Zero-*trans* uptake rates determined with starved yeast suspensions underestimate the activity of sugar-proton symporters

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Zero-*trans* rates of sugar uptake into microbes are typically measured using cells harvested, washed to remove growth media, stored in nutrient-free buffer at 0 °C and then very briefly incubated with radio-labelled sugar. We studied lactose transport by *Kluyveromyces lactis* CBS2359 and two *Saccharomyces cerevisiae* recombinants expressing *K. lactis LAC* genes for lactose permease (a proton symporter) and β-galactosidase. For cells harvested in mid-fermentation, zero-*trans* uptake rates of ^{14}C-lactose were 4 to 7-fold lower than lactose consumption rates during fermentation, although conditions (temperature, pH, lactose concentration, etc) were similar. We did not detect extracellular β-galactosidase activity that could help explain this discrepancy. Pre-incubation of the yeast suspension with 0.2-0.5 % glucose or fructose for about 5 min at assay temperature (20 or 30 °C) immediately before the transport assays caused a 2-fold increase in zero-*trans* uptake rates. The energy charge of the starved cells was low (0.5-0.6), but rapidly rose to > 0.9 during the incubation with glucose, due to increases in yeast ATP levels and decreases in ADP and AMP levels. Zero-*trans* uptake of ^{14}C-maltose by a brewer's yeast (also a proton-symport mechanism) was similarly increased by pre-incubation with glucose. We conclude that zero-*trans* uptake assays performed, as usually done, with starved yeast suspensions, can underestimate the uptake capacity of ATP-dependent sugar proton symporters.