Journal of Biomolecular NMR, **13:** 303–304, 1999.
KLUWER/ESCOM

Letter to the Editor: Sequence-specific ^{1}H, ^{13}C and ^{15}N assignment of the single-stranded DNA binding protein of the bacteriophage ϕ29

Antonio Pineda-Lucena, Geerten W. Vuister & Cornelis W. Hilbers
NSR Center for Molecular Structure, Design and Synthesis, Laboratory of Biophysical Chemistry, University of Nijmegen, 6525 ED Nijmegen, The Netherlands

Received 21 October 1998; Accepted 16 November 1998

Key words: CHAPS, NMR assignments, ϕ29, ssDNA

Biological context

Single-stranded DNA (ssDNA) binding proteins (SSBs) are expressed in a wide variety of organisms (Kneale, 1992). They are involved in processes vital for the life cycle of these organisms, such as DNA replication, recombination and repair. SSBs bind to ssDNA as monomers (bacteriophage T4 gp32, AdSSB, ϕ29 SSB), dimers (Pf3 SSB, M13 gene-V protein), heterotrimers (human RPA) and homotetramers (HmstSSB, *E. coli* SSB).

The gene V of the bacteriophage ϕ29 encodes an SSB of 124 amino acids which stimulates viral DNA replication. ϕ29 SSB binds to ssDNA with relatively low affinity and moderate cooperativity, covering 3–4 nucleotides per monomer, probably as a single array of protein units along the DNA (Soengas et al., 1994). The low sequence homology with other SSBs has hampered the identification of the ϕ29 domain(s) and protein-protein contacts involved in the cooperative binding to ssDNA.

Methods and results

Escherichia coli PC-V3059 cells (Phabagen Collection, Utrecht, The Netherlands), transformed with the pGM26 expression vector encoding the SSB of ϕ29, were grown at 303 K. Protein expression was induced raising the temperature to 315 K for 3 h when reaching $OD_{600} = 0.2$. ^{15}N and ^{13}C labeling was carried out by growing in minimal medium using $^{15}NH_4Cl$ and ^{13}C-glucose as the sole nitrogen and carbon sources. The protein was purified using differential ammonium sulfate fractionation and further phosphocellulose and DEAE-cellulose chromatography. The yield was 40–50 mg L^{-1} in minimal media.

SSBs have a strong tendency to aggregate, even in the absence of the ssDNA (Casas-Finet et al., 1992). Our initial studies indicated that ϕ29 is also not soluble at concentrations required for NMR studies. It was observed that ϕ29 SSB forms a gel at concentrations larger than 0.4 mM. To get insight into the forces modulating the ϕ29 SSB self-association, the influence of pH, salt, urea and non-denaturating detergent was examined. The apparent lack of significant effects from variations in pH and salt concentration suggest minimal contributions to the aggregation processes from charge-charge interactions. In contrast, at non-denaturating concentrations of urea (0.25–0.40 M), ϕ29 SSB oligomers seem to break down suggesting that hydrophobic and polar residues are responsible for ϕ29 SSB association. Experiments with the non-denaturating detergent CHAPS, which also disrupts hydrophobic interactions, confirmed these results. In the presence of CHAPS, the solubility of ϕ29 SSB increased to 0.85 mM.

Four samples, one unlabeled, one uniformly ^{15}N labeled, and the other two (^{15}N and $^{15}N/^{13}C$) reverse labeled for the Thr and Leu residues, were prepared as 0.85 mM protein solutions in 90%/10% (v/v) H_2O/D_2O or 100% D_2O, 10 mm Na_2HPO_4, 5 mM CHAPS, pH 6.0. The following experiments were acquired to assign the backbone resonances: 3D HNCO, 3D HN(CO)CA, 3D HCACO, 3D HNCA, and 3D ^{15}N-separated TOCSY- and NOESY-HSQC (Grzesiek et al., 1997). Pulsed field gradient versions of the experiments, with water flip-back pulses and sensitivity enhancement (Kay et al., 1994) were recorded on Varian Unity *Inova* 500 and *Inova* 750 spectrometers at 298 K.

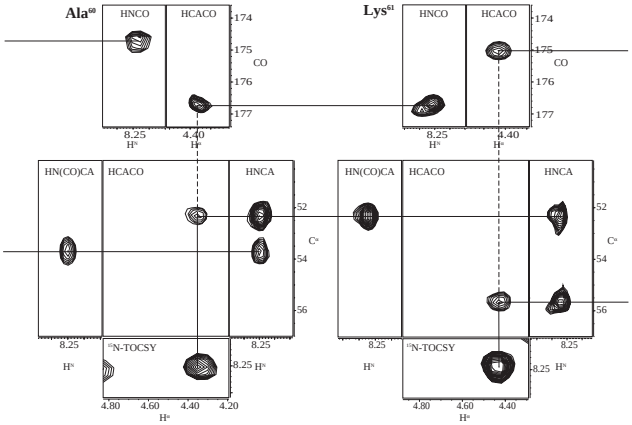

Figure 1. Sequential assignment of the Ala60-Lys61 fragment in the ϕ29 SSB sequence based on the connectivities found in the 3D spectra. The solid lines correlate connectivities found in the different experiments. The dashed lines connect identical peaks in different cross-sections of the same experiment.

The program NMRPipe (Delaglio et al., 1995) was used for transformation of all data whereas the program XEASY (Bartels et al., 1996) was used for the analysis. The assignment strategy was initially based upon the usage of ^{15}N-^{1}H detected triple-resonance spectra in conjunction with ^{15}N-separated NOESY spectra following the now common protocol. However, it proved impossible to transfer magnetization beyond the C^{α} nucleus, presumably because of the short T_2 resulting from higher molecular weight aggregates, conformational exchange, and an inherent low sample concentration. Sequential assignment using the C^{α} nucleus alone proved problematic because of the insufficient dispersion. Therefore, we resorted to the initially proposed procedure by Ikura et al. (1990). Using ^{15}N-separated TOCSY, HNCA, and HCACO experiments, the intra-residual CO, ^{15}N, and $^{1}H^{N}$ resonances were correlated. Comparison with the HNCO provided an additional sequential link. The assignment procedure is illustrated in Figure 1.

The assignments of the non-aromatic side chains were obtained using 3D HC(C)H- and (H)CCH-TOCSY in conjunction with a 2D constant-time ^{1}H/^{13}C HSQC (Vuister and Bax, 1992). Aromatic spin systems were assigned using a 2D ^{1}H-TOCSY acquired on a non-labeled sample in D_2O.

Extent of assignments and data deposition

112 out of 124 residues were almost fully assigned, with exception of the amide side chain resonances. Of the unassigned residues three are located at the C-terminus and eight correspond to reverse labeled (Thr, Leu) residues. For most of the latter residues, ^{1}H spin systems have been identified but not mapped sequentially yet.

The ^{1}H, ^{13}C, and ^{15}N chemical shifts of the single-stranded DNA binding protein of the bacteriophage ϕ29 recorded at 298 K, pH 6.0 have been deposited at BioMagResBank (http://www.bmrb.wisc.edu) under BMRB accession number 4239.

Acknowledgements

This research was supported by an European Molecular Biology Organization (EMBO) fellowship and a Training and Mobility Research Grant/Marie Curie fellowship of the European Commission (contract number: FMBICT950493) to A.P.-L.

References

Bartels, C., Xia, T.H., Billeter, M., Güntert, P. and Wüthrich, K. (1995) *J. Biomol. NMR*, **6**, 1–10.

Casas-Finet, J.R., Fischer, K.R. and Karpel, R.L. (1992) *Proc. Natl. Acad. Sci. USA*, **89**, 1050–1054.

Delaglio, F., Grzesiek, S., Vuister, G.W., Zhu, G., Pfeifer, J. and Bax, A. (1995) *J. Biomol. NMR*, **6**, 277–293.

Grzesiek, S., Bax, A., Hu, J.-H., Kaufman, J., Palmer, I., Stahl, S.J., Tjandra, N. and Wingfield, P.T. (1997) *Protein Sci.*, **6**, 1248–1263.

Ikura, M., Kay, L.E. and Bax, A. (1990) *Biochemistry*, **29**, 4659–4667.

Kay, L.E., Xu, G.-Y. and Yamakazi, T. (1994) *J. Magn. Reson.*, **A109**, 129–133.

Kneale, G.G. (1992) *Curr. Opin. Struct. Biol.*, **2**, 124–130.

Murzin, A.G. (1993) *EMBO J.*, **12**, 861–867.

Soengas, M.S., Esteban, J.A., Salas, M. and Gutierrez, C. (1994) *J. Mol. Biol.*, **239**, 213–226.

Vuister, G.W. and Bax, A. (1992) *J. Magn. Reson.*, **98**, 428–435.