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Flexibility and Adaptability in Binding of *E. coli* Cytidine Repressor to Different Operators Suggests a Role in Differential Gene Regulation

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Interactions between DNA-bound transcription factors CytR and CRP regulate the promoters of the *Escherichia coli* CytR regulon. A distinctive feature of the palindromic CytR operators is highly variable length central spacers (0–9 bp). Previously we demonstrated distinct modes of CytR binding to operators that differ in spacer length. These different modes are characterized by opposite enthalpic and entropic contributions at 25 °C. Of particular note were radically different negative ΔC_p values suggesting variable contribution from coupled protein folding and/or DNA structural transitions. We proposed that the CytR DNA binding-domain adopts either a more rigid or flexible DNA-bound conformation in response to the different spacer lengths. More recently, similar effects were shown to contribute to discrimination between operator and non-specific DNA binding by LacR, a CytR homolog. Here we have extended the thermodynamic analysis to the remaining natural CytR operators plus a set of synthetic operators designed to isolate spacing as the single variable. The thermodynamic results show a broad and monotonic range of effects that are primarily dependent on spacer length. The magnitude of effects suggests participation by more than the DNA-binding domain. ^{15}N HSQC NMR and CD spectral analyses were employed to characterize the structural basis for these effects. The results indicate that while CytR forms a well-ordered structure in solution, it is highly dynamic. We propose a model in which a large ensemble of native state conformations narrows upon binding, to an extent governed by operator spacing. This in turn is expected to constrain intermolecular interactions in the CytR-CRP-DNA complex, thus generating operator-specific effects on repression and induction of transcription.

Keywords: protein–DNA interaction; thermodynamics; van't Hoff analysis; operator sequences; repressors

Introduction

The *Escherichia coli* cytidine repressor protein (CytR) is a member of the LacR family of bacterial repressors,[1] a family that shares an unusually high degree of structural and functional similarity. In contrast to other family members that regulate single operons, CytR functions as a differential regulator of multiple operons. These encode enzymes and transport proteins involved in nucleoside catabolism and are referred to as the CytR regulon.[2] In combination, CytR and the *E. coli* cAMP receptor protein (CRP), an activator of over 100 *E. coli* genes, confer variable extents of activation, repression and induction, even among promoters with similar arrangement of regulatory elements.[2,3]

Whereas other LacR family members mediate repression by competitive DNA-binding with affinity controlled by an allosteric ligand, CytR-

Abbreviations used: CRP, *E. coli* cAMP receptor protein; CRP1 and CRP2, promoter proximal and promoter distal CRP binding sites on DNA; CytO, cytidine responsive operator in CytR-regulated promoter; CytR, *E. coli* cytidine repressor protein; DBD, DNA-binding domain; HSQC, heteronuclear single quantum correlation; OG, Oregon Green 514; RNAP, *E. coli* RNA polymerase.

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regulated operons exhibit a relatively rich diversity of regulatory patterns. Key to this diversity are protein–protein interactions between CytR and other components of the transcriptional machinery, particularly CRP. This raises a question of interest from both gene regulatory and protein chemistry perspectives: how does CytR achieve a level of sophistication in its interactions with these other proteins and as a consequence in its regulatory mechanism(s) that other LacR family members do not?

The most common arrangement of regulatory elements in CytR-regulated promoters features two CRP operators arranged in tandem. The promoter proximal and promoter distal sites, designated CRP1 and CRP2, respectively, flank an intervening CytR operator, which we denote, CytO (Figure 1). CytR alone has no repressor activity; instead it modulates CRP-mediated activation.[4] CytR and CRP bind cooperatively to form a DNA-bound regulatory complex in which one CytR dimer bound to CytO forms a protein bridge between the two CRP dimers bound to CRP1 and CRP2.[5,6]

CRP activates transcription *via* two distinct kinetic mechanisms; by associating to form the three-protein, CRP-CytR-CRP cooperative complex, CytR interferes with both. First, interactions between the α-subunit of RNA polymerase (RNAP) and the CRP dimers bound to both CRP1 and CRP2 recruit RNAP to bind DNA between CRP1 and CRP2, in competition with CytR binding to CytO. There is also competition between CytR and the α-subunit of RNAP for interaction with CRP, because the surfaces for these interactions overlap one another on CRP.[7,8] A second set of interactions between RNAP and CRP bound to CRP1 mediates an allosteric activation of the rate of open complex.[9] Although CytR does not compete directly with these interactions,[7] the interaction between CytR and CRP bound to CRP1 is reported to transform CRP from an allosteric activator to an allosteric inhibitor of RNAP.[10] This plurality of mechanisms for modulation of CRP-mediated activation is suited well to a role in differential gene regulation.

The significance of CytR-CRP cooperativity is highlighted by the fact that cooperativity is lost, and repression reversed, when CytR binds cytidine. Thus, CytR-CRP cooperativity underlies induction as well as repression. In contrast to other LacR family repressors for which effector binding is linked to DNA binding, cytidine binding to CytR has little effect on the intrinsic affinity or specificity for CytR binding to DNA.[6,11] Addressing the question how cytidine binding is coupled to CytR-CRP cooperativity we showed that cytidine is also not a direct allosteric effector of CytR-CRP interactions.[12] Instead, cytidine binding favors a CytR conformation that restricts the ability to simultaneously bind the operator and form the protein bridge between DNA-bound CRP dimers. Thus, allosteric control of a geometric constraint underlies induction, rather than control of interactions between any specific pair of macromolecular partners.

Given the structural similarity between LacR family members similar mechanisms of allostery might also be anticipated. The mechanism is understood for LacR and PurR based on high resolution structures of both DNA-bound and free repressors.[13–15] In these cases ligand binding is linked to a conformational transition of the large C-terminal, ligand binding domain referred to as the core. The transition disrupts the interface between the core dimer and a linker peptide connecting the core and N-terminal DNA binding domain (DBD). In the DNA-bound form, this linker forms a helix, referred to as the hinge helix. This comprises the dimer interface between the DBDs and also contacts the minor groove at the center of the operator. Disruption of the hinge helix–core domain interface destabilizes the hinge helices, which consequently are unfolded in the operator-free repressors.

The hinge peptides in CytR must accommodate a unique feature of its sequence-specific DNA-

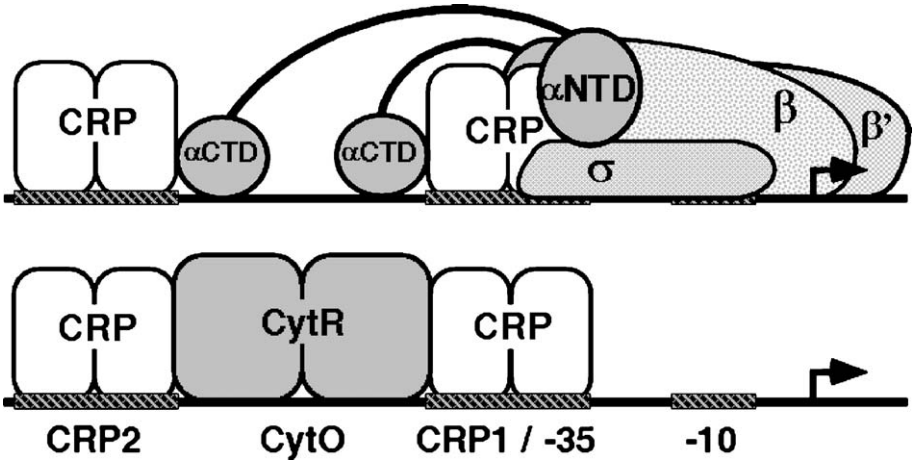

Figure 1. Arrangement of CytR-regulated promoters showing alternate configurations with RNA polymerase bound (top) and with CytR bound (bottom). A linker connecting the C-terminal (αCTD) and N-terminal (αNTD) domains of the α-subunit of RNAP provides the flexibility to allow the former to compete for CytR association, both with CytO and with CRP bound to CRP1 and CRP2.