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Backbone NMR chemical shift assignment for the substrate binding domain of *Escherichia coli* HscA

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Abstract HscA is a Hsp70-type chaperone protein that plays an essential role to mediate the iron-sulfur (Fe-S) cluster biogenesis mechanism in *Escherichia coli*. Like other Hsp70 chaperones, HscA is composed of two domains: the nucleotide binding domain (NBD), which can hydrolyze ATP and use its chemical energy to facilitate the Fe-S cluster transfer process, and the substrate binding domain (SBD), which directly interacts with the substrate, IscU, the scaffold protein of an Fe-S cluster. In the present work, we prepared the isolated SBD construct of HscA (HscA(SBD)) and conducted the solution-state nuclear magnetic resonance (NMR) experiments to have its backbone chemical shift assignment information. Due to low spectral quality of HscA(SBD), we obtained all the NMR data from the sample containing the peptide LPPVKIHC, the HscA-interaction motif of IscU, from which the chemical shift assignment could be done successfully. We expect that this information provides an important basis to execute detailed structural characterization of HscA and appreciate its interaction with IscU.

Keywords HscA, Hsp70-type chaperone, NMR spectroscopy, iron-sulfur cluster biogenesis

Introduction

The Fe-S cluster biogenesis is an essential process of supplying Fe-S clusters to diverse proteins within a cell.[1,2] Because of potential toxicity of free iron and sulfur ions, Fe-S cluster assembly and transfer mechanisms are tightly regulated by several protein factors and their interactions.[3] Among them, HscA is an Hsp70-like chaperone that mediates the Fe-S cluster transfer mechanism. It was reported that HscA can interact with IscU, the scaffold protein where an Fe-S cluster is assembled, and this interaction stabilizes the dynamic state of IscU and facilitates the transfer of an Fe-S cluster from IscU to other apo-proteins.[4,5] The two different domains of HscA play critical roles in this Fe-S cluster transfer process; the NBD has an ATPase activity that may contribute to an efficient cluster transfer, while the SBD interacts with the substrate, IscU, and stabilizes its dynamic state.[6,7] Previous studies reported that this interaction involves the highly conserved motif of IscU, ^{99}LPPVKIHC^{106}.[8] Despite these extensive studies, however, the detailed mechanisms regarding how HscA interacts with IscU and how this interaction can result in transfer of an Fe-S cluster are still elusive. To contribute to resolving this issue, we conducted solution NMR spectroscopic studies and succeeded to obtain the chemical shift information of HscA(SBD) that was bound to the ^{99}LPPVKIHC^{106} peptide. We believe that this basic

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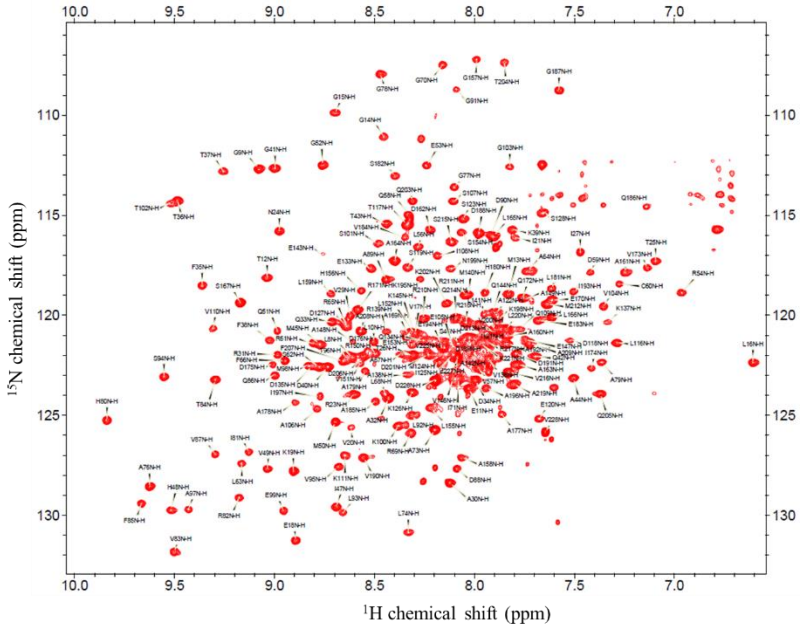

Figure 1. The NMR signal assignment results of [frac-^{2}H; U-^{13}C; U-^{15}N]-HscA(SBD) that was bound to the LPPVKIHC peptide. The assignment information is shown on its ^{1}H-^{15}N TROSY-HSQC spectrum.

information may contribute to subsequent structural studies to appreciate the interaction of HscA with IscU and elucidate the Fe-S cluster biogenesis mechanism.

Experimental procedures

Recombinant *E. coli* HscA(SBD) samples, which contain the residues 389-616 of HscA, were prepared as described previously.[9] For backbone NMR signal assignment of HscA(SBD), we needed to take fractional deuteration procedure ([frac-^{2}H]) for improved spectral resolution and sensitivity. In addition, we added 1-fold of the ^{99}LPPVKIHC^{106} peptide (unlabeled) to the sample of HscA(SBD) to further increase overall spectral quality. The sample for NMR experiments, therefore, contained 1 mM of [frac-^{2}H; U-^{13}C; U-^{15}N]-HscA(SBD) and 1 mM of the ^{99}LPPVKIHC^{106} peptide along with the buffer consisting of 50 mM Tris·HCl, 150 mM NaCl, 0.5 mM EDTA, 5 mM dithiothreitol, 0.01 % NaN_3, and 7% D_2O. For NMR experiments, 300 μL of the sample was inserted to a shigemi tube, and the experimental condition was maintained to pH 7.5 and 25 °C.

NMR data was acquired with a 600-MHz NMR spectrometer (Bruker) equipped with a cryogenic HCN probe. The following NMR spectra was obtained: 2D ^{1}H-^{15}N TROSY-HSQC, 3D TROSY-HNCO, 3D TROSY-HN(CA)CO, 3D TROSY-HNCA, 3D TROSY-HN(CO)CA, and 3D TROSY-HNCACB. The raw data were processed with TopSpin 3 (Bruker) and were subsequently analyzed with POKY software suite.[10,11]

Results and discussion

Our initial trial to conduct the backbone signal assignment of HscA(SBD) was not successful due to its low spectral quality, suggesting heterogeneous structural states of HscA(SBD) in the absence of a substrate. We therefore added the well-known substrate for HscA(SBD), the ^{99}LPPVKIHC^{106} peptide,[6] to the sample of [frac-^{2}H; U-^{13}C; U-^{15}N]-HscA(SBD), which indeed significantly improved the quality of the NMR spectra. Subsequently, we collected 2D and 3D NMR experiments to conduct the backbone signal assignment. Possibly due to a relatively large size of the complex (~25 kDa) and some remaining dynamic regions, the signal assignment could not be done fully; no assignment could be found for a few regions, such as the residues 1~6, 112~114, 129~131 and 222~223 (the residue number was renumbered from 1 for the amino acid

sequence of HscA(SBD)), and there were also partially missing assignments, particularly for $^{13}C_{\beta}$, which could be also attributed to heterogeneous deuteration around $^{13}C_{\beta}$ atoms. Still, we could assign most of ^{1}H-^{15}N HSQC signals of HscA(SBD), as shown in Figure 1 and Table 1.

Our study indicates that the substrate peptide may stabilize a certain conformation of HscA(SBD) among several heterogeneous structural states in the absence of a substrate. Our data may provide a useful information to further elucidate this structural transition of HscA upon responding to the addition of substrates. We are currently conducting subsequent studies to appreciate detailed binding interaction of HscA with IscU and the related Fe-S cluster transfer mechanisms.

Table 1. The backbone chemical shift assignment results (in ppm) of HscA(SBD) complexed with the LPPVKIHC peptide.

Residue	^{13}CO	$^{13}C_{\alpha}$	$^{13}C_{\beta}$	$^{1}H_N$	$^{15}N_H$	Residue	^{13}CO	$^{13}C_{\alpha}$	$^{13}C_{\beta}$	$^{1}H_N$	$^{15}N_H$	Residue	^{13}CO	$^{13}C_{\alpha}$	$^{13}C_{\beta}$	$^{1}H_N$	$^{15}N_H$	Residue	^{13}CO	$^{13}C_{\alpha}$	$^{13}C_{\beta}$	$^{1}H_N$	$^{15}N_H$
S7	172.7	59.0	62.4	-	-	R61	175.8	54.8	-	8.81	121.4	D118	-	57.3	-	7.52	121.2	D175	180.3	57.0	39.0	9.01	122.5
L8	177.2	52.0	44.9	8.61	122.1	S62	175.1	58.6	-	8.78	122.6	S119	-	60.7	61.9	8.34	117.6	D176	179.1	57.0	39.4	8.49	121.9
G9	170.9	45.6	-	9.08	112.7	L63	177.0	55.2	43.1	9.17	127.4	E120	-	58.8	29.4	7.67	125.2	A177	181.0	54.4	16.7	7.86	125.0
L10	174.9	52.6	45.5	8.51	121.3	A64	175.1	51.7	-	7.74	117.8	I121	-	65.3	-	7.94	121.2	A178	179.1	54.7	-	8.89	124.4
E11	176.0	55.5	29.4	8.03	123.7	R65	174.2	55.0	33.1	8.64	120.6	A122	-	54.6	17.2	7.89	119.9	A179	180.8	54.5	17.2	8.55	122.8
T12	174.8	59.6	71.4	9.04	118.1	F66	171.4	55.6	-	8.95	122.3	S123	-	61.2	62.1	8.06	115.2	H180	176.9	59.1	28.9	7.95	118.9
M13	175.3	55.8	30.2	7.83	119.0	A67	176.1	50.0	-	8.37	121.9	M124	180.4	59.1	30.8	8.17	122.4	L181	176.8	57.1	39.5	7.61	118.7
G14	174.9	44.4	-	8.45	111.1	L68	174.5	53.4	-	8.45	124.0	I125	179.1	64.6	-	8.11	121.9	S182	176.7	61.2	62.0	8.40	113.1
G15	174.5	45.5	-	8.70	109.9	R69	175.4	54.3	-	8.32	125.9	K126	179.5	59.4	31.3	8.31	123.8	E183	180.6	59.0	28.9	7.68	120.2
L16	175.6	53.1	43.9	6.61	122.4	G70	175.1	45.2	-	8.16	107.5	D127	178.0	56.6	40.1	8.65	120.3	V184	179.7	64.3	-	8.35	116.0
V17	175.3	61.3	31.6	8.30	120.9	I71	174.0	59.6	-	8.08	123.2	S128	-	-	-	7.66	114.9	A185	177.2	53.6	16.6	8.49	124.3
E18	176.1	53.4	30.1	8.89	131.3	P72	175.8	62.4	-	-	-	A132	179.9	56.0	17.9	-	-	Q186	177.2	55.5	28.4	7.14	114.6
K19	175.7	56.7	30.7	8.91	127.8	A73	175.5	52.4	-	8.20	125.7	E133	179.4	59.1	-	8.52	117.7	G187	172.8	44.0	-	7.58	108.8
V20	175.6	63.1	32.3	8.62	125.6	L74	173.9	52.5	-	8.33	130.8	Q134	179.0	58.6	-	8.25	121.3	D188	175.2	52.6	41.1	7.98	115.9
I21	-	57.0	-	7.79	116.1	P75	176.1	62.5	-	-	-	D135	-	56.6	39.0	8.81	122.6	D189	176.7	52.3	41.4	8.03	121.1
P22	177.4	61.7	-	-	-	A76	178.8	52.9	-	9.63	128.6	V136	179.1	66.3	30.9	7.93	122.2	V190	176.5	65.6	-	8.56	127.1
R23	174.5	56.3	-	8.60	124.0	G77	175.7	45.2	-	8.10	113.6	K137	179.1	59.0	31.2	7.33	120.3	D191	178.2	56.8	40.6	7.79	121.9
N24	174.1	54.7	-	8.98	115.8	G78	173.6	45.7	-	8.47	107.9	A138	181.2	54.4	-	8.34	123.0	A192	181.6	54.2	-	7.85	121.5
T25	173.6	63.8	-	7.09	117.3	A79	176.3	50.4	-	7.36	122.3	R139	177.3	58.9	-	8.44	120.8	I193	176.9	65.5	37.0	7.51	118.8
T26	173.1	62.6	69.0	8.31	122.0	H80	173.4	54.2	32.6	9.84	125.2	M140	179.1	58.6	-	8.03	119.0	E194	179.9	60.1	-	8.17	121.4
I27	174.3	57.7	-	7.47	116.8	I81	174.4	58.6	-	9.13	126.8	L141	-	57.7	-	7.95	120.0	K195	179.3	59.2	31.9	8.27	118.2
P28	175.3	61.9	-	-	-	R82	176.5	54.9	-	9.18	129.1	A142	180.9	54.9	-	8.00	121.7	A196	181.2	54.4	19.8	7.94	123.6
V29	170.8	60.4	-	8.62	120.0	V83	174.8	60.6	-	9.50	131.8	E143	180.7	59.4	-	8.76	116.9	I197	178.0	66.4	37.5	8.77	124.0
A30	176.9	50.7	-	8.12	128.4	T84	174.1	61.6	-	9.30	123.2	Q144	178.6	57.6	-	7.91	119.8	K198	179.6	58.8	-	7.88	120.2
R31	173.2	54.0	-	8.99	122.0	F85	173.4	55.2	-	9.67	129.4	K145	180.4	60.9	-	8.31	120.8	N199	177.4	56.1	38.3	8.12	117.7
A32	176.8	49.8	-	8.43	124.2	Q86	174.0	54.0	-	9.00	123.0	V146	177.8	66.4	-	8.11	123.1	V200	178.3	67.3	-	7.90	120.7
Q33	172.6	54.4	-	8.71	120.3	V87	176.2	60.2	-	9.30	126.9	E147	178.8	58.3	-	7.75	121.2	D201	178.2	57.3	40.5	8.23	122.0
D34	174.6	53.1	-	8.00	123.2	D88	176.8	52.5	41.3	8.09	127.7	A148	178.4	54.9	18.1	8.61	121.3	K202	180.0	58.9	31.4	8.14	119.4
F35	173.8	56.6	-	9.36	118.5	A89	177.7	53.9	17.8	8.44	118.3	A149	180.8	54.6	-	7.74	119.1	Q203	178.0	55.9	26.9	8.31	114.3
T36	174.1	58.7	70.3	9.48	114.3	D90	176.7	53.5	-	7.91	116.0	R150	179.6	-	-	8.53	121.7	T204	174.8	61.7	69.2	7.85	107.5
T37	173.4	59.4	-	9.26	112.8	G91	174.0	44.8	-	8.09	108.7	V151	179.4	65.8	-	8.58	122.4	Q205	178.6	59.3	27.7	7.38	123.9
F38	174.1	58.0	-	9.03	121.2	L92	175.7	55.5	41.2	8.31	125.0	L152	178.8	57.7	40.3	8.29	121.0	D206	178.5	57.1	39.3	8.56	122.0
K39	175.4	52.9	-	7.81	115.7	L93	174.5	53.1	42.4	8.66	129.9	E153	180.1	58.9	-	8.31	121.4	F207	176.7	58.1	-	8.65	122.3
D40	178.1	54.7	-	8.73	122.6	S94	173.9	56.5	63.5	9.55	123.1	S154	177.5	60.8	-	8.07	115.9	A208	180.5	54.7	17.0	8.57	120.8
G41	173.1	45.6	-	9.00	112.7	V95	174.5	60.2	-	8.68	127.5	L155	178.2	57.3	40.7	8.22	124.7	A209	180.6	54.5	17.2	7.72	121.4
Q42	176.5	56.5	-	7.70	122.1	T96	172.9	60.5	70.9	8.64	122.0	H156	179.5	59.5	-	8.57	118.8	R210	179.4	58.5	30.0	8.25	120.2
T43	174.0	60.9	-	8.44	115.4	A97	174.0	49.5	-	9.43	129.7	G157	176.4	46.6	-	7.99	107.2	R211	178.4	58.6	29.3	8.04	119.0
A44	176.6	50.0	-	7.51	123.2	M98	174.5	53.0	-	8.83	122.5	A158	180.3	55.0	18.6	8.05	127.1	M212	177.4	56.7	31.3	7.64	119.5
M45	173.7	54.0	-	8.77	121.4	E99	177.6	56.0	-	8.96	129.8	L159	178.9	56.8	40.9	8.72	119.0	D213	177.6	55.6	40.7	7.90	120.8
S46	173.0	57.1	63.1	8.08	121.4	K100	178.3	60.4	-	8.38	125.5	A160	179.5	54.1	17.2	7.84	121.1	Q214	177.4	57.4	28.2	8.10	120.1
I47	172.4	57.6	32.2	8.69	129.6	S101	176.9	61.6	-	8.48	116.4	A161	179.7	53.7	19.4	7.24	117.9	S215	176.3	60.4	62.3	8.12	116.3
H48	173.7	51.8	-	9.52	129.7	T102	177.0	62.0	71.1	9.52	114.4	D162	176.2	55.1	42.0	8.22	115.8	V216	177.6	64.1	31.2	7.83	122.8
V49	174.3	62.1	-	9.04	127.7	G103	-	45.6	-	7.82	112.6	A163	179.1	55.5	18.1	7.79	122.5	R217	178.0	57.5	29.2	7.93	121.4
M50	174.1	53.1	-	8.70	125.3	V104	174.1	62.9	30.9	7.36	119.6	A164	178.5	53.3	17.6	8.40	117.3	R218	177.1	57.1	29.4	8.04	120.4
Q51	174.5	52.7	-	8.99	120.8	E105	175.4	54.1	-	8.10	121.8	L165	176.7	55.2	41.3	7.91	116.1	A219	178.4	52.6	18.0	7.81	123.5
G52	172.6	43.2	-	8.76	112.5	A106	174.9	51.5	-	8.79	124.7	L166	177.1	52.8	43.6	7.61	120.1	L220	177.8	55.1	41.2	7.84	120.7
E53	177.7	54.3	-	8.24	112.5	S107	172.8	56.4	66.7	8.11	114.3	S167	174.9	56.4	64.5	9.17	119.4	K221	177.2	56.1	31.9	7.97	121.4
R54	174.5	54.8	-	6.96	118.9	I108	173.3	59.0	-	8.19	117.0	A168	180.9	55.2	16.9	-	-	S224	174.5	57.6	63.2	-	-
E55	175.4	57.3	-	-	-	Q109	175.3	54.0	-	7.78	120.7	A169	180.9	54.4	17.7	8.34	121.0	V225	175.8	61.8	31.7	8.15	121.6
L56	179.4	53.0	-	8.28	116.6	V110	172.6	59.5	-	9.31	120.6	E170	179.8	57.9	30.3	7.61	119.2	D226	175.9	54.1	40.6	8.20	123.3
V57	178.1	65.1	-	7.98	122.6	K111	-	53.2	-	8.65	127.0	R171	177.1	57.2	28.2	8.58	119.7	E227	175.5	56.0	29.7	8.08	122.1
Q58	175.6	57.0	-	8.33	115.0	G115	173.3	45.0	-	-	-	Q172	177.6	58.4	27.7	7.76	119.2	V228	181.1	63.1	32.3	7.65	125.8
D59	175.5	53.9	-	7.42	117.8	L116	177.1	53.7	43.2	7.29	121.4	V173	179.8	65.5	31.0	7.14	117.7						
C60	171.7	58.6	-	7.28	118.5	T117	-	-	-	8.33	115.5	I174	176.9	65.8	37.4	7.41	122.7						

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