Characterizing the Inherent Dynamics of Cyclophilin-A

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Introduction

While our understanding of protein sequence and structure has grown exponentially within the last 20 years, the role of dynamics in protein function has only recently begun to be studied at atomic resolution. Advancements in NMR relaxation experiments that probe protein motions on the micro-millisecond (μs-ms) timescale have allowed us to begin studying a diverse array of dynamic functions that include the dynamics associated with enzyme conformational changes (*1*, *2*). Here, we have used multiple temperatures and multiple static field strengths to characterize the inherent dynamics of Cyclophilin-A (CypA) using R2-CPMG experiments.

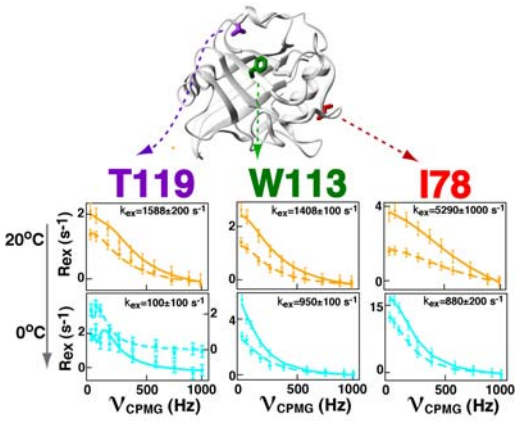

Figure 1. Several chemical exchange processes occur within free CypA as exemplified for T119 (magenta), W113 (green), and I78 (red). The R2-CPMG dispersion profiles are shown for these residues at 20°C (orange) and 0°C (light blue) at both 600 MHz (dashed line, ■) and 900 MHz (straight line, ●). The extracted exchange rates, k_{ex}, are shown for each simultaneous fit of the data at both static magnetic fields. For T119 the axis for each field at 0°C and 10°C are offset for 600 MHz (right axis) for clarity since there is no static magnetic field dependence for this residue at lower temperatures.

Experimental

Transverse Relaxation Optimized SpectroscopY (TROSY)-based ^{15}N-R2-CPMG pulse sequences were applied for relaxation dispersion experiments on either a Varian 600 MHz (at NHMFL) or 900 MHz spectrometer (at the Rocky Mountain 900) collected at 0°C, 10°C, and 20°C. Exchange rates (kex) were extracted from R2-CPMG dispersion profiles using the program CPMG_FIT.

Results and Discussion

We have discovered that multiple exchange processes occur within free CypA and thus cannot be described simply by a single cooperative dynamic exchange process previously proposed (*2*). These exchange processes are highly localized and range from relatively fast motions on one side of the protein to much slower motions on the other side with corresponding exchange rates that vary by approximately six-fold (i.e. from 1000-6000 s^{-1}). Moreover, while many residues exhibit different temperature dependencies as shown by their R2-CPMG dispersion profiles, the central core residues of CypA exhibit the least changes in their μs-ms motions with temperature (Fig. 1, W113 for example).

Conclusions

The inherent μs-ms motions of CypA include numerous dynamic events that exhibit markedly different temperature dependencies. This work implies that CypA does not simply undergo a global exchange event, but a much more complicated exchange.

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References

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2. E. Z. Eisenmesser *et al.*, *Nature* **438**, 117 (Nov 3, 2005).