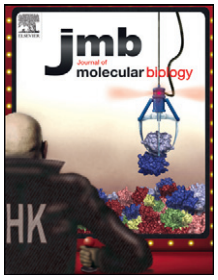

Conformational Transitions that Enable Histidine Kinase Autophosphorylation and Receptor Array Integration

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Abstract

During bacterial chemotaxis, transmembrane chemoreceptor arrays regulate autophosphorylation of the dimeric histidine kinase CheA. The five domains of CheA (P1–P5) each play a specific role in coupling receptor stimulation to CheA activity. Biochemical and X-ray scattering studies of thermostable CheA from *Thermotoga maritima* determine that the His-containing substrate domain (P1) is sequestered by interactions that depend upon P1 of the adjacent subunit. Non-hydrolyzable ATP analogs (but not ATP or ADP) release P1 from the protein core (domains P3P4P5) and increase its mobility. Detachment of both P1 domains or removal of one within a dimer increases net autophosphorylation substantially at physiological temperature (55 °C). However, nearly all activity is lost without the dimerization domain (P3). The linker length between P1 and P3 dictates *inter*subunit (*trans*) *versus intra*subunit (*cis*) autophosphorylation, with the *trans* reaction requiring a minimum length of 47 residues. A new crystal structure of the most active dimerization-plus-kinase unit (P3P4) reveals *trans* directing interactions between the tether connecting P3 to P2–P1 and the adjacent ATP-binding (P4) domain. The orientation of P4 relative to P3 in the P3P4 structure supports a planar CheA conformation that is required by membrane array models, and it suggests that the ATP lid of CheA may be poised to interact with receptors and coupling proteins. Collectively, these data suggest that the P1 domains are restrained in the off-state as a result of cross-subunit interactions. Perturbations at the nucleotide-binding pocket increase P1 mobility and access of the substrate His to P4-bound ATP.

Introduction

Kinases are essential sensors and transducers of cellular signals [1–4]. Regulation of their enzymatic activity often depends upon large-scale domain motions [1,5]. CheA is a multi-domain dimeric histidine kinase that acts as the primary enzyme in the bacterial chemotaxis signal transduction pathway [6,7]. During chemotaxis, signals propagate through membrane-incorporated chemoreceptor arrays composed of methyl-accepting chemotaxis proteins (MCPs), histidine kinases (CheA), and coupling proteins (CheW). Through complex interactions, these proteins initiate an intracellular phosphorelay that ultimately regulates flagella rotational switching [8–10]. Central issues in understanding the mechanism of chemotaxis signaling concern how CheA autophosphorylation is regulated and, specifically, what conformational transitions convert the kinase from an inactive into an active form. Many biochemical and structural studies have been conducted to elucidate the organization of the receptor arrays, as well as identify the specific interactions between the MCPs, CheA, and CheW in the extended lattice [11–15]. Electron cryo-tomography images [13,14,16,17] of the receptor arrays within intact cells reveal a hexagonal arrangement of proteins with features supported by the crystal structure of a CheA/CheW ring in complex with a Tm14 MCP dimer [18]. Previously determined *Thermotoga maritima* CheA structures [18–24] provide building blocks to understand the CheA domain arrangements within the lattice. However, domain positions in the most complete structure of CheA to date (comprising the core P3P4P5 domains or Δ289) [20] must be altered about the linker joining the P3 and P4 domains to fit the constraints of the lattice [12].

Each CheA subunit contains five distinct domains (P1–P5), which have discrete functions and display

variable mobility [25]. The P1 domain contains the substrate histidine residue (His45 in *T. maritima* or His48 in *Escherichia coli*), the P2 domain docks the response regulator CheY for phosphotransfer from P1, the P3 domain dimerizes the two subunits, the P4 domain acts as the ATP-binding kinase module, and the P5 domain couples CheA to CheW and chemoreceptors [6,26–28]. CheA differs from sensor histidine kinases in several ways: CheA does not contain a transmembrane domain, relying instead on P5 and CheW for interaction with transmembrane components; it has the phosphorylatable His residue on a separate domain (P1) instead of the dimerization domain (P3); and it utilizes a separate docking domain (P2) for CheY. P2 is not necessary for phosphotransfer to the response regulator CheY *per se* [29] but variants lacking the P2 domain (ΔP2) exhibit a reduced phosphotransfer rate relative to full-length CheA ($CheA_{FL}$) and support a lower extent of chemotaxis [29,30]. The linkers between the CheA domains have also been shown to play important roles in CheA activity [31,32]. For example, the P3-to-P4 linker influences the dynamical properties and kinase activity of the P4 domain [32]. NMR structures of *E. coli* and *T. maritima* CheA P1 assign the 20 linker residues C-terminal to the P1 4-helix bundle as an additional α-helix that runs along the other four helices [24,33,34]. This additional helix constrains the P1 domain and may restrict the movement and spatial orientation of activated CheA [24,34]. Notably, *E. coli* CheA autophosphorylates in *trans*, with one subunit phosphorylating the other [35,36].

Structural determinants for ATP binding and P1 phosphorylation are well defined within the P4 [19] and P1 [37] domains, although the manner and regulation of their interaction remains unclear. Nonetheless, recent work has elucidated possible contact points between these domains with one specific interaction identified between *E. coli* CheA Glu38 and Lys346 [38–42]. Additional studies indicate directional steering of the P1 domain toward the *trans* P4 subunit [36] and allosteric behavior of the two kinase active sites [42,43]. Negative cooperativity for nucleotide binding [44], which implies interaction between the P4 domains, is not easily reconciled with the large P4 separation found in the crystal structure of P3P4P5 [19]. Domain interactions within the full-length kinase may also limit motions of the substrate domains important for phosphorylation. Indeed, cryo-electron microscopy (cryo-EM) images of native arrays in different activity states suggest that the P1 and P2 domains are more ordered in the inhibited state [11].

Herein, we study the consequence of covalent attachment of the P1 substrate domain to the CheA kinase module using the structurally characterized CheA from *T. maritima* as our primary subject of investigation. The minimal linker length for *trans* autophosphorylation is defined; surprisingly, shorter linkers switch to *cis* phosphorylation and impart less activity. Small-angle X-ray scattering (SAXS) reveals that binding of non-hydrolyzable analogs of ATP releases the P1 domains from a constrained environment. Targeted disulfide cross-linking between the P1 and P4 reactive centers correlates kinase activity with access of P1 to the P4 ATP pocket. Severing the P1 domains from the kinase core (P3P4P5) activates autophosphorylation at higher temperatures, as does the formation of heterodimers with only one P1 module. P4 alone has nearly no reactivity toward P1, but addition of the P3 dimerization greatly enhances kinase activity. The structure of the minimal active fragment, P3P4, exhibits a planar arrangement of the domains, consistent with the CheA conformation required by the receptor arrays. Coupling crystallographic and biochemical data, we generate an updated working model of *T. maritima* CheA within the context of the hexagonal receptor lattice that includes a productive association between P1 and P4. Overall, these data underscore the importance of specific interactions between CheA subunits that control accessibility of the histidine substrate domain to the ATP-binding kinase module. Rearrangement about interdomain linkers appears critical for functional assembly of CheA into the receptor arrays.

Results

Generation of ΔP2 variants

To generate CheA variants with altered linkages between the kinase core (P3P4P5, also known as Δ289) and the substrate P1 domain, we fused P1 to P3P4P5 with linkers that retain various sections of the native P1–P2 connection along with several short non-native sequences added to adjust length and flexibility (Fig. 1a). The ΔP2 variant nomenclature was assigned according to the number of residues that connect P3 residue 293 to P1 residue 100, the latter of which ends the fourth helix of the P1 domain of *T. maritima* CheA (Fig. 1a). The longest variants (90AA and 85AA) contains all but the last residue (K177) of the native P1–P2 linker. The shorter variants (36AA, 41AA, and 47AA) retain the 33 residues C-terminal to the fourth P1 helix. The 41AA and 47AA variants also include a 5- to 14-residue spacer to facilitate linker flexibility.

Activity of ΔP2 variants compared to $CheA_{FL}$

CheA autophosphorylation activity generally decreases as the linker length in the ΔP2 variants shortens. ΔP2 variants with long linkers (90AA and 85AA) undergo similar degrees of autophosphorylation compared to *T. maritima* full-length CheA at