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Specificity and Affinity of Lac Repressor for the Auxiliary Operators *O2* and *O3* Are Explained by the Structures of Their Protein–DNA Complexes

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The structures of a dimeric mutant of the Lac repressor DNA-binding domain complexed with the auxiliary operators *O2* and *O3* have been determined using NMR spectroscopy and compared to the structures of the previously determined Lac–*O1* and Lac–nonoperator complexes. Structural analysis of the Lac–*O1* and Lac–*O2* complexes shows highly similar structures with very similar numbers of specific and nonspecific contacts, in agreement with similar affinities for these two operators. The left monomer of the Lac repressor in the Lac–*O3* complex retains most of these specific contacts. However, in the right half-site of the *O3* operator, there is a significant loss of protein–DNA contacts, explaining the low affinity of the Lac repressor for the *O3* operator. The binding mode in the right half-site resembles that of the nonspecific complex. In contrast to the Lac–nonoperator DNA complex where no hinge helices are formed, the stability of the hinge helices in the weak Lac–*O3* complex is the same as in the Lac–*O1* and Lac–*O2* complexes, as judged from the results of hydrogen/deuterium experiments.

Keywords: lac headpiece; *lac* operators; NMR; protein–DNA recognition

Introduction

The *Escherichia coli* Lac repressor controls the expression of genes involved in lactose metabolism.[1,2] Effective downregulation of these genes is achieved by the presence of multiple Lac repressor operator binding sites within the *lac* operon. The main operator *O1* overlaps with the *lac* promoter and is essential for the function of the *lac* operon. Furthermore, the two auxiliary operators *O2* and *O3*, located 401 bp downstream of *O1* and 92 bp upstream of *O1*, respectively, contribute significantly to transcriptional repression. *O1* is indispensable, since mutation or deletion of *O1* leads to an almost complete loss of repression even in the presence of both auxiliary operators.[3,4] Inactivation of either *O2* or *O3* results in a slight decrease in repression, while combined loss of both *O2* and *O3* leads to a significant (~70-fold) decrease in repression.[4] This cooperativity can be well explained as the tetrameric protein functions as a dimer of dimers that can simultaneously bind to the *O1* operator and to either of the auxiliary *O2* and *O3* operators,[5,6] creating one of two alternative DNA loops.[7,8]

Mutational studies revealed that the Lac repressor has different affinities for various operators, resulting in distinct repression efficiency.[9] The differences in the operator affinities of the Lac repressor can be ascribed to the variation in their sequences. *O1* and *O2* operators have similar base-pair compositions, while the *O3* sequence differs significantly (Fig. 1). Natural *lac* operators are pseudo-palindromic sequences, where symmetry is broken by variations in the sequence between the two half-sites and by

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Abbreviations used: HTH, helix–turn–helix; NOD, nonoperator DNA; H/D, hydrogen/deuterium; NOE, nuclear Overhauser enhancement; 2D, two-dimensional; NOESY, NOE spectroscopy; RDC, residual dipolar coupling; 3D, three-dimensional; TOCSY, total correlated spectroscopy; HSQC, heteronuclear single quantum coherence.

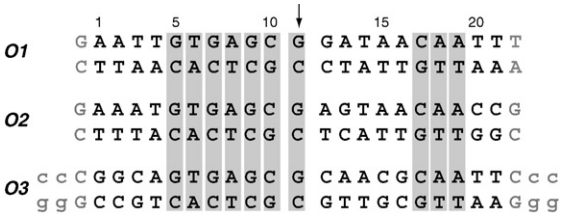

Fig. 1. Sequences of naturally occurring *lac* operators. The three natural operators *O1*, *O2*, and *O3* are aligned with the numbering referred to in the text and figures. Arrow denotes the central base pair. The bases conserved in all natural operators are highlighted. The two binding sites within each operator are referred to as the left (base pairs 1–10) and the right (base pairs 12–21). The extension of operator sequences is shown in gray.

insertion of the central G:C base pair (Fig. 1). The two half-sites differ significantly in their affinity for the Lac repressor when considered separately.[10] Mutational analysis has suggested a greater overall contribution to binding by the left operator site because mutations in the left site sequence are more deleterious to repressor binding than those in the right site sequence.[3,11]

The amino-terminal headpiece (residues 1–62) of the Lac repressor is responsible for operator recognition and binding. Residues 1–49 of the headpiece form a stable fold consisting of three helices. The first two helices comprise a canonical helix–turn–helix (HTH) DNA-binding motif. The second (or recognition) helix of the HTH motif contains several residues that make contacts with DNA. The C-terminal residues Arg50-Gly58 of the headpiece form a so-called hinge region, which is unstructured in the absence of DNA. Initially, NMR structural studies were hindered by the unfavorable dynamics of the isolated monomeric Lac headpiece in complexes with natural operators probably due to its low affinity for them.[12] Inspired by the work of Falcon *et al.*,[13] who introduced the mutation of Val by Cys at position 52 in the full-length Lac repressor, Kalodimos *et al.*[14] prepared a covalently linked dimeric Lac headpiece (HP62V52C). This dimeric headpiece had an affinity for the natural operator in the picomolar range, comparable to that of the intact dimeric Lac repressor.[14] Analysis of the structure of the HP62V52C–*O1* complex revealed a distinct binding mechanism of headpiece to the left and right halves of the operator.[15] The global positioning of the dimer on the operator was dramatically asymmetric, resulting in a different pattern of specific contacts between the two half-sites demonstrating the intrinsic plasticity of the Lac headpiece. The structure of the left site of the complex was similar to that of the Lac headpiece bound to the fully symmetrical operator.[16] In agreement with the crystal structure,[17] hinge helices were folded and bound in the minor groove between base pairs 10 and 11, thereby introducing a kink in the DNA. This also confirmed a study by Spronk *et al.*, who showed

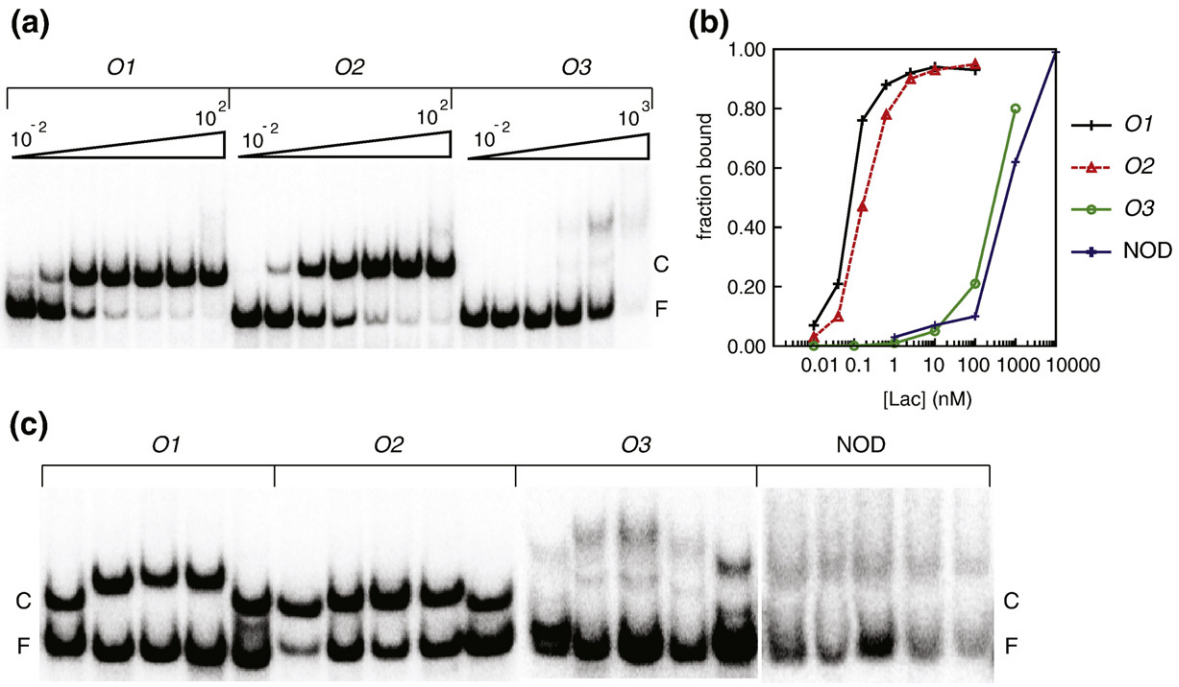

Fig. 2. DNA binding and bending experiments. (a and b) Representative DNA binding experiment using the indicated operators with a serial dilution of dimeric HP62V52C. The highest and lowest protein concentrations (nM) are indicated above each experiment. Different operator fragments are described in Fig. 1. (c) DNA bending experiment using the circular permutation method with the dimeric HP62V52C mutant. The free probe and the protein–DNA complex are indicated with F and C, respectively.