CONFORMATIONAL DYNAMICS OF THE CYTOCHROME P450CAM-PUTIDAREDOXIN COMPLEX PROBED VIA 2D IR SPECTROSCOPY

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Protein conformational dynamics are at the root of many biological processes but are difficult to characterize experimentally because they occur at timescales that can range from picoseconds to milliseconds and longer. Sophisticated techniques must be used to measure dynamics occurring on fast timescales (fs or ps), such as dynamics of protein side chains or solvent in protein microenvironments. Two-dimensional infrared (2D IR) spectroscopy has emerged as a powerful tool for the measurement of protein dynamics and conformational heterogeneity at the picosecond timescale due to its high temporal and spatial resolution. However, the IR spectrum of a protein is typically severely congested due to the large number of similarly bonded atoms. For this reason, protein 2D IR is paired with site-specific incorporation of spectrally resolved IR probes that are active in the transparent frequency region (1800-2500 cm^{-1}) and thus act as vibrational reporters. Putidaredoxin is known to play an effector role on cytochrome P450cam, however the conformation of the cytochrome P450cam-putidaredoxin (P450-Pdx) complex is currently debated. The conformational dynamics of the P450-Pdx complex were measured using heme-bound CO as a vibrational probe of local environment. To further examine the proposed conformational state of the P450-Pdx complex, the dynamics of a P450cam mutant (L358P) thought to behave similarly to the putidaredoxin complex were also measured. The information gathered from 2D IR experiments has provided new insight into the conformational states exhibited by the P450-Pdx complex.