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# GLYCOGEN PHOSPHORYLASE ACTIVITY IN PERMEABILIZED CELLS OF *SACCHAROMYCES CEREVISAE*

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## Summary

The velocity of formation of glucose 6-phosphate and glucose by protamine-treated permeabilized yeast cells was dependent on the concentration of inorganic phosphate. Fluoride inhibited the reaction. Only small amounts of glucose 6-phosphate were produced in presence of ATP and glucose. We conclude that we are measuring the glycogen phosphorylase reaction inside the permeabilized cells.

We have investigated the possibility of measuring the activity of glycogen phosphorylase (α-1,4-glucan:orthophosphate glucosyltransferase, EC 2.4.1.1 [1,2]) in permeabilized cells of *Saccharomyces cerevisiae* prepared by protamine treatment [3]. These structures, although not viable, retain essentially all of the macromolecular components. Their low molecular weight compounds are lost and their permeability and other surface properties are altered, so that specific enzymic reactions can be measured by incubating them with appropriate substrates and measuring the products in the medium. It is presumed that some of the intracellular relationships are not totally altered, so that information relevant to real intracellular events can be obtained. Such an approach has been attempted with both yeast [3,4] and bacterial [5,6] permeabilized cells prepared by various means.

*S. cerevisiae* (Guiness Strain 1338) was grown for 48 h in bacto-peptone (5 g/l), yeast extract (4 g/l), malt extract (4 g/l), glucose (1%) at

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room temperature without aeration. Cells were collected by centrifugation, washed twice with distilled water, lyophilized, and stored in the cold. Permeabilized cells were prepared by incubation with protamine, as previously described [3]. Under phase-contrast microscopy many of the cells exhibited a mosaic-like structure on their outer surface and invaginations of the membrane (Fig. 1). They were not viable, and did not ferment glucose.

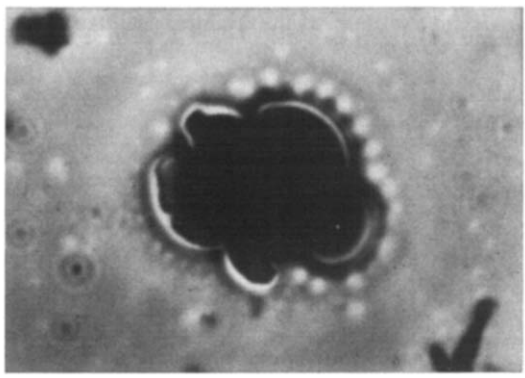

**Fig.1. Group of permeabilized yeast cells exhibiting a mosaic-like arrangement on their outer surface. An invagination of the membrane can be seen in one of the cells.**

Phosphorylase was assayed in the direction of glycogen degradation by shaking a cell suspension (8.0 mg/ml final concentration) in 10 mM Tris, pH 7.5 or 10 mM histidine, pH 6.5 or 6.8 buffers, and 1.0 mM $P_i$ at 30 °C. Aliquots of 5.0 ml were removed at intervals (usually 0, 30, and 60 min), rapidly centrifuged, and the supernatants frozen immediately. Glc-6-*P* was determined in a coupled assay system by measuring the formation of NADPH at 340 nm in presence of excess glucose-6-phosphate dehydrogenase. The actual system used is a slight modification of that used for the direct assay of phosphorylase [7]. Phosphoglucomutase was added to this system for the determination of glucose 1-phosphate. Glucose was determined with the glucostat reagent. Glycogen was extracted from yeast cells with 5 M NaOH followed by 0.5 M $HCl_4$ (modified from [8]), and assayed with the phenol–sulfuric acid reagent [9]. Part of the glycogen becomes extracted by the base, and must be precipitated with 3 vols of ethanol and assayed separately.

Table I shows that considerable amounts of Glc-6-*P* and glucose are formed when these cells are incubated with $P_i$, but that hardly any products are formed in its absence. There was no detectable formation of glucose 1-phosphate, indicating that the equilibrium of the phosphoglucomutase reaction inside the cells favors Glc-6-*P* formation.

Although it did not seem likely that the Glc-6-*P* could be formed from $P_i$ through the formation of ATP, since these cells are largely depleted of intracellular small molecular weight compounds, we investigated this

TABLE I

FORMATION OF GLUCOSE 6-PHOSPHATE AND GLUCOSE IN PERMEABILIZED YEAST CELLS

The cell suspension (8.0 mg/ml final concentration) was incubated at 30°C in 10 mM Tris buffer, 7.5. Additions were made to the following final concentrations: $P_i$, 1.0 mM; sodium fluoride, 0.1 M; ATP—$MgCl_2$, 0.1 mM; glucose, 0.1 mM. Velocities of formation of glucose 6-phosphate and glucose are expressed as nanomoles formed/ml of reaction mixture/h.

| Additions to system | Glc-6-*P* | Glucose |
|---|---|---|
| None | 0 | 0 |
| NaF | 0 | 0 |
| $P_i$ | 116 | 180 |
| Pi+NaF | 38 | 0 |
| Glucose+ATP—Mg | 28 | — |
| Glucose+ATP—Mg+NaF | 0 | — |

possibility. As Table I shows only small amounts of Glc-6-*P* were formed in presence of glucose, ATP, and magnesium ions.

The fact that fluoride inhibits the formation of glucose completely indicate that the free sugar is formed by the action of phosphatases. In addition, fluoride causes a partial inhibition of the phosphorylase reaction, as it also does in vitro [10].

There was no reaction when the supernatants from the ghost suspensions were incubated with $P_i$ alone or in combination with glycogen; thus, the enzymatic activity resides inside the cells. Furthermore, there was no reduction of NADP in the assay system unless glucose-6-phosphate dehydrogenase was added, which showed that the results are not due to the formation of unspecific reducing compounds.

When the concentration of $P_i$ was varied from 0.75—4.0 mM, the velocity of product formation (the sum of Glc-6-*P* and glucose) varied following hyperbolic kinetics. $K_m$ values for $P_i$ at pH values of 6.5—7.5 were in the range of 1.0—2.0 mM. These values are close to those obtained for other phosphorylases in vitro [7] and for Glc-1-*P* in the yeast enzyme [1,2].

Since the reaction proceeds in the presence of $P_i$ alone, we conclude that the enzyme is acting on intracellular glycogen, which is present at a concentration of about 9%. This compares well with the level in the intact yeast wich is 10.5%. It appears that the immediate environment of the enzyme can be modified by altering the external environment, Thus, we feel that these permeabilized structures can be used for studies on some of the factors which control glycogen degradation in yeast [5].

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## References

1 Sagardia, F., Gotay, I.C. and Rodriguez, M. (1971) Biochem. Biophys. Res. Commun. 42, 829—835
2 Fossett, M., Muir, L.W., Nielsen, L.D. and Fischer, E.H. (1971) Biochemistry 10, 4105—4113
3 Schlenk, F. and Zydek-Cwik, C.R. (1970) Arch. Biochem. Biophys. 138, 220—225

4 Adams, B.G. (1972) Anal. Biochem. 45, 137—146
5 Liersch, M.H. and Preiss, J. (1967) Bacteriol. Proc. p. 110
6 Reeves, R.E. and Sols, A. (1973) Biochem. Biophys. Res. Commun. 50, 459—466
7 Sagardia, F. and Green, J.W. (1969) Biochim. Biophys. Acta 185, 80—87
8 Trevelyan, W.E. and Harrison, J.S. (1956) Biochem. J. 63, 23—32
9 Dubois, M.K.A., Gilles, K.K., Hamilton, P.A. and Smith, F. (1956) Anal. Chem. 28, 350—357
10 Rodriguez, M. (1968) M.S. Thesis, University of Puerto Rico, Rio Piedras, Puerto Rico

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