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STRUCTURAL–FUNCTIONAL ANALYSIS OF BIOPOLYMERS AND THEIR COMPLEXES

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Interface of the Interaction of the Middle Domain of Human Translation Termination Factor eRF1 with Eukaryotic Ribosomes

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Abstract—Translation termination in eukaryotes is governed by the interaction of two, class 1 and class 2, polypeptide chain release factors with the ribosome. The middle (M) domain of the class 1 factor eRF1 contains the strictly conserved GGQ motif and is involved in hydrolysis of the peptidyl-tRNA ester bond in the peptidyl transferase center of the large ribosome subunit. Heteronuclear NMR spectroscopy was used to map the interaction interface of the M domain of human eRF1 with eukaryotic ribosomes. The protein was found to specifically interact with the 60S subunit, since no interaction was detected with the 40S subunit. The amino acid residues forming the interface mostly belong to long helix α1 of the M domain. Some residues adjacent to α1 and belonging to strand β5 and short helices α2 and α3 are also involved in the protein–ribosome contact. The functionally inactive G183A mutant interacted with the ribosome far more weakly as compared with the wild-type eRF1. The interaction interfaces of the two proteins were nonidentical. It was concluded that long helix α1 is functionally important and that the conformational flexibility of the GGQ loop is essential for the tight protein–ribosome contact.

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Key words: eukaryotic ribosome, middle domain of eRF1, translation termination, NMR spectroscopy, protein–ribosome interaction

INTRODUCTION

Eukaryotic polypeptide chain release factor 1 (eRF1) is a translation termination factor that is responsible for the recognition of the stop codon at the end of the mRNA coding sequence and subsequent hydrolysis of peptidyl-tRNA [1]. X-ray analysis of eRF1 has revealed three domains: N-terminal (N), middle (M), and C-terminal (C) [2]. The main function of the N domain is to recognize the stop codon in the decoding center of the small subunit of the ribosome [3–6]. The C domain interacts with the class 2 termination factor eRF3 [7–9], which acts as a ribosome- and eRF1-dependent GTPase and stimulates the eRF1 activity [10]. The key role of the M domain is hydrolysis of peptidyl-tRNA [11]. The M domain contains the invariant tripeptide Gly–Gly–Gln (GGQ motif, residues 183–185 in human eRF1), which is common for all translation termination factors, including prokaryotic and mitochondrial RF1 and RF2, eRF1, and aRF1 [2, 11, 12]. The GGQ motif is in the peptidyl transferase center of the large subunit of the *Escherichia coli* ribosome on evidence of X-ray analysis [13] and cryoelectron microscopy [14, 15]. Any substitution for either Gly residue in the GGQ motif dramatically reduces the ability of eRF1 to hydrolyze the peptidyl-tRNA bond in vitro and in vivo in both prokaryotes and eukaryotes [2, 11, 16, 17]. For instance, the GAQ mutants of RF1 and RF2 are four- to fivefold less efficient in translation termination as compared with the corresponding wild-type proteins, although the mutation does not affect the binding of the termination factor to the ribosome [18]. Structural and biochemical data indicate that the GGQ minidomain plays a universal role in both prokaryotes and eukaryotes, being crucial for hydrolysis of peptidyl-tRNA in the peptidyl transferase center.

When the NM domain is used in place of eRF1, RF activity is preserved in the absence of the C domain in vitro [19]. This finding indicates that the C domain does not appreciably contribute to the eRF1 binding

† Deceased.

with the ribosome. At the same time, eRF1 devoid of its N domain is still capable of inducing the GTPase activity of eRF3 in vitro [20]. Since this activity is only stimulated in the ternary complex eRF3•eRF1•ribosome [10, 21], the MC fragment of eRF1 efficiently binds with the ribosome. Thus, it is possibly the M domain of eRF1 that binds with the large ribosome subunit.

The structure and dynamic properties of the isolated M domain of eRF1 in solution have been studied by NMR spectroscopy [22]. NMR provides a unique opportunity to monitor the intermolecular interactions, including the interactions of proteins with the ribosome or its subunits. For instance, NMR studies have shown that bacterial (*E. coli*) initiation factor 3 (IF3) interacts with the 30S subunit. When 30S subunits are added to IF3 at a molar ratio of 1–5%, some signals are selectively broadened in the 1D NMR spectrum [23]. This observation has been used to identify the interface of interactions with the *E. coli* 30S subunit in IF1 [24] and IF3 [25]. These studies have been performed with the ^{15}N-labeled proteins and heteronuclear correlation spectroscopy to identify the amino acid residues whose signals are most sensitive to the presence of ribosome subunits. The residues have been identified by changes arising in their signals in ^{15}N-^{1}H HSQC spectra during protein titration with 30S subunits. It has been demonstrated that the residues identified on the basis of HSQC spectra are indeed involved in the centers of interactions of IF1 and IF3 with the ribosome.

The objectives of this work were to examine whether the isolated M domain of human eRF1 specifically binds to the 60S ribosome subunit and to identify the amino acid residues that form the interface of eRF1 interactions with the ribosome.

EXPERIMENTAL

Preparation of the ^{15}N-labeled M domain of human eRF1. To produce the M domain of human eRF1, *E. coli* cells were transformed with a recombinant plasmid, which was based on pET23b(+) (Novagen, United States) and coded for region 140–275 of eRF1 [20]. The M-domain gene was overexpressed in *E. coli* BL-21 (DE3) cells. The recombinant M domain contained a His_6 tag at the C terminus according to the vector structure.

To obtain the ^{15}N-labeled M domain of human eRF1, *E. coli* cells were grown in the M9 minimal medium, which contained $^{15}NH_4Cl$ as the only nitrogen source. One liter of M9 contained 6 g of Na_2HPO_4, 3 g of KH_2PO_4, 0.5 g of NaCl, and 1 g of $^{15}NH_4Cl$; pH was adjusted to 7.4; the medium was autoclaved. A *E. coli* cell culture was grown in M9 at 37°C to A_{600} = 0.8–1.0, supplemented with 0.8 mM IPTG, and incubated at 30°C for 16–20. Cells were collected by centrifugation and sonicated. The M domain of eRF1 was isolated by affinity chromatography on Ni-NTA agarose (Qiagen, United States) as described previously for the full-size eRF1 [19]. Then, the M domain was purified by ion exchange chromatography, using an AKTA system (GE Healthcare, United Kingdom) and a 1-ml HiTrap HP SP column. A protein solution in 20 mM potassium-phosphate (pH 6.9) containing 5 mM β-mercaptoethanol and 50 mM NaCl was loaded on the column, and the protein was eluted with a linear gradient of NaCl (50 mM to 1 M). The resulting preparation was dialyzed against 20 mM Tris-HCl (pH 6.5), 50 mM NaCl, 2.5 mM $MgCl_2$.

Isolation and purification of 60S and 40S ribosome subunits. We isolated 80S ribosomes from rabbit reticulocytes. A reticulocyte lysate was centrifuged at 245000 *g* at 6°C for 4.5 h. The pellet was resuspended in 30 mM Tris-HCl (pH 7.5), 50 mM KCl, 6 mM $MgCl_2$, 2 mM DTT, 0.25 M sucrose. After adding 4 M KCl dropwise to the final concentration 0.5 M, the suspension was centrifuged at 245000 *g* for 4.5 h. The pellet was resuspended in 20 mM Tris-HCl (pH 7.5), 50 mM KCl, 4 mM $MgCl_2$, 2 mM DTT. The suspension was supplemented with 1 mM puromycin and incubated in ice for 10 min and at 37°C for 10 min. After adding 4 M KCl dropwise to the final concentration 0.5 M, the preparation was centrifuged through a sucrose concentration gradient (10–30%) at 112500 *g* for 13 h. The sucrose gradient was fractionated, the optical density was measured, and the fractions with the maximal contents of the material were pooled. Ribosome subunits were transferred into 20 mM Tris-HCl (pH 7.5), 2.5 mM $MgCl_2$, 2 mM DTT, 0.1 mM EDTA, 50 mM KCl, 0.25 M sucrose and centrifuged at 245000 *g* for 7 h. The pellet of ribosome subunits was dissolved in the same buffer to a necessary concentration (no less than 100 optical units/ml in the case of 60S subunits and no less than 70 optical units/ml in the case of 40S subunits).

NMR experiments were performed on Varian Inova 600 and 800 MHz and Bruker Avance 600 MHz spectrometers, which were equipped with triple resonance cryoprobes to record the ^{1}H nuclear resonance and ^{15}N and ^{13}C excitation. The M domain of human eRF1 (30–200 μM, 600 μl) was titrated with either 40S or 60S subunits. Direct and reverse titrations were performed at the following molar ratios of 60S subunits to the M domain: 1 : 15000, 1 : 9000, 1 : 8000, 1 : 7500, 1 : 5000, 1 : 4500, 1 : 4250, 1 : 4000, 1 : 3500, 1 : 2500, and 1 : 2000. To check the specificity of the interaction, the M domain was titrated with 40S subunits at 40S–M molar ratios of 1 : 3500 and 1 : 1750. To study the effect of ionic strength on the interaction of the M domain with 60S subunits, the NaCl concentration in the final mixture was varied from 50 to 75 mM. The interaction of the G183A mutant of the M domain with 60S ribosomes was studied at 60S–M

ratios of 1 : 8000, 1 : 5000, 1 : 4000, 1 : 3000, and 1 : 1500.

All spectra were recorded at 298 and 278 K. To monitor the changes in the width of resonance bands, we used ^{15}N-^{1}H HSQC spectra [26], which were recorded at 278 K.

Analysis of the NMR spectra and visualization of the results. NMR spectra were transformed using the nmrPipe software package [27]. The transformation employed a Gaussian weighting function in ^{1}H and ^{15}N dimensions. After the Fourier transform in both dimensions, the 2D spectrum matrix was 2048×1024. The results were visualized, the spectra were analyzed, and the NMR signals were integrated using the programs nmrDraw [27] and Sparky (http://www.cgf.ucsf.edu/home/sparky).

To measure the integrated signal intensity, the parameters of the Gaussian line shape for permitted signals of HSQC spectra were estimated by nonlinear regression, using the standard algorithms of the Sparky program. Signal assignments in NMR spectra of the wild-type M domain of human eRF1 and its G183A mutant were based on earlier data [22, 28].

The results were visualized using the molecular graphics programs InsightII (Accelrys, United States) and VMD [29]. To compute the protein molecular surface accessible for the solvent, we applied the InsightII program and Connolly's algorithm [30].

RESULTS

Monitoring the interactions of the M domain of eRF1 with the 60S ribosome subunits, we did not observe any changes in the intensity and width of protein signals in HSQC spectra at M–60S molar ratios of more than 5000 : 1. As the 60S concentration was further increased, the signal intensity decreased in proportion to the change in M–60S molar ratio. When the M–60S molar ratio was lower than 3000 : 1, all signals were almost completely broadened and, in fact, disappeared from the HSQC spectra. The most significant changes in the spectra were observed within a narrow range of M–60S ratios, from 4500 : 1 to 4000 : 1 (Table 1). Figure 1 shows a fragment of the ^{15}N-^{1}H HSQC spectrum obtained for the M domain of eRF1 in the absence of ribosomes (Fig. 1a) and during titration with 60S subunits (Figs. 1b–1d). Most signals from the M domain decreased within the above range of M–60S ratios. Some signals displayed dramatic changes in intensity. For instance, several amide signals of the protein were absent from the spectra at M–60S ratios 4000 : 1 (Fig. 1c) and 4250 : 1 (Fig. 1d); the corresponding resonance positions in the spectra are shown with circles.

When the M–60S molar ratio was lower than 3000 : 1, almost no signals were detectable in the HSQC spectrum. However, the signal intensity in the spectrum was restored as the ionic strength was increased by changing the NaCl concentration from 50 to 75 mM. An increase in ionic strength weakened the association of the protein with the ribosome subunit, and the free protein was released. In addition, the signal intensity was gradually restored with time: the HSQC spectra obtained 5 h after adding 60S subunits to the M domain were almost identical to the control spectrum, obtained in the absence of ribosomes. This effect was most likely explained by time-dependent degradation of 60S ribosome subunits. The changes in signal intensity in the HSQC spectra were reversible. When the M domain was added to an M–60S mixture whose HSQC spectrum had almost no signals, the signals appeared again. A similar effect was observed during reverse titration of 60S subunits with the M domain; i.e., the signal intensity decreased at the same molar ratios as in direct titration.

In addition, NMR was used to study the interaction of the M domain of eRF1 with 40S ribosome subunits. When 40S subunits were added to a protein solution at an M–40S molar ratio of 3500 : 1 or even 1750 : 1, detectable changes did not appear in the ^{15}N-^{1}H HSQC spectrum.

The functionally inactive G183A mutant of the M domain of eRF1 was also tested for interactions with 60S ribosome subunits. Changes in signal intensities in the HSQC spectrum were monitored at M–60S molar ratios of 8000 : 1, 5000 : 1, 4000 : 1, 3000 : 1, and 1500 : 1. A decrease in signal intensities was only observed when the relative concentration of 60S subunits was more than 0.025% of the protein concentration, i.e., at M–60S molar ratios of 4000 : 1, 3000 : 1, and 1500 : 1. Only a few signals changed in intensity at a ratio of 4000 : 1 (Table 2). The number of such signals just exceeded ten at a ratio of 3000 : 1. The amino acid residues whose backbone amide protons displayed the greatest changes included Ala183, Gly184, Ser186, Thr208, Gly216, Thr234, and Ser239. In the case of Arg198, Asn220, and Arg245, we additionally observed a decrease in the intensity of signals from side-chain NH groups. It should be noted that an appreciable number of signals were detectable in the spectrum at an M–60S ratio of 1500 : 1 in the case of the G183A mutant, whereas almost all signals had already disappeared from the spectrum of the wild-type M domain at a ratio of 3000 : 1.

DISCUSSION

The Nature of a Decrease in Signal Intensity Observed in the NMR Spectrum of the M Domain upon Its Interaction with 60S Ribosome Subunits

As already mentioned, NMR spectroscopy has been used in several studies to identify the interaction interfaces of ribosome subunits with ribosomal pro-

Table 1. Relative integrated intensities of the signals from amino acid residues of the eRF1 M domain upon its interaction with eukaryotic 60S ribosome subunits at various 60S–M ratios

Residue		60S–M ratio:			Residue		60S–M ratio:		
		1/4500	1/4250	1/4000			1/4500	1/4250	1/4000
S	144	0.55	0.45	0.30	Q	211	0.37	0.44	0.51
K	145	0.43	0.39	0.29	**L**	**212**	**0.30**	**0.24**	**0.00**
F	**146**	**0.03**	**0.00**	**0.00**	**F**	**213**	**0.62**	**0.36**	**0.07**
G	147	0.37	0.25	0.16	I	214	0.36	0.39	0.21
F	148	0.47	0.44	0.31	S	215	0.34	0.31	0.31
V	150	0.27	1.17	0.37	G	216	0.27	0.56	0.17
D	152	0.38	0.85	0.17	K	218	0.50	0.36	0.26
G	153	0.15	0.50	0.90	N	220	0.19	0.35	0.22
S	154	0.50	0.35	0.30	V	221	0.25	0.27	0.90
G	155	0.53	0.34	0.21	A	222	0.28	0.28	0.47
G	159	0.34	0.48	0.28	G	223	0.29	0.49	0.32
T	160	0.33	0.41	0.20	L	224	0.30	0.58	0.26
L	161	0.22	0.42	0.78	**V**	**225**	**0.09**	**0.07**	**0.00**
Q	**162**	**0.33**	**0.28**	**0.00**	**L**	**226**	**0.46**	**0.30**	**0.00**
G	163	0.51	0.36	0.21	G	228	0.33	0.50	0.35
N	164	0.36	0.50	0.21	A	230	0.56	0.49	0.35
T	165	0.38	0.30	0.21	**K**	**233**	**0.06**	**0.00**	**0.00**
R	166	0.37	0.28	0.26	**T**	**234**	**0.22**	**0.00**	**0.00**
E	167	0.34	0.49	0.34	**L**	**236**	**0.00**	**0.15**	**0.08**
V	168	0.39	0.51	0.94	S	237	0.41	0.16	0.27
L	**169**	**1.02**	**0.23**	**0.00**	Q	238	0.33	0.28	0.25
H	**170**	**0.50**	**0.38**	**0.00**	M	241	0.40	0.22	0.44
F	172	0.46	0.32	0.25	F	242	0.36	0.61	0.24
T	173	0.45	0.46	0.21	Q	244	0.28	0.47	0.21
V	174	0.07	0.16	0.21	R	245	0.29	0.31	0.31
D	175	0.40	0.48	0.23	S	248	0.38	0.37	0.35
K	178	0.42	0.36	0.21	K	249	0.37	0.29	0.28
G	181	0.38	0.44	0.23	**V**	**250**	**0.33**	**0.52**	**0.00**
G	183	0.60	0.53	0.39	**L**	**251**	**0.34**	**0.00**	**0.00**
G	**184**	**0.47**	**0.30**	**0.10**	K	252	0.37	0.32	0.37
S	186	0.71	0.46	0.21	L	253	0.42	0.29	0.12
A	187	0.50	0.33	0.69	V	254	0.24	0.38	0.17
R	192	0.06	0.36	0.18	D	255	0.37	0.22	0.18
L	193	0.52	0.32	0.18	S	257	0.41	0.27	0.31
E	196	0.14	0.49	0.28	Y	258	0.31	0.12	0.49
K	**197**	**0.37**	**0.21**	**0.10**	G	259	0.36	0.30	0.22
H	199	0.42	0.29	0.14	G	260	0.40	0.47	0.93
N	200	0.27	0.21	0.22	E	261	0.39	0.66	0.37
Y	201	0.38	0.27	0.29	N	262	0.22	0.17	0.18
R	**203**	**0.32**	**0.29**	**0.11**	**I**	**268**	**0.58**	**0.04**	**0.00**
K	**204**	**0.51**	**0.35**	**0.00**	E	269	0.20	0.26	0.27
V	**205**	**0.31**	**0.00**	**0.00**	L	270	0.44	0.31	0.18
E	207	0.93	0.36	0.27	S	271	0.17	0.63	0.52
T	**208**	**0.17**	**0.00**	**0.00**	T	272	0.56	0.63	0.34
A	209	0.14	0.32	0.20	V	274	0.25	0.32	0.30
V	**210**	**0.26**	**0.29**	**0.00**	His_6		0.91	0.78	0.70

Note: Here and in Table 2, the intensity of the corresponding signals from the protein in the absence of ribosomes was taken as unity. Amino acid residues whose signal intensities changed to the greatest extent are in bold. The residues are numbered as in the full-size human eRF1. Only the residues whose integrated signal intensities in HSQC spectra could be reliably estimated are shown.

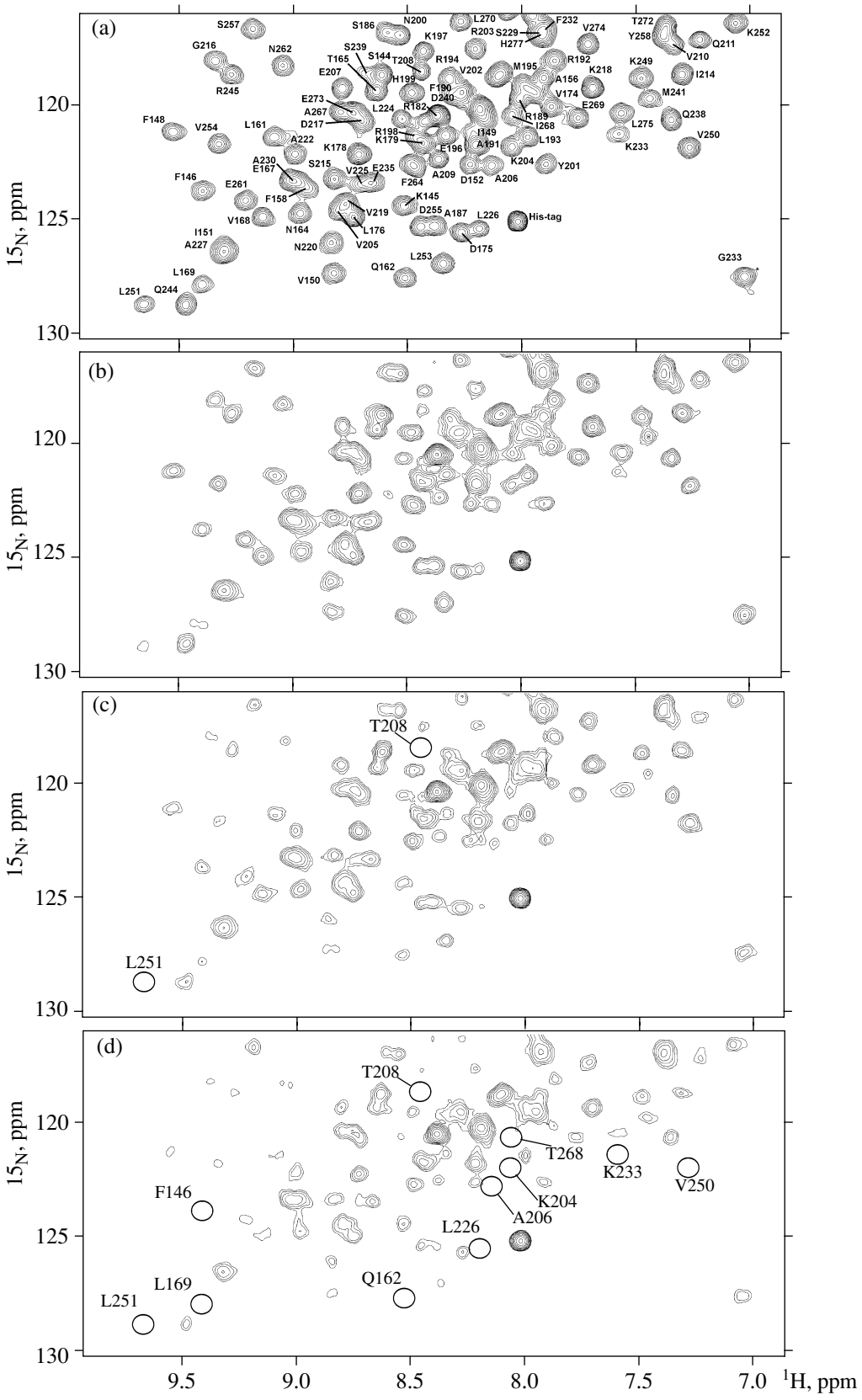

Fig. 1. Fragment of the ^{15}N-^{1}H HSQC spectrum obtained for the M domain of human eRF1 in the (a) absence or (b–d) presence of eukaryotic 60S ribosome subunits at 60S–M ratios of (b) 1 : 4500, (c) 1 : 4250, and (d) 1 : 4000. Signals that disappeared or decreased in intensity by one order of magnitude upon the interaction with the ribosome are shown with circles.

Table 2. Relative integrated intensities of the signals from amino acid residues of the G183A mutant of the eRF1 M domain upon its interaction with eukaryotic 60S ribosome subunits at various 60S–M ratios

Residue		60S–M ratio:			Residue		60S–M ratio:		
		1/5000	1/4000	1/3000			1/5000	1/4000	1/3000
S	144	0.72	0.70	0.69	F	213	0.83	0.58	0.54
F	146	0.78	0.77	0.54	I	214	0.76	0.69	0.50
G	147	0.90	0.95	0.96	S	215	0.86	0.83	0.54
F	148	1.02	0.83	0.71	**G**	**216**	**0.75**	**0.41**	**0.00**
V	150	0.96	0.80	0.81	K	218	0.81	0.68	0.56
G	153	0.80	0.80	0.80	N	220	0.91	0.93	0.62
S	154	0.91	0.74	0.70	V	221	0.88	0.80	0.73
G	155	0.83	0.82	0.71	A	222	0.86	0.80	0.63
G	159	0.87	0.74	0.57	G	223	0.85	0.78	0.62
T	160	0.82	0.70	0.66	L	224	0.87	0.60	0.43
L	161	0.88	0.78	0.71	L	226	1.09	0.70	0.87
Q	162	0.87	0.69	0.58	G	228	0.89	0.81	0.73
G	163	0.82	0.57	0.59	A	230	0.66	0.57	0.41
N	164	0.78	0.64	0.59	**T**	**234**	**0.81**	**0.51**	**0.00**
T	165	0.87	0.72	0.47	E	235	0.53	0.49	0.30
R	166	0.81	0.70	0.53	L	236	0.88	0.77	0.56
V	168	0.77	0.75	0.56	S	237	0.88	0.72	0.60
L	169	0.69	0.57	0.81	Q	238	0.75	0.73	0.52
H	170	1.03	0.77	0.74	**S**	**239**	**0.45**	**0.12**	**0.00**
F	172	0.75	0.66	0.47	M	241	1.06	0.82	0.63
T	173	0.85	0.80	0.59	F	242	0.82	0.68	0.69
D	175	0.63	0.59	0.38	Q	244	0.84	0.84	0.67
L	176	0.86	0.67	0.56	R	245	0.83	0.79	0.68
K	178	0.91	0.72	0.53	S	248	0.97	0.80	0.74
G	181	0.79	0.61	0.53	K	249	0.96	1.02	0.74
A	**183**	**0.60**	**0.50**	**0.29**	V	250	0.99	0.96	0.66
G	184	0.77	0.71	0.47	L	251	1.10	0.73	1.01
S	**186**	**0.74**	**0.63**	**0.00**	K	252	0.94	0.87	0.80
F	190	0.70	0.57	0.55	L	253	1.01	0.94	0.76
R	192	0.72	0.67	0.52	V	254	0.85	0.73	0.58
L	193	0.84	0.81	0.57	D	255	0.76	0.67	0.51
E	196	1.01	0.61	0.50	S	257	0.89	0.69	0.58
H	199	0.79	0.58	0.47	Y	258	0.44	0.73	0.64
N	200	0.89	0.62	0.61	G	259	0.74	0.70	0.46
Y	201	0.72	0.67	0.47	G	260	0.81	0.89	0.68
R	203	0.78	0.51	0.50	E	261	0.85	0.71	0.60
K	204	0.88	0.80	0.58	N	262	0.77	0.50	0.51
E	207	0.82	0.66	0.65	A	267	0.92	0.61	0.51
T	**208**	**0.77**	**0.20**	**0.25**	E	269	0.77	0.71	0.50
A	209	0.75	0.77	0.61	S	271	0.83	0.75	0.68
V	210	1.08	0.80	0.40	E	273	0.45	0.38	0.35
Q	211	0.80	0.77	0.58	V	274	1.03	0.82	0.81
L	212	0.90	0.77	0.48	His_6		0.92	0.84	0.78

teins [24, 25]. It is essential for a successful application of NMR that the free and ribosome-bound forms of a protein exchange quite rapidly (on the NMR time scale). In such a case, the observed NMR signals contain average information about both of the protein forms, and the contribution of each form is proportional to its content in the mixture. When a target spectral parameter substantially differs between the bound and free forms, a minor amount of the free form can dramatically affect the result. This circumstance makes it possible to observe the binding effects, even with a large molar excess of the protein over ribosomes. It has been assumed that a chemical shift is the main parameter whose changes indicate that the corresponding residue belongs to the interface of the protein–ribosome interaction [23]. However, calculations demonstrate that, even if a chemical shift substantially changes upon protein binding, its change cannot explain the observed decrease in signal intensity when the protein is in a 1000-fold or greater excess over ribosomes. We think that the main factor in this case is a change in nuclear relaxation parameters, first and foremost, a considerable increase in transverse relaxation rate R_2 [31]. In the case of ribosome subunits, whose molecular weight amounts to millions of Daltons, the correlation time of rotational diffusion is several hundred nanoseconds; consequently, transverse relaxation rates can reach several hundred Hertz. Hence, NMR pulse sequences, based on the transfer of magnetization via spin–spin interactions, are inefficient in this case, and signals consequently disappear from the spectrum. The amino acid residues that directly contact the ribosome would display a maximal effect. The effect may be lower in the case of more distant residues, since R_2 decreases with an increasing distance from the interface of the interaction with the ribosome owing to the intrinsic flexibility of the protein chain. When the flexibility of a protein region is low (e.g., in the case of β-strands), a substantial decrease in signal intensity may also be observed for amino acid residues involved in the rigid structural region rather than exposed on the protein surface.

The M Domain of eRF1 Specifically Binds to the 60S Ribosome Subunit

Our NMR experiments indicate that the M domain of human eRF1 specifically binds with 60S subunits of eukaryotic ribosomes but does not bind with 40S subunits. In the case of 60S subunits, the intensity of signals from the amide protons already decreased at an M–60S molar ratio of 4500 : 1, which was due to the binding of the M domain with high-molecular-weight particles (Table 1). Almost all protein signals disappeared at a ratio of 3000 : 1. On the other hand, 40S subunits added to the M domain at a molar ratio of 3500 : 1 or even 1750 : 1 did not cause visible changes in the HSQC spectrum. These findings strongly suggest a lack of a specific binding of the M domain of eRF1 to the 40S ribosome subunit.

Amino Acid Residues of the eRF1 M Domain That Form the Interface of Its Interaction with the 60S Subunit

Table 1 shows the changes observed for the signal intensity of amide protons in the ^{15}N-^{1}H HSQC spectrum of the M domain upon its titration with 60S ribosome subunits. It should be noted that changes in signal intensity were only monitored for well-resolved signals. Since such signals account for about 70% of all amide signals in the HSQC spectra, our sample can be considered to be representative. As the content of 60S subunits was increased from 0.022% (M–60S ratio 4500 : 1) to 0.025% (4000 : 1), the intensity of all protein signals decreased. The decrease was rather insignificant in the case of some signals and substantial in the case of some others. For instance, the signals from the agile terminal His_6 tag decreased only slightly, in contrast to the signals from F146, Q162, L169, H170, and some other residues (shown in bold in Table 1). Most of the residues whose amide protons displayed a maximal decrease in signal intensity upon the interaction of the protein with 60S subunits are exposed on the protein surface and cluster predominantly on one of the hemispheres (Fig. 2). A decrease in the intensity of signals from F146, V255, and L226, which are in the β-core rather than on the protein surface, can be explained by their low flexibility and proximity to the interface of the interaction with the ribosome.

The structure of the human eRF1 M domain is shown as a combination of secondary structure elements in Fig. 3a. The Cα atoms of the residues that were most strongly affected by the ribosome in titration experiments are shown with spheres. It is noteworthy that most of these residues belong to extended helix α1. Two other clusters of such residues are in the region of short helix α2 (residues 233–235) and at the boundary between helix α3 and strand β5 (residues 250 and 251). It is possible to assume that these regions, first and foremost, helix α1, are involved in the interaction of the M domain of eRF1 with the 60S subunit of the eukaryotic ribosome.

An extended α-helix, similar to α1, is found in the third domain of prokaryotic RF1 and RF2; this domain contains the GGQ loop and interacts with the large ribosome subunit [13, 32]. A similar helix is found in the central domain of the eRF1 homolog Dom34, which also binds to the large ribosome subunit [33]. Although the prokaryotic and eukaryotic factors have almost no homology [32], the positions of their long α-helices coincide. For instance, a superimposition of the Cα coordinates of helix α1 of the eRF1 M domain with the corresponding coordinates estab-

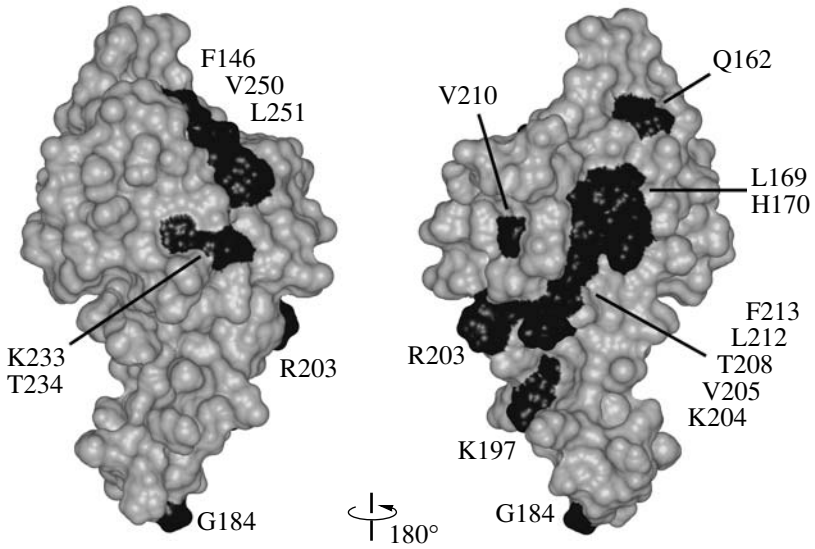

Fig. 2. Molecular surface accessible to the solvent as constructed for the M domain of human eRF1 according to Connolly's algorithm [30]. Amino acid residues whose signal intensities most dramatically changed upon the interaction with the 60S ribosome subunit are shown black.

lished for RF1 in the termination complex of *Thermus thermophilus* ribosome [13] shows that the long α-helices of the two factors are similar in arrangement relative to the GGQ loop and that the positions of their GGQ tripeptides nearly coincide (Fig. 4).

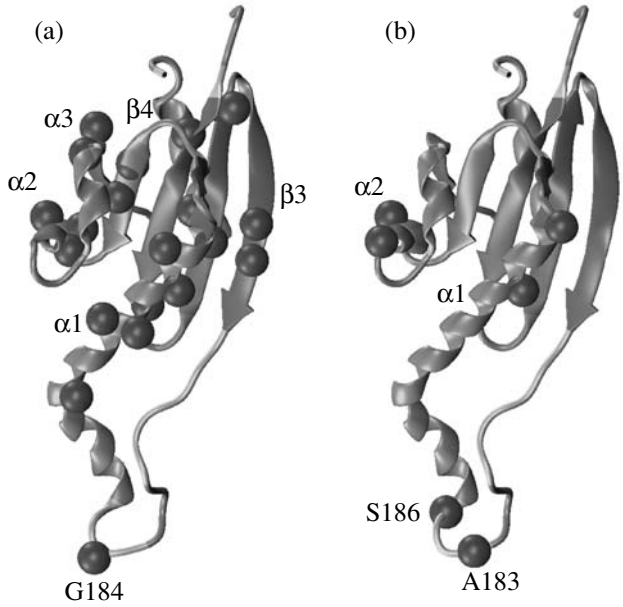

Fig. 3. Amino acid residues whose signal intensities most substantially changed upon the interaction of the M domain of human eRF1 with the 60S ribosome subunit. The positions of the Cα atoms of these residues are indicated with large spheres on the backbone of the wild-type structure. (a) The wild-type protein and (b) the G183A mutant.

Several inferences can be made from a comparison of the interaction with the 60S subunit between the wild-type M domain of human eRF1 and its G183A mutant. First, the mutant is similar to the wild-type protein in interacting specifically with 60S ribosome subunits. Second, the interaction with the ribosome is far weaker in the case of the mutant than in the case of the wild-type protein. It is impossible to exactly quantify the extent to which the mutation reduces the protein–ribosome binding constant, but the reduction itself is beyond question. The intensity of most signals from the M domain dramatically decreased upon interaction with the ribosome at an M–60S ratio of 4000 : 1 in the case of the wild-type M domain, while a similar decrease was observed at a ratio of 1500 : 1 in the case of the G183A mutant; i.e., the ribosome concentration was almost threefold higher. Third, the set of amino acid residues affected upon interaction with the ribosome was changed in the G183A mutant. Such residues were fewer in the mutant than in the wild-type protein. Figure 3c shows the arrangement of the residues whose signals most substantially changed when the G183A mutant was titrated with 60S ribosome subunits. It is seen that the geometry of interactions with the ribosome slightly differs between the G183A mutant and the wild-type protein. In particular, the interacting residues do not cluster in extended helix α1 in the mutant. The G183A mutation restricts the conformational flexibility of the GGQ loop without appreciably affecting the structure of the eRF1 M domain [22]. It is possible to assume that the restriction prevents a tighter binding of the M domain to the

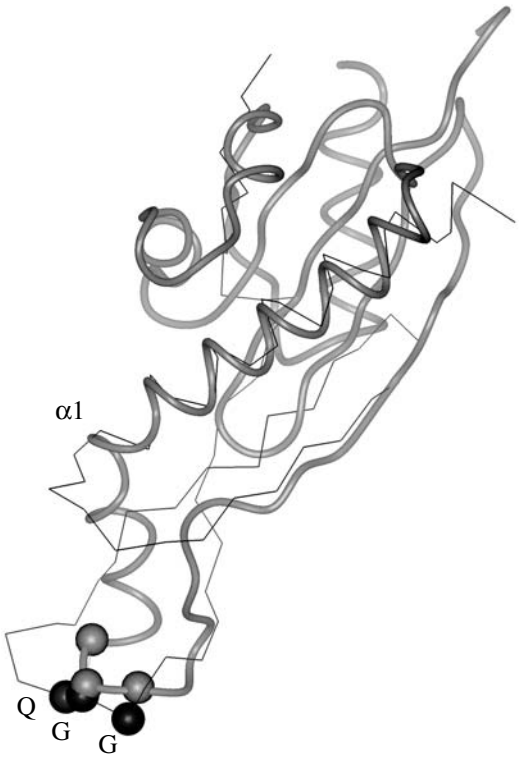

Fig. 4. Superimposition of the Cα coordinates established for the M domain of human eRF1 in solution [22] and for RF1 contained in the termination complex of the *Thermus thermophilus* ribosome in crystal [13]. A fragment of the long α-helix (α1 in the case of eRF1) was taken from each structure for superimposition. The eRF1 chain is shown as a gray tube; the RF1 conformation is shown as thin black lines connecting Cα atoms. The Cα atoms of the strictly conserved GGQ tripeptide are shown with spheres.

60S subunit through helix α1 and, consequently, a proper positioning of the M domain on the ribosome.

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