METABOLITE REGULATION OF THE I AND D FORM OF RAT MUSCLE GLYCOGEN SYNTHETASE[1]

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We have reported recently (Rothman and Cabib, 1966, 1967) that ATP and ADP can act under certain conditions as powerful inhibitors of yeast glycogen synthetase (UDP-D-glucose:glycogen α-4-glucosyltransferase). Glucose 6-phosphate, while relatively poor as an activator of this enzyme, reverses the inhibition efficiently and specifically. In view of the possible physiological implications of these results, it seemed interesting to find out whether similar effects occur with the glucose 6-phosphate-independent (I) and dependent (D) forms of the muscle glycogen synthetase.

EXPERIMENTAL PROCEDURE

The I and D forms of glycogen synthetase were obtained from rat muscle, following a procedure essentially similar to that of Villar-Palasí *et al.* (1966). The assay mixture contained 0.1 M succinate-cacodylate buffer, pH 6.6, 10 mM EDTA, 1.5% shell fish glycogen, 0.4 mM UDP-C^{14}-glucose (uniformly labeled in the glucose moiety, specific activity 350,000 cpm/μmole), and enzyme in a total volume of 0.05 ml. Incubations were carried out at 30° and the reaction was stopped by adding 66% ethanol-0.1 M ammonium acetate. Glycogen was reprecipitated with 66% ethanol, plated on stainless steel planchets, and counted in a gas flow counter.

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RESULTS AND DISCUSSION

The results of Table I show that ATP and ADP are potent inhibitors for both forms of the enzyme. AMP and P_i are poorer inhibitors, and the former may be disregarded as a functional effector, because of its low concentration in muscle (Parker, 1954). Addition of glucose-6-P reverses the inhibitions to a great extent. In contrast, the inhibition produced by UDP, a substrate analogue, is not removed by the phosphoric ester.

TABLE I

Effect of different metabolites on muscle glycogen synthetase activity

Inhibitor	Concentration (mM)	Inhibition (%)			
		I		D	
		-	10 mM glucose-6-P	0.5 mM glucose-6-P[a]	10 mM glucose-6-P
ATP	6	91	19	88	15
ADP	6	79	19	84	38
AMP	6	36	0	30	0
P_i	10	18	10	49	0
UDP	0.5	86	72	66	58

[a] The activity of the D form cannot be measured in the absence of glucose-6-P. Therefore, a concentration of glucose-6-P that gives almost full activity was employed.

Fig. 1 depicts the effect of varying glucose-6-P concentration on the enzymatic activity in the absence and in the presence of ATP. Two relevant features of these curves should be pointed out. One is the sigmoid character of the plot obtained with the D enzyme in the presence of ATP. The other is that glucose-6-P reverses the inhibition at much lower concentrations with the I than with the D form.

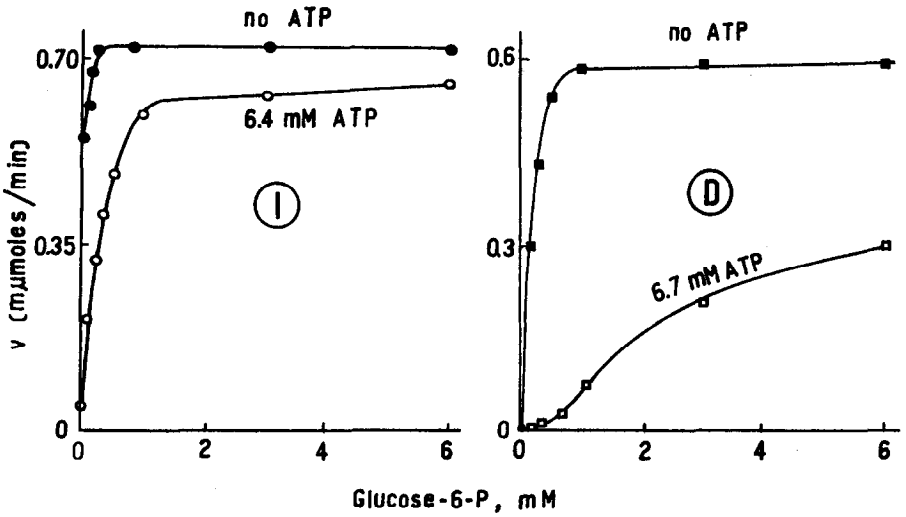

Fig. 1. Effect of increasing glucose-6-P concentration on the activity of the I and D form of muscle glycogen synthetase in the absence and in the presence of ATP.

TABLE II

Kinetic constants of I and D forms for UDP-glucose, in the presence or absence of glucose-6-P and ATP

Additions		I		D	
Glucose-6-P (mM)	ATP (mM)	Km (mM)	Vm (μmoles/min/mg)	Km (mM)	Vm (μmoles/min/mg)
-	-	0.34	0.16	-	-
0.5	-	-	-	0.30	0.21
10.0	-	0.25[a]	0.17	0.25	0.20
-	6	5	0.18	-	-
0.5	6	0.37	0.16	6.6	0.23
10.0	6	-	-	0.26	0.23

[a] Rosell-Pérez et al. (1962) have reported a five-fold decrease in the Km of the I form upon addition of glucose-6-P, at pH 7.8. Our experiments were carried out at pH 6.6. When working at pH 7.8, we obtained results essentially similar to those of Rosell-Pérez et al. (1962).

Inspection of Table II reveals that the ATP inhibition is formally of the competitive type with both the I and D forms, as shown by the constancy of the maximum velocity, in spite of large variations in the Km. Addition of glucose-6-P restores the Km to the value obtained in the absence of effectors.

The similarity of these results with those already reported for the yeast enzyme (Rothman and Cabib, 1966, 1967), particularly the lack of reversion of the UDP inhibition by glucose-6-P, suggests that in this case too the adenine nucleotides and inorganic phosphate act as allosteric effectors. An efficient procedure for the desensitization of the muscle enzyme has not yet been found, although photooxidation of the I form in the presence of methylene blue caused a marked decrease in ATP inhibition. The recovery of activity was, however, small.

Rosell-Pérez and Larner (1964) also found inhibition of the D form of dog muscle synthetase with ATP, ADP and inorganic phosphate, but the effects were relatively small, probably owing to the high pH value and UDP-glucose concentration used by these authors. The results of the present study were obtained under conditions similar to those supposedly prevailing in vivo, that is in the pH range 6.6-7.1 (Caldwell, 1956), at 0.4 mM UDP-glucose (Caputto et al. 1950) and in the presence of 6-7 mM ATP[a]. In this way it could be shown that not only the D, but also the so-called independent activity of glycogen synthetase may depend completely on glucose-6-P. Thus, both enzymatic activities behave qualitatively according to the same general pattern of inhibition and reactivation. On the other hand, their quantitative dissimilarity may have important consequences. If the concentration of glucose-6-P in muscle attains the small value sufficient to saturate the D-synthetase, both forms would be equally active, and a D to I interconversion would not affect the rate of glycogen synthesis. Since, however, in the presence of ATP (plus ADP) the D form is strongly inhibited when the I form is fully active (see Fig. 1), it follows that a transformation of D into I or viceversa, may indeed be attended by a major change in activity. Thus, the differential inhibition by ATP provides a rationale for the functionality of the I-D interconversion.

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[a] This concentration corresponds to the sum ATP + ADP, as found in rat muscle (Parker, 1954), which is the relevant value, since both nucleotides inhibit to about the same extent.

Finally, by comparing the situation in yeast and muscle, it would appear that the latter, belonging to a more evolved organism, possesses both the metabolite regulation of the yeast cell, and, in addition, a superimposed mechanism of transformation (I and D forms), subject to hormonal control (Leloir, 1965). Both mechanisms may be functional under the appropriate conditions. A similar situation seems to exist with the phosphorylase a and b system (Morgan and Parmeggiani, 1964).

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