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Backbone assignment of the intrinsically disordered N-terminal region of Bloom syndrome protein

Min June Yang and Chin-Ju Park*

Department of Chemistry, Gwangju Institute of Science and Technology, Gwangju 61005, Republic of Korea

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Abstract Bloom syndrome protein (BLM) is a pivotal RecQ helicase necessary for genetic stability through DNA repair processes. Our investigation focuses on the N-terminal region of BLM, which has been considered as an intrinsically disordered region (IDR). This IDR plays a critical role in DNA metabolism by interacting with other proteins. In this study, we performed triple resonance experiments of $BLM_{220-300}$ and presented the backbone chemical shifts. The secondary structure prediction based on chemical shifts of the backbone atoms shows the region is disordered. Our data could help further interaction studies between $BLM_{220-300}$ and its binding partners using NMR.

Keywords Bloom syndrome protein, backbone assignment, intrinsically disordered region

Introduction

The Bloom syndrome protein (BLM), an important RecQ helicase[1], safeguards gene stability by participating in DNA repair processes[2]. Notably, BLM plays a central role in homologous recombination (HR) during DNA double-strand break repair[3]. Collaborating with DNA topoisomerase 3-α (TOP3α), RecQ-mediated genome instability protein 1 (RMI1), RMI2, and BLM forms a replication fork complex crucial for managing replication stress and ensuring accurate replication[4].
BLM has a helicase core consisting of an ATPase domain, an RQC domain, and an HRDC domain at the C-terminal (Figure 1)[5, 6], but no structural domain exists at the N-terminal. The N-terminal region is a flexible and intrinsically disordered region (IDR) that interacts with the N-terminal region of Replication protein A 70 kDa (RPA70N)[7], mini-chromosome maintenance protein subunit 6 (MCM6)[8], and

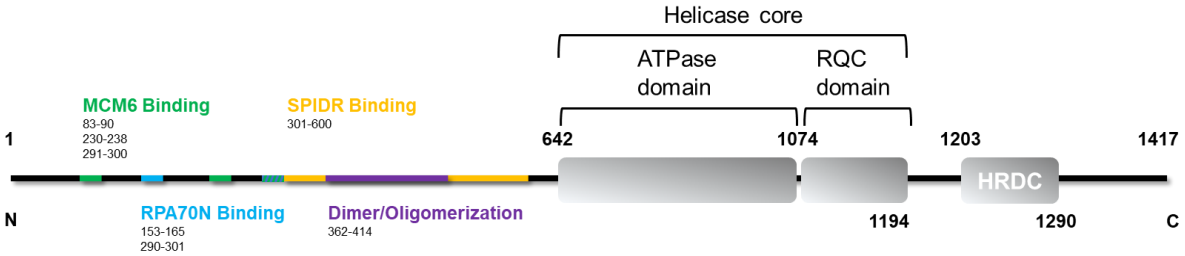

Figure 1. The domain structure of BLM. BLM has 3 structured domains (ATPase, RQC, and HRDC domains) and IDR at N-terminal. N-terminal IDR interacts with MCM6 (Green) RPA70N (Cyan), SPIDR (Orange), and themselves (Violet). Interacting amino acid residue numbers are shown below, respectively.

* Address correspondence to: **Chin-Ju Park,** Department of Chemistry, Gwangju Institute of Science and Technology, Gwangju 61005, Republic of Korea, Tel: 82-62-715-3630; Fax: 82-62-715-2866; E-mail: cjpark@gist.ac.kr

scaffolding protein involved in DNA repair (SPIDR)[9] proteins, crucial for DNA metabolism processes (Figure 1). BLM's N-terminal region also promotes dimerization and interactions with other BLM molecules[10]. Although the N-terminal of BLM is involved in various protein-protein interactions, the chemical shifts assignment has not yet been done. Our study employs NMR spectroscopy, including 2D and 3D spectra, along with automated assignment *via* PINE-SPARKY.2 plugins[11]. Successful chemical shift assignments of backbone atoms of the $BLM_{220-300}$ region are expected to help interaction studies between the BLM N-terminal and its partners.

Experimental Methods

Sample preparation – BLM residue number 220-300 ($BLM_{220-300}$) was cloned into the pET His 6 GST TEV LIC cloning vector (Addgene, USA) and transformed into BL21(DE3)pLysS cells (Novagen, USA). The cells were grown in lysogeny broth (LB) medium 37℃ until they reached an optical density at 600 (OD_{600}) of 0.6. Following induction with 1.0 mM IPTG, the cells were incubated at 18℃ for 20 hours. The produced proteins were initially purified using a Ni-NTA column (Cytiva, USA) and gel filtration chromatography. The GST tags were removed using Tobacco Etch Virus (TEV) protease. The $BLM_{220-300}$ region was separated from the cleaved GST connected with the six-histidine tag using the Ni Sepharose™ 6 Fast Flow (Cytiva, USA). The purified protein samples were prepared in the NMR buffer (20 mM 2-(N-morpholino) ethane sulfonic acid (MES), 50 mM NaCl, 1 mM 4-dithiothreitol (DTT), 1 mM ethylenediaminetetraacetic acid (EDTA), pH 6.5).

Protein samples labeled with ^{15}N and ^{13}C isotopes were obtained by cultivating cells in M9 minimal media containing ammonium chloride (^{15}N, 99%) and D-glucose (U-$^{13}C_6$, 99%; Cambridge isotope laboratories, USA). These samples underwent the same overexpression and purification steps as those cultured in LB media.

NMR experiments and secondary structure prediction – Freshly prepared ^{15}N- and ^{13}C-labeled $BLM_{220-300}$ samples were used for chemical shift assignments. The protein sample was dissolved to a final concentration of 500 μM in the NMR buffer and then transferred to a salt-tolerant Shigemi NMR tube (Shigemi, Allison Park, PA) for subsequent NMR experiments. NMR spectra were acquired using a Bruker AVANCE III (Bruekr, Germany) spectrometer operating at 800 MHz for ^1H, equipped with a cryogenic triple- resonance probe (KBSI, Ochang). All experiments were conducted at a temperature of 298 K.

Several NMR experiments were performed for backbone assignments, including 2D ^1H-^{15}N HSQC, 3D HNCO, 3D HN(CA)CO, 3D HN(CO)CACB, and 3D HNCACB, as detailed in Table 1. Data processing of NMR information was carried out using NMRPipe software[12] and TOPSPIN software from Bruker. Analysis of the processed spectra was done using the NMRFAM-SPARKY software package[13], with the aid of (semi-)automated tools and verification functions provided by NMRFAM-SPARKY.

The initial peak-picking was executed by the APES automation plugin, and the identified peaks were subsequently validated by two strip plots. For further validation of C_α/C_β peaks, strip plots were generated from HN(CO)CACB and HNCACB spectra. Verification of C_O peaks was achieved by generating strip plots from HNCO and HN(CA)CO spectra. Peaks that were confirmed from the backbone spectra were then inputted into the PINE-SPARKY.2 plugin[11] to interact with the I-PINE web server[14] for automated assignments.

Overall secondary structure was predicted from TALOS-N[15] based on combined N^H, ^{15}N, $^{13}C_\alpha$, $^{13}C_\beta$, and $^{13}C_O$ backbone chemical shifts.

Results and Discussion

Backbone assignment of $BLM_{220-300}$ – Assigned chemical shifts of backbone ^1H, ^{15}N, and ^{13}C resonances for $BLM_{220-300}$ have been deposited in the

BioMagResBank (http://www.bmrb.wisc.edu/, accession number 52084). Figure 2 shows a 2D ^{1}H-^{15}N HSQC spectrum of $BLM_{220-300}$, with assigned peaks showing sequence details and residue numbers. The peaks have a similar ^{1}H chemical shift and are narrowly distributed. This is a common feature of IDRs[16]. The ^{1}H, ^{15}N, and ^{13}C assignments obtained for $BLM_{220-300}$ are listed in Table 2. Despite the large dynamic range of peak intensities, peak splitting, and peak overlaps, we could assign 72 of 82 backbone amide groups between 220-300, excluding the two proline residues and including the three GST-tag remaining residues. Residues at the terminal (S217 and N218) and overlapped peaks (E245, H261, E263, D264, E265, E270, E277, and S282) were unassigned. Assignment statistics: 72 of 82 backbone ^{15}N-$^{1}H^{N}$ (87.8%), 81 of 84 $^{13}C_{\alpha}$ (96.4%), 80 of 83 $^{13}C_{\beta}$ (96.4%), and 81 of 84 $^{13}C_{O}$ (96.4%) backbone atoms were assigned.

$BLM_{220-300}$ has no secondary structure – TALOS-N[15] was performed to analyze assigned and referenced backbone chemical shifts. Random coil index order parameter result from TALOS-N is shown in Figure 3. As already known, $BLM_{220-300}$ has no secondary structure because it is IDR. However, this IDR is a significant region to study as an interaction with other proteins and the dimer/multimerization region with NMR.

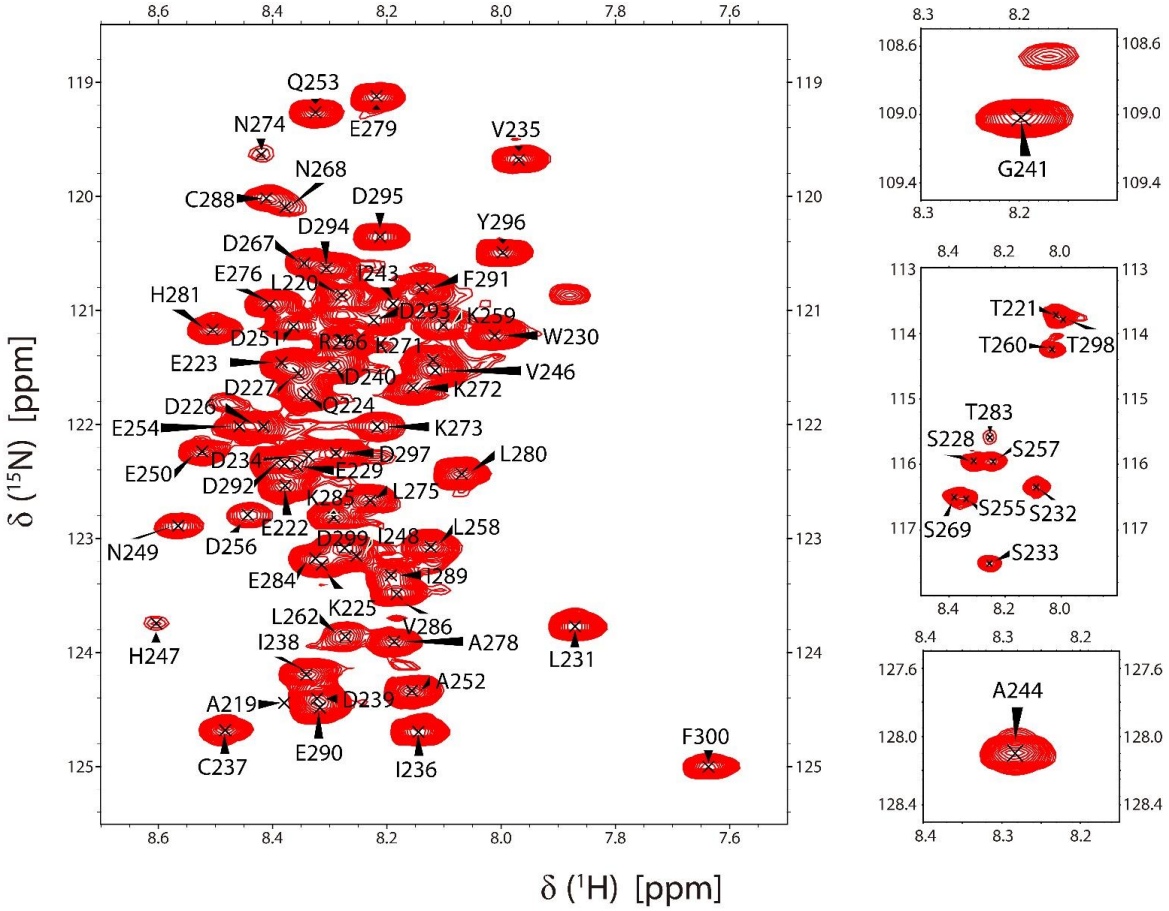

Figure 2. 2D ^{1}H-^{15}N HSQC spectrum of 500 μM $BLM_{220-300}$ with NMR buffer (20 mM MES, 50 mM NaCl, 1 mM DTT, 1 mM EDTA, pH 6.5) and 10% D_2O. This experiment was performed at 298 K on BRUKER AVANCE III 800 MHz. Assignments are labeled on the spectrum.

Table 1. 2D and 3D NMR spectra experiment information for backbone assignments. All experiments were performed at 298 K with Bruker AVANCE III 800 MHz.

Experiment	Number of scans	Spectral width (ppm) $^{1}H \times ^{13}C \times ^{15}N$	Number of points $^{1}H \times ^{13}C \times ^{15}N$	Offset (ppm) ^{1}H, ^{13}C, ^{15}N	NUS Amount (%)
^{1}H-^{15}N-HSQC	4	13.02 × 24	800 × 192	4.7, 119	100
HNCO	16	11.49 × 7 × 24	698 × 128 × 192	4.701, 173.5, 119	25
HN(CA)CO	16	11.49 × 7 × 24	698 × 128 × 192	4.701, 173.5, 119	25
HN(CO)CACB	32	11.49 × 64 × 24	698 × 192 × 256	4.701, 43, 119	12.5
HNCACB	16	11.49 × 64 × 24	698 × 192 × 256	4.701, 43, 119	12.5

Table 2. Backbone C_O, C_α, C_β, H^N, and N chemical shifts of $BLM_{220\text{-}300}$. (unit : ppm)

Residue	C_O	C_α	C_β	H^N	N	Residue	C_O	C_α	C_β	H^N	N
217 SER						259 LYS	176.8	56.37	32.68	8.078	121
218 ASN	174.6	53.08	38.85			260 THR	174.4	61.98	69.65	8.015	114.2
219 ALA	177.6	52.54	19.11	8.36	124.4	261 HIS	174.6	55.6	29.25		
220 LEU	177.7	55.29	42.11	8.261	120.9	262 LEU	177.5	55.42	42.21	8.269	123.8
221 THR	174.6	61.54	69.71	8.001	113.7	263 GLU					
222 GLU	176.4	56.64	30.15	8.359	122.5	264 ASP					
223 GLU	176.3	56.62	30.18	8.361	121.4	265 GLU	176.8	56.92	29.85		
224 GLN	175.7	55.59	29.18	8.325	121.7	266 ARG	176.4	56.52	30.57	8.256	121.2
225 LYS	176.1	55.92	33.21	8.298	123.1	267 ASP	176.3	54.46	41.03	8.317	120.5
226 ASP	176	54.14	41.23	8.411	121.9	268 ASN	175.9	53.43	38.66	8.356	120
227 ASP	176.6	54.32	40.87	8.328	121.5	269 SER	175.1	59.61	63.54	8.367	116.4
228 SER	175	59.17	63.58	8.291	115.9	270 GLU	176.9	57.05	29.75		
229 GLU	176.3	56.91	29.83	8.341	122.3	271 LYS	176.9	56.58	32.53	8.106	121.4
230 TRP	176	57.09	29.17	7.988	121.2	272 LYS	176.7	56.53	32.72	8.141	121.6
231 LEU	177	54.86	42.44	7.852	123.7	273 LYS	176.4	56.55	32.88	8.198	122
232 SER	174.7	58.16	63.7	8.073	116.3	274 ASN	175.4	53.35	38.55	8.402	119.5
233 SER	174.2	58.32	63.77	8.236	117.4	275 LEU	177.6	55.66	42.07	8.214	122.6
234 ASP	175.9	54.38	40.97	8.313	122.2	276 GLU	177	56.98	29.84	8.378	120.9
235 VAL	175.9	62.18	32.57	7.948	119.7	277 GLU	176.6	56.94	29.97		
236 ILE	175.8	60.77	38.57	8.138	124.7	278 ALA	178.1	52.93	19.02	8.168	123.8
237 CYS	174.4	58.06	27.76	8.475	124.7	279 GLU	176.6	56.58	29.98	8.194	119
238 ILE	175.7	61.02	38.77	8.332	124.2	280 LEU	177.4	55.35	42.2	8.051	122.3
239 ASP	175.6	54.18	41.27	8.304	124.3	281 HIS	176.3	56.79	29.89	8.496	121.1
240 ASP	176.4	54.11	41.14	8.273	121.4	282 SER	174.9	58.4	63.65		
241 GLY	178.8	44.65		8.176	108.9	283 THR	174.5	61.81	69.5	8.246	115.5
242 PRO	177.2	62.93	31.88			284 GLU	176.1	56.4	30.19	8.309	123.1
243 ILE	176	60.92	38.58	8.179	120.9	285 LYS	176.2	55.86	32.88	8.283	122.8

244 ALA	176.1	52.13	19.23	8.272	128.1	286 VAL	174.5	59.74	32.3	8.173	123.5
245 GLU	176.1	56.21	30.37			287 PRO	176.5	63.14	31.97		
246 VAL	175.6	62.01	32.75	8.106	121.6	288 CYS	174.3	58.06	27.85	8.403	120
247 HIS	174.3	54.92	29.41	8.603	123.7	289 ILE	175.7	61.07	38.62	8.182	123.3
248 ILE	175.6	60.85	38.75	8.248	123.1	290 GLU	175.6	56.16	30.37	8.301	124.4
249 ASN	175.2	53.12	38.77	8.557	122.8	291 PHE	175.1	57.14	39.83	8.13	120.8
250 GLU	176.2	56.79	30.11	8.509	122.2	292 ASP	175.6	53.95	41.28	8.37	122.3
251 ASP	176.1	54.31	40.97	8.345	121	293 ASP	176	54.23	41.25	8.203	121
252 ALA	177.7	52.49	18.99	8.139	124.3	294 ASP	175.8	54.35	41.12	8.28	120.5
253 GLN	176.3	55.82	29.29	8.308	119.2	295 ASP	175.8	53.96	40.95	8.187	120.2
254 GLU	176.8	56.69	30	8.441	121.9	296 TYR	175.5	57.65	38.74	7.975	120.4
255 SER	174.8	58.6	63.73	8.335	116.5	297 ASP	176	54.05	41.11	8.267	122.1
256 ASP	176.8	54.77	40.93	8.424	122.7	298 THR	174.1	61.43	69.74	7.986	113.7
257 SER	174.9	59.04	63.52	8.221	115.9	299 ASP	174.8	54.19	41	8.257	123
258 LEU	177.6	55.49	41.89	8.102	123	300 PHE	173.3	58.9	40.11	7.613	124.9

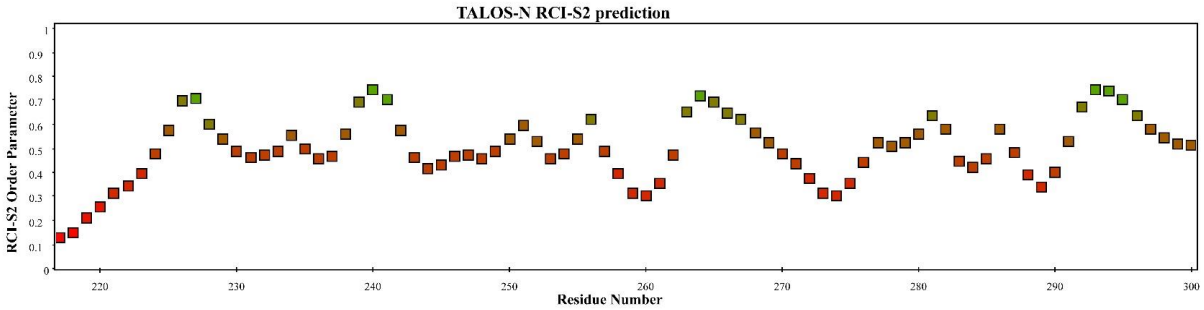

Figure 3. TALOS-N[15] results were calculated from the backbone chemical shift data of $BLM_{220-300}$. Random coil index order parameters ($RCI-S^2$) calculated from TALOS-N (Red to green, ordered to rigid). No secondary structure was predicted from the TALOS-N $RCI-S^2$ calculation.

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