Structural and dynamical studies of the autoinhibtory domain of E. coli NusA

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ABSTRACT

The *Escherichia coli* NusA protein is involved in transcription pausing, termination, and antitermination by associating with the transcription complex. It consists of three major domains, an amino-terminal RNA-Polymerase binding domain, a central RNA binding domain, and an approximately 160 amino-acid carboxyterminal domain, regulating the RNA-binding activity of full-length NusA. RNA binding to NusA could be observed for a deletion mutatant with the carboxyterminal 79 amino-acids missing or a full-length NusA in the presence of the lambda N protein or the alpha subunit of the RNA-Polymerase, but not for full-length NusA (Mah et al, 2000).

To address questions in molecular detail about the interaction of carboxyterminal domain of *E.coli* NusA with its binding partners a high-resolution structure of the 17 kDa domain, *E.coli* NusA (339-495), is required. To this end multidimensional heteronuclear NMR spectra were acquired. So far we have analyzed the chemical shifts of backbone and side chain resonances indicating that the carboxyterminal domain of NusA is an all-alpha protein. Dynamical features of the carboxyterminal domain of NusA were investigated by NMR relaxation measurements, showing two regions with different correlation times connected by a flexible linker.

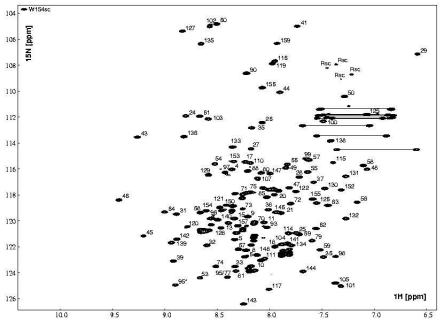

Fig 1. [^{1}H,^{15}N] HSQC spectrum of uniformly labeled *E.coli* NusA (339-495). The amide resonances are labeled with their corresponding residue numbers. NH_2 sidechains of asparagine and glutamine are connected by lines, the arginine ε-NH, and the tryptophane NH are marked by 'sc'. Asterisks indicate minor conformations.

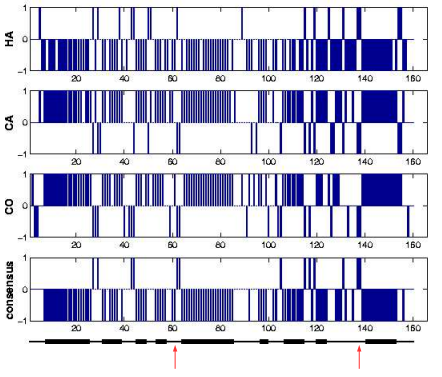

Fig 2. Chemical shift index (CSI) of HA, CA, and CO nuclei of *E.coli* NusA (339-495). A grouping of four or more negative values not interrupted by a positive value in the consensus indicates a helical conformation (analog to Wishart and Sykes, 1994). The identified helices are represented as black bars. Profile based sequence analysis predicts two helix -hairpin-helix motifs (Shao and Grishin, 2000) in *E.coli* NusA (339-495) with the hairpins at G61-L62-D63, and G136-L137-T138, respectively (Mah et al, 2000). The position of the predicted hairpins (red arrows) is in agreement with the experimental CSI data.

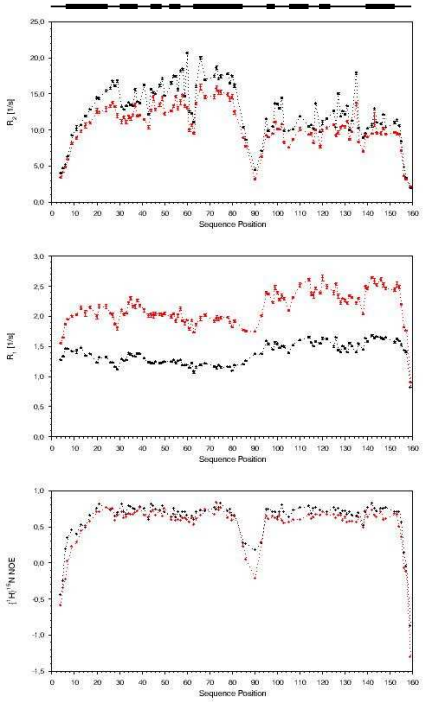

Fig 3. Relaxation data at proton frequencies of 400 MHz (red) and 600 MHz (black) indicate that *E.coli* NusA (339-495) consists of two regions with different correlation times connected by a flexible linker.
Transverse (R_2) and longitudinal (R_1) relaxation rates provide information about the rotational reorientation of the protein in solution, for example the characteristic time constant of the reorientation τ or anisotropc motion. For well-structured segments as regular secondary structure elements the correlation time is proportional to the R_2/R_1 ratio. The different average R_2/R_1 values (residues deviating more than one standard deviation from the average were omitted) of 6.5 ± 0.5 (400 MHz) and 12.9 ± 1.1 (600 MHz) for residues 16-82, and 3.9 ± 0.3 (400 MHz) and 7.2 ± 0.9 (600 MHz) for residues 95-154 indicate that *E.coli* NusA (339-495) consists of two segments reorienting at least partially independent. Low values of the heteronuclear NOE as can be seen for the N-and C-termini and residues 85-93 of E.coli NusA (339-495) give evidence of flexible regions. The black bars represent helices predicted by CSI analysis.

Analysis of relaxation data

Relaxation data were interpreted using the reduced spectral density mapping method (Farrow et al, 1995). In this approach, the spectral density terms at high frequencies ($J(\omega_H)$, $J(\omega_H + \omega_N)$, $J(\omega_H - \omega_N)$), are approximated by a single term $J(0.87\omega_H)$. The three remaining spectral density values $J(0)$, $J(\omega_N)$, and $J(0.87\omega_H)$ can be calculated analytically and at each field strength separately from R1, R2, and heteronuclear NOE (NOE) data according to

$$J(0.87\omega_H) = 4 / (5d^2) (\gamma_H/\gamma_N) (\text{NOE-1}) R_1 \quad (1)$$

$$J(\omega_N) = (R_1 - 7d^2/4\, J(\omega_H)) / (3d^2/4 + c^2) \quad (2)$$

with $d = \mu_0 h \gamma_H \gamma_N / (8\pi^2)\ \langle 1/r^3 \rangle$ and $c = \omega_N / \sqrt{3}\ (\sigma_\parallel - \sigma_\perp)$.
(γ: gyromagnetic ratio; r: N-H distance; μ_0: permeability of free space; h: Plank's constant; $\sigma_\parallel/\sigma_\perp$: parallel and perpendicular components of the axially symmetric chemical shift tensor)

Data acquired at two field strengths allow the determination of an additional parameter R_{ex} that describes conformational or solvent exchange processes on a micro to milli second time scale, and affects the transversal relaxation rates and $J(0)$.

$$R_2^{tot} = R_2 + R_{ex} \quad (3)$$

The R_{ex} values and the corrected $J(0)$ value are evaluated using the following equations (Farrow et al, 1995)

$$J(0) = 1/\beta\, [(R_2^{600} - \kappa R_2^{400}) - 3d^2/8\, (J(\omega_H^{600}) - \kappa J(\omega_H^{400})) - c^2/2\, (J(\omega_N^{600}) - J(\omega_N^{400})) - 13d^2/8\, (J(0.87\omega_H^{600}) - \kappa J(0.87\omega_H^{400}))] \quad (4)$$

$$R_{ex}^{400} = R_2^{400} - (d^2/2 + 2c^2_{400}/3)\, J(0) - (3d^2/8 + c^2_{400}/2)\, J(\omega_H^{400}) - 13d^2/8\, (J(0.87\omega_H^{400})) \quad (5)$$

$$R_{ex}^{600} = \kappa\, R_{ex}^{400} \quad (6)$$

where $\kappa = (\omega_H^{400}/\omega_H^{600})^2$ and $\beta = d^2/2\ (1-\kappa)$.

The spectral densities were further interpreted in terms of the Lipari - Szabo model (Lipari and Szabo, 1982) in which the total motion of the N-H vector is separated in the overall tumbling of the protein and internal motions. Equations (7) and (8) provide an estimate for the the generalized squared order parameter S^2 and the residual rotational correlation time τ for well-structured residues (Bracken et al, 1999)

$$\tau = \omega_N^{-1} (J(0) - J(\omega_N)) / (J(\omega_H))^{1/2} \quad (7)$$

$$S^2 = 5\, (J(0) - J(\omega_N))\, (1 + 2\omega_N^2 \tau^2)) / (2\omega_N^2 \tau^3) \quad (8)$$

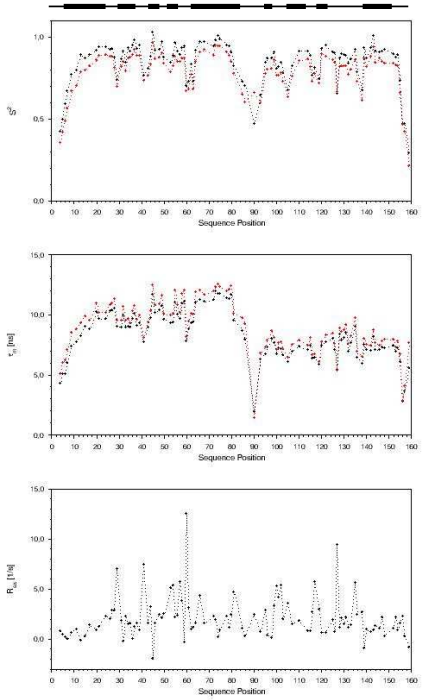

Fig 4. The order parameter S^2, the local correlation time τ, and the exchange rate correlate with secondary structure elements determined by the CSI method. Data at 400 MHz are shown in red, data at 600 MHz in black.

The squared order parameter S^2 can be understood as the amplitude of the internal NH vector motions. High order parameters ($S^2 > 0.8$) are commonly found in regions of regular secondary structure or rigid loops. Here the correlation of high order parameters and the helices predicted by CSI analysis in Fig. 2 (represented as black bars) is clearly visible though order parameters evaluated from data at 400 MHz are slightly lower than order parameters evaluated from data at 600 MHz. The field dependency of the order parameter and the correlation time τ is due to nanosecond time scale motions (Korshnev et al, 1997).
The local correlation time τ also correlates with secondary structure. The variation of the average correlation time for each predicted helix suggests that the overall tumbling of the protein is anisotropic and that the helices are oriented differently with respect to the diffusion tensor. Furthermore the different average correlation times for residues 16-82 (~10 ns) and residues 95-154 (~8 ns) as already evident from the R_2/R_1 ratio (Fig. 3) indicate that *E.coli* NusA (339-495) is composed of two regions with different mobility.
The exchange contribution R_{ex} to the transversal relaxation rate contains information about conformational or solvent exchange processes. Residues exhibiting exceptionally high values of R_{ex} are situated in regions of nonregulary secondary structure.

CONCLUSION

The well-dispersed amide resonances in the [^{1}H,^{15}N] HSQC as well as the high values of the {^{1}H}^{15}N heteronuclear NOE suggest that the carboxyterminal domain of *E.coli* NusA is a stable and properly folded protein. Though the tertiary structure has not been elucidated yet, secondary chemical shifts provide evidence that *E.coli* NusA (339-495) is an all-alpha protein. Profile based sequence analysis predicts two helix-hairpin-helix motifs which are in agreement with the location of the secondary structure elements. Further confirmation of the location secondary structure elements is provided by high order parameters which correlate with the position of the helices determined by CSI analysis.
Different average correlation times of ~10 ns, and ~8 ns for residues 16-82, and residues 95-154, respectively, suggest that *E.coli* NusA (339-495) is composed of two more or less independently reorienting domains connected by a flexible linker as evident from low values of the {1H}15N heteronuclear NOE in the intermediate region. These results also agree with biological data, since the 79 carboxyterminal residues (residues 80-159) are involved in the autoinhibition of full-length NusA.
The anisotropy seen in the varying average values of the correlation times for the predicted helices may be helpful in the ongoing structure calculations particularly with regard to the determination of the mutual orientations of the helices.

LITERATURE

[1] Lipari and Szabo, J Am Chem Soc, 1982, 104: 4546-4559
[2] Mah et al, 2000, Genes Dev, 14: 2664-2675
[3] Shao and Grishin, 2000, Nucleic Acids Res, 28: 2643-2650
[4] Wishart and Sykes, 1994, Methods Enzymol, 239: 363-392
[5] Farrow et al, 1995, J BioMol NMR, 6: 153-162
[6] Bracken et al, 1999, J Mol Biol, 285: 2133-2146
[7] Korzhnev et al, 1997, J Magn Reson, 127:184-91