Structural changes between signaling states of bacterial chemoreceptors probed by solid -state NMR and hydrogen exchange

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Abstract

The limitations of structural studies of membrane proteins imposed by the lipid environment has made it difficult to determine the molecular mechanism of transmembrane signaling. For the bacterial chemoreceptor family there are crystal structures of the internal and external domains, structural models of the transmembrane domain, and evidence for subtle ligand-induced conformational changes, but the signaling mechanism remains controversial. We have used a novel site-directed solid-state NMR distance measurement approach, using $^{13}C^{19}F$ REDOR, to measure a ligand-induced change of 1.0 ± 0.3 Å in the distance between helices α1 and α4 of the ligand-binding domain in the *intact, membrane-bound* serine receptor. This distance change is shown not to be due to motion of the sidechain and thus is due to motion of either the α1 or α4 helix. Additional distance measurements can be used to determine the type of backbone motion and to follow it to the cytoplasm, to test and refine current proposals for the mechanism of transmembrane signaling. This is a promising general method for *high-resolution* measurements of local structure in intact, membrane-bound proteins. ^ Also, we have probed structural difference between different signaling states of the cytoplasmic domain using soluble c-fragments of the aspartate receptor. Previous studies of mutant c-fragments have demonstrated that oligomeric state is correlated with signaling state: smooth swimming (kinase-inactivating) mutants are predominantly oligomeric whereas wild-type and tumble (kinase-activating) mutants are monomeric. The current hydrogen exchange studies reveal a small stable core of amides in the wildtype c-fragment which is expanded in the dimer-forming S461 L c-fragment. Comparison of the pH dependence of hydrogen exchange and dimer dissociation rates for S461 L reveal the expanded core is present in the monomer, dimer and transition states indicating the increased core stabilizes both the monomeric and dimeric structure. We speculate that such intradimer stabilization in the intact receptor's cytoplasmic domain is key to the kinase-inactivating state and therefore represents the structural change propagated by ligand binding. ^

Subject Area

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