

## Employing electron paramagnetic resonance spectroscopy to the study of protein dynamics

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Electron paramagnetic resonance (EPR) spectroscopy was employed to study the dynamics in the substrate binding site of papain. The EPR spectrum of the spin labeled protein encodes information on the dynamics of the spin label and its surroundings. The dynamics are a combination of three modes of motion; tumbling of the macromolecule in solution, internal motions of the nitroxide and protein backbone fluctuations. By minimizing tumbling and assuming that internal nitroxide motions are largely independent from the factors being tested, differences in backbone fluctuations can be observed. The research project was intended to serve as testing and preparation for future research into the cold-adaptation of enzymes. The nitroxide spin label (1-oxyl-2,2,5,5-tetramethyl- $\Delta^3$ -pyrroline-3-methyl) methanethiosulfonate (MTS) was made to react with Cys-25 of papain to yield a disulfide bridge.

The effects of temperature, viscosity, denaturants and inhibitors on the EPR spectrum of papain were measured. Spin label mobility was reduced at low temperatures and in 40% sucrose solution, as expected. Not expected was the reduced mobility due to urea, at molarities insufficient for denaturation. This indicates a more closed structure of the substrate binding site, at least in the vicinity of Cys-25, or urea crosslinking the spin label with the protein backbone, f.ex. through hydrogen bonding. The urea molarities measured had little effect on the viscosity of solution. Despite overall protein structure being maintained at these molarities, protease activity was completely lost. The binding of a *p*-aminobenzamidine inhibitor reduced mobility considerably. The inhibitor binds directly to the substrate binding site of papain where the spin label resides, and thus likely reduces possible movements of both the spin label itself and the protein backbone.

The results imply that local backbone dynamics as well as interactions with surrounding main-chain atoms can be measured with EPR spectroscopy.