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Backbone Assignment of the N-terminal Domain of Human Replication Protein A 70 kDa

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Abstract Replication Protein A (RPA) is the eukaryotic single-stranded DNA binding protein. It involves in DNA replication, repair, and damage response. Among three subunits, RPA70 has a protein-protein binding domain (RPA70N) at the N-terminal. It has known that the domain recruits several damage response proteins to the damaged site. Also, it is suggested that there are more candidates that interact with RPA70N. Even though several studies performed on the structural aspects of RPA70N and its ligand binding, the backbone assignments of RPA70N is not available in public. In this study, we present the backbone assignments of RPA70N.

Keywords Replication Protein A (RPA), Backbone Assignments, NMR

Introduction

Replication Protein A (RPA) is the single-stranded DNA binding protein which involves in various DNA metabolisms such as DNA replication, repair, and damage response.[1,2] Among three subunits (70 kDa, 32 kDa and 14 kDa) of human RPA, 70 kDa contains two main DNA binding domains (70A and 70B) and the protein interacting domain (70N) at its N-terminal. Previously, DNA binding properties of RPA70A and 70B studied extensively.[3,4]

It has been known that RPA70N interacts with several DNA damage response proteins such as p53, ATRIP, RAD9, MRE11 and SV40.[5-7] Through the interaction, damage response proteins are recruited to the damage site.[8] Also, the three-dimensional (3D) structure of RPA70N alone[9,10] and several crystal structures of RPA70N with its potent inhibitors are available.[11] Recently, there are growing interests in the RPA inhibition for the anticancer target.[12] Even though many in-depth structural studies of RPA70N in the aspect of the protein-protein interaction, the backbone assignment of the protein is not available in public. In order to investigate the protein-protein interaction of RPA70N with other candidates besides damage response proteins, we performed triple resonance experiments for the backbone assignment of RPA70N.

Experimental Methods

Construct cloning – Full-size gene of human RPA70 was obtained from Promega. The DNA encoding RPA 70N domain (1-120) was amplified by polymerase chain reaction (PCR) from RPA full gene. The PCR product was cloned into pET15b vector which has N-terminal 6xHis by using NdeI/XhoI restriction sites. DNA construct was confirmed by the

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DNA sequencing. It was transformed into *Escherichia coli* strain BL21 (DE3) strain.

Protein expression and purification – RPA70N was produced as described previously.[13] Cells were grown in the M9 media which containing ^{15}N labeled NH_4Cl and ^{13}C labeled glucose at 37 °C until the 600 nm optical degree is reached 0.5. The protein induction was performed with 1.0 mM isopropyl-1-thio-β-D-galactopyranoside (IPTG). The cells were further incubated at 18 °C for 18 hours after induction.

Cells were harvested by 15 minutes of the centrifuge at 9,000 rpm at 4°C and resuspended in the buffer containing 50 mM NaH_2PO_4 and 300 mM NaCl at pH 8.0. After the sonication, the protein was purified with Ni-NTA column. Further purification was performed with gel permeation chromatography using AKTA pure and Hi-Load 16/600 75pg (GE Healthcare) with 20 mM Tris, 100 mM NaCl, 2 mM DTT at pH 7.4.

NMR Spectroscopy – The sample contained 1.0 mM ^{13}C, ^{15}N-labeled RPA70N in 20 mM Tris-HCl, 100 mM NaCl, 2 mM DTT at pH 7.4. ^{1}H-^{15}N HSQC, ^{1}H-^{13}C HSQC, HNCACB, HNCO, and HN(CA)CO experiments[14] were performed using 800 MHz Bruker Avance II NMR spectrometer equipped with a cryogenic probe at 298 K (Korea Basic Science Institute, Ochang). All NMR spectra were processed with Topspin (Bruker) software and analyzed with Sparky.[15]

The chemical shifts were used for analyzing secondary structure based on the backbone angle predictions with TALOS^{+}[16].

Results and Discussion

The ^{1}H, ^{15}N and ^{13}C assignment obtained for RPA70N are listed in Table 1. Among 120 total residues, 107 amide resonances were assigned. Considering seven prolines, the completeness is 94.7 %. G36, N38 and S38 which is followed by two consecutive prolines in the β1/ β2 loop were not assigned. It might be caused by the flexibility in the loop. Also, S54 in the β3, K88 in the β4/ β5 loop, and V116 in the C-terminal were not assigned. For the carbons, 94.2 % of Cα, 93.7 % of Cβ, and 94.2 % of CO were assigned. Based on these chemical shifts, we analyzed the secondary structure of the protein with TALOS^{+}[16]. Figure 1(A) shows the sequences with the secondary structure. The unassigned residues are colored in gray. Compare to the previously reported 3D structures[9,10], the secondary structure is well matched. (Figure 1B) Figure 2 Shows the ^{1}H-^{15}N HSQC spectra of RPA70N. Each peak was labeled with assigned amino acids. Previously, parts of ^{1}H-^{15}N HSQC spectra of RPA70N has been reported.[4,5,11] Our assignments are all consistent with that information.

In summary, we present the backbone assignment of human RPA70N. We expect that it could facilitate to examine protein-protein or protein-ligand interactions of RPA70N.

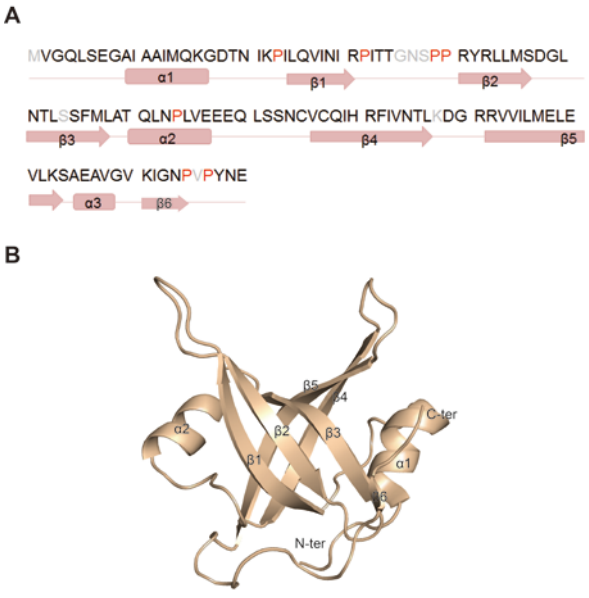

Figure 1. (A) Amino acid sequence and the secondary structure of human RPA70N. The unassigned residues are colored in gray and prolines are colored in red. The secondary structure is assigned based on TALOS^{+} analysis[16] (B) 3D structure of the RPA 70N domain.[10] (PDB ID : 2b29)

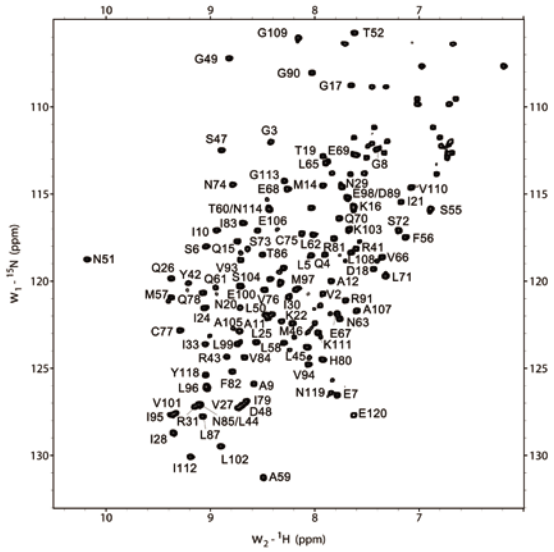

Figure 2. ^{1}H-^{15}N HSQC spectra of RPA70N.

Table 1. Backbone CA, CB, CO, N and HN chemical shifts of human RPA70N. (unit: ppm)

Residue	CA	CB	CO	N	HN
M1	52.75	-	173.2	-	-
V2	60.33	29.68	173.7	120.8	7.966
G3	42.86		170.8	112	8.417
Q4	53.28	27.32	173.1	118.5	7.926
L5	51.33	39.58	175.7	118.5	8.044
S6	53.43	58.71	170.7	118	9.051
E7	55.95	27.11	175.8	126.5	7.791
G8	42.33		172.3	112.5	7.416
A9	52.35	17.6	176.7	125.9	8.589
I10	63.79	33.44	174.1	117.1	8.949
A11	52.52	15.03	176.8	122.1	8.454
A12	53.06	15.21	178	120	7.85
I13	63.11	36.06	175.6	119.2	8.306
M14	53.22	29.55	175.8	114.5	7.93
Q15	54.74	26.65	175	117.7	8.747
K16	53.38	30.5	174.9	115.7	7.632
G17	43.85		171.4	108.7	7.652
D18	51.91	38.71	173.4	119.3	7.442
T19	58.5	66.32	172.2	112.8	7.929
N20	50.8	36.14	172.7	121.5	8.718
I21	58.58	37.84	171.6	115.5	7.179
K22	50.64	30.06	172	122.3	8.325
P23	60.08	30.43	171.9		
I24	58.59	34.86	172.8	121.5	9.066
L25	50.14	45.02	171.9	123.5	8.567
Q26	50.58	29.42	173.5	119.8	9.381
V27	60.39	29.45	172.7	127.3	8.737
I28	57.51	33.09	173.7	128.7	9.358
N29	50.51	39.67	169.6	114.6	7.745
I30	58.58	38.14	171.9	120.9	8.255
R31	49.8	30.29	170.5	127.2	9.153
P32	59.42	29.29	174.3		
I33	57.78	36.12	173.5	123.6	9.054
T34	57.81	67.43	171.5	119.5	8.344
T35	58.14	67.67	172.1	117	8.365
G36	-		-	-	-
N37	-	-	-	-	-
S38	-	-	-	-	-
P39	-	-	-		
P40	60.54	30.01	172.8		
R41	50.35	30.26	172.7	118.1	7.606
Y42	55.98	37.6	171.3	120.2	9.214
R43	51.69	30.6	173.2	124.3	8.848
L44	51.89	41.73	170.8	127.1	9.103
L45	50.76	41.16	173.9	123.8	8.076
M46	51.33	34.09	171.2	122.4	8.22
S47	51.61	64.11	172.2	112.5	8.9
D48	50.22	39.34	171.9	127.1	8.703
G49	42.71		170.6	107.2	8.826
L50	53.6	41.56	175.6	121.9	8.478
N51	49.47	42.83	171.5	118.8	10.18

T52	56.56	68.36	170.8	105.8	7.623	L87	51.55	39.13	176.6	127.7	9.078
L53	52.57	43.93	173.6	119.9	8.437	K88	-	-	174.2	-	-
S54	56.19	61.94	172.5	-	-	D89	50.61	37.4	174.4	115.3	7.686
S55	54.38	59.94	170.4	115.9	6.898	G90	42.22		171.9	108	8.032
F56	54.56	38.54	173	117.5	7.136	R91	54.35	28.24	172.4	121.1	7.71
M57	50.26	32.48	172.7	120.9	9.383	R92	52.56	30.06	173.4	120.1	8.335
L58	52.06	40.59	175.4	123.5	8.301	V93	57.16	32.69	170.5	118.7	8.711
A59	50.96	16.62	176.1	131.3	8.498	V94	58.46	29.35	172.2	124.7	8.065
T60	62.93	65.84	175.3	115.8	8.449	I95	57.27	34.81	173.8	127.6	9.374
Q61	55.78	24.67	173.8	120.4	8.956	L96	51.64	39.8	171.7	126	9.046
L62	51.51	39.36	173.7	117.3	8.017	M97	53.44	31.22	172.9	120.5	8.18
N63	54.71	32.84	172.4	122.2	7.768	E98	53.48	31.6	172.8	115.2	7.686
L65	55.63	39.87	177.8	113.2	7.901	L99	52.05	43.54	169.7	123.6	8.748
V66	62.02	29.67	177.4	118.6	7.364	E100	51.59	31.67	172.4	120.2	8.702
E67	56.73	26.31	175.5	121.9	7.786	V101	61.55	28.32	172.9	127.5	9.336
E68	53.25	26.4	173.1	114.7	8.269	L102	53.19	40.82	174.8	129.5	8.909
E69	55.2	23.82	172.5	112.8	7.61	K103	52.01	34.19	172.2	117.1	7.68
Q70	55.27	26.46	174.1	116.4	7.771	S104	55.08	62.19	170.9	120.3	8.707
L71	50.98	41.53	171	119.7	7.326	A105	53.01	17.42	177.9	122.9	8.722
S72	54.23	62.65	170.6	117.1	7.206	E106	56	26.4	174.8	117.1	8.547
S73	59.38	60.36	170.5	118.1	8.644	A107	51.02	16.16	176	121.7	7.606
N74	53.39	33.53	174	114.5	8.791	V108	62.41	30.25	176	118.4	7.657
C75	56.22	24.71	168.9	117.3	8.127	G109	44.34		170.5	106.1	8.165
V76	59.08	31.78	172.8	120.5	8.493	V110	56.8	33.2	171	114.7	7.082
C77	52.92	30.11	168.2	122.8	9.294	K111	54.39	29.69	173.6	123	7.978
Q78	51.51	27.91	171.7	120.6	9.078	I112	59.25	35.82	173.6	130.1	9.191
I79	57.19	33.86	171.4	127	8.669	G113	41.96		169.4	114.3	8.294
H80	55.16	30.87	173.2	124.4	7.959	N114	48.39	36.73	-	115.9	8.437
R81	54.23	31.27	171.4	117.5	7.825	P115	-	-	-		
F82	53.51	39.03	169.1	125.2	8.794	V116	-	-	-	-	-
I83	57.27	40.07	171.3	116.6	8.691	P117	61.37	29.27	174.4		
V84	58.82	30.04	173	124.3	8.674	Y118	56.74	37.38	172.5	125.4	9.051
N85	48.75	39.01	171.3	127.1	9.117	N119	50.71	36.12	169.9	126.4	7.851
T86	59.71	66.75	172	118.5	8.507	E120	55.53	28.79	173	127.6	7.625

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