Quantification of μs-ms Dynamics Responsible for the Kinetic Cooperativity Effect in Human Pancreatic Glucokinase

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Introduction

Quantification of dynamics responsible for generating kinetic cooperativity in glucokinase are critical in understanding the complex molecular mechanism by which this enzyme maintains glucose concentrations constant. Previously, we assigned the 2D ^{1}H-^{13}C HMQC-TROSY spectrum of glucokinase specifically labeled in the δ position of Ile with CH_3 via site-directed mutagenesis and developed a protocol for producing the enzyme in highly deuterated background.

Experimental

Glucokinase 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 pulse sequences for relaxation dispersion experiments were implemented on a 800 MHz spectrometer and relaxation dispersion profiles were recorded in different liganded states as well as PHHI-inducing variants of glucokinase. Each profile resulted from 20 time points of 2D ^{1}H-^{13}C HMQC-TROSY spectra recoded for approximately 2 hours.

Results and Discussion

Most of the unliganded glucokinase cross-peaks display relaxation dispersions with the k_{ex} and population distribution in agreement with the previously proposed functional model [1].

Conclusions

This study represents the first quantification of site-specific dynamics on wild-type and activated states of glucokinase using CPMG NMR.

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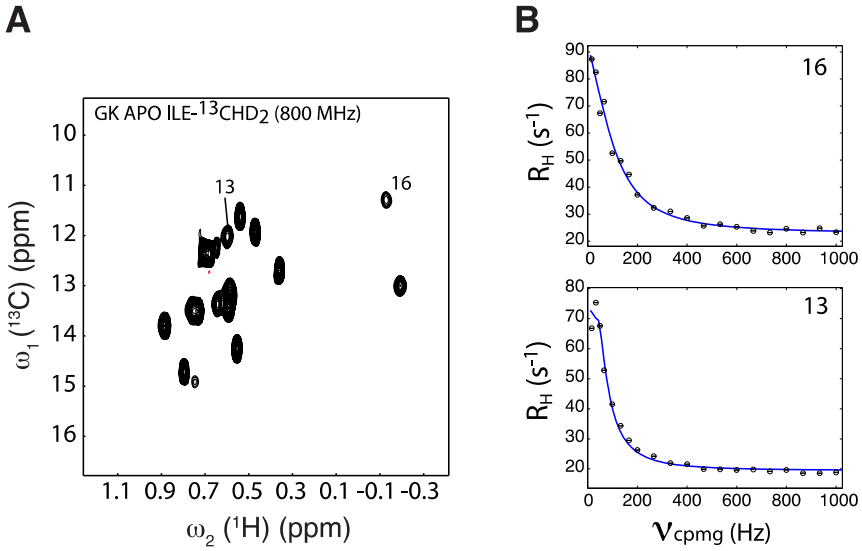
References

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



(A) 2D ^{1}H-^{13}C HSQC-TROSY spectrum of unliganded glucokinase labeled with $^{13}CHD_2$ (δ) Ile. (B) Representative examples of relaxation dispersion profiles coming from cross-peaks 16 (upper panel) and 13 (lower panel).