Mechanism of latent allostery in *E. Coli* dihydrofolate reductase (DHFR)

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Allostery regulates the activity of a protein by changes at a site away from the active site. This regulation typically occurs through ligand binding, but the effect of a distal mutation on the activity of a protein can be seen as latent allostery. Since 1964, several models have been proposed to describe allostery. Some involve global conformational changes, such as the Monod-Wyman-Changeux (MWC) model[1], while others rely on changes in the vibrational modes of the protein, such as the dynamic allostery model proposed by Cooper and Dryden[2]. However, there is still no consensus and the theoretical explanation of allostery remains elusive. In this study, we focus on *E. coli* DHFR, in which distal mutations at site G121 have been shown to affect hydride transfer between the cofactor NADPH and the ligand H3F[3]. Using replica-exchange molecular dynamics simulations, we show that the effect of G121V on hydride transfer can be explained by a conformational equilibrium shift due to steric interactions, from conformations in which the NADPH and H3F cycles are parallel to conformations in which they are perpendicular. Using Oanca et al.'s implementation of EVB for Gromacs[4], we show that parallel conformations are much more reactive than perpendicular ones, and that the main effect of the Met20 loop on reactivity is to stabilize the parallel conformation, rather than to provide a specific electrostatic environment as previously thought[5]. The equilibrium shift is consistent with a MWC-type model of allostery. Using out-of-equilibrium MD simulations, we then propose a mechanism for the transition pathway from the parallel conformation of WT to the perpendicular conformation of G121V, where the typical time is hundreds of ns, ruling out the hypothesis of dynamic allostery. This mechanism could be considered as a mechanism for latent allostery in *Ec*DHFR.

KEYWORDS: Allostery ; Monod-Wyman-Changeux ; DHFR ; Protein

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