COMPLEMENTATION OF 3D STRUCTURE OF DELTA SUBUNIT OF RNA POLYMERASE FROM *Bacillus subtilis* WITH DESCRIPTION OF INTERNAL MOTIONS IN TERMS OF REDUCED SPECTRAL DENSITY MAPPING

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Abstract

RNA polymerases of Gram positive and Gram negative bacteria differ. The subunit composition in Gram positive bacteria includes two additional subunits. One of them is denoted -subunit. The structure of -subunit from *Bacillus subtilis* has been solved recently using methods of solution NMR [1] (PDB ID = 2KRC). Here, we extend the information about the structure by the basic characterization of its dynamics studied at two temperatures. The standard relaxation experiments (R_1, R_2, ssNOE) were performed and data were analyzed using reduced spectral density mapping as the most straightforward approach. The analysis reveals flexible residues in the central part of the sequence. It confirms expected independent stochastic movements in the C-terminal part of the molecule. Results also yield an evidence of a slow conformational exchange of several residues in the well-structured region of the protein.

Introduction

Currently, the structural biology relies mostly on the description of static models of biomolecular structures. However, such a description may not be sufficient although it defines the relevant most populated conformation. Biomolecules are highly dynamic systems and the overall energy function might be very structured with shallow minima. It allows a coexistence of many conformations with a similar potential energy. Some of them might be crucial for the function of biomolecules even if they are not the most favorable ones. The internal structural variability of biomolecules is sometimes overlooked and mechanistic studies can be negatively influenced by the lack of the dynamic information.

Nuclear magnetic resonance, X-ray crystalography, and cryo-electron microscopy are the most widely used methods for structure determination of biomolecules. X-ray crystalography and cryo-electron microscopy are not well suited for a dynamic description of biomolecules. However, a more comprehensive description including information about the internal motions of biomolecules is accessible by NMR.

The dynamic information obtained by NMR methods should not be confused with a diversity within the ensemble of structures defining various solutions of structure calculations based on the NMR data. The latter is caused by the uncertainty of the determined structure due to the lack of structural restraints.

Different NMR methods have been developed to study motions at various timescales. Thus, the techniques cover all timescales starting from ps-motions as the fastest ones, which can be studied by NMR. The analysis of auto- and cross-relaxation rates of excited spin magnetization [2] serves to the description of the fast motions up to the timescale given by the global Brownian tumbling of the studied molecule in a solution. Residual dipolar couplings (RDCs) [3] may be used as a source of information about slower motions up to approximately micro to millisecond level. Motions on the micro to millisecond timescale can be studied by the relaxation dispersion experiments [4]. Slower motions are accessible by ZZ-exchange [5] experiments and peak shape analysis [6][7].

Several methods have been developed to analyze the relaxation rates in order to extract the information about the pico- to nano-second motions. The Model free approach [8][9] is the most popular approach, later extended to include even more than one internal motion mode [10]. No particular model of motion needs to be specified within this approach. Nevertheless, the fitted parameters might be further interpreted if some particular models of motions are reasonable to assume. The analysis faces the problem of separating the internal motion contribution to the relaxation rates from the contribution of a free molecular tumbling in the solution. The correct treatment might be difficult and several approaches have been proposed to overcome this problem [11, 12]. Hence, it is often worth taking advantage of a more straightforward approach, providing parameters less intuitive for interpretation but less sensitive to misinterpretation. Spectral density mapping represents such an approach. It is based on a simple recalculation of measured relaxation data and it thus avoids

complications arising from the necessity to utilize nonlinear fitting procedures. Moreover, it does not require assumptions the Model free approach is based on. It assumes that the studied relaxing system fulfills the condition of an isolated spin pair, only single dipole-dipole and CSA interactions contribute to the relaxation, and the CSA interaction is axially symmetric and collinear with the dipole-dipole interaction. To compensate the lack of data, *reduced spectral density mapping* was introduced, i.e., a single effective value of the spectral density function is used instead of the spectral density function values evaluated at the hydrogen frequency and at frequencies increased and decreased by the ^{15}N frequency [13].

Materials and methods

Preparation of sample

The sample of -subunit of RNA polymerase from *Bacillus subtilis* was prepared using the expression vector pET22b. The gene coding the structured part of the -subunit was inserted in between NdeI and XhoI sites. The protein was expressed using 2 l of minimal media M9 enriched by $^{15}NH_4Cl$ to uniformly label the protein with isotope ^{15}N. The protein was purified by ion-exchange chromatography and dialyzed twice against 20 mM phosphate buffer, pH = 6.6, and 10 mM NaCl. The final concentration of the sample was 0.8 mM. The sample contained 99 residues including a His tag at the C-terminus. The N-terminal initial methionine was cleaved off after expression.

NMR experiments

The standard relaxation measurements of longitudinal auto-relaxation rate (R_1), transverse auto-relaxation rate (R_2) and cross-relaxation rate using the steady state NOE experiment (ssNOE) were performed. The whole set of data was acquired at 7 C and 27 C. The temperature was carefully calibrated using dry methanol sample [14]. NMR experiments were carried out at 500 MHz Varian spectrometers except the ssNOE measurement at 7 C, which was performed at 500 MHz Bruker spectrometer. Both spectrometers were equipped with a standard triple resonance probe. The interscan delay was set to 2s in the measurements of auto-relaxation rates. The series of 0.0*, 0.1, 0.15, 0.25*, 0.35, 0.5*, 0.65, 0.75*, 0.9, 0.95, 1.0s and 0.0*, 0.0192, 0.0384, 0.0576, 0.0768, 0.096*, 0.1152*, 0.1344, 0.1536, 0.1728, 0.192* relaxation delays were used for the determination of R_1 and R_2 relaxation rates at 27 C, respectively. The series of 0.0**, 0.1, 0.15*, 0.25**, 0.35, 0.4, 0.5**, 0.65*, 0.75*, 0.9, 0.95, 1.0*s and 0.0**, 0.0192*, 0.0384*, 0.0576*, 0.0768*, 0.096**, 0.1152*, 0.1344, 0.1536*, 0.1728* relaxation delays were used for the determination of R_1 and R_2 relaxation rates at 27 C, respectively. The delays denoted by a star and double star were measured two and three times, respectively. The ssNOE experiment was set up with a length of the saturation period 7s followed after 3 s of interscan delay and 5 s followed after 11 s of interscan delay at 27 C and at 7 C, respectively.

Analysis of relaxation experiments

The spectra were processed using the program *NMRPipe* [15] and analyzed in the program *Sparky* [16]. The previously assigned amide resonances [1] were used to identify peaks in the spectra at 27 C, while the assignment of spectra measured at 7 C was determined following the trends of changes in the peak positions within the sequence of HSQC spectra measured from 37 C to 7 C with a 10 C-step. The decay of the peak intensities has been fitted to a monoexponential decay using the program *Relax* [17]. The error of the ssNOE parameter has been estimated from the noise in the both reference and saturated spectra. The extracted relaxation rates were analyzed by the reduced spectral density mapping, using an in-house written script.

Results and discussion

76 and 67 peaks were suitable for the analysis at 27 C to 7 C. The results of the calculated spectral density function at zero frequency J(0), nitrogen spin precession frequency J($_N$), and hydrogen spin precession frequency J($_H$) are shown for individual residues in Fig. 1, Fig. 2, and Fig. 3, respectively. The differences between data obtained at 27 C and 7 C are caused by slowing down the overall tumbling of the molecule and increasing the internal motion restrictions at the lower temperature. The increase in the values of spectral density function J($_H$) accompanied by very low values of spectral density function J(0) is observed for the C-terminal residues. It suggests that residues with higher residue number than 85 move rapidly and independently of the overall molecular motion of the rest of the molecule. The effect is observed at both temperatures. The N-terminal part of the molecule exhibits larger internal motions as well. It is proved by the higher values of J($_H$). However, it is more compactly attached to the core of the protein and follows its overall motions because the values of spectral density function J(0) are similar to the values of the majority of the residues. The residues surrounding the central part of the molecule show the same pattern. It im-

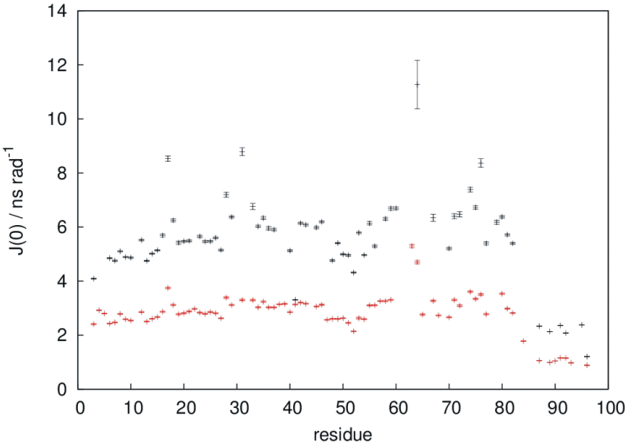

Figure 1. The dependence of the spectral density function at zero frequency on the residue number. Data shown in red (bottom) and black correspond to the measurements at 27 C and 7 C.

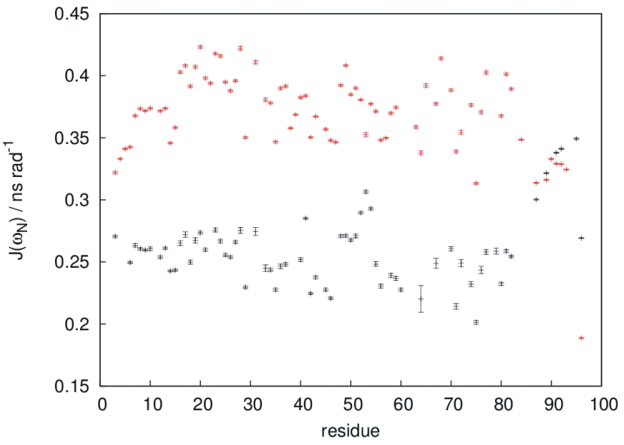

Figure 2. The dependence of the spectral density function at nitrogen spin frequency on the residue number. Data shown in red and black (bottom) correspond to the measurements at 27 C and 7 C.

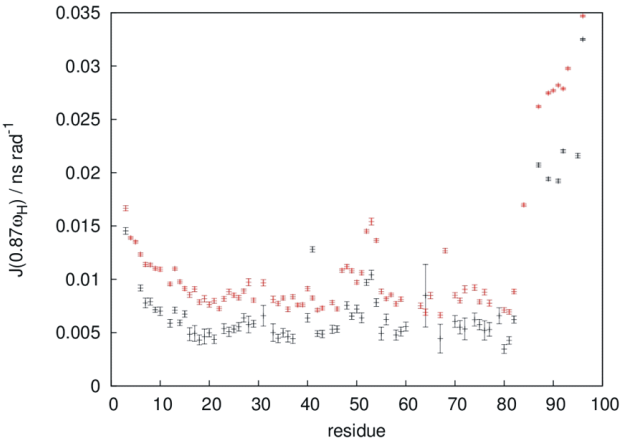

Figure 3. The dependence of the spectral density function at effective frequency (defined as 0.87 H) on the residue number. Data shown in red and black (bottom) correspond to the measurements at 27 C and 7 C.

plies that the structure is rather disordered in this region although not completely free. Hence, the higher structural variability should be taken into account if some interactions in this region are expected. The residues characterized by significantly higher values of the spectral density function J(0) are supposed to undergo chemical exchange at s – ms timescale. J(H) values typical for well structured proteins were obtained throughout the molecule except the C-terminal part.

In conclusion, relaxation measurements analyzed by spectral density mapping complemented the recently determined 3D structure of -subunit of RNA polymerase from *Bacillus subtilis* with the description of internal dynamics of the protein. The most extensive fast motions were observed in the disordered C-terminal tail. More interestingly, residues 52—54 exhibited higher flexibility compared to the remaining residues of the structured part of the molecule. Finally, high values of J(0) found for V17, V31, N63, I64, and W76 revealed slow motions of these residues. Relaxation dispersion measurements, aimed at further quantitative analysis of the slow conformational dynamics are currently in progress.

References

1. V. Motáčková, H. Šanderová, L. Žídek, J. Nováček, P. Padrta, A. Švenková, J. Korelusová, J. Jonák, L. Krásný, V. Sklenář, *Proteins*, **78**, (2009), 1807.
2. D.M. Korzhnev, M. Billeter, A.S. Arseniev, V.Y. Orekhov, *Prog. NMR Spec.*, **38**, (2001), 197.
3. J.R. Tolman, *J. Am. Chem. Soc.*, **124** , (2002), 12020.
4. A.G. Palmer, C.D. Kroenke, J.P. Loria, *Methods in Enzymology*, Academic Press, London, **339**, (2001), 204.
5. J. Cavanagh, W. Fairbrother, A.G. Palmer, M.Rance, N.J. Skelton, *Protein NMR Spectroscopy*, Academic Press, London.
6 B.D.N. Rao, *Methods in Enzymology*, Academic Press, London, **176**, (1989), 279.
7 J. Sandstrom, *Dynamic NMR Spectroscopy* , Academic Press, London, (1982).
8. G. Lipari, A. Szabo, *J. Am. Chem. Soc.*, **104** , (1982), 4546.
9. G. Lipari, A. Szabo, *J. Am. Chem. Soc.*, **104** , (1982), 4559.
10. G.M. Clore, A. Szabo, A. Bax, L.E. Kay, P.C. Driscoll, A.M. Gronenborn, *J. Am. Chem. Soc.*, **112** , (1990), 4989.
11. J.A. Butterwick, P.J. Loria, N.S. Astrof, C.D. Kroenke, R. Cole, M. Rance, A.G. Palmer, *J. Mol. Biol.*, **339**, (2004), 855.
12. E. d'Auvergne, P. Gooley, *J. Biomol. NMR*, **40**, (2008), 121.
13. N.A. Farrow, O.W. Zhang, A. Szabo, D.A. Torchia, L.E. Kay, *J. Biomol. NMR*, **6**, (1995), 153.
14. N.R. Krishna, L.J. Berliner, *Biological Magnetic Resonance*, Kluwer Academic, New York, (1999).
15. F. Delaglio, S. Grzesiek, G.W. Vuister, G. Zhu, J. Pfeifer, A. Bax, *J. Biomol. NMR*, **28**, (2004), 69.
16. T.D. Goddard, D.G. Kneller, SPARKY 3, University of California, San Francisco.
17. E. d'Auvergne, P. Gooley, *J. Biomol. NMR*, **40**, (2008), 107.

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