

Evidence for Partially Unfolded Intermediates after the Rate-limiting Transition State of Apocytochrome b₅₆₂

R. Chu, R. Knowlton, M. Andrykovitch, X. Ji, and Y. Bai (NCI)

Abstract No. chu0467

Beamline(s): X9B

Introduction: It has been suggested that the unfolding of N- and/or C-terminal helices of apocytochrome *b*₅₆₂, a four-helix bundle protein, occurs earlier than that of the two middle helices and leads to partially unfolded forms under native conditions. To see whether these partially unfolded forms populate before or after the rate-limiting step of folding, the kinetic folding of apocytochrome *b*₅₆₂ and its stable mutants were elucidated.

Methods and Materials: Stopped-flow and equilibrium fluorescence and CD; single crystal X-ray diffraction.

Results: The folding is apparently a two-state process. Importantly, the rate-limiting transition state has the two-middle helices folded and the N- and C-terminal helices unfolded.

Conclusions: These results suggest that the partially unfolded forms of apocytochrome *b*₅₆₂ populate after the rate-limiting step of folding.

Acknowledgments: We thank Dr. Zbigniew Dauter for his kind assistance and suggestions during data collection and processing at synchrotron beamline X9B in Brookhaven National Laboratory.