Millisecond Dynamics are Abolished in PHHI-like Activated Glucokinase Variants

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Introduction

Although activation of human pancreatic glucokinase (GCK) by small molecules has been pursued since early 1990 and the discovery of small molecules that can activate this enzyme were a great promise, the clinical results have been quite disappointing [1]. These results revealed undesirable side effects such as: increased circulating triglyceride levels, increased blood pressure and hypoglycemic episodes, suggesting limitations in their use. Quantification of dynamics responsible for activation of GCK will shred some light into the molecular mechanisms by which this enzyme can be activated and will contribute to the design of better activators. Here, we assigned the 2D ^{1}H-^{13}C HMQC-TROSY spectrum of a model activating variant, the α13 helix variant, specifically labeled in the δ position of Ile with $^{13}CH_3$ via site-directed mutagenesis and quantified the dynamics associated with GCK activation.

Experimental

GCK was produced recombinantely in *Escherichia coli* BL21(DE3) strain in highly deuterated background and labeled specifically with $^{13}CH_3(\delta)$ Ile or $^{13}CHD_2(\delta)$ Ile, respectively. In collaboration with Dr. Vitali Tugarinov (NIH) pulse sequences for relaxation dispersion experiments were implemented on a 800 MHz spectrometer and relaxation dispersion profiles were recorded in different liganded states for the α13 variant of GCK. Each dispersion profile resulted from 20 time points of 2D ^{1}H-^{13}C HMQC-TROSY spectra recorded for approximately 4 hours.

Result and Discussion

Most of the unliganded α13 helix variant cross-peaks display faster dynamics than the wild-type enzyme suggesting a mechanism of activation via an acceleration of conformational exchange processes in agreement with the previously proposed functional model [2].

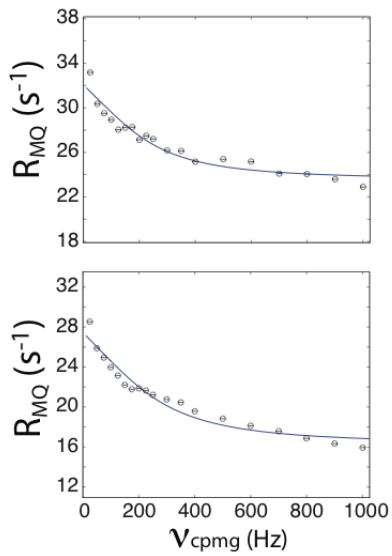

Figure 1. Representative examples of relaxation dispersion profiles coming from cross-peaks of α13 helix.

Conclusion

This study represents the first quantification of site-specific dynamics on activated states of GCK using CPMG NMR.

Acknowledgements

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References

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[2] Larion, M., *et al.,* PLOS Biology, **10(12),** 1-9 (2012).