# Allosteric Properties of Yeast Glycogen Synthetase. I. General Kinetic Study*

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ABSTRACT: Yeast glycogen synthetase (uridine diphosphate glucose:glycogen $\alpha$-4-glucosyltransferase, EC 2.4.1.11) is moderately stimulated by glucose 6-phosphate at neutral pH. Addition of certain anions at relatively high concentrations (0.1–0.2 M) inhibits the enzyme, but glucose 6-phosphate reverses the inhibition. Consequently, the relative stimulation by the phosphoric ester is much larger in the presence of inhibitors. Chloride was used as model inhibitor in the following study. Substrate kinetics are Michaelian both without and with added inhibitor. The inhibition is of the mixed or of the competitive type. On the other hand, sigmoid curves are obtained when the reaction rate is represented as a function of glucose 6-phosphate concentration in the presence of chloride, or as a function of chloride concentration in the presence of glucose 6-phosphate. Treatment of the enzyme with fluorodinitrobenzene with uridine diphosphate glucose as protector leads to a total loss of sensitivity against chloride, while the inhibition by uridine diphosphate, a product of the glycogen synthetase reaction, is maintained. It is concluded that chloride and other inhibitors are allosteric, *i.e.*, bind to a site different from that of uridine diphosphate glucose. The direct activating effect of glucose 6-phosphate is also obtained with a number of other substances, but the reversal of inhibition by anions is only shared by glucosamine 6-phosphate.

A tentative model of the enzyme, compatible with the results, includes a single site for each substrate, an unspecific site for an activating ion, and several sites for both glucose 6-phosphate and the allosteric inhibitor.

The activation of glycogen synthetase (uridine diphosphate glucose:glycogen $\alpha$-4-glucosyltransferase, EC 2.4.1.11) by glucose 6-phosphate has attracted much attention because of its possible physiological significance. Since glucose-6-P[1] is in the metabolic pathway from glucose to glycogen, this is a case of "precursor activation," perhaps the first reported (Leloir *et al.*, 1959); the situation is reciprocal to the more commonly found "end-product inhibition." Nevertheless, the fact that glycogen synthetases of different sources vary widely in their dependence from glucose-6-P (Goldemberg, 1966), cast some doubts about the generality and importance of this phenomenon *in vivo*. The yeast enzyme (Algranati and Cabib, 1962) is one of the synthetases in which the effect of the phosphoric ester is relatively small, although it is more marked at high, unphysiological pH values. A further study of this problem has disclosed that many anionic substances inhibit the enzyme, and that glucose-6-P is capable of reversing the inhibition. Kinetic analysis shows this effect to be of an allosteric nature.

## Experimental Section

*Materials.* UDP-[$^{14}$C]glucose was synthesized according to Roseman *et al.* (1961) or purchased from the International Chemical and Nuclear Corp. Yeast glycogen synthetase was purified according to Algranati and Cabib (1962).

*Enzyme Assay.* The incubation mixture contained 0.1 M glycylglycine (pH 7.5), 0.4 mM UDP-[$^{14}$C]glucose (uniformly labeled in the glucose moiety, sp act. 375,000 cpm/$\mu$mole), 3% glycogen, and enzyme, in a total volume of 0.05 ml. After incubating for 15 min at 30°, 0.25 ml of 33% KOH was added and the tubes were kept for 10 min in a boiling water bath. After adding 0.25 ml of water, glycogen was precipitated with 0.7 ml of 96% ethanol. After reprecipitation, glycogen was redissolved in water, plated on stainless-steel or glass planchets, and counted in a gas-flow counter.

UDP-glucose was used at 0.4 mM, rather than at 5 mM as done previously (Algranati and Cabib, 1962), because at the lower concentration, the inhibition and reactivation effects were more marked.

To check for contamination with amylolytic activity, samples of $^{14}$C-labeled glycogen were incubated with the

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[1] Abbreviations used: UDP, uridine diphosphate; glucose-6-P, glucose 6-phosphate; 3-P-glyceric acid, 3-phosphoglyceric acid.

TABLE I: Inhibitory Effect of Different Anions and Reversal by Glucose-6-P.[a]

| Addn to Reaction Mixture | Concn (M) | Activity | | Activation Ratio +Glucose-6-P: −Glucose-6-P |
|---|---|---|---|---|
| | | − Glucose-6-P | + Glucose-6-P | |
| None | — | 100 | 171 | 1.71 |
| Chloride | 0.2 | 40 | 160 | 4 |
| Bromide | 0.1 | 55 | 162 | 2.95 |
| Iodide | 0.1 | 13 | 78 | 6 |
| Fluoride | 0.2 | 111 | 205 | 1.85 |
| Nitrate | 0.1 | 44 | 142 | 3.2 |
| Sulfate | 0.1 | 36 | 140 | 3.9 |
| Phosphate | 0.2 | 35 | 140 | 4.0 |
| Pyruvate | 0.2 | 68 | 170 | 2.7 |
| Lactate | 0.2 | 114 | 166 | 1.46 |
| Oxalate | 0.2 | 15 | 163 | 10.8 |
| Malonate | 0.2 | 55 | 148 | 2.7 |
| Succinate | 0.2 | 100 | 148 | 1.48 |
| Maleate | 0.2 | 19 | 170 | 7.9 |
| Fumarate | 0.2 | 70 | 185 | 2.65 |
| Oxalacetate | 0.2 | 37 | 162 | 4.4 |
| UDP | $5 \times 10^{-4}$ | 32 | 72 | 2.25 |

[a] Glucose-6-P, when added, was 10 mM. The potassium salts were used for all the compounds tested. The activity in the absence of glucose-6-P and inhibitors is taken as 100.

enzyme in the conditions of the assay (but omitting UDP-glucose), with or without 0.1 M KCl. Glycogen was then reisolated and counted as above described. No loss of radioactivity was detected in these experiments.

## Results

*Kinetic Aspects of Enzyme Inhibition and of Its Reversal.* The results shown in Table I illustrate the inhibition found with several anionic substances. Although the concentration needed for a substantial inhibition was high, a simple effect of ionic strength is ruled out, because some of the anions tested, such as succinate, fluoride, and lactate, were noninhibitory.[2] Close analogs of succinate and lactate, *i.e.*, maleate and pyruvate, respectively, depressed the enzymatic activity, thus showing a certain specificity of the effect. This specificity also demonstrates that the inhibition could not be caused by the cation, since all the substances were tested as the potassium salts. Moreover, sodium and potassium chloride showed the same inhibitory potency. In the presence of 10 mM glucose-6-P, the inhibition was practically abolished in most cases. Therefore, the relative activation caused by the phosphoric ester becomes very large when calculated taking the inhibited activity as starting point. Glucosamine-6-P was also effective as reactivator. In one experiment the activation ratio with 10 mM glucosamine-6-P was 1.4 in the absence and 3.4 in the presence of 0.3 M chloride. The corresponding values for 10 mM glucose-6-P were 1.42 and 3.5.

Chloride was chosen as model effector for the following series of experiments. Table II shows that the inhibition was essentially reversible, even at very high chloride concentrations. Preincubation of the enzyme with or without chloride, followed by dilution and addition of glucose-6-P, led to an almost complete recovery of activity.

TABLE II: Reversibility of Chloride Inhibition.[a]

| Addn to Preincubn Mixture (M) | Addn to Assay Mixture | Activity |
|---|---|---|
| — | — | 100 |
| — | Glucose-6-P (10 mM) | 175 |
| KCl (0.4) | KCl (0.4 M) | 5 |
| KCl (0.4) | Glucose-6-P (10 mM)[b] | 150 |

[a] The preincubation mixture contained 0.1 M glycylglycine (pH 7.5) and enzyme. KCl was added where indicated. After 15 min at 30°, an aliquot of each tube was added to the assay mixture. The final concentrations were 0.1 M glycylglycine (pH 7.5), 0.4 mM UDP-[$^{14}$C]glucose (375,000 cpm/$\mu$mole), and 3% glycogen. KCl and glucose-6-P were added where indicated. The activity in the absence of glucose-6-P and potassium chloride is taken as 100. No loss of activity was found after preincubation. [b] Potassium chloride was carried from the preincubation mixture so that its final concentration in the assay mixture was 0.12 M.

The inhibition appears to be partially competitive with UDP-glucose, as shown in Figure 1. However, a fully competitive inhibition was obtained with other batches of enzyme, like the one used in the experiment of Figure 2. Both in the absence and in the presence of chloride, hyperbolic substrate–velocity curves and rectilinear inverse plots were obtained. Addition of glucose-6-P reversed completely chloride inhibition, changing back the $K_m$ to the value found in the absence of inhibitor (see Figure 2). For comparison, it is shown in Figure 3 that UDP, a close structural analog of UDP-glucose, gave a competitive type of inhibition, with a $K_i$ of 0.2 mM in

[2] A previous report (Rothman and Cabib, 1966) that 0.2 M phosphate was noninhibitory, was later found to be in error. On the other hand, acetate, malate, glycolate, and $\beta$-hydroxybutyrate, all at 0.2 M concentration, were without effect on the activity.

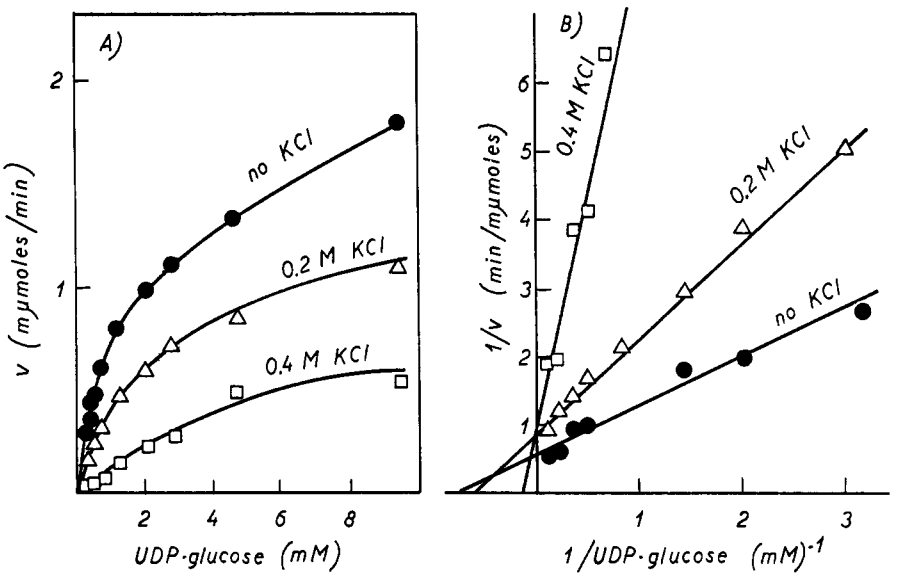


FIGURE 1: Effect of UDP-glucose concentration on reaction rate in the absence and in the presence of KCl. Assay conditions as under Experimental Section.

the absence of glucose-6-P. As shown in Table I, glucose-6-P was almost without effect on the inhibition by UDP.

When the effect of glucose-6-P on the rate was examined, sigmoid curves were obtained in the presence of chloride, as shown in Figure 4A. Accordingly, inverse plots gave rise to curves with upward concavity (Figure 4B).

Cooperative effects were also found when chloride was varied at different fixed levels of glucose-6-P, as can be seen in Figure 5A,B. Hill plots (Atkinson, 1966) for both cases are shown in Figure 6A,B.

*Desensitization of the Enzyme to Inhibitors.* The fact that glucose-6-P was able to relieve the inhibition by anionic substances, but had little effect on a competitive inhibitor such as UDP, and the cooperative effects shown in Figures 4 and 5 indicated that the inhibition by the anions might be of the allosteric type. Several unsuccessful attempts were made to destroy the inhibitor site by heating, addition of mercuric salts, or *p*-mercuribenzoate. The last two reagents were found to inhibit the enzyme very strongly: $10^{-6}$ M $Hg(NO_3)_2$ gave 81% and $10^{-5}$ M *p*-mercuribenzoate 94% inhibition.

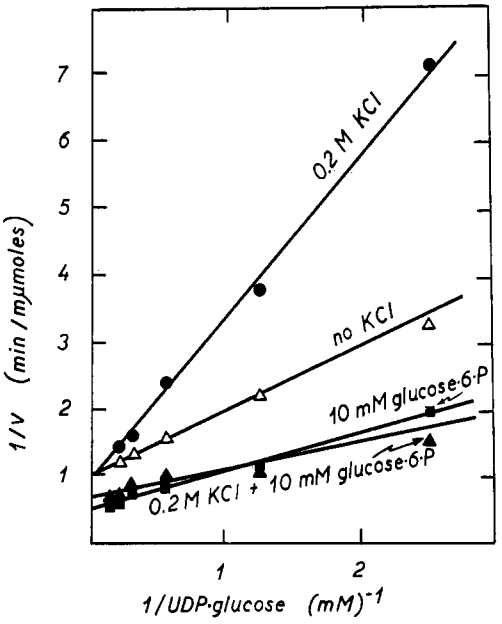


FIGURE 2: Reversal of KCl effect by glucose-6-P. Conditions as in Figure 1.

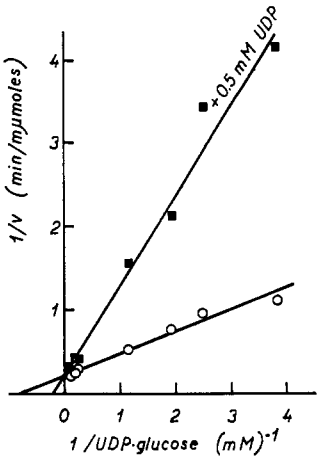


FIGURE 3: Inhibition by UDP. Assay conditions as under Experimental Section.

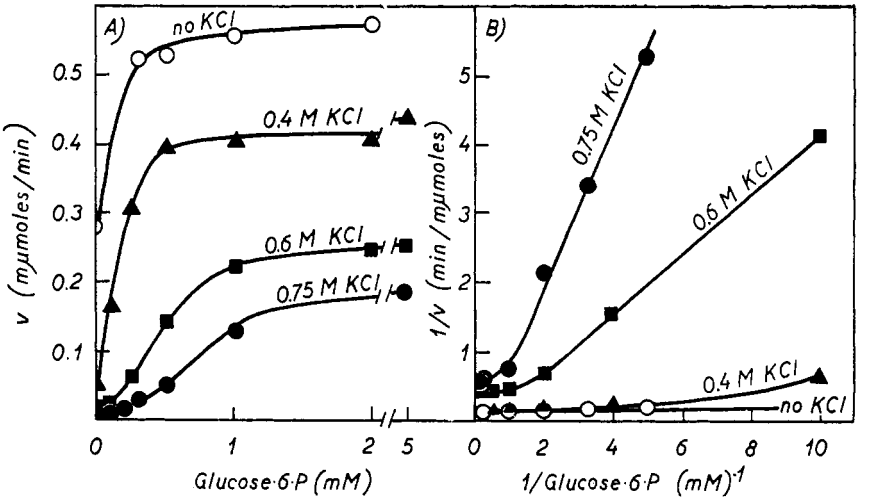


FIGURE 4: Effect of glucose-6-P on reaction rate in the absence and in the presence of KCl. Conditions as in Figure 1.

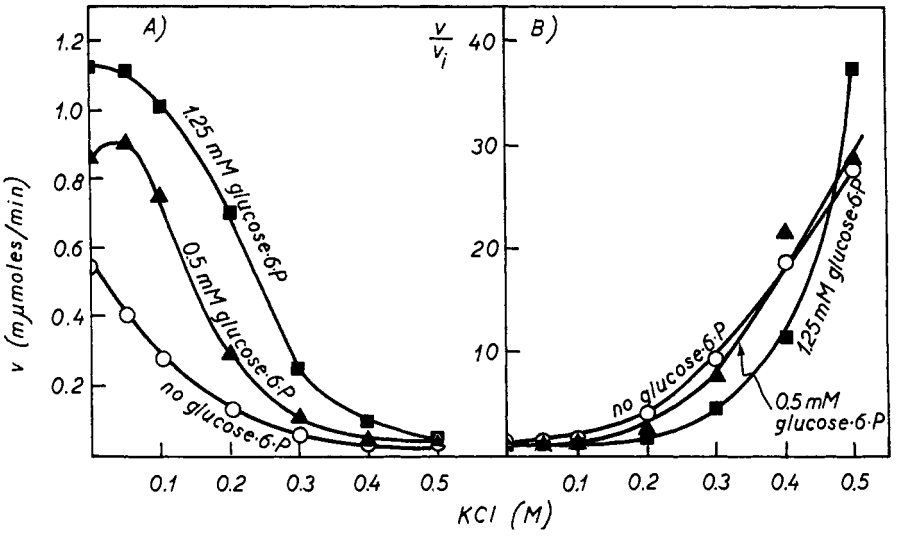


FIGURE 5: Effect of KCl on reaction rate in the absence and in the presence of glucose-6-P. Conditions as in Figure 1. $v$ and $v_i$ indicate the initial velocity without and with chloride, respectively.

Finally, desensitization of the enzyme was achieved by fluorodinitrobenzene treatment (Pontremoli *et al.*, 1965; Rosen and Rosen, 1966) at pH 8 and in the presence of UDP-glucose as protector of the active site. Figure 7 shows that the activity in the absence of inhibitor declined slowly during dinitrophenylation, whereas the activity measured in the presence of chloride increased rapidly, so that after about 15 min, the enzyme had practically lost its sensitivity to the inhibitor. On more prolonged treatment, chloride gave rise to a substantial activation. A possible explanation for the latter effect will be discussed below. When the substrate site was not protected with UDP-glucose, the activity was rapidly lost. It is remarkable that in this case no desensitization took place, as long as the enzyme activity was detectable.

As indicated in Table III, the inhibition by UDP at two different levels remained unchanged after dinitrophenylation, whereas the sensitivity to both chloride and maleate was lost. In these experiments, dinitrophenylation was stopped by adding an excess of glycylglycine, and incubation was carried out without separating the dinitrophenyl dipeptide. To confirm that the effects observed were due to a permanent change in the enzyme, the latter was separated from the other components of the dinitrophenylation mixture by passage through a Sephadex column. As shown in Table IV, line 1, the results of this experiment were similar to those previously obtained.

The concentration of fluorodinitrobenzene for optimal desensitization varied, according to the enzyme preparation, between 0.75 and 1.5 mM. Treatments at pH lower

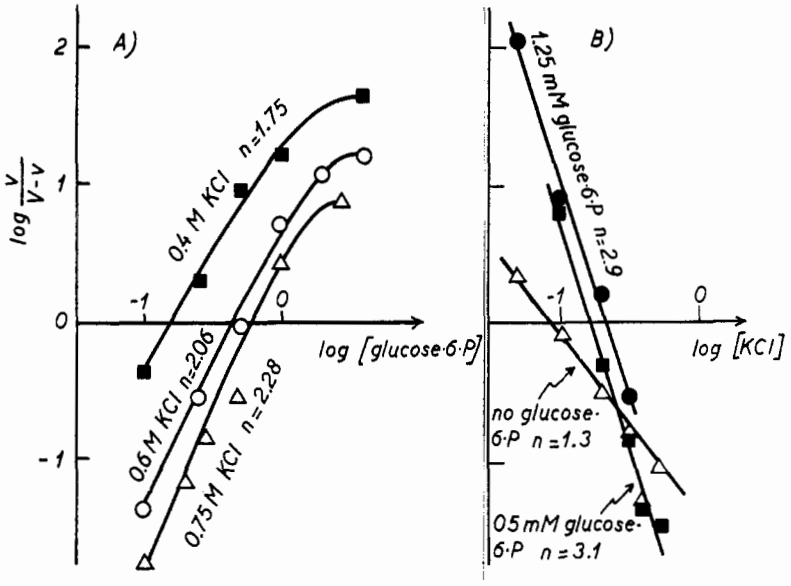


FIGURE 6: Hill plots (Atkinson, 1966) of the data shown in Figures 4A and 5A.

than 8 did not produce desensitization, while a higher pH led to a low recovery of activity.

The dinitrophenylated enzyme could still be activated by glucose-6-P, but the dependence of this activation on pH was markedly altered, as illustrated by Figure 8. In the absence of the phosphoric ester, the pronounced decrease in the activity at an alkaline pH was no longer observed. A change in the pH–activity curve upon dinitrophenylation was also observed with fructose diphosphatase (Pontremoli *et al.*, 1965).

As shown in Table IV, addition of glucose-6-P to the dinitrophenylation mixture did not change the results appreciably. An attempt to protect the inhibitor site with chloride or maleate led instead to an almost total inac-

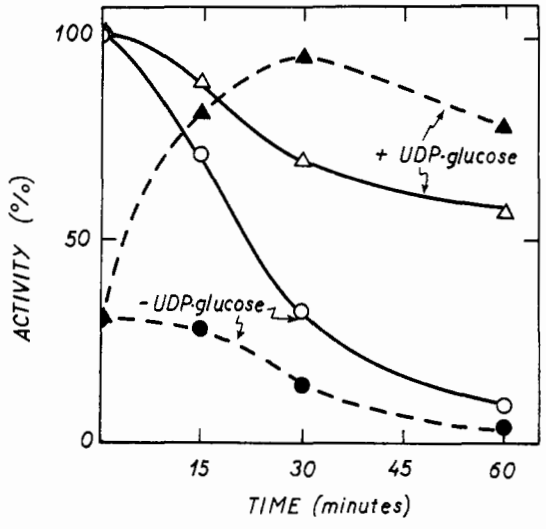


FIGURE 7: Time course of enzyme desensitization with 2,4-fluorodinitrobenzene in the presence and in the absence of UDP-glucose. Conditions as in Table III, except that UDP-glucose was omitted where indicated. Full lines: activity measured in the absence of KCl. Broken lines represent activity measured with 0.25 M KCl.

TABLE III: Inhibition of Dinitrophenylated Enzyme.[a]

| Inhibitor Added | Concn (mM) | Inhibition (%) | |
|---|---|---|---|
| | | Native Enzyme | Dinitrophenylated Enzyme |
| KCl | 200 | 45[b] | 0 |
| Maleate | 200 | 43[b] | 2 |
| UDP | 0.5 | 39 | 37 |
| UDP | 2 | 67 | 69 |

[a] The dinitrophenylation mixture contained 25 mM Tris-succinate buffer at pH 8.5, 12.5 mM UDP-glucose, 1.5 mM 2,4-fluorodinitrobenzene, 4 mM EDTA, and enzyme. The final pH of the mixture was 8. After shaking for 15 min in the dark at room temperature, the mixture was cooled in ice, and aliquots were withdrawn and added to the incubation mixture. The final composition of the latter was 0.1 M g'ycylglycine buffer, pH 7.5, 5 mM UDP-[$^{14}$C]glucose (sp act. 50,000 cpm/$\mu$mole), and 3% glycogen, in a total volume of 0.1 ml. [b] The inhibition is lower than in Table I, because of the higher concentration of UDP-glucose in this experiment.

TABLE IV: Effect of Different Additions to Dinitrophenylation Mixture.[a]

| Expt | Addn | Act. Recovd after FDNB (%) | % Inhibition by 0.2 M KCl | |
|---|---|---|---|---|
| | | | Before Treatment | After Treatment |
| 1 | — | 53 | 45 | 0 |
| | Glucose-6-P (7 mM) | 35 | 44 | 0 |
| | KCl (0.3 M) | 7 | 45 | — |
| 2 | — | 27 | 52 | 0 |
| | Maleate (0.25 M) | 5 | 52 | — |
| | UDP[b] (10 mM) | 36 | 52 | 15 |

[a] Dinitrophenylation was carried out as in Table III. The dinitrophenylation mixture (1 ml) was passed through a Sephadex G-25 column (1.4 × 7 cm) equilibrated with 0.025 M Tris-succinate buffer (pH 8.5) and aliquots of the effluent were assayed for enzyme activity as described under Experimental Section except that the UDP-glucose concentration was 4 mM and the sp act. 62,000 cpm/μmole. FDNB, fluorodinitrobenzene. [b] No UDP-glucose was added to the dinitrophenylation mixture in this case.

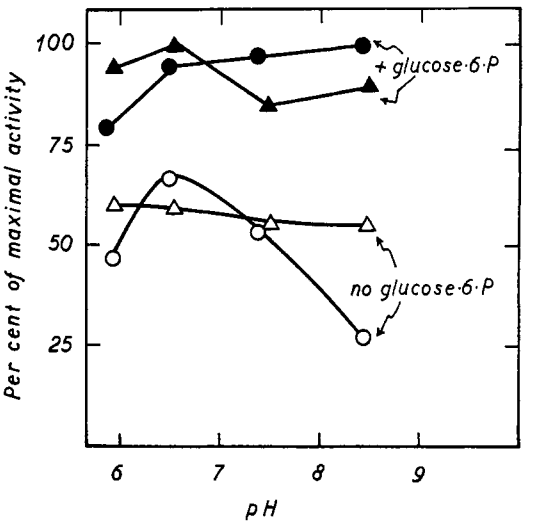


FIGURE 8: pH–activity curve of native (○, ●) and dinitrophenylated (△, ▲) enzyme. The enzyme was treated with fluorodinitrobenzene and passed through a Sephadex column as described in Tables III and IV. Succinate–cacodylate buffer (0.1 M of each) was used at pH 6 and 6.5, and 0.1 M glycyclglycine in the more alkaline range. Other assay conditions as under Experimental Section.

tivation of the enzyme (see Table IV). On the other hand, UDP could substitute for UDP-glucose as protector of the active site during dinitrophenylation.

It may be of interest to mention that a preparation of the enzyme which had been stored for a week in the refrigerator, rather than at the usual temperature of −20°, was found to have lost spontaneously its sensitivity to chloride inhibition while the pH dependence of the activity with or without glucose-6-P was normal. Another preparation, when kept under the same conditions, remained sensitive to the inhibitor.

*The Effect of Activators.* A kinetic study of the activating effect of glucose-6-P on the enzyme at two different pH values (see Figure 9) indicated that the ester only increased the maximum velocity, but had no effect on the Michaelis constant. The latter did not change appreciably with the pH. The lack of effect of glucose-6-P on the apparent affinity of the enzyme for UDP-glucose is to be contrasted with the opposite behavior reported for the muscle glycogen synthetase (Villar-Palasi *et al.*, 1966).

When the effect of a large number of compounds on the enzymic activity was tested at a concentration of 10 mM, it was found that several of them gave a stimulation. Some examples are given in Table V. Other activators were 2-P-glyceric acid and 2,3-diphosphoglyceric acid.

It is remarkable that two of the effective compounds, phosphate and sulfate, are inhibitors rather than activators when tested at higher concentrations (see Table I). At pH 9.1, where the stimulation by glucose-6-P is much larger (*cf.* Algranati and Cabib, 1962), 3-P-glyceric acid was a relatively poor activator.

The effect of glucose-6-P and of other activators was not additive. In one experiment, 10 mM glucose-6-P activated 77%, 10 mM 3-P-glyceric acid 81%, and a mixture of the two, 100%. An important observation was that only glucose-6-P (and glucosamine-6-P, as already mentioned) was able to reverse the inhibition by chloride (see Table V).

*The Effect of Magnesium Ions.* The above-reported experiments were carried out in the absence of $Mg^{2+}$. Magnesium acetate at 10 mM concentration activated the enzyme about 50%, whether glucose-6-P was included or omitted in the incubation mixture. The effect of magnesium might be partially due to the formation of a non-inhibitory complex with UDP. In fact, it was found that 0.5 mM UDP inhibited 68% in the absence and 29% in the presence of 10 mM $Mg^{2+}$, when UDP-glucose was 0.4 mM. Nevertheless, some activation by $Mg^{2+}$ was observed even when no inhibition by the reaction product was apparent. On the other hand, chloride inhibited to the same extent both with or without $Mg^{2+}$. From these experiments it was concluded that, although $Mg^{2+}$ has a stimulating effect on the enzyme, its omission in the reaction mixture does not change appreciably the kinetic results.

*Reproducibility of the Results.* It must be mentioned that, although the results with the same batch of the enzyme were reproducible, there was a certain variability from one preparation to another, especially in the extent of the activation by glucose-6-P and of the inhibition by

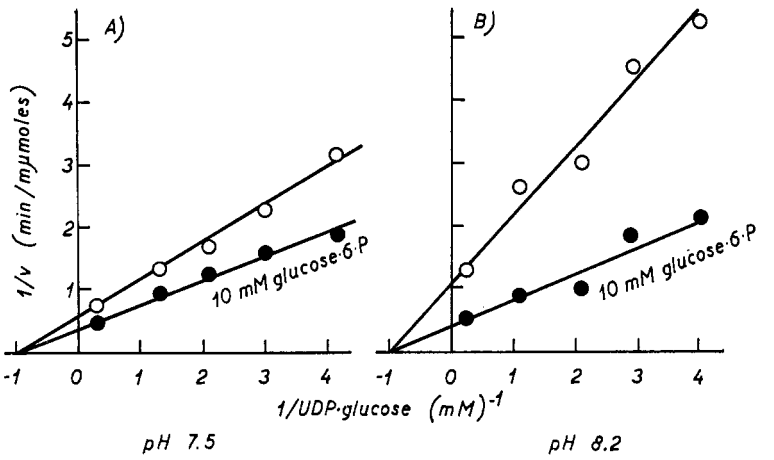


FIGURE 9: Effect of glucose-6-P on reaction kinetics at two different pH values. Glycylglycine was used as buffer in both cases. Other assay conditions as under Experimental Section. Open symbols: without glucose-6-P. Closed symbols: with 10 mM glucose-6-P.

TABLE V: Effect of Chloride in the Presence of Different Activators.[a]

| pH | Activator | Concn (mM) | Activity −KCl | Activity + 0.2 M KCl | Inhibn (%) |
|---|---|---|---|---|---|
| 7.5 | None | — | 100 | 48 | 52 |
| | Glucose-6-P | 10 | 157 | 156 | 0 |
| | Glucose-1-P | 12 | 121 | 47 | 61 |
| | Trehalose-P | 10 | 140 | 79 | 44 |
| | 3-P-glyceric acid | 10 | 158 | 73 | 54 |
| | Sulfate | 10 | 147 | 85 | 42 |
| | Phosphate | 10 | 129 | 63 | 51 |
| 9.1 | None | — | 100 | 20 | 80 |
| | Glucose-6-P | 10 | 510 | 350 | 31 |
| | 3-P-glyceric acid | 10 | 178 | — | — |

[a] Assay conditions as described in Experimental Section except that glycylglycine at two different pH values was used. Activity in the absence of activator and of potassium chloride was taken as 100 at each pH value. The activity at pH 9.1 was 35% of that at pH 7.5.

anions. The qualitative pattern, however, did not change appreciably.

## Discussion

In the following discussion, it will be assumed that glycogen was saturating the enzyme at all times and that the results were influenced by variation in the UDP-glucose site only. At the present time, it is not easy to conduct experiments in which the kinetic properties of the enzyme toward glycogen are studied, since the purified preparations contain a large excess of the polysaccharide (Algranati and Cabib, 1962).

The results of several experiments indicate that the anionic inhibitors and UDP-glucose bind to different sites of the enzyme. There is no obvious similarity between the structure of the inhibitors and that of UDP-glucose. The inhibition is, at least in some cases, of the partially competitive type, while that obtained with UDP is fully competitive. Glucose-6-P relieves to a great extent the inhibition in most cases, whereas UDP, presumably a true competitive inhibitor, depresses the activity to about the same extent in the presence or in the absence of glucose-6-P.

Finally, the desensitization of the enzyme brought about by treatment with fluorodinitrobenzene, seems to give a decisive argument in favor of the allosteric nature of the inhibition. Again, the behavior of UDP is at variance from that of the other anions. The fact that its inhibitory power at two different concentrations is un-

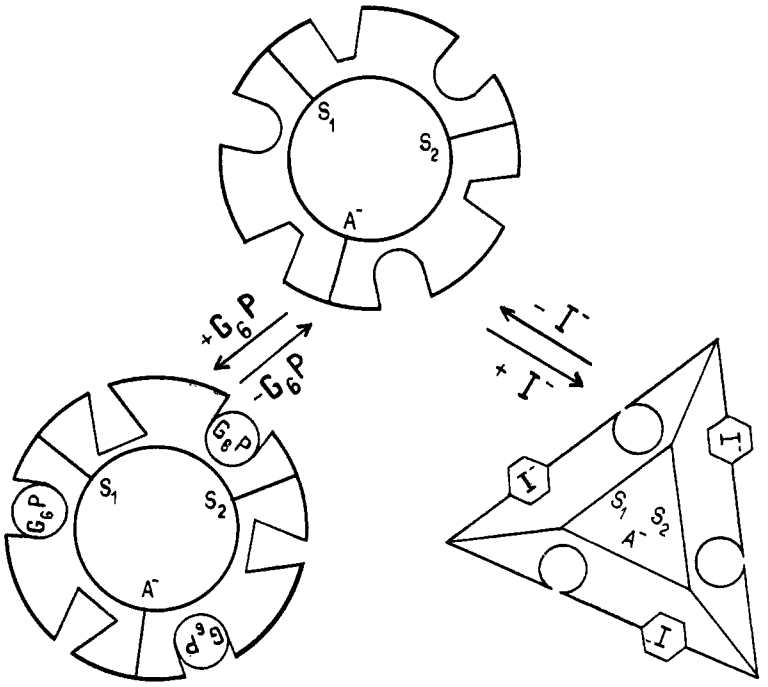


FIGURE 10: Tentative scheme of the interaction between enzyme and allosteric effectors. $S_1$ and $S_2$, substrate sites for UDP-glucose and glycogen, $A^-$, unspecific site for anionic activator; G-6-P, glucose-6-P; $I^-$, allosteric inhibitor.

affected by dinitrophenylation would indicate that the substrate site has not been greatly changed by the treatment.

The sigmoid curves obtained with glucose-6-P in the presence of chloride, or *vice versa*, suggest that both effectors bind to the enzyme at several sites. The slopes of Hill plots indicate a minimum of three sites in each case. It remains to be established whether glucose-6-P and chloride compete for the same sites. A direct competition would seem to be excluded by the fact that, in the presence of high concentrations of chloride, glucose-6-P was unable to reverse the inhibition completely (see Figure 4A). The results of Table II show that this behavior could not be ascribed to partial inactivation of the enzyme by chloride. In addition, glucose-6-P failed to protect the inhibitor site from dinitrophenylation (see Table IV).

The two effects of glucose-6-P, *i.e.*, the activation in the absence of inhibitors, and the reversal of inhibition, seem to be largely unrelated. In the first case, glucose-6-P only modifies the maximum velocity of the reaction, whereas, as a reactivator, it also affects the $K_m$, as shown in Figure 2. Furthermore, the activating effect is shared by a number of substances[3] (Table V) while the relief of inhibition is much more specific. As a first approximation, therefore, a special site for an unspecific, anionic activator may be postulated.

The existence of an activator site may explain why in some cases the dinitrophenylated enzyme, in which the inhibitor site was not longer functional, was actually stimulated by chloride. Occasionally, the native enzyme was also found to be activated by moderate concentrations of chloride, in the presence of glucose-6-P, which makes the inhibitor site less accessible to the anion (*cf.* Figure 5A). It may also be mentioned that sulfate and phosphate activate at 10 mM but inhibit at higher concentrations (see Table I). It seems, therefore, that the action of each anion on the enzyme will depend on its concentration and on the relative affinity for the activator and the inhibitor site.

If one attempts to apply the theory of Monod *et al.* (1965) to the results of this study, a discrepancy becomes evident. According to that theory the enzyme can exist in two interconvertible forms, and the effectors modify the equilibrium between the two forms by combining preferentially with one of them. The mathematical treatment of the model requires any such effector to show homotropic cooperative effects, *i.e.*, sigmoid curves. In the case of yeast glycogen synthetase, UDP-glucose appears to be an effector of this type, since its affinity for the enzyme is changed in the presence of inhibitors (see Figures 1 and 2). Nevertheless no homotropic cooperative effects were observed with UDP-glucose, either in the presence, or in the absence of chloride. One possible explanation for this behavior could be that the enzyme has several sites for glucose-6-P and the inhibitor (which show cooperative effects) but a single site for UDP-glucose. With this model, the results would also agree with the Koshland–Atkinson theory (Koshland, 1964; Atkinson *et al.*, 1965), according to which each new

[3] It should be noted, however, that at pH 9, 3-P-glyceric acid was less effective as activator than glucose-6-P (Table V).

molecule of effector that binds to the enzyme causes a further change in its conformation. In this case an additional requirement should be introduced, *i.e.*, that substrate can only bind to the free enzyme, in order to explain the competitive type of inhibition and the linearity of Lineweaver–Burk plots. Such a hypothesis would agree with the observation that treatment with fluorodinitrobenzene in the presence of inhib tors 'ed to irreversible loss of activity (see Table IV). In fact, under those conditions, substrate might be unable to combine with the enzyme and to protect the active site against dinitrophenylation.

Several examples have appeared in the literature, of regulatory enzymes in which the inhibitor behaves in an apparently competitive way, and the Lineweaver–Burk plots are rectilinear both in the presence or in the absence of inhibitor, as obtained in the present work (Umbarger and Brown, 1958; Sturani *et al.*, 1963; Caskey *et al.*, 1964; Kornfeld *et al.*, 1964). A more thorough study of some of these cases, including determinations of the rate dependence on inhibitor concentration, may provide interesting data for the interpretation of allosteric effects.

Although it may be useful and stimulating to emit hypotheses to fit the experimental data, we share the opinion of Atkinson *et al.* (1965), that the time is not yet ripe for a comprehensive theory of regulatory enzymes. Therefore, the tentative model shown in Figure 10, is only given to summarize and unify the results obtained. In this model, the substrates would occupy a single site each. For the sake of simplicity, only one site is indicated for the unspecific activator, which could combine with any of the enzyme forms. There would be more than one regulatory subunit. Three are shown, for illustrative purposes only. Of the possible conformations of the enzyme, only three extreme cases have been included: the enzyme in the absence of effectors, saturated with glucose-6-P, and saturated with inhibitor, respectively. The binding of glucose-6-P would modify the inhibitor sites, making more difficult, but not necessarily impossible, for the inhibitor to combine with them. The inhibitor would do the same for the glucose-6-P sites. Thus many enzyme complexes would be possible, bearing variable numbers of glucose-6-P and inhibitor molecules.

The inhibition described in this paper is rather unspecific; the inhibitors are only effective at relatively high concentrations and many of them can be found in the cell only in small amounts or not at all. Nevertheless, the reactivation by glucose-6-P is specific and the effect can be quite large. It may, therefore, be suspected that there exist in the cell substances, capable of inhibiting the enzyme under conditions found *in vivo* and that the action of glucose-6-P can be instrumental in regulating the activity of the enzyme. This topic will be considered in the following paper (Rothman and Cabib, 1967).

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