

R1 side chain mobility and solvent accessibility were scanned using cw-EPR and the power saturation technique. Both parameters show significant changes at multiple sites which are widely scattered over the peripheral domain of cdb3 indicating that the binding interface involves multiple discontinuous patches rather than the single binding motif revealed by previous mutation deletion studies. The global structure of the complex has been investigated by determining multiple inter-molecular distance constraints using DEER (double electron electron resonance) between selected sites on the peripheral domain of cdb3 and surface sites on the backbone region of ankD34. The measured distances are not consistent with the previous docking model reported in the literature (Michaely et al., *EMBO J.* 21(23):6387-96, 2002). The EPR and DEER data are now being utilized in concert with molecular modeling approaches to construct a new structural model for the cdb3•ankD34 complex. Supported by: NIH P01 GM080513.

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Structural Dynamics Of Myosin'S Light Chain Domain In A Pre-power Stroke Conformation

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Cross-linking the two most reactive Cys of the myosin catalytic domain (CD) (SH1 and SH2) inhibits force production and ATP hydrolysis and locks myosin in a weak actin-binding conformation. Recent work using a bifunctional spin label (BSL) to crosslink SH1 and SH2 has shown that the CD is immobilized and orientationally disordered, suggesting that cross-linking traps myosin in an intermediate state that primes the myosin head to generate force (Thompson et al., BJ, in press). In the present study, we measured light chain domain (LCD) structural dynamics in muscle fibers as a function of SH crosslinking. If the CD really is orientationally disordered by crosslinking and the two domains are structurally coupled, some of this disorder should be propagated to the LCD. To measure LCD structural dynamics, we used site-directed spin labeling to label chicken gizzard regulatory light chain (RLC), and then exchanged this labeled RLC for the native RLC in rabbit psoas fibers. Prior to crosslinking, EPR spectra acquired with the fiber axis parallel and perpendicular to the external field were very different, but cross-linking decreases this difference, indicating increased disorder. Saturation transfer EPR on these fibers showed that the heads remained immobile and thus attached to actin. These results support our hypothesis that SH1-SH2 crosslinking traps an actomyosin complex, possibly the first force-generating state in the power stroke, in which the CD is highly disordered and LCD is partially disordered, indicating a partially flexible linkage. A secondary goal of this research is to improve the technology for EPR on muscle fibers by developing a novel high-sensitivity EPR resonator for analysis of spin-label mobility, orientation, and force on labeled fibers.

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Interactions of Lipoygenase with Paramagnetic Substrate Analogs

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How fatty acid substrates and inhibitors enter and reside in the internal cavity of lipoygenases is still unresolved. A medium-resolution, solution structure of lipoygenase is obtained by positioning spin labels on a grid of five sites near the protein surface, all 20-30 Å distant from iron. Cysteine substitutions, for subsequent spin labeling, are made on a background of soybean lipoygenase in which serine replaces four native cysteines. Dynamics and solvent exposure of the spin labeled side chains are characterized by solution electron paramagnetic resonance (EPR) spectra in the presence and absence of viscosogens and fatty acid substrate analogs. Additionally, more detailed structural information is obtained from site directed spin labeling of helix-2 to examine how

binding of inhibitors influences the conformation of this critical helix. Examples of changes in separation of outer hyperfine extrema in the EPR spectra when 30% sucrose is added include the following. For helix-2: spin labeled mutants T259C (change=0.17mT); K260C (mobile); F270C (0.11mT); and for grid points: L480C (0.16mT); L541C (0.16mT); and A569C (mobile). This study precedes determining the location of paramagnetic substrate analogs by examining dipolar-determined distances between the protein labeled sites and spin labeled substrate analogs.

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Solute Effects on Spin Label Mobility for Aqueous Exposed Sites on HIV-1 Protease

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We investigated the effects of four solutes on the protein rotational correlation time, the local dynamics of the protein backbone, and the mobility of labels covalently attached to multiple aqueous exposed sites in the HIV-1 protease (HIV-1 PR) using X-band continuous wave (CW) and pulsed electron paramagnetic resonance and fluorescence anisotropy. The HIV-1 PR contains two Beta-hairpin flaps that sit over the active site and are believed to undergo a large conformational change to allow the substrate access to the active site. The solutes used in this study were glycerol, sucrose, PEG3000 and Ficoll400 providing for a comparison between hydrophilic solutes (sucrose and Ficoll) and solutes that can interact with hydrophobic patches on the protein (glycerol and PEG3000) as well as a comparison between small viscosogens and macromolecular crowders. The CW-EPR line shapes for four commonly used nitroxide spin labels attached to the Beta-hairpin flap region of HIV-1 protease were found to have to largest change in the presence of glycerol and PEG3000 whereas sucrose and glycerol have the larger effects on the steady-state fluorescence anisotropy. Comparison of the CW line shapes and distance distribution profiles from the pulsed EPR experiments for HIV-1 protease in the absence and presence of inhibitor reveals that the presence of solutes affects the line shape by perturbing the spin label mobility via preferential interactions between the solutes and the spin label.

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Biochemical and Biophysical Characterization of the Dysfunctional E506Q MsbA Protein

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MsbA is a 65kDa ABC transporter found in the inner membrane of Gram-negative bacteria. The ABC transporter superfamily is one of the largest known and is responsible for the transport of a variety of substrates from lipids to antibiotics. ABC transporter dysfunction is involved in a range of human pathologies from cystic fibrosis to Stargardt's macular dystrophy. MsbA functions as a homodimer comprised of two nucleotide binding domains and two transmembrane domains and transports lipid A across the inner membrane of Gram-negative bacteria. MsbA is an essential protein in *E. coli* and its deletion or dysfunction results in the accumulation of lipid A in the inner membrane causing membrane instability and cell death. The E506Q mutation in the nucleotide binding domain is dysfunctional in MsbA and is a well-known mutation within the ABC transporter superfamily. To characterize this dysfunctional mutation for the first time in MsbA, *in vivo* growth assays, *in vitro* ATPase activity assays, and EPR spectroscopy studies throughout the ATP hydrolysis cycle were conducted. The E506Q mutation was paired with nine different reporter residues, each in or near a key nucleotide binding domain motif. Each pair has been characterized by site-directed spin labeling, ATPase assays, and an *in vivo* growth assay and compared to the reporter residues alone. To identify the stage in the ATP hydrolysis cycle in which the E506Q mutation is dysfunctional, the local tertiary interactions before, during, and after ATP hydrolysis were monitored by EPR spectroscopy at each stage of the ATP hydrolysis cycle, using ATP, ADP, and ATP and vanadate. With the ATPase activity, growth rate, and EPR spectroscopy compared between the reporters alone and paired with the E506Q mutation, the functional step at which the E506Q mutation dysfunction has been identified in MsbA.

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Calculation of the EPR Spectrum of a Small Nitroxide from Molecular Dynamics Simulations

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Algorithms have been developed for calculating the continuous wave electron paramagnetic resonance (EPR) spectrum of a nitroxide spin label from a free-induction decay (FID) derived from a molecular dynamics (MD) simulation. These algorithms have been successfully used to simulate the EPR spectra of two spin-labeled mutants of T4 lysozyme, T4L F153R1 and T4L K65R1

