Order–Disorder Transitions Govern Kinetic Cooperativity and Allostery of Monomeric Human Glucokinase

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Introduction
Glucokinase is a key metabolic enzyme that functions as the body’s principal glucose sensor. Glucokinase regulates the rate at which insulin is secreted by the pancreas by using a unique but poorly understood cooperative kinetic response to increasing glucose concentrations. The physiological importance of this enzyme is underlined by the fact that mutations in the glucokinase gene lead to a specific form of diabetes known as maturity-onset diabetes of the young type II (MODY II). In this study, we use solution NMR of all 17 specifically $^{13}$C$^{\delta_{1}}$-labeled isoleucines and the 3 $^{15}$N$^{\varepsilon}$-labeled tryptophan side chains to understand how the kinetic properties of glucokinase contribute to glucose homeostasis. We also address how a class of recently discovered small-molecule drugs, which hold promise as therapeutics for type 2 diabetes, function to enhance glucokinase activity.

Experimental
All NMR data were collected at 800 MHz proton field and equipped with a TCI cryogenic probe. The GCK NMR samples were prepared in potassium phosphate buffer (25 mM, pH 8.0), containing KCl (25 mM), DTT (10 mM), deuterated glycerol (5% v/v), and D2O (10% v/v). $^{1}$H- $^{13}$C methyl-TROSY HMQC and $^{1}$H- $^{15}$N HSQC experiments were recorded as matrices of 2048×390 and 2048×128 complex data points, respectively.

Results and Discussion
We find that the small domain of unliganded GCK is intrinsically disordered and samples a broad conformational ensemble (Figure 1A). However, in the presence of glucose or a small-molecule activator, the enzyme population shifts towards a more narrow, well-structured ensemble of states (Figure 1B). Our findings provide a new model for glucokinase cooperative kinetics, which relies on a slow order–disorder transition in response to glucose concentrations. Our results also reveal a universal mechanism of glucokinase activation, which may help the development of new antidiabetic agents.

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References
[1] Larion, M., et al., PLOS Biology, 10, in press.

Figure 1. Mechanism of GCK kinetic cooperativity. (A) The small domain of unliganded GCK is intrinsically disordered, giving rise to a broad conformational ensemble. (B) Glucose binding, activator binding, or an activating PHHI-associated mutation promotes folding of the disordered regions in the small domain, narrowing the conformational distribution. Upon formation of the GCK–glucose binary complex, ATP binds and catalysis proceeds with little additional reorganization. (C) Following product release, ordered unliganded GCK persists until the small domain undergoes an order–disorder transition on the millisecond time scale, allowing access to the “time delay loop” (red): Under low glucose concentrations, the delay loop is operational, leading to slow turnover and kinetic cooperativity. Under high glucose concentrations (or when GCK is activated), the delay loop is effectively bypassed, turnover is fast, and cooperativity is eliminated (green).