Uptake of D-Glucosamine by *Saccharomyces cerevisiae*

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**ABSTRACT.** D-Glucosamine does not serve as a metabolic substrate in *Saccharomyces cerevisiae* although it stimulates by 15% endogenous respiration. It is taken up by a system or systems shared with D-glucose, D-fructose and D-xylose but apparently not fully with 2-deoxy-D-glucose. Its half-saturation constant is $38 \pm 14 \text{ mmol/L}$, in agreement with its inhibitor constant versus D-glucose and D-xylose uptake. Its maximum rate is $69 \pm 17\mu\text{mol per g dry mass per min}$. The transport is thermodynamically passive but D-glucosamine distribution follows the membrane potential, reaching ratios of $80:1$ at pH 7.5 and about $1:1$ at pH 4.0. These ratios decrease with increasing D-glucosamine concentration as well as with increasing suspension density, and are affected by metabolic inhibitors.

The only studies on D-glucosamine uptake by yeast are those of Höfer's laboratory (Niemitz *et al.* 1981), done on the actively transporting yeast *Rhodotorula gracilis*, the haploid form of *Rhodosporidium toruloides*. As D-glucosamine lends itself to derivatization (such as dansylation) better than nitrogen-free sugars do, it was examined here whether and to what extent D-glucosamine is transported by the previously described (Kotyk 1967) systems of monosaccharide transport in *Saccharomyces cerevisiae*.

**MATERIAL AND METHODS**

Yeast strain and its cultivation. *Saccharomyces cerevisiae* K (CCY 21-4-60) grew in a yeast extract-glucose medium as described before (Kotyk and Michaljaničová 1979). After harvesting, the cells were washed in distilled water and aerated in suspension on a magnetic stirrer for 2 h.

Uptake of D-glucosamine. Yeast suspension was prepared generally to a density of 2 - 3 mg dry mass per mL and the pH was maintained at 6.5 with a triethanolamine-phthalic acid buffer (0.1 mol/L). Incubation was done in 25-mL Erlenmeyer flasks agitated in a Dubnoff water bath. Samples (0.15 mL) were
removed at suitable time intervals (starting with 30 s and exponentially proceeding up to 64 min), filtered through a Synpor 5 membrane filter (0.65 μm pores; *Synthesia*, Czechoslovakia), and washed with 2.5 mL ice-cold water. The filters with adhering cells were counted in a toluene scintillation cocktail in a Varian scintillation spectrometer.

The membrane potential was estimated from the distribution of labelled tetraphenylphosphonium bromide, using the Nernst equation (cf. Vacata *et al.* 1981).

Reagents. 14C-Labelled D-glucosamine was from the Institute for Research, Production and Uses of Radioisotopes (Czechoslovakia). It was checked by thin-layer chromatography that it contained less than 2 % radioactive compounds other than D-glucosamine, all of them in the high-molar-mass range. Still, the glucosamine strip was eluted and the eluate used in all experiments. Uniformly 14C-labelled D-glucose was from the same institute while uniformly 14C-labelled D-xylose was from Amersham International (United Kingdom). 14C-Labelled tetraphenylphosphonium bromide was a custom-made sample from Hoechst (FRG).

All nonlabelled compounds were provided by Lachema (Czechoslovakia) and were marked as analytical-grade reagents. Again, it was made sure that the D-glucosamine used was free of any traces of other sugars.

**RESULTS AND DISCUSSION**

**Absence of D-glucosamine metabolism**

It was first established to what extent D-glucosamine can serve as respiration substrate for this yeast strain and thus be converted to other metabolic intermediates. This was done in two ways.

1. **By measuring the QO₂ of the yeast in the presence of D-glucosamine manometrically, using concentrations of 50 mmol/L. This yielded values indistinguishable from endogenous respiration in any of the three experiments carried out.**

2. **By following the production of 14CO₂ from universally labelled D-glucosamine in a double-sidearm Warburg vessel as it was trapped in a KOH-soaked filter paper. Using various sugars for comparison the production was as follows (in cpm per mg dry mass per unit radioactivity in 30 min): D-glucose 3365, D-fructose 1855, D-xylose (nonmetabolized) 4, D-glucosamine 8.**

Still, when using universally labelled 14C-D-glucose-grown yeast and measuring its CO₂ production, the same sugars as above stimulated its “endogenous rate” by a factor of 1.95, 2.87, 1.03 and 1.15, respectively, the last value being significantly different from 1.00. This may be due to D-glucosamine drawing on the energy of the yeast cells by dissipating slightly the membrane potential which is immediately re-established. The absence of such stimulation in the manometric experiments was apparently caused by the low accuracy and high scatter of the relatively low QO₂ values (between 5.4 and 7.8 μL O₂ per mg dry mass per h).