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Structural insights into the inhibited state of Glycogen Synthase

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Glycogen is an osmotically inert polymer of glucose, synthesized during times of nutritional sufficiency so that it can be rapidly catabolized when there is an energy demand[1]. Glycogen synthase (GS) is responsible for the bulk of its synthesis by transferring glucose from UDPG to an existing glucose polymer[1]. Eukaryotic GS is allosterically activated by glucose-6-phosphate (G6P) and negatively regulated by covalent phosphorylation[2]. A cluster of six arginine residues are conserved across all eukaryotic species which determine the enzyme's ability to respond to these activating and inhibitory signals[2]. Prior structural studies from our lab had shed light on the dephosphorylated and activated state of the enzyme[3]. However, little is known on the phosphorylated state of the enzyme.

For structural studies on the inhibited state, we used the yGsy2R589/592A mutant as a surrogate since it has a basal activity state similar to the inhibited phosphorylated state. We solved the structure of the mutant to a resolution of 3.3 Å. While the overall structural arrangement of the tetramer is similar to the basal state enzyme, the interfaces are more closed. In particular, the N-terminal Rossmann-fold domain is rotated toward the interface by 5.9˚, limiting access to the active site by the acceptor end of the glycogen chain. Coincident with this domain closure, we also observed that the the distance between the regulatory helices of adjacent monomers are moved closer to one another. Based on this observation, we hypothesized we could develop a reversible redox regulatory feature in the enzyme by substituting cysteine residues for arginines 581 and 592, which lie across from each other at the interface. Consistent with our hypothesis, the yGsy2R581/592C double mutant exhibited very low activity, and could not be activated by G6P. However, normal function of the enzyme could be restored in the presence of reducing agents like DTT, BME and TCEP.

Taken together, our mutational work demonstrates that the conserved arginine cluster in the regulatory helix, both regulates the enzyme's response to signaling inputs and keeps the enzyme in a basal state conformation that is poised to respond to the activating and inhibitory inputs.

References:

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3. Structural basis for glucose-6-P activation of glycogen synthase. *Proc Natl Acad Sci U S A.* 2010 Oct 12;107(41):17563-8.