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Letter to the Editor: Backbone chemical shift assignments of the LexA catalytic domain in its active conformation

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Biological context

The LexA repressor controls the SOS response of *E. coli* to conditions that damage DNA or inhibit DNA replication (Little and Mount, 1982). Upon activation by RecA, LexA undergoes self-cleavage within a linker region between its N-terminal DNA-binding and dimeric C-terminal protease domains, thereby leading to expression of SOS response genes. The proteolytic mechanism involves S119 as a nucleophile that is activated by a neutral general base, K156. Based on genetically screened mutants with elevated rates of autocleavage, it was proposed that LexA exists in two distinct conformational states (L* and L), only one of which (L*) is competent for catalysis. Recent crystallographic studies of several catalytically inactive mutant forms of LexA showed two conformations, termed C and NC that were hypothesized to correspond to L* and L, respectively (Luo et al., 2001). In the C form of the isolated catalytic domain, stabilized by four mutations (L89P, Q92W, E152A, and K156A), the cleavage site region (CSR; residues G75–Y98) is proximal to the catalytic center, whereas in the NC form, containing only the S119A substitution, it is displaced by ~20 Å. The CSR is composed of a β-hairpin (β3-loop-β4) and contains the target A84–G85 peptide. In a third variant, evidence for both conformations of the CSR was found, highlighting the dynamic nature of the structural transition and raising the possibility that the observed NC and C conformations may also reflect crystal packing constraints. Thus, to probe further the structural and energetic features of the L/L* conformational switch, we have used NMR spectroscopy to characterize LexA in solution.

Methods and experiments

Uniformly ^{15}N- and ^{13}C/^{15}N-labeled LexA with the mutations L89P, Q92W, E152A, and K156A (plus a benign D150H substitution) were expressed in *E. coli* grown in M9 media, and purified as described (Luo et al., 2001). After tryptic cleavage and elution from a monoQ column (Amersham), the resulting catalytic domain (residues 68–202), denoted LexA-ΔQM, was dialyzed against 20 mM potassium phosphate, pH 7.0, and concentrated to ~1 mM in 5% D_2O for NMR data collection at 30 °C. Sequential assignment were obtained using sensitivity-enhanced HNCA-TROSY, HNCACB, (HB)CBCA(CO)NH, HNCO-TROSY, and ^{15}N-edited NOESY spectra, recorded with a Varian Inova 600-MHz spectrometer. ^{15}N relaxation measurements (Farrow et al., 1994) were carried out with a Varian Unity 500-spectrometer. Data were analyzed in NMRView (Johnson and Blevins, 1994) with SmartNoteBook (Slupsky et al., 2003).

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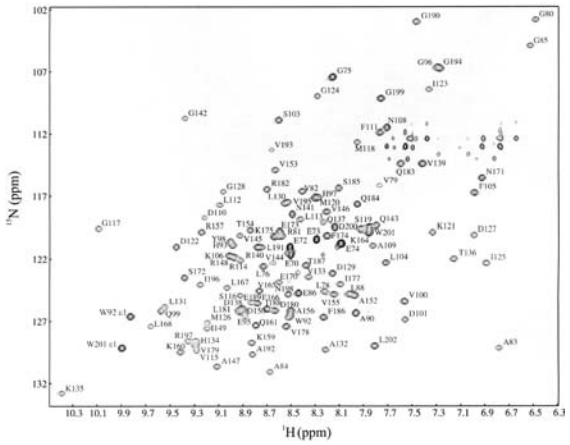

Figure 1. A 600 MHz ^{1}H–^{15}N TROSY-HSQC spectrum of ^{13}C/^{15}N-labeled LexA-ΔQM.

Extent of assignments and data deposition

Complete resonance assignments for the $^{1}H^{N}$, $^{13}C'$, $^{13}C^{\alpha}$, and, when applicable, amide ^{15}N and $^{13}C^{\beta}$ nuclei from 119 out of 135 residues in LexA-ΔQM were obtained using a standard suite of triple resonance experiments (Figure 1). $^{1}H_{N}$ and ^{15}N signals were not detected for L68, L69, E71, G162, and N163. From ^{15}N relaxation measurements, the effective correlation time for the global tumbling of LexA-ΔQM was found to be 24.0 ± 0.2 ns. Although biophysical and crystallographic studies indicate that the protein is a symmetrical dimer (Luo et al., 2001), this correlation time is higher than expected for a 30 kDa complex (~16 ns; Daragan and Mayo, 1997) and thus suggestive of additional self-association under the experimental conditions.

Based on chemical shift and NOE information, LexA-ΔQM adopts a predominant conformation in solution similar to the crystalline C form. Backbone dihedral angles predicted by TALOS (Cornilescu et al., 1999) are in a good agreement with those in the crystal structure of this protein and consistent with a secondary structure of 9 β-stands. NOE connectivities between the amide protons of L76–Y98, as well as between those of L78–G96, demonstrate that the CSR retains a β3-loop-β4 topology in solution. More importantly, NOE interactions between the amide protons of V79–L112, and V82–R114, as well as strong sequential NOEs between V79–G80, confirm the extended pairing of parallel strands β3 and β5 and the presence of β-bulge involving the latter residues. This β-bulge is absent in the NC conformation. Further evidence for the close correspondence between the solution and the crystal structures of LexA-ΔQM is the proximity of the CSR, with the scissile A84–G85 bond, to the catalytic residues 119 and 156. This proximity is reflected by observed NOE connectivities between the amide protons of A83–V155 (in the C and NC conformations, their separations are 4.3 Å vs. 14.1 Å), A84–S116 (2.7 Å vs. 13.5 Å), G85–V155 (3.5 Å vs. 13.5 Å), L88–V153 (3.4 Å vs. 9.4 Å), and A90–V153 (4.1 Å vs. 11.8 Å), In parallel, ^{15}N relaxation data reveals that the backbone of the CSR, including A84 and G85, is well-ordered, with only the exposed amides of A90 and Q91 showing evidence for enhanced mobility on the ns–ps timescale. Together these data support the hypothesis that the L* form of LexA-ΔQM adopts a stable C conformation in solution.

The ^{1}H, ^{13}C, and ^{15}N backbone chemical shifts of LexA-ΔQM have been deposited in the BioMagResBank database (http://www.bmrb.wisc.edu) under BMRB accession number 6373.

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References

Cornilescu, G. Delaglio, F. and Bax, A. (1999) *J. Biomol. NMR*, **13**, 289–302.

Daragan, V.A. and Mayo, K.H. (1997) *Prog. Nucl. Magn. Reson. Spectrom.*, **31**, 63–105.

Johnson, B.A. and Blevins, R.A. (1994) *J. Biomol. NMR*, **4**, 603–614.

Farrow, N.A. Muhandiram, R. Singer, A.U. Pascal, S.M. Kay, C.M. Gish, G. Shoelson, S.E. Pawson, T. Foreman-Kay, J.D. and Kay, L.E. (1994) *Biochemistry*, **33**, 5984–6003.

Little, J.W. and Mount, D.W. (1982) *Cell*, **29**, 11–22.

Luo, Y. Pfuetzner, R.A. Mosimann, S. Paetzel, M. Frey, E.A. Cherney, M. Kim, B. Little, J.W. and Strynadka, N.C.J. (2001) *Cell*, **106**, 585–594.

Slupsky, C.M. Boyko, R.F. Booth, V.K. and Sykes, B.D. (2003) *J. Biomol. NMR*, **27**, 313–321.