Activity and localization of maltodextrin binding site mutants of glycogen synthase in *Saccharomyces cerevisiae*

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Background

Glycogen is a glucose polymer formed by the enzyme glycogen synthase and is used in many organisms to store chemical energy. *Saccharomyces cerevisiae* (baker's yeast) was used as a model genetic organism to study the activity and localization of glycogen synthase. Glycogen synthase is encoded by the genes *GSY1* and *GSY2*. *GSY2* is the gene responsible for the formation of Gsy2p, whose action accounts for ~90% of glycogen synthase activity; the remainder of total glycogen synthase activity stems from Gsy1p. Because glycogen synthase binds to glycogen, it can be used to determine glycogen localization. Glycogen synthase can appear in various locations throughout the cells, giving distinct patterns. Gsy2p has been shown to be regulated by phosphorylation. Phosphorylation of Gsy2p leads to inactivation of the enzyme, a decrease in glycogen storage, and a more localized pattern of glycogen synthase. Conversely, lowering the phosphorylation state of Gsy2p will result in increased glycogen production and delocalization of glycogen synthase throughout the cell. Glucose-6-P (glucose-6-phosphate) has the effect of activating glycogen synthase regardless of its phosphorylation state.

We obtained a set of plasmids from a collaborator that encoded Gsy2p mutated at sites believed to be involved with maltodextrin binding. Maltodextrin is a chain of 20 or fewer dextrose (dextrorotatory glucose) molecules with α(1→4) glycosidic bonds. A protein sequence involved in maltodextrin binding likely would also bind to glycogen. Our task was to discover the localization pattern shown by the maltodextrin binding site mutants of glycogen synthase using a GFP (green fluorescent protein) tag on *GSY2*. The goal of this study was to determine the effects of these Gsy2p maltodextrin binding mutants on glycogen synthase activity and localization as well as glycogen accumulation.

Materials and Methods

Materials

A number of yeast strains were used in these studies: *gsy1 gsy2* + PRS316 (empty vector), *gsy1 gsy2* + WT (wild type), *gsy1 gsy2* + S1A3, *gsy1 gsy2* + S2A3, *gsy1 gsy2* + S1/S2 AC-1, and *gsy1 gsy2* + S1/S2 AC-2.

Methods

Glycogen Synthase Assay[1]

Glycogen synthase activity was measured through a radiochemical assay incorporating [^{14}C] UDP glucose into glycogen.

Glycogen Determination Assay[2]

Glycogen content was determined by comparison to a standard curve of known amounts of glycogen, following digestion of glycogen into glucose by a coupled enzyme assay (hexokinase and glucose 6 dehydrogenase).

Iodine Staining[3]

Iodine staining was used as a qualitative colorimetric analysis where yeast colonies were exposed to iodine vapors, and darker brown stains correlated with higher amounts of glycogen present.

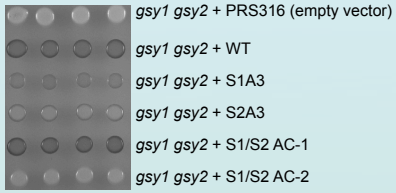
Glycogen Synthase Localization Studies[4]

Images taken from a Zeiss Axioskop 2 Plus equipped with a 100x oil immersion lens and an AxioCam MRm camera configured for epifluorescence were used to find the localization of glycogen synthase in sample cells by way of GFP marked plasmids. Hoechst 33342 was used to stain the nuclei of sample cells. The filters used for the bright field, nuclear stain, and *gsy2* + GFP were DIC, DAPI, and FITC, respectively.

Results and Discussion

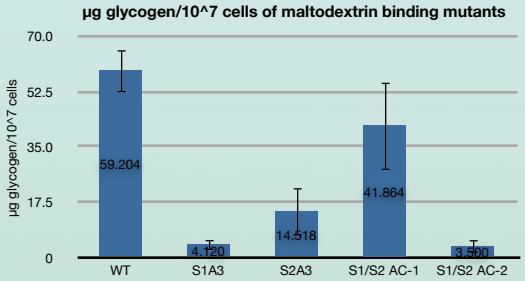
Iodine Staining

An iodine stain showed the strain with the empty vector making no glycogen and the wild type making a comparatively significant amount of glycogen. The *gsy1 gsy2* + S1A3 and *gsy1 gsy2* + S1/S2 AC-2 strains appeared to make little to no glycogen. The *gsy1 gsy2* + S1A3 mutant appeared darker than *gsy1 gsy2* + S2A3, which indicates a higher level of glycogen. However, quantitative data (see Figure 2) show this to be false. This suggests a different branching pattern, which affects the perceived intensity of the iodine staining.



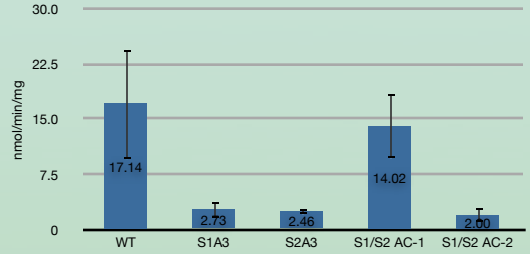
Glycogen Determination Assay

The levels of *gsy1 gsy2* + S1A3 and *gsy1 gsy2* + S1/S2 AC-2 were very low compared to wild type. All constructs were transformed into a *gsy1 gsy2* mutant.



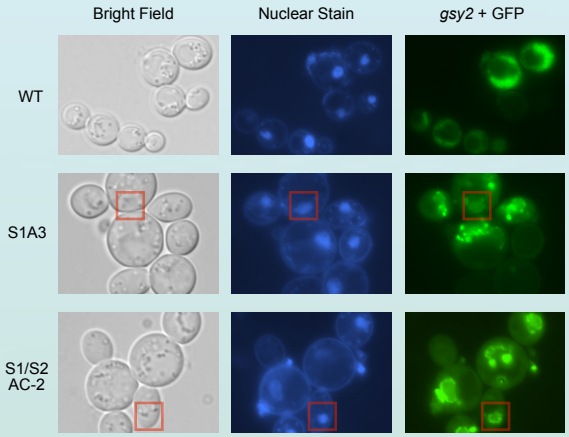
Glycogen Synthase Assay

Culture lysates were assayed with the addition of glucose-6-P to ensure full activation of Gsy2p to measure its activity. The data show a correlation between glycogen synthase activity and cellular glycogen accumulation. All constructs were transformed into a *gsy1 gsy2* mutant.



Glycogen Localization Studies

The *gsy1 gsy2* + S1A3 and *gsy1 gsy2* + S1/S2 AC-2 mutants expressed glycogen synthase activity concentrated around the nuclear periphery as opposed to the more diffuse pattern typically expressed by the wild type.



Conclusion

The amount of glycogen present in our yeast cells was essentially proportional to the activity of glycogen synthase. Our maltodextrin binding mutants synthesized varying degrees of glycogen, with the *gsy1 gsy2* + S1A3 and *gsy1 gsy2* + S1/S2 AC-2 mutants making very small, but measurable, amounts and *gsy1 gsy2* S2A3 making a reasonable amount of glycogen when compared to the wild type.

Localization studies showed that the mutants *gsy1 gsy2* + S1A3 and *gsy1 gsy2* + S1/S2 AC-2 exhibited glycogen synthase activity localized in the nuclear periphery. When the mutants were examined with a *gac* background (data not shown), the localization was more defined around the nucleus. The cause of this is unknown and is a topic for future studies.

[1] Thomas, J.A. Schlender, K.K., and Larner, J. (1968) Anal. Biochem. 25 486-499
[2] Chester, V.E. J. Gen. Microbiol. (1968) 51 49-56
[3] Wang, Z. Wilson W.A., Fujino, M.A., and Roach P.J. (2001) Mol. Cell. Biol. 21 5742-5752
[4] Invitrogen, Carlsbad, Calif., USA