REGULATORY PROPERTIES OF YEAST GLYCOGEN SYNTHETASE[1,2]

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An early finding in the study of muscle glycogen synthetase (UDP-D-glucose:glycogen α-4-glucosyltransferase) was that glucose-6-P, a glycogen precursor, activates this enzyme (Leloir et al., 1959). Further work showed that the stimulation by the phosphoric ester differed greatly, according to the source of enzyme (Goldemberg, 1966). The possible physiological significance of the activation thus remained in doubt.

The synthetase from yeast is only moderately dependent on glucose-6-P for maximal activity (Algranati and Cabib, 1962). It has now been found that several anions inhibit the enzyme, and that glucose-6-P reverses this inhibition, giving rise to very large stimulations of the reaction rate, under the appropriate conditions. A detailed study has shown that the anionic substances act as allosteric inhibitors (Monod et al., 1965).

MATERIALS AND METHODS

Yeast glycogen synthetase was purified according to Algranati and Cabib (1962). UDP-glucose-C^{14} (uniformly labeled in the glucose moiety) was purchased from the International Chemical and Nuclear Corporation

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[2]Taken from a thesis to be submitted by Lucía B. Rothman to the University of Buenos Aires in partial fulfillment of the requirements for the degree of Doctor in Chemistry.

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(City of Industry, Calif.). The assay mixture contained 0.1 M glycyl-glycine, pH 7.5 or 0.1 M cacodylate-succinate buffer, pH 5.9, 0.4 mM UDP-glucose-C^{14} (specific activity 375,000 cpm/μmole), 3% glycogen, and enzyme, in a total volume of 0.05 ml. After 15 minutes incubation at 30°, the reaction mixture was digested with alkali, glycogen was precipitated twice with ethanol, plated on glass or stainless steel planchets and counted in a gas flow counter.

RESULTS

The inhibitory effect of several anions is shown in Table I.

TABLE I

Inhibition of glycogen synthetase by different substances and its reversion by glucose-6-P

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

Additions to reaction mixture	Concentration (M)	Activity		Activation ratio
		- glucose-6-P	+ glucose-6-P	+ glucose-6-P / -glucose-6-P
None	-	100	172	1.72
Chloride	0.2	35	168	4.7
Nitrate	0.1	44	142	3.2
Sulfate	0.1	36	140	3.7
Phosphate	0.2	100	-	-
Maleate	0.2	19	170	7.9
Succinate	0.2	100	148	1.48
Pyruvate	0.2	68	170	2.7
Lactate	0.2	114	166	1.46

Although relatively high concentrations were needed, some specificity of the effect is evident. Glucose-6-P had a strong reactivating action, but it did not reverse the inhibition of a true competitive inhibitor, such as UDP (see Table III).

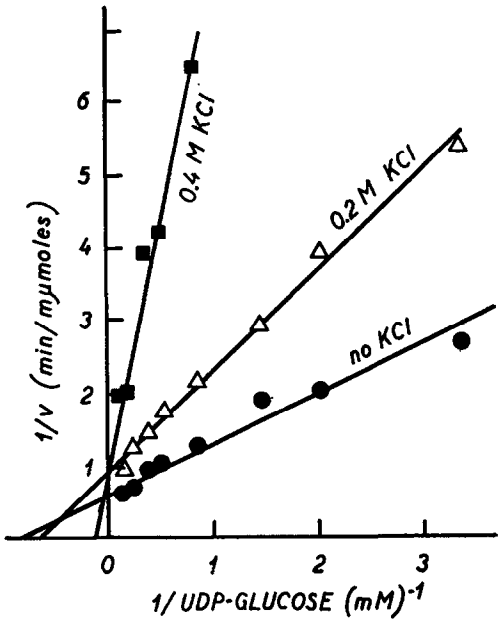

Figure 1. Inverse plots of the rate as a function of UDP-glucose concentration in the absence and in the presence of chloride.

In further experiments, chloride was chosen as standard modifier. Kinetic analysis of the effect is depicted in Figures 1 and 2. Inverse plots of the rate as a function of UDP-glucose concentration gave straight lines, thus showing Michaelian kinetics, both in the absence and in the presence of inhibitor. The inhibition itself was of the mixed type. When the concentration of glucose-6-P was varied, maintaining chloride constant, sigmoid curves were obtained, as shown in Figure 2A. Representation of the rate as a function of chloride concentration, at fixed levels of glucose-6-P, also showed cooperative effects (Figure 2B).

These results suggested that chloride was acting as an allosteric inhibitor, and several efforts were made to desensitize the enzyme to it. Treatment of glycogen synthetase with 2,4-dinitrofluorobenzene (Pontremoli et al., 1965) in the presence of UDP-glucose, was an effective procedure, as shown in Table II. While inhibition by chloride was abolished, the dinitrophenylated enzyme still responded to activation by glucose-6-P and inhibition by UDP (not shown), thus indicating an essentially intact substrate site.

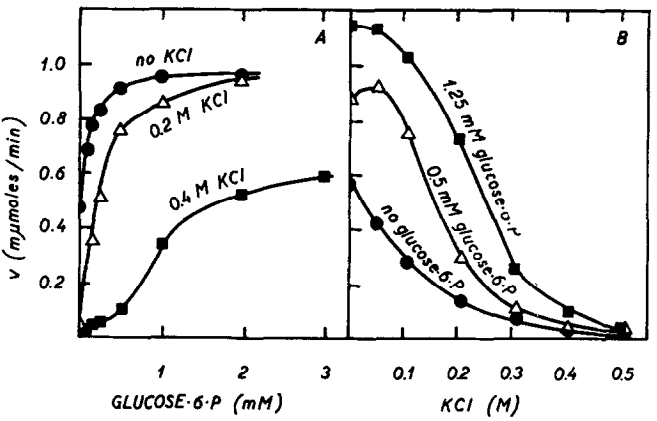

Figure 2. A. Effect of changing the concentration of glucose-6-P, in the presence of different amounts of chloride.
B. Effect of changing the concentration of chloride at fixed concentration of glucose-6-P.

TABLE II

Effect of dinitrophenylation on enzyme inhibition by chloride

The dinitrophenylation mixture contained: 25 mM Tris-succinate buffer at pH 8.5, 12.5 mM UDP-glucose, 2,4-dinitrofluorobenzene and enzyme. The final pH of the mixture was 8. After shaking for 15 minutes in the dark, at room temperature, the mixture was cooled in ice, aliquots were withdrawn and added to the assay mixture. The final composition of the latter was 0.1 M glycylglycine buffer, pH 7.5, 5 mM UDP-glucose-C^{14} (specific activity 50,000 cpm/μmole), and 3% glycogen, in a total volume of 0.1 ml. When added, KCl was in a final concentration of 0.2 M. After 30 minutes of incubation at 30°, the incorporation of labeled glucose in glycogen was measured as described under "Materials and Methods". Activity of the untreated enzyme in the absence of KCl is taken as 100.

Dinitrofluorobenzene concentration (mM)	Activity		KCl inhibition (%)
	KCl	+ KCl	
-	100	59	41[a]
0.25	97	63.2	34
0.5	83	63.8	23
1	66	65	2
1.5	61	63.5	0
2.5	52	55	0

[a] The inhibition is lower than in Table I, because of the higher concentration of UDP-glucose in this experiment.

The pH-activity curve had been previously determined (Algranati and Cabib, 1962) with Tris-maleate buffer, which was now found to be an inhibitor (see Table I). When the experiment was repeated using non-inhibitory buffers, two facts became apparent: first, that the enzyme was fully active at pH 6, near the value reported for the yeast cell (Eddy, 1958); second, that maleate was more inhibitory at pH 6 than at pH 7.5. As shown in Table III, the same effect was obtained with several other substances.

TABLE III

Effect of pH on enzyme inhibition

Glucose-6-P, when added, was 10 mM. The activity in the absence of glucose-6-P (G-6-P) and inhibitors is taken as 100.

Inhibitor	Concentration (mM)	Enzyme activity					
		pH 7.5			pH 5.9		
		- G-6-P	+ G-6-P	+ G-6-P / - G-6-P	- G-6-P	+ G-6-P	+ G-6-P / - G-6-P
None	-	100	173	1.7	100	145	1.45
KCl	200	40	160	4	46	126	2.7
Maleate	100	50	-		10.5	-	
Citrate	100	87	-		5.5	83	15.1
Phosphoenolpyruvate	10	86	-		55	46	2.7
ATP	5	61	146	2.4	10	95	9.5
	1				60	142[a]	2.4
ADP	5	48	160	3.3	14	90	6.4
GTP	5	89	178	2	13	90	6.9
UDP	0.5	32	72	2.25	42	59	1.4

[a] With 0.1 mM glucose-6-P

By working at the lower pH, it was possible to find some very strong inhibitors. Phosphoenolpyruvate was almost 20 times as effective as chloride, while ATP, ADP and GTP surpassed phosphoenolpyruvate by an order of mag-

nitude. AMP, GMP and GDP were rather poor inhibitors. The fact that glucose-6-P produces large reactivating effects with ATP and the other substances, indicates that the nature of the inhibition is similar to that produced by chloride. Further evidence in favor of this supposition came from measurements of the rate as a function of glucose-6-P concentration, in the presence of ATP, at pH 5.9. Sigmoid curves were obtained, as with chloride at pH 7.5 (see Figure 2A). At the acidic pH, 0.2 mM glucose-6-P was sufficient to give maximal reactivation.

DISCUSSION

The allosteric nature of the inhibition by anions is indicated by the reactivating effect of glucose-6-P, not found with competitive inhibitors, and is confirmed by the disappearance of the inhibition upon dinitrophenylation of the enzyme. The sigmoid curves obtained when the concentration of the activator or the inhibitor was varied, suggest that these substances bind to more than one site of the enzyme. Conversely, the lack of cooperative effect with UDP-glucose, in the presence or in the absence of inhibitor, may be interpreted by supposing that the substrate binds to a single site.

The inhibition by ATP and other nucleotides at pH 6, at concentrations near those found *in vivo* (Betz and Chance, 1965; Kopperschläger and Hofmann, 1964), and the reactivation by small amounts of glucose-6-P, may be of importance in the regulation of glycogen synthesis. If the combined concentrations of the inhibitory nucleotides are always large enough to keep the enzymatic activity low in the absence of glucose-6-P, then the concentration of the latter may be the decisive factor in determining the rate of glycogen formation. The level of glucose-6-P is probably regulated, in turn, by the phosphofructokinase reaction, which in yeast is inhibited by ATP and activated by AMP (Ramaiah *et al.*, 1964). The interconversion between the adenine nucleotides, which depends on the energy state of the cell, would then influence the rate of synthesis of glycogen indirectly through the phosphofructokinase step.

An analogous situation would develop when nitrogen is supplied in the form of ammonium ion. Phosphofructokinase would also be activated,

thus leading to a diminished synthesis of glycogen (Trevelyan and Harrison, 1956) and channelling the carbon of glucose towards the nitrogenous cell constituents necessary for growth.

Whether similar mechanisms might be operative in animal tissues will be the subject of further study.

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