juice was decreased by active serum with the exception of the samples obtained from pernicious-anaemia patients.

In these experiments exposure to anti-intrinsic factor serum was found to decrease the vitamin B_{12}-binding capacity only of materials having intrinsic factor activity *in vivo*. Apparently anti-intrinsic factor serum can be used for the differentiation of non-specific and specific intrinsic factor binding.

It should be pointed out that one has to add different amounts of vitamin B_{12}, when testing samples with unknown vitamin B_{12}-binding capacity because it has been found that the percentage depression of vitamin B_{12} power in normal human gastric juice is more marked when its binding capacity is nearly saturated.

The vitamin B_{12}-binding capacity of serum itself may influence the results to a limited degree. This can be avoided by the use of a 34% ammonium sulphate precipitate of serum, which contains all anti-intrinsic factor activity in the precipitated γ-globulins and does not bind the vitamin B_{12} (ref. 4).

The estimation of vitamin B_{12}-binding power susceptible to inhibition by anti-intrinsic factor serum provides a more specific method for detecting intrinsic factor *in vitro*, which can be used for screening fractions obtained in purification procedures. The assay of human gastric juice by this method shows promise as a convenient diagnostic technique in pernicious anaemia, because it has the advantage of avoiding the administration of radioactive material to the patient, which is required for the performance of the presently available diagnostic tests based on the absorption of labelled vitamin B_{12}.

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Received November 9th, 1962
Revised manuscript received January 30th, 1963

PN 1225

Gene-controlled facilitated diffusion and active transport of α-thioethylglucopyranoside in *Saccharomyces cerevisiae*

HAWTHORNE[1] has established that the complementary gene pairs MG_1MG_2, MG_2MG_3, or MG_4MA_1 control α-methyl glucoside fermentation in *Saccharomyces cerevisiae*. MG_1 and MG_3 are regulatory genes for isomaltase synthesis[2,3] and MA_1 a structural gene for α-glucosidase (EC 3.2.1.20).

Abbreviation: TEG, α-thioethylglucopyranoside.

The uptake of non-fermentable sugars by yeast, which has been studied by a number of workers, has been characterized by an paucity of active transport systems[4]. Sugar uptake is stereospecific and the rate of entry of sugar into the cell obeys saturation kinetics. In the case of fermentable sugars, SOLS AND DE LA FEUNTE[5] have observed that the rate of uptake of sugar is faster than would be expected for facilitated diffusion. On the other hand, in *Escherichia coli* the uptake of β-galactosides involves active transport and can under certain conditions reach concentrations 3000–10000 times that of the external concentration[6].

It is the main aim of this communication to report the existence of both facilitated-diffusion and active-transport mechanisms in *S. cerevisiae* for the uptake of α-thioethylglucopyranoside. These two uptake systems are both controlled by the MG_2 gene.

Strain 5002-5C ($maMG_1MG_2mg_3$) was pre-grown in a medium containing 0.5 % $(NH_4)_2SO_4$, 0.2 % KH_2PO_4, 0.05 % $MgSO_4 \cdot 7H_2O$, 0.2 % peptone, 0.1 % yeast extract with either 5 % glucose or 2 % α-methylglucoside and then transferred to a medium containing 10^{-3} M ^{35}S TEG (1.0 mC/mmole) and 10^{-1} M acetate at 30° or 0°. At intervals samples were withdrawn, filtered on a millipore filter, washed with cold water and partially dried at room temperature. After separation of the yeast mass from filter paper, cells were distributed on planchets and counted.

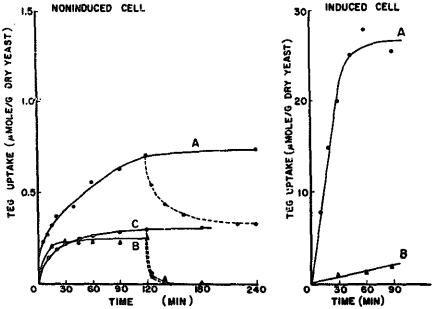

Fig. 1. Uptake of [^{35}S]TEG in non-induced and induced cells. Yeast strain 5002–5C was grown in a medium containing either glucose (non-induced) or α-methylglucoside (induced), washed and resuspended in acetate medium containing 1 mM [^{35}S]TEG and incubated at either 30° (curve A) or 0° (curve B). At 120 min a portion of the cell suspension was filtered, washed and resuspended in the original volume of acetate medium without TEG. As a control TEG uptake in glucose grown cells of 5001-2B ($mg_1mg_2mg_3$) was also followed at 30° (curve C).

The kinetics of TEG uptake in non-induced cells is shown in Fig. 1. In glucose-grown cells (5002-5C) TEG uptake reaches a maximum of 0.8 μmole/g dry wt. after 90–120 min at 30°, 40 % of which is lost upon removal of TEG. At 0° the maximum level was 40 % of that of at 30°; all radioactivity was lost following withdrawal of TEG. The latter is probably due to a free diffusion into a space between cell wall and cytoplasmic membrane. In all recessive strains (mg_2) the uptake of TEG is

maximal at 0.4 μmole/g dry yeast, is completely lost by washing and the K_m for the rate of TEG uptake is infinite. On the other hand the difference between the amount of TEG taken up at 30° and 0° is consistent with a facilitated diffusion process. The internal concentration of TEG does not exceed the external concentration. A reciprocal plot of uptake rate *versus* concentration is linear and has a K_m of 0.05 M. The process is stereospecific since TEG uptake is inhibited by α-methylglucoside and glucose. The TEG uptake, however, is insensitive to azide and dinitrophenol.

In cells which are grown in α-methylglucoside (Fig. 1), TEG accumulation at 30° reaches 100 times the concentration of that in glucose-grown cells. The internal TEG flows out according to a monomolecular reaction at 30° when TEG is withdrawn. This accumulation involves active transport since the internal concentration of TEG under certain conditions can reach 30 times that of the external medium, and inhibitors of energy-yielding reactions (azide, monoiodoacetate, arsenate, arsenite and *p*-chloromercuribenzoate) inhibit the process. The K_m for active transport of TEG (1.4 mM) is lower than that for facilitated diffusion. TEG as well as α-methylglucoside can induce the accumulation system. The induced active transport system is also stereospecific since maltose, trehalose, α-methylglucoside and low concentrations of glucose competitively inhibited the uptake, whereas melibiose, cellobiose, β-methylglucoside, lactose and galactose had no effect.

TABLE I

UPTAKE OF TEG BY SELECTED GENOTYPES

For experimental conditions see legend Fig. 1. Non-induced cells were grown in glucose medium and induced cells in glucose medium containing TEG. Total uptake of 1 mM TEG was measured after 120 min at 30° and the non-washable uptake, 15 min after withdrawal of TEG. The cytoplasmic volume (2.1 ml/g dry wt. of cells) was determined by direct microscopic measurements. From this concentration ratio of TEG in the cytoplasm to that in the medium (C_i/C_{ex}) was calculated.

Strain	*Genotype*	*Non-induced*			*Induced*	
		Total μmoles/g	*Non-washable μmoles/g*	C_i/C_{ex}	*Total μmoles/g*	C_i/C_{ex}
5002-5C	$MG_1MG_2mg_3$	0.83	0.56	0.27	23.1	10.8
5001-1B	$mg_1MG_2MG_3$	0.91	0.47	0.22	34.4	16.2
5001-2B	$mg_1mg_2mg_3$	0.35	0.03	0.01	0.41	0.01
5001-2C	$mg_1MG_2mg_3$	0.65	0.39	0.19	27.8	13.0
5001-5A	$mg_1MG_2mg_3$	0.72	0.45	0.21	13.3	6.1
5002-1B	$MG_1mg_2mg_3$	0.29	0.01	0.00	0.33	0.01
5001-5D	$mg_1mg_2MG_3$	0.31	0.04	0.01	0.26	0.00

The entrance of TEG into cells with various genotypes is summarized in Table I. Facilitated diffusion in non-induced cells is observed only in strains carrying MG_2. In addition only these same strains have an inducible active transport mechanism for TEG accumulation. MG_1 and MG_3, although regulatory genes for isomaltase synthesis[2,3], have no effect on the induction of the accumulation system.

In summary, *S. cerevisiae* possesses two mechanisms for TEG uptake, facilitated diffusion and active transport, both of which are controlled by the MG_2 gene.

This work was aided by grant from National Institutes of Health (E-1459). One of us (H.O.) was a postdoctoral trainee on National Institutes of Health Training Grant (2G-686).

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Received December 28th, 1962

Biochim. Biophys. Acta, 71 (1963) 229–232

PN 1219

Apparent uncoupling of the Na^+ and K^+ activation of the human erythrocyte membrane adenosine triphosphatase

Human erythrocyte membrane contains a Mg^{2+}-dependent ATPase activity which can be stimulated by the presence of Na^+ and K^+ (refs. 1, 2). Many properties of this enzymic activity and those of the active transport of cations in intact erythrocytes and "reconstituted ghosts" have been correlated[1–5]. It has been established, for example, that cardiac glycosides at the same concentrations which inhibit the pump flux of cations can also inhibit the increase in ATPase activity due to the presence of Na^+ and K^+. Thus there is sufficient evidence indicating that this enzyme is intimately involved in the process of active transport of Na^+ and K^+ across the erythrocyte membrane.

A common feature of the enzyme preparations reported previously, on which some of the proposed models for active transport have been based[3], is the need for the simultaneous presence of Na^+ and K^+ for the increase in the activity of the enzyme. The ATPase of the ghosts and fragmented ghosts, prepared by POST *et al.*[1], was not activated by either Na^+ or K^+ alone, while several ghost preparations reported by DUNHAM AND GLYNN[2] were slightly activated by K^+ but not at all by Na^+. This communication presents preliminary data on the preparation and properties of an ATPase associated with erythrocyte-membrane fragments which can be activated by either Na^+ or K^+ alone.

Ghosts were prepared from washed erythrocytes by hemolysis in water and repeated washing in dilute Tris buffer according to POST *et al.*[1]. The properties of these ghosts were similar to those of the preparations reported by DUNHAM AND GLYNN[2]. Slight activation of the ATPase was observed with K^+. Sodium alone did not stimulate the activity. A suspension of ghosts in $5 \cdot 10^{-4}$ M Tris buffer (pH 7.4) was treated with ultrasonic waves generated by a Branson Model S-75 Sonifier for