Biliverdin Reductase B is Inherently Dynamic

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Introduction

Human Biliverdin Reductase B (BLVRB) has recently emerged as a critical redox regulator that can control hematopoietic cell fate and we have shown that human BLVRB is highly abundant in red blood cells that are in turn the most highly abundant cell in the human body. However, little is known in regard to how this ubiquitously expressed enzyme functions, much less the role of dynamics in function. However, our very interest in the dynamics of enzymes is clouded by the difficulty in actually studying enzymes due to line broadening and poor coherence transfers in 3D experiments. Thus, we have utilized differential temperature dependencies to assign and fully characterize BLVRB as a tool to pinpoint how critical residues within the BLVRB active site "clamp" over its coenzyme.

Experimental

HNCA and HN(co)CA were collected at 10° C and 20° C for apo BLVRB on a Bruker 800 at the High Magnetic Field Laboratory (NHMFL) along with temperature titrations. R2-CPMG dispersion collected at the Rocky Mountain 900 facility.

Results and Discussion

We have utilized temperature to successfully assign the backbone resonances of BLVRB (Fig. 1a), which has facilitated R2-CPMG studies that have identified motions within the active site (Fig. 1b). While this indicates the presence of μs-ms timescale motions, many of these active site loops exhibit elevated R2 relaxation rates even at the highest applied field, which also indicates faster timescale motions in the μs timescale. These studies have elucidated the role of inherent enzyme motions for coenzyme binding, as we have recently reported [1].

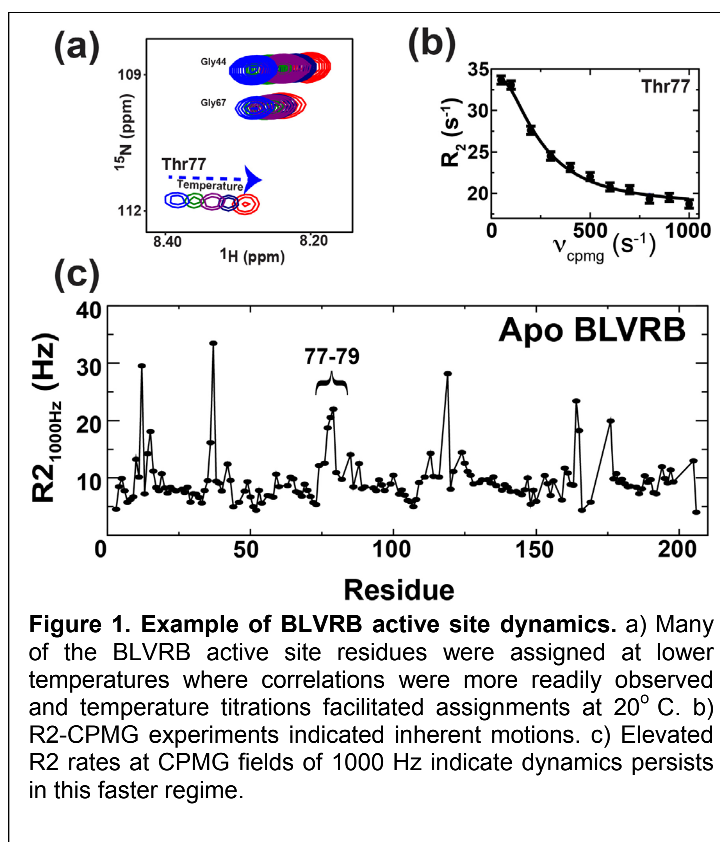

Figure 1. Example of BLVRB active site dynamics. a) Many of the BLVRB active site residues were assigned at lower temperatures where correlations were more readily observed and temperature titrations facilitated assignments at 20° C. b) R2-CPMG experiments indicated inherent motions. c) Elevated R2 rates at CPMG fields of 1000 Hz indicate dynamics persists in this faster regime.

Conclusions

BLVRB dynamics are on multiple timescales and represent evolutionarily fine-tuned motions to facilitate coenzyme binding.

Acknowledgements

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References

[1] Paukovich, N., *et al*., Journal of Molecular Biology, **430**, 3234-3250 (2018).