

Abstract

A number of different proteins are regulated by the second messenger 3'-5'-cyclic adenosine monophosphate (cAMP). Some of these proteins harbor a conserved cyclic nucleotide-binding domain (CNBD). Ion channels (CNG/HCN), nucleotide-exchange factors (EPACs), protein kinases (PKA/PKG), and transcription factors (CAP) are regulated by a CNBD in their activity. My aim was to study the dynamics of the CNBD of a cyclic nucleotide-gated ion channel from the bacterium *Mesorhizobium loti* (mlCNG-channel).

First, I have investigated the kinetics of ligand binding using the stopped-flow technique as well as flash photolysis of the novel caged compound BCMACM-8-NBD-cAMP. I can show that the on-rate of ligand binding for cAMP and 8-NBD-cAMP is lower than the diffusion limit. The low affinity mutant R348A is characterized by a 350-fold lower affinity towards 8-NBD-cAMP compared to the wild-type CNBD. In kinetic terms this is due to an increased off-rate of the mutant, whereas the on-rate is similar to that of the wild-type. Taken together with recent results from NMR spectroscopic studies of the CNBD, I conclude that structural changes induced by ligand binding do follow the *induced fit* mechanism, i. e. structural changes are a direct consequence of ligand binding.

However, to get a more detailed picture of the dynamics of secondary structure rearrangements, I wanted to measure distance changes within the CNBD in a time-resolved manner. Intramolecular distances of unpaired electrons are eventually measured by the double electron-electron resonance (DEER) method. Even though the DEER method is a very slow technique it can be applied to study fast relaxation process, if interstages are trapped. To accomplish this I have built a microsecond freeze-hyper quenching device capable of freeze quenching protein samples 80 μ s to 20 ms after they have been mixed with a ligand. Unpaired electrons were introduced in the protein by site directed-spin labeling. I have used the unnatural amino acid p-acetylphenylalanin, in addition to the labeling of cysteines. Up to now, only a single interspin distance could be obtained. Nevertheless, some further improvements of the labeling protocols will enable us to study the dynamics of the CNBD comprehensively.