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Colocalization of Fast and Slow Timescale Dynamics in the Allosteric Signaling Protein CheY

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Abstract

It is now widely recognized that dynamics are important to consider for understanding allosteric protein function. However, dynamics occur over a wide range of timescales, and how these different motions relate to one another is not well understood. Here, we report an NMR relaxation study of dynamics over multiple timescales at both backbone and side-chain sites upon an allosteric response to phosphorylation. The response regulator, *Escherichia coli* CheY, allosterically responds to phosphorylation with a change in dynamics on both the microsecond-to-millisecond (μs-ms) timescale and the picosecond-to-nanosecond (ps-ns) timescale. We observe an apparent decrease and redistribution of μs-ms dynamics upon phosphorylation (and accompanying Mg^{2+} saturation) of CheY. Additionally, methyl groups with the largest changes in ps-ns dynamics localize to the regions of conformational change measured by μs-ms dynamics. The limited spread of changes in ps-ns dynamics suggests a distinct relationship between motions on the μs-ms and ps-ns timescales in CheY. The allosteric mechanism utilized by CheY highlights the diversity of roles dynamics play in protein function.

Introduction

Allostery is widespread in biology as an effective means for regulating protein activity. While the existence of protein dynamics over a range of timescales is well established, how these dynamics contribute to allosteric protein function is poorly understood. The mechanism(s) underlying long-range communication necessary for allostery may include not only large changes in the conformation but also changes in the dynamic fluctuations about a mean conformation.[1,2] Allosteric mechanisms have been shown to utilize the now familiar conformational change,[3–5] but more recent cases have been shown to solely use fast (i.e., picosecond to nanosecond, or ps-ns) internal dynamics.[6–8] It has been suggested that fast fluctuations are involved in facilitating the slower motions.[9] This connection between different timescales of motion and function may be an important mechanism in allostery. However, to our knowledge, only for adenylate kinase has there been any discussion of the relationship between timescales.[9] Allosteric proteins often possess both slow and fast dynamics, but the relationship between the two remains unclear.

Escherichia coli CheY is an ideal protein for the study of protein motions as they relate to function since it is an allosteric signaling protein that displays significant motions on multiple timescales. CheY is a response regulator (RR) from the two-component system that regulates chemotaxis.[10,11] Upon phosphorylation at D57,[12] CheY undergoes a conformational change that enables tight binding to the flagellar motor protein FliM (and minor interactions with FliN[13]) at a distal surface. CheY binding to FliM and FliN promotes a conformational change in the flagellar protein FliG, which results in a switch from counterclockwise to clockwise flagellar rotation.[14,15]

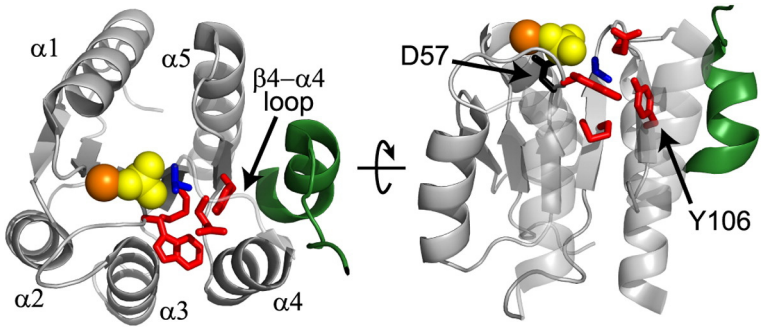

Fig. 1. Crystal structure of CheY displaying residues involved in the conformational switch. CheY is shown bound to Mg^{2+} (orange), BeFx (yellow) and a peptide derived from FliM (green). The site of phosphorylation, D57, is highlighted in black. Allosteric signaling quartet residues W58, M85, E89, and Y106 are shown as red sticks, and T87 is shown as blue sticks. Data Bank ID: 1F4V.

Because of the necessity for a quick chemotactic response, the autodephosphorylation rate of CheY is 2.5 min^{-1}.[16] Therefore, the ability to study the phosphorylated state of CheY is difficult and it has become common to use the phosphoryl mimic BeF_x.[17–20]

The CheY conformational switch has been extensively studied as a model for understanding phosphorylation-induced activation. Phosphorylation in CheY is sensed by T87, A88, and K109, which form hydrogen bonds with the phosphoryl group and are important in the initial allosteric signaling.[20,21] The phosphorylation-induced conformational change in CheY includes motion of the β4–α4 loop and a quartet of residues (E89, W58, M85, and Y106)[22] whose motions result in the rotation of Y106 from an "out" solvent-exposed rotamer to an "in" buried one (Fig. 1).[18,20]

Not only is CheY an excellent model RR for conformational change due to phosphorylation, RRs have become favored models for understanding conformational allostery. Previous studies of RRs focused on the conformational switch between inactive and active conformations on the slow timescale by monitoring the inherent equilibrium in the unphosphorylated protein.[3,22–24] While fast backbone dynamics have also been reported, they have not been shown to be involved in the allosteric transition.[3,24,25] Here, we report the first multi-timescale study of both an unphosphorylated and phosphorylated RR including measurement of fast side-chain dynamics.

We previously reported motions on the microsecond-to-millisecond (μs-ms) timescale that correlate with the intrinsic switching between inactive and active-like conformations in CheY in the absence of the activating phosphoryl group.[22] Here, we observe the slow dynamics dampen and shift to the FliM binding interface upon phosphorylation (and accompanying Mg^{2+} saturation) of CheY. Additionally, we show that large changes in methyl dynamics on the ps-ns timescale upon phosphorylation of CheY correlate with areas that undergo conformational change. Additional small significant changes are located in other regions known to affect CheY function. The dynamics on the slow and fast timescales localize to the same areas, indicating a possible connection between timescales that may be necessary for the allosteric transition in CheY.

Results and Discussion

(μs-ms) dynamics are dampened and shift toward the FliM binding interface upon phosphorylation of CheY

^{15}N Carr–Purcell–Meiboom–Gill (CPMG) relaxation dispersion experiments[26] were used to measure motions on the μs-ms timescale for unphosphorylated CheY (CheY-unP)[22] and BeF_x-bound CheY (CheY-P). This experiment measures the contribution from a conformational exchange process on the μs-ms timescale (R_{ex}) to the effective transverse relaxation rate ($R_{2,eff}$) as a function of the spacing between 180° pulses in the CPMG train (τ_{cp}),

$$R_{2,\mathrm{eff}} = R_2^{\circ} + R_{\mathrm{ex}}\left(1/\tau_{\mathrm{cp}}\right), \qquad (1)$$

where R_2° is the intrinsic relaxation rate.[27] Full relaxation dispersion experiments can be fit to reveal the kinetic and thermodynamic parameters of the exchange. For CheY-P, full relaxation dispersion experiments were carried out, but only a few residues have a high enough R_{ex} ($>2\ s^{-1}$) to enable accurate fitting, and it is therefore more beneficial to only consider the level of R_{ex} in each CheY state (details in Materials and Methods). The complete curves (Fig. S1a) and fit parameters (Table S1) for all CheY-P residues shown in Fig. 2 can be found in the Supplementary Information.

Here, we compare μs-ms dynamics in CheY-P to CheY-unP. CheY sample buffers with 10 mM Mg^{2+} were used, even though CheY-unP is not fully saturated with Mg^{2+} at this concentration[22] (CheY-P is), as partial saturation is expected under normal cellular conditions. Overall, there is a decrease in R_{ex} upon phosphorylation of CheY (Fig. 2a) compared to CheY-unP in the presence of 10 mM Mg^{2+}. Surprisingly, R_{ex} is not zero for CheY-P. In CheY-