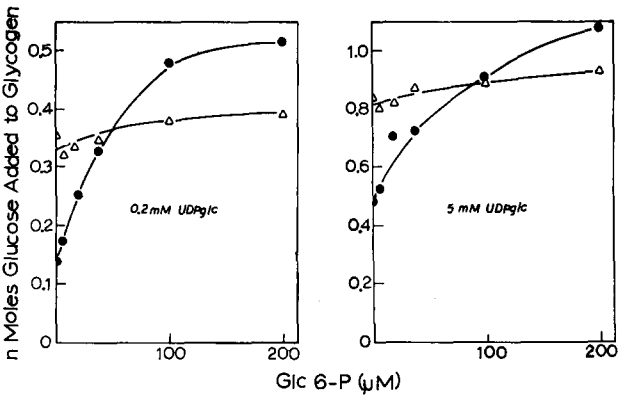


Fig. 3. The effect of $Na_2SO_4$ and glucose-6-*P* on assays of a partially purified heart glycogen synthase preparation. A partially purified enzyme prepared as described under Methods was assayed at 0.2 mM and 5 mM UDPglucose with (△—△) and without (●—●) 14 mM $Na_2SO_4$. The enzyme preparation, assayed with 14 mM $Na_2SO_4$ and 10 mM glucose-6-*P*, gave a ratio of synthase *I*/total synthase of approximately 0.55.

less effect of any contaminating glucose-6-*P* when glycogen synthase *I* is assayed in the presence of $Na_2SO_4$.

It is known that ATP inhibits skeletal muscle glycogen synthase *I* at acid pH and that glucose-6-*P* decreases this inhibition[5]. The data in Table VIII show that $Na_2SO_4$ can also prevent ATP inhibition of purified glycogen synthase *I* when assayed at pH 6.5. ATP was not inhibitory at pH 7.6 or in the presence of $Mg^{2+}$.

TABLE VIII

EFFECT OF ATP AND ACTIVATORS ON GLYCOGEN SYNTHASE *I*

| *pH** | *Addition (2 mM)* | *Enzyme activity*** | | | | | |
|---|---|---|---|---|---|---|---|
| | | *Skeletal muscle* | | | *Heart* | | |
| | | *No addition* | *10 mM $Na_2SO_4$* | *6.7 mM Glucose-6-P* | *No addition* | *10 mM $Na_2SO_4$* | *6.7 mM glucose-6-P* |
| 6.5 | Water | 9.30 | 10.8 | 11.9 | 11.2 | 14.9 | 15.9 |
| | ATP | 6.37 | 10.1 | 12.1 | 8.00 | 14.8 | 15.9 |
| | ATP + $MgCl_2$ | 8.89 | — | — | 9.59 | — | — |
| 7.6 | Water | 7.92 | 12.8 | 13.7 | 8.66 | 16.2 | 17.7 |
| | ATP | 7.92 | 12.9 | 13.8 | 9.13 | 16.1 | 18.0 |
| | ATP + $MgCl_2$ | 8.74 | — | — | 9.27 | — | — |

* Assays were run in 50 mM Tris–maleic acid.
** nmoles glucose added to glycogen in 10 min.

## DISCUSSION

The data reported here in Tables I–V show that glycogen synthase *I* from both bovine heart and rabbit skeletal muscle has a broad specificity for activation. Glucose-6-*P* was effective at the lowest concentration, but any molecule that could be considered as a divalent anion at pH 7.6 was able to completely activate this enzyme

when assayed at 5 mM UDPglucose. However, glucose-6-*P* decreased the $K_m$ for UDPglucose more than other activators[11]. The results are in contrast to the specificity of glycogen synthase *D* from dog skeletal muscle as reported by Rosell-Perez and Larner[1]. Their data show that glycogen synthase *D* was activated by glucose-6-*P* and less efficiently by several other phosphorylated carbohydrates. Sulfate and phosphate were incapable of activating their enzyme. The present paper also shows that heart glycogen synthase *D* is not activated by $Na_2SO_4$ even if added at a concentration of 145 mM (Table VII). Additionally, activation of muscle glycogen synthase *D* by glucose-6-*P* was inhibited by $Na_2SO_4$ or $Na_2HPO_4$[1] whereas, our results show (Table V) that $Na_2SO_4$ and glucose-6-*P* are not antagonistic with respect to glycogen synthase *I* activation.

In assaying glycogen synthase one is usually interested in the molecular ratio of glycogen synthase *I* to *D* in the preparation. In the past an assay at 5 mM UDPglucose with no activator has been used as a measure of the amount of glycogen synthase *I* and an assay with 5–10 mM glucose-6-*P* present as a measure of the total synthase activity. However, it is now well established by these results, and by those of DeWulf *et al.*[8], Mersman and Segal[9], and Piras *et al.*[5], that glycogen synthase *I* must also be activated by any of several compounds, to compare the amount of enzyme to that of glycogen synthase *D* assayed with glucose-6-*P*. Many of the activators of the *I* form are present in tissue extracts and, therefore, may influence assays of glycogen synthase that are performed in the absence of glucose-6-*P*. The most important of these endogenous activators is glucose-6-*P* since the activation constant of glycogen synthase *I* for glucose-6-*P* is very low (Table III) and small amounts may cause partial activation of the *I* form during assay. Therefore, we propose that assays of glycogen synthase with 5 mM UDPG carried out with saturating concentrations of $Na_2SO_4$, more accurately represent the content of glycogen synthase *I*, for the following reasons. First, it is shown in Fig. 3 that the assay of glycogen synthase without activators is less influenced by the concentration of glucose-6-*P* when assayed with 14 mM $Na_2SO_4$. Secondly, since the $Na_2SO_4$ completely activates the glycogen synthase *I* (Table IV), the ratio of the activity obtained with $Na_2SO_4$ to that with glucose-6-*P* more nearly represents the molecular ratio of glycogen synthase *I* to total synthase. Further evidence for this conclusion is the fact that purified glycogen synthase *I* gives a ratio of approximately 0.6–0.7 when assayed with no activator and with glucose-6-*P*, but a ratio of 1.0 when the assay is done with 10 mM $Na_2SO_4$ and with 10 mM glucose-6-*P* (Table VI). Third, it is known that ATP can inhibit glycogen synthase *I* in the absence of any activator (Table V and Piras *et al.*[5]), but $Na_2SO_4$ can overcome this inhibition (Table V). Therefore, the inhibition affect of any ATP in a crude fraction assayed for glycogen synthase, can be alleviated by the use of $Na_2SO_4$ at saturating concentrations. A similar proposal has already been made by DeWulf *et al.*[8].

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