CONTROL PROPERTIES OF YEAST GLYCOGEN PHOSPHORYLASE

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Summary -- Yeast glycogen phosphorylase is not AMP-sensitive and exists in enzymatically non- interconvertible forms. It is competitively inhibited in vitro by G-6-P and UDPG. Glucose also inhibits. These effectors, however, do not seem to affect the enzyme in vivo. We suggest that this is due to saturation of the enzyme with the substrates intracellularly.

The most important mechanisms for control of glycogen degradation in animal cells appear to be: a) activation of glycogen phosphorylase by AMP[2], and b) conversion of phosphorylase from an AMP-independent to an AMP-dependent form (1). In addition, other mechanisms exist which could be thoretically useful to the cell. These include competitive inhibition of the phosphorylase by G-6-P, UDPG, and glucose (2). The in vivo importance of these, however, is not clear.

Yeast (Saccharomyces cerevisiae) and potato phosphorylase are reported to exist as only one, AMP-independent, type (3,4). Thus, the two most important control mechanisms of other systems are absent in these two cases. We have studied the properties of yeast phosphorylase further in partially purified preparations, and have found that other types of control properties are present. However, these do not seem to operate in vivo.

Footnotes- 1- Present adress: Dept. of Biology, University of Santo Domingo, Dominican Republic

2- Abbreviations: AMP- adenosine- 5' phosphate; G-6-P glucose -6 - phosphate; G-1-P - glucose -1- Phosphate; Pi- inorganic phosphate; UDPG- uridine diphosphate glucose; EDTA- ethylene diaminetetra- acetate.

A hypothesis is proposed to explain these observations.

Materials and Methods

Bakers yeast phosphorylase (Fleischman's) was assayed as described previously for the blue crab muscle phosphorylase (5), except that no AMP was required, and 10 minute intervals and a pH of 5.5 (3), were used. Activities are expressed in international units. Protein was determined with the Folin-Phenol reagent (6). Carbohydrates were determined with the Phenol- Sulfuric acid reagent (7). The cells were desintegrated with a prolonged cold acetone treatment, or in a Bronwill M S K cell homogenizer. Phosphorylase was further purified as follows (full details to be published): Phosphorylase was separated from glycogen in the extract by adsorption on calcium phosphate gel and washing, as described for yeast-glycogen synthetase (8). No amylase treatment was necesary to remove traces of the glycogen. Washing the gel with 20% ammonium sulfate (w/v) removed a substantial amount of inactive protein. The gel was dissolved in 0.1M EDTA, and the enzyme precipitated with ammonium sulfate at 45% saturation. The enzyme was dissolved in 0.01M Histidine, pH 5.5, and chromatographed in a 40 x 2.5 cm Sephadex G-200 column. The activity peak eluted slightly behind the void volume whereas the bulk of the protein eluted several fractions behind the phosphorylase. The enzyme was lyophilized for storage. Table 1 shows a typical purification scheme.

Results

We had previously reported the presence of only one form of the enzyme in yeast (3). Fractionation on Sephadex G-200 yielded in some cases two fractions with activity. By calibration of the column with protein markers, these were found to correspond to molecular weights of approximately 400,000 and 195,000 moles/g. (These were done on 70 x 1.5 cm columns). Fractionation by polyacrylamide gel disc electrophoresis in a pH 8.5 system (9)

Table 1

Partial Purification of Yeast Glycogen Phosphorylase

Fraction	Total Activity	Total Protein, mg.	Sp. Activity	Purification Factor	Recovery
Crude Extract	199.2	1130	0.19	—	100
Fraction dissolved in 0.01 M Histidine (After $(NH_4)_2 SO_4$ precipitation)	42.0	19.7	2.12	11	21
Combined fractions from Sephadex G-200 Chromatography	38.4	1.4	27.3	140	19

Details in text. Only the activity corresponding to a 400,000 molecular weight was obtained in this experiment. Specific activity: units per mg of protein.

and staining at pH 5.5, yielded four protein bands, three of which had enzymatic activity. The 3 phosphorylase bands accounted for about 50-60% of the total protein. However, both on Sephadex G-200 and polyacrylamide gel disc electrophoresis separation, all of the forms were equally active in presence or absence of AMP. Thus, they do not correspond to the enzymatically-interconvertible forms with differential AMP sensitivity of animal systems. They could correspond to changes in quaternary structure with concentration, a phenomenon which has been found with rabbit muscle phosphorylase a (10). It is also possible that they could be isozymes.

We have worked with the high molecular weight form obtained by Sephadex G-200 gel filtration, the form always present. This is the one which gives 3 activity peaks on disc electrophoresis. Thus, no discrimination between the different forms has been attempted. However,

Table 2

Effects of Glucose, G-6- P, and UDPG on Yeast Glycogen Phosphorylase

Effector	Concentration	Sp. Activity	% Inhibition
None	-	26.1	-
Glucose	50m M	24.5	6
G-6-P	3m M	11.2	57
UDPG	3.3m M	9.6	63

Phosphorylase was assayed as described in the text. Effectors were added to the assay medium at the final concentrations shown.

they are all AMP-insensitive and not enzymatically interconvertible.

Table 2 shows the effect of glucose, UDPG, and G-6- P on the activity of the enzyme. Inhibition by glucose is so low that we could not unequivocally determine its kinetic nature. UDPG and G-6-P, as in other systems (11, 12) are competitive inhibitors of G-1-P (not shown).

We previously reported a substantial affinity of the enzyme for glycogen, with Km values of 0.5 mg/ml (3). This value is considerably less than that of Neurospora (Km, 9 mg/ml) and E. coli (Km, 6.7 mg/ml) phosphorylase (11, 12). Further indication of this high affinity is the fact that the yeast enzyme precipitates at 45% ammonium sulfate saturation, but in crude extracts with high glycogen concentration (ca. 27 mg/ml), it precipitates, together with glycogen, at 75% saturation.

Discussion

Free glucose concentration inside a yeast cell is practically zero, since the permeation step is limiting with respect to its metabolism (13). Therefore, a 6% inhibition of yeast phosphorylase by 0.05 M glucose is of no consequence in cellular metabolism.

The inhibition shown by G-6-P and UDPG, however, must be analyzed more carefully. Studies on the levels of some intracellular metabolites in yeast under conditions leading to changes in rate of glycogen synthesis (14) have shown that under N limitation (which leads to net glycogen synthesis) G-6-P levels were as high as 3m M. Disregarding the possibility of compartmentation, such a level could cause a considerable inhibition of phosphorylase. However, no change in the rate of glycogen degradation was observed. UDPG levels did not reach 1m M in these experiments (14), and were usually around 0.2m M. Values reported for strain 4236 of Saccharomyces (15), yield concentrations of less than 1.0m M. In strain 1338 we have obtained values of 2.4m M in the early stationary period due to C limitation (manuscript in preparation). This last value would inhibit phosphorylase about 50%. In the stationary period in this case, however, glycogen content is decreasing rapidly, and little net synthesis is taking place since N abundance presumably maintains G-6-P levels low (14). This suggests that there is little effect of UDPG on glycogen phosphorylase in vivo.

Since G-6-P and UDPG are inhibitors of phosphorylase in vitro, but seem to have little or no effect in vivo, there may be some cellular parameter which reduces their effect. Both substances are competitive inhibitors of G-1-P, probably because of direct active site competition. However, G-1- P is a product of the reaction in the cell; the substrates are glycogen and Pi. Since the inhibition of both effectors is non-competitive with respect to glycogen in the synthetic reaction, it appears reasonable that the inhibition in the degradative reaction is due to one of two mechanisms: a) the glucose moiety of the inhibitors block the site at which the terminal glucose residue of glycogen combines in degradation, or b) the phosphate

groups of the inhibitors block Pi. A combination of both effects is also possible, especially in th case of UDPG. Thus, competitive effects with respect to the substrate (s) are likely.

It is highly probable that in vivo the enzyme is bound to glycogen particles, where the end-groups concentration is high. The intracellular Pi concentration in yeast has a value of around 0.02 M (16). Therefore, the enzyme in vivo appears to be present in an environment of high substrate saturation, and this counteracts the effects of competitive inhibitors.

The possibility that phosphoglucomutase is the rate-limiting step in glycogen degradation, thus masking the effects of phosphorylase inhibitors, can be eliminated by the observation, quoted before, that no increase in glycogen degradation occurs when the G-6-P concentration decreases sharply (14).

In order to account for anomalies in the levels of UDPG of glycogen deficient mutants of strain 4236, which we have also observed in mutants of strain 1338 (manuscript in preparation), it has been proposed that recycling of G-1-P, formed by phosphorylase, into UDPG and glycogen takes place (15). In the apparent absence of control of phosphorylase activity by intracellular metabolites, we feel that recycling of G-1-P may be a mechanism of major importance in controlling the overall rate of glycogen degradation in yeast cells. The degree of recycling may be determined by the intracellular concentration of G-1-P, and by the action of intracellular metabolites on the pyrophosphorylase and/or synthetase.

These observations, of course, do not eliminate the possibility of control of phosphorylase levels in yeasts, nor the possibility that the different forms of the enzyme may have different substrate and/or effector affinities and that their relative levels may be varied. Both of these, however, would be long-range mechanisms.

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