Allostery and Hysteresis are coupled in human UDP-glucose dehydrogenase

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Human UDP-glucose dehydrogenase (hUGDH) is regulated by an atypical allosteric mechanism in which the feedback inhibitor UDP-xylose (UDP-Xyl) competes with substrate for the active site. Binding of UDP-Xyl triggers the T131-Loop/α6 allosteric switch, which converts the hexameric structure of hUGDH into an inactive, horseshoe-shaped complex (E^{Ω}). This allosteric transition buries residue A136 in the protein core to produce a subunit interface that favors the E^{Ω} structure. Here we use a methionine substitution to prevent the burial of A136 and trap the T131-Loop/α6 in the active conformation. We show that $hUGDH_{A136M}$ does not exhibit substrate cooperativity, which is strong evidence that the methionine substitution prevents the formation of the low UDP-Glc affinity E^{Ω} state. In addition, the inhibitor affinity of $hUGDH_{A136M}$ is reduced 14 fold, which most likely represents the K_i for competitive inhibition in the absence of the allosteric transition to the higher affinity E^{Ω} state. hUGDH also displays a lag in progress curves, which is caused by a slow, substrate-induced isomerization that activates the enzyme. Stopped flow analysis shows that $hUGDH_{A136M}$ does not exhibit hysteresis, which suggests that the T131-Loop/α6 switch is the source of the slow isomerization. This interpretation is supported by the 2.05 Å resolution crystal structure of $hUGDH_{A136M}$, which shows that the A136M substitution has stabilized the active conformation of the T131-loop/α6 allosteric switch. This work shows that the T131-Loop/α6 allosteric switch couples allostery and hysteresis in hUGDH.