Mechanistic Origins of Enzyme Activation in Human Glucokinase Variants Associated with Congenital Hyperinsulinism

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Introduction

Human glucokinase (GCK) acts as the body's primary glucose sensor and plays a critical role in glucose homeostatic maintenance. It is uniquely capable of serving this role because displays an ultra-sensitive, positive cooperative response to its substrate glucose at physiological concentrations. Gain-of-function mutations in the *gck* gene produce hyperactive enzyme variants that cause congenital hyperinsulinism. Prior biochemical and biophysical studies suggest that activated disease variants can be segregated into two mechanistically distinct classes, termed α-type and β-type. Our results provide a detailed description of the molecular origins and functional alterations that occur in each of these activated, disease-associated GCK variants.

Experimental

Here, we report the results of comparative hydrogen−deuterium exchange mass spectrometry (HDX-MS) of wild-type GCK and representative activated variants. HDX-MS was conducted on the 21 T Fourier transform ion cyclotron resonance mass spectrometer housed at the NHMFL. We combine these structural investigations with viscosity variation studies and chemical quench-flow kinetic analyses to investigate mechanistic differences associated with steps that contribute to the catalytic rate (k_{cat}) for each enzyme.

Results and Discussion

Our combined viscosity variation and chemical quench-flow data indicate that β-type activation occurs via an acceleration of the product release step, such that it no longer partially limits the value of kcat. HDX-MS analysis suggests that β-type activation is not accompanied by significant differences in enzyme backbone structure and/or dynamics. In contrast, α-type activation results from a shift in the conformational ensemble of unliganded GCK in favor of a state that resembles the glucose-bound wildtype enzyme. This structural alteration does not appear to impact the partially rate-limiting process of product release or the presence of a burst phase, which is characteristic of both wild-type and α-type variants. Our HDX-MS results provide an improved level of understanding regarding the structural and dynamic origins of both activation mechanisms, as they substantially expand the data available from prior NMR investigations to include >90% of the polypeptide backbone for each enzyme. Both types of activated variants investigated herein reduce the kinetic cooperativity of wild-type human GCK. In particular, our results demonstrate that GCK can be activated by altering the enzyme's conformational ensemble or by mutations that perturb the kinetics of the partially rate-limiting product release step.

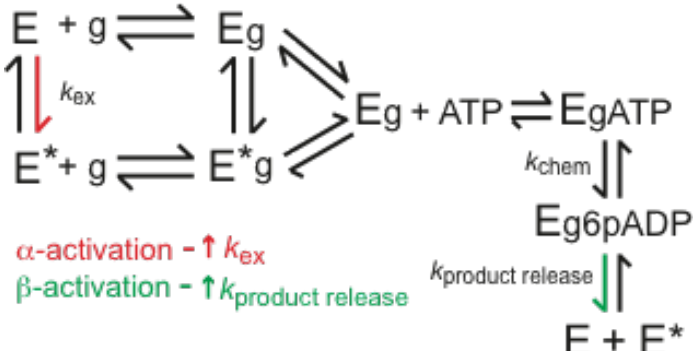

Fig. 1 The mechanism of GCK catalysis is highly complex. α-type activation arises when the rate of conformational exchange (k_{ex}) is shifted in favor of the compact GCK conformation. In contrast, the β-type activation mechanism relies on a shift in the rate of product release ($k_{product\ release}$), such that it is no longer rate-limiting for catalysis. Both activation mechanisms are operational in disease-causing GCK mutants.

Conclusions

The findings presented here confirm the existence of two distinct mechanisms of activation for human GCK and establish the molecular basis for the α- and β-activation processes. In particular, our results demonstrate that GCK can be activated by altering the enzyme's conformational ensemble or by mutations that perturb the kinetics of the partially rate-limiting product release step. Because both types of activated GCK variants contribute to human disease, our ability to uncover the underlying molecular origins of activation could facilitate the development of targeted therapeutic approaches for treating hyperinsulinemia unique to each type of activation mechanism.

Acknowledgements

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References

[1] Sternisha, S.M., *et al*. *Biochemistry*, **57**, 1632-1639 (2018).