Release Factors eRF1 and RF2

A UNIVERSAL MECHANISM CONTROLS THE LARGE CONFORMATIONAL CHANGES∗§

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Class I release factors 1 and 2 (RF1 and RF2) terminate protein synthesis by recognizing stop codons on the mRNA via their conserved amino acid motifs (NIKS in eRF1 and SPF in RF2) and by the conserved tripeptide (GGQ) interactions with the ribosomal peptidyltransferase center. Crystal structures of eRF1 and RF2 do not fit their ribosomal binding pocket (73 Å). Cryoelectron microscopy indicates large conformational changes in the ribosome-bound RF2. Here, we investigate the conformational dynamics of the eRF1 and RF2 using molecular dynamics simulation, structural alignment, and electrostatic analysis of domain interactions. We show that relaxed eRF1 has a shape remarkably similar to the ribosome-bound RF2 observed by cryoelectron microscopy. The similarity between the two release factors is as good as between elongation factor G and elongation factor Tu-guanosine-5′(β,γ-imido)triphosphate-tRNA. Further, the conformational transitions and dynamics of eRF1 and RF2 between the free and ribosome-bound states are most likely controlled by protonation of conserved histidines. For eRF1, the distance between the NIKS and GGQ motifs shrinks from 97.5 Å in the crystal to 70–80 Å. For RF2, the separation between SPF and GGQ elongates from 32 Å in the crystal to 50 Å. Coulombic interaction strongly favors the open conformation of eRF1; however, solvation and histidine protonation modulate the domain interactions, making the closed conformation of eRF1 more accessible. Thus, RF1 and RF2 function like molecular machines, most likely fueled by histidine protonation. The unified conformational control and the shapes of eRF1 and RF2 support the proposition that the termination of protein synthesis involves similar mechanisms across species.

Protein synthesis on the ribosome has four steps: initiation, elongation, termination, and recycling. Class I release factors (RF1 and RF2) terminate protein synthesis by recognizing stop codons on the mRNA (1) via their conserved amino acid motifs (NIKS in eRF1 and SPF in RF2) and by the conserved tripeptide (GGQ) interactions with the ribosomal peptidyltransferase center (2, 3). Prokaryotes have both RF1 and RF2, whereas eukaryotes have only one omnipotent factor, eRF1. Class II release factors RF3 and eRF3 have different functions, including the release of the Class I factors.

Despite the fact that polypeptide release does not depend on tRNAs, it was suspected that Class I release factors (RF) mimic tRNAs (2, 4–9), due to the functions of the two important motifs: stop codon recognition and peptide release activity. Several translation factors like the ribosomal recycling factor (RRF) and elongation factor G (EF-G) have shapes similar to that of tRNA (10–12). However, unlike the tRNA conformation (13, 14), where some structural features make the tRNAs equivalent and thus recognized by the translation apparatus (13, 15, 16), the available structures of the Class I release factors indicate that the RF conformity requires significant conformational changes.

The distance between the ribosomal decoding center and the peptidyltransferase center is ~73 Å (2, 6, 7, 16). However, in the release factor crystal structures (4, 5), the separation between the NIKS and the GGQ motifs in eRF1 is 97.5 Å (measured from the Ile-Cα to Gln-Cα), and the distance between the SPF and GGQ in RF2 is only 32 Å (Pro-Cα to Gln-Cα). Two cryoelectron microscopy (cryo-EM) studies revealed large conformational changes upon binding of the RF2 to the ribosome (6, 7). The separation between SPF and GGQ in RF2 extended to 61 Å in one cryo-EM study (6) and to 73 Å in another cryo-EM study (7). The overall binding of the RF2 does not mimic tRNA, and it appears that the functional mimicry of the protein and the RNA is more convincing than a simple structural mimicry (2, 8, 9). Still, the distances between the two RNA signal binding motifs have to be around 73 Å for the Class I release factors. It was suggested that the eRF1 structure should also be adjusted to fit this range (6). The question is then how do the termination release factors change their conformations to achieve conformity in their reversible ribosome binding? If the termination of protein synthesis involves similar mechanisms across species, there should probably be a similar mechanism controlling the conformational changes of Class I release factors.

Here we have carried out molecular dynamics (MD) simulations at room temperature to investigate the driving force of the conformational changes for the eRF1 and RF2. The simulations show remarkable differences between the conformational behavior with neutral and with protonated histidine (n-His and p-His) residues for both eRF1 and RF2. Sequence analysis indicates that these histidines are highly conserved. Our results clearly show that the conformations of both eRF1 and RF2...
The protonation contribution of the domain interactions is evaluated complex), and eRF3.

The proposed mechanism of the conformational change for the eRF1. Domain 1 contains residues 8–141, domain 2 includes residues 145–275, and domain 3 has residues 276–437. The hinge region is composed of residues 1–7 and 142–144. The opening and closing of domain 2, with respect to domain 1, characterize the major dynamics. The position of domain 2 is controlled by the balance of the electrostatic interaction with other domains, modulated by histidine protonations.

MATERIALS AND METHODS

Molecular dynamics simulations were performed using the Charmm package (17) and Charmm 27 force field (18). Long range electrostatic interactions were calculated with the PME method (19). The systems were kept in constant pressure ensembles (NPT) with the Hoover temperature control (20). The reference pressure was 1 atm, and the temperature was 300 K unless specified. The temperature of the simulation of eRF1 with neutral histidines was raised from 300 to 310 K after 3.6 ns in order to accelerate the conformational change. Still, the NIKS-GGQ separation is larger in the n-His form than in the p-His form of eRF1. The time step used was 2 fs, with a SHAKE constraint on all bonds containing hydrogen atoms. The protein crystal structures were used as starting point for the simulations. Sodium atoms were added to the water to make the overall simulation system free of net charge, which is required to properly compute electrostatic effects using the particle mesh Ewald method. The eRF1 structure (Protein Data Bank code 1dt9) has missing residues (positions 1–4, 334–342, 359–369, and 423–437), which were added into the system as random coils. The N-terminal (positions 1–4) and C-terminal (positions 423–437) residues are added into the system as random coils. The miss-
ing loop (positions 334–342 and 359–369) residues are added, restricted by the loop connectivity, and minimized with the rest of the protein fixed. The simulated eRF1 systems include residues 1–437 and 21,143 TIP3 water molecules. The RF2 system includes the protein (Protein Data Bank code 1gqe) solvated with 14,125 water molecules. The eRF1 and RF2 molecules are partitioned (Fig. 1). The molecules are partitioned by the loop connectivity, and minimized with the rest of the protein fixed. The simulated eRF1 systems include residues 1–437 and 21,143 TIP3 water molecules. The RF2 system includes the protein (Protein Data Bank code 1gqe) solvated with 14,125 water molecules. The crystal waters were included in the initial systems for both the eRF1 and RF2 simulations.

Protein sequences were retrieved using the NCBI Blast server (available on the World Wide Web at www.ncbi.nlm.nih.gov/BLAST), searching against the eRF1 and RF2 sequences. Sequences with identity higher than 90% were discarded in order to obtain a balanced conservation among the various species. The final sequences for eRF1 and RF2 (with an identity range of 25–90%) were realigned with ClustalX (21).

The eRF1 is partitioned into three large domains and a hinge region (Fig. 1). Domain 1 contains residues 8–141, domain 2 includes residues 145–275, and domain 3 has residues 276–437. The hinge region is composed of residues 1–7 and 142–144. The interactions between two domains, domain A and domain B, are calculated as follows.

\[
\Delta G(A, B) = (E_{col}(A + B) + E_{all}(A + B)) - (E_{col}(A) + E_{all}(A)) - (E_{col}(B) + E_{all}(B)) \quad (\text{Eq. 1})
\]

The protonation contribution of the domain interactions is evaluated according to the equation,

\[
\Delta \Delta G(\text{protonation}) = \Delta G(\text{protonated histidine A, B}) - \Delta G(\text{neutral histidine A, B}) \quad (\text{Eq. 2})
\]

where \(E_{col}\) is the coulombic interaction energy, and \(E_{all}\) is the electrostatic contribution to the solvation energy. The solvation energies are calculated using the Generalized Born method with (standard) molecular volume (GBMV) (22). In order to evaluate the interaction as accurately as possible, no distance cut-off is used, and the grid-based GBMV module is used. In the GBMV calculation, the dielectric constant of water is set to 80, and the Debye-Huckel ionic term is 0.2 to reflect the salt effect.

The molecular similarity distance is evaluated using procedures adapted from Ankerst et al. (23) (Fig. 2). The molecules are partitioned using combined models of shells and sectors (23). The atomic positions are converted to a polar coordinate system. The shell radius \(R_i\) is calculated by the equation,

\[
R_i = R \times \frac{i}{N} \quad (\text{Eq. 3})
\]

where \(R\) is the radius of the overall system, and \(S\) is the shell number. We use five and 10 shells. \(R\) is the distance from the average center to the most distant point in the molecular system. The angular space is
Conformational Changes of Release Factors eRF1 and RF2

The Conformational Changes of eRF1—The structure of eRF1 has three domains (4) (Figs. 1 and 3A). The NIKS motif locates in domain 1, in a loop linking two α-helices. The GGQ motif locates at the tip of domain 2, connecting a strand and a helix. Domains 1 and 2 are connected by a hinge around residue Asp142. N-terminal residues 1–7 also link domain 1 and contact with domain 2. Thus, the hinge area includes residues 1–7 and 142–144. Domain 3 is less structured than domains 1 and 2, with missing loops around residue His189 and the C terminus. Domain 3 connects with domain 2 via a kinked helix 8 (Fig. 3A).

The separation of the NIKS and GGQ motifs in the eRF1 crystal structure is 97.5 Å, measured from the Ile-Cα to Gln-Cα. In our simulations in aqueous solutions, eRF1 with either neutral or charged histidine groups shows considerable conformational changes compared with the crystal conformation (Figs. 3 and 4). Domains 1 and 2 tend to bend closer to each other; however, the extent of bending depends on the charged states of the histidine groups. For eRF1 with n-His, the distance between the NIKS and GGQ motifs fluctuates around 90 Å (Figs. 3B and 4A, red line). For the p-His form, the separation may shrink to 70–75 Å (Figs. 3C and 4A, green line), which fits into the range needed for its functional binding with the ribosome (73 Å).

The structural features that accompany the large fluctuations of domains 1 and 2 are very interesting. Consistent with the large separation of the NIKS-GGQ motifs, the conformation of the n-His form (Fig. 4B, red line) is closer to the crystal structure, with a smaller r.m.s. deviation. Although the p-His form (Fig. 4B, green line) of eRF1 has much larger global motions than the eRF1 n-His, the change in the individual domains in the p-His form is similar to that of the n-His or even less (Supplementary Fig. 1). Domain 1 is very rigid for both n-His and p-His eRF1, with the r.m.s. deviation being around 1.5–2.0 Å (Supplemental Fig. 1A). Notably, domain 2 has a larger structural change in the n-His eRF1 (r.m.s. deviation 3 Å) than in the p-His eRF1 (r.m.s. deviation 2 Å; Supplemental Fig. 1B). Domain 3 has a large disordered region and is flexible in both (r.m.s. deviation 5 Å; Supplemental Fig. 1C).

A “spring breaker” mechanism restricts the hinge motion of domain 2 in the n-His form of eRF1, as indicated in Fig. 3. Structurally, the motion of domain 2 is controlled by two flexible regions, one in the hinge strand of residues 140–143 and another in the middle of helix 8. The hinge strand is insensitive to the protonation of histidines, since it has similar dynamic behavior for both neutral and protonated forms (Supplemental
Fig. 1E). In the crystal, helix 8 has a large kink in the middle, resulting in a bent helix. However, the distorted helix 8 gradually restores its perfect helical conformation in the neutral histidine form (red line) effectively locking the hinge. The stretched helix now acts as a spring breaker to restrict the conformational fluctuation of the domain motion. If the histidines are protonated, the kinked region remains in the distorted state (green line).

The Conformational Changes of RF2—In contrast to the expanded binding epitopes of eRF1, the crystal structure of the bacterial polypeptide release factor RF2 is tightly packed (5) (Fig. 5A). Domain 1 of RF2 contains only a helical bundle. Domains 2 and 4 are one integrated unit with β-sheets wrapped around helix 5. Domain 3 is well packed with the core domain. The anticodon motif SPF and the peptidyltransferase center binding motif GGQ are located at the tips of the core domain and domain 3, respectively. We measure the separation of SPF and GGQ using the distance between Pro-Cζ and Gln-Cζ (32 Å). The nearest contact between the two motifs, measured between the side chain of Phe207 and Gly250, is only 23 Å.

Simulation of the RF2 with neutral histidines leads to an even more compact conformation (Fig. 5B). Initially, there is a slight increase in the SPF-GGQ distance. However, it shrinks from 40 to 25 Å and fluctuates around the 30-Å range (Fig. 4C, red line). The crystal structure of the GGQ loop (residues 247–257) was modeled based on crystal packing, due to the poor electron density resolution (5). The fluctuation observed in our MD simulations could reflect its native state property. Other parts of the RF2 are quite stable, with only a slight twist away from the crystal structure.

Again, a profound structural change occurs for the RF2, when the histidines are protonated (Fig. 5C). The SPF-GGQ distance constantly increases from the beginning of the simulation and reaches 50 Å in about 2.5 ns (Fig. 4C, green line). There is a simultaneous change in the SPF-GGQ distance and a large twist of domain 1 at around 1 ns (Supplemental Fig. 2).

The final shape of the extended RF2 is halfway between the crystal structure (5) and the structure observed in the cryo-EM (6, 7), as indicated in the superposition of the three structures in Fig. 6 (crystal structure (blue ribbon), cryo-EM structure (green ribbon), and structure from MD (red ribbon)). The structural extension of the SPF-GGQ observed in our simulation is...
achieved mainly through a large flip of the loop containing the GGQ motif. The helix arm is twisted only slightly and keeps its compact contact with the core domain. The movement of domain 1 is achieved mainly by bending the $\alpha_1$, $\alpha_2$, and $\alpha_4$ helices. A large kink links $\alpha_1$ and $\alpha_2$. The $\alpha_4$ helix is hinge-linked with the core domain. Thus, in our MD simulations, $\alpha_2$ and half of $\alpha_3$ still keep their original position, whereas $\alpha_1$, $\alpha_4$, and half of $\alpha_3$ move to the conformation observed in the cryo-EM (6, 7).

Conservation of Histidine in eRF1 and RF2—Conservation of Histidine in eRF1 and RF2—The conformational sensitivity of eRF1 and RF2 to the protonation state of histidine may be traced to their enrichment in charged residues. 27% of the eRF1 residues are charged. For RF2, charged residues constitute as much as 32%. The charge distributions in the proteins are uneven. The eRF1 domain 1 and the GGQ minidomain are positive, whereas part of domain 2 and most of domain 3 have a larger number of negative charges (Fig. 1). RF2 domain 1 is highly negative. Sequential alignments of the RF1 and RF2 proteins indicate that many of the charged residues are conserved (Fig. 7).

The highly uneven charge distribution between the domains indicates that the interdomain electrostatic interactions may affect the proteins’ function (24). It is known that mutating a conserved Glu changes the function of the release factors (25). Therefore, the protonation state of histidines can be crucial, by modulating the charge distributions in the domains. Indeed, sequence alignments show that certain critical histidines within the charged residues’ environments are conserved in both RF1 and RF2 (Fig. 7). For RF1, three are highly conserved (Figs. 1 and 3A). His$^{132}$ is located in the center of domain 1. His$^{160}$ and His$^{169}$ are near the GGQ motif in domain 2. His$^{180}$ is only two residues away from the Gly$^{183}$-Gly$^{184}$-Gln$^{185}$ motif. The less conserved His$^{170}$ is also in domain 2. Domain 3 has three unconserved His residues located in the disordered region. Histidines are even more conserved in the RF2. All five histidines in the RF2 are highly conserved (Fig. 5A) and are located near the two critical SPF and GGQ motifs. Again, GGQ is directly connected to a histidine (His$^{253}$). Histidine conservation suggests direct functional roles, and it was postulated that His$^{132}$ in eRF1 may be involved in codon binding as well (26). Here we propose that conserved histidines may also modulate the protein conformation.

Mechanism of Conformational Change of eRF1: Electrostatic Analysis of a Molecular Machine—To understand the mechanism and driving force of the conformational changes, we compute the histidine protonation energies and the interaction energies among the domains of eRF1 (Figs. 1 and 8–10). We choose 170 structures from our MD trajectory of eRF1 with protonated histidines, to sample the conformational distributions from the open to the closed forms. Among these conformations, there are 52 open conformers (with NIKS-GGQ distance of $>90$ Å), 58 intermediates (with NIKS-GGQ distances between 80 and 90 Å), and 60 closed conformers (with NIKS-GGQ distance of $<80$ Å). For each structure, we compute the effect of protonation on the overall energy, the electrostatic contribution to the domain-domain interaction ($\Delta G$; Equation 1), and the protonation effect on the interdomain interaction.
We also studied 49 conformations from the MD trajectory of eRF1 with neutral histidines, for which most of the structures are in the open state (Fig. 3B). First, we look at the correlation between the electrostatic energy ($E_{\text{coulombic}} + E_{\text{solvation}}$) and the NIKS-GGQ distance. Fig. 8A plots the total electrostatic energy of eRF1 with neutral histidines. Fig. 8B plots of the total electrostatic energy of eRF1 with protonated histidines. Fig. 8C is the change of the total electrostatic energy upon histidine protonation. As may be seen in Fig. 8A, for the eRF1 with neutral histidines, there is only a weak correlation between the electrostatic energy and the NIKS-GGQ distance. However, when the histidines are protonated, we observe the strong dependence of the electrostatic energy on the NIKS-GGQ distance, with closed conformations more favored (Fig. 8B). Histidine protonation strongly increases the stabilizing effect of electrostatic interactions (more negative). The overall effects of the protonation are shown in Fig. 8C and Table I. In general, the overall protonation effects are favored for the close conformations. In Fig. 8C, we see two clusters of points. The highlighted (circled) region is the conformations from the trajectories of the MD simulations of eRF1 with neutral histidines. The histidine protonation energies in this highlighted cluster are notably less negative. The structural characteristics of this cluster of conformations are (i) they are all highly open, and (ii) the kinked helix is locked (Fig. 3B). Table I shows the detailed analysis of the histidine protonation effect. For the 170 conformations from the MD simulation trajectory with protonated histidines, the closed conformations are about $-13$ kcal/mol more stable than the open conformations.

The above results nicely explain the dynamic difference between the eRF1 with neutral and protonated histidines. Consistent with the molecular dynamics simulations, the electrostatic analyses also show that histidine protonation should drive the eRF1 toward closed conformations. Below, we attempt to understand the mechanism (i.e. how this electrostatic effect may lead to the conformational changes of the eRF1).

As indicated in Fig. 1, we partition the eRF1 into three large domains and a hinge region. Domains 1 and 2 are mostly positively charged, and the hinge region and domain 3 are negatively charged. Histidine protonation should modulate the electrostatic interactions among the domains. Intuitively, coulombic interaction will be repulsive for the domain 1-domain 2 interactions and attractive between domain 2-domain 3 interactions. In reality, the interactions among the domains are complex, such as domain 1-domain 2, domain 2-domain 3, domain 2-hinge, domain 1-domain 3, domain 2-(domain 1 + domain-3), etc. The preferred protein conformations should be the result of the subtle balance of these domain interactions. Since the open and closed conformations of the eRF1 mainly involve

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**Fig. 6. Superposition of conformations of RF2.** The conformations are taken from the crystal structure, from molecular dynamics simulations with protonated histidines, and from cryo-EM (Fig. 4, A, C, and D, respectively). SPF motifs for the three structures are superimposed together, whereas different locations of GGQ motifs reflect the structural difference. It can be seen that the structure from the MD simulation (red ribbon) is positioned between that of crystal structure (blue ribbon) and the cryo-EM structure (green ribbon).
FIG. 7. Sequence alignments of related species for RF1 and RF2. Only regions with conserved histidines are shown. Other marked residues are conserved charged residues. A, RF1; B, RF2.

FIG. 8. Electrostatic energy ($E_{\text{coulombic}} + E_{\text{solvation}}$) and the changes upon protonation of histidines in eRF1. A, the electrostatic energies for eRF1 with neutral histidines are weakly dependent on the NIKS-GGQ distance. B, the electrostatic energies for eRF1 with protonated histidines are strongly dependent on the NIKS-GGQ distance. C, electrostatic energy changes upon protonation of histidines are strongly dependent on the NIKS-GGQ distance, energetically more favorable for the closed conformation. D, change of the electrostatic interaction between domain 1 and domain 2 is slightly more negative; there is no correlation with distance. E, change of the electrostatic interaction between domain 2 and domain 3 is slightly more positive; there is no correlation with distance.
the domain 2 motion relative to other domains, we focus on the
domain 1-domain 2 interactions and domain 2-domain 3
interactions.

Fig. 9 shows the electrostatic contributions to the domain 1-domain 2 interaction in eRF1. Neutral histidine forms are shown as follows: coulombic interaction (A); solvation contribution (B); overall electrostatic interaction, \( \Delta G = \Delta E_{\text{coulombic}} + \Delta E_{\text{solvation}} \) (C). Protonated forms are as follows: coulombic interaction (D); solvation contribution (E); overall electrostatic interaction (F). The interactions shown in C, D, E, and F are highly correlated with the NIKS-GGQ separation.

Fig. 10 shows the electrostatic contributions to the domain 2-domain 3 interaction in eRF1. Neutral histidine forms are shown as follows: coulombic interaction (A); solvation contribution (B); overall electrostatic interaction, \( \Delta G = \Delta E_{\text{coulombic}} + \Delta E_{\text{solvation}} \) (C). Protonated forms are as follows: coulombic interaction (D); solvation contribution (E); overall electrostatic interaction (F). The interactions shown in D and E are highly correlated with NIKS-GGQ separation.

As indicated in Fig. 9, due to the overall positive charges on both domain 1 and domain 2, the coulombic contributions are always repulsive. However, the solvation shows an attractive contribution for the domain 1 and domain 2 interaction. For the neutral histidine forms, the correlations of the individual terms
The change of electrostatic energies upon eRF1 histidine protonation

| NIKS-GGQ distances | >90 Å (open) | 80–90 Å | <80 Å (closed) | All average |
|--------------------|-------------|---------|----------------|-------------|
| Number of conformations | 52 | 58 | 60 | |
| Histidine protonation energy (kcal/mol) | $-207^b$ | $-213$ | $-220$ | $-213$ |
| $\Delta G$ (domain 1–domain 2) (kcal/mol) | $1.09$ | $1.05$ | $0.64$ | $0.91$ |
| $\Delta G$ (domain 2–domain 3) (kcal/mol) | $1.27$ | $1.68$ | $1.99$ | $1.67$ |

$^a$ Taken from the MD simulations with protonated histidines.
$^b$ The average histidine protonation energy for 49 conformations from the trajectory of the simulations with neutral histidines is $-94$ kcal/mol

(\(\Delta E_{\text{ele}}\) or \(\Delta E_{\text{solv}}\)) with the NIKS-GGQ distance are weak, with very small correlation coefficients. However, the total electrostatic contribution shows a NIKS-GGQ distance-dependent behavior, with \(R^2\) of 0.27 (Fig. 8, A–C). For the protonated histidine form, there are strong correlations of the coulombic interaction, solvation contribution, and overall electrostatic contributions with the NIKS-GGQ distances (Fig. 9, D–F). Generally, the domain 1-domain 2 interactions are attractive for the open conformations and repulsive for the closed conformations. Since the overall electrostatic contributions are correlated with the NIKS-GGQ distance, the change of domain 1-domain 2 interaction probably changes protein conformation.

The \(\Delta G\) of the overall protonation effects on the domain 1-domain 2 interaction are reported in Fig. 8D and Table I. There is no correlation with the NIKS-GGQ distance (Fig. 8D). However, histidine protonation increases the domain 1-domain 2 attraction in the open state and decreases the repulsion in the closed state (Table I). The average \(\Delta G\) for the 170 conformations is $-0.9$ kcal/mol.

Similar analyses were performed for the domain 2-domain 3 interactions, and the results are shown in Fig. 10. Similar to the domain 1-domain 2 interactions, the domain 2-domain 3 interactions show an increased distance-dependent behavior for the protonated histidine in the coulombic interaction and solvation contribution. As indicated in Fig. 10, for the protonated histidine forms, the coulombic interaction strongly prefers the open conformation, with a correlation coefficient as high as \(R^2\) of 0.51. However, again, the solvation contribution compensates for this trend. Thus, overall, the electrostatic contribution for the domain 2-domain 3 interaction is directionless, for both neutral histidine form (Fig. 10C) and the protonated histidine form (Fig. 10F). Thus, we do not expect that the domain 2-domain 3 interactions would affect the open-closed conformational shift. Therefore, although the \(\Delta G\) for the domain 2-domain 3 is positive (Fig. 8E, Table I), histidine protonation does not affect the domain 2-domain 3 positions.

In summary, electrostatic analyses suggest that the histidine protonation should drive the eRF1 conformation to the closed state. The conformational preference of the eRF1 may be controlled by the balance of interactions among the three domains. Among these interactions, histidine protonation increases the domain 1-domain 2 attraction in the open state and decreases the repulsion in the closed state, making the closed state more accessible.

**Molecular Shapes of eRF1 and RF2: Comparison with Related Macromolecules**—The large conformational changes observed in our simulations prompted us to investigate the molecular shapes of eRF1, RF2, and their related macromolecules. We devised a histogram-based structural comparison algorithm to quantitatively compare the shape similarity of the proteins and RNA (Figs. 1 and 11; see “Materials and Methods”). The similarity distance measures the similarities across the macromolecules. Identical molecules have a distance of 0.0. The similarity distance increases with a decrease in the similarity. The results are reported in Table II.

First, we examined the shape similarity between the two structures of RRF (Protein Data Bank codes 1dd5 and 1eh1; Fig. 11A) with an r.m.s. deviation of 3.0 Å. The similarity distance we measured was 2.5. At the other extreme, as a negative control, we compared the eRF3 and the EF-Tu-tRNA complex. The structure of the eRF3 has been solved recently (27). Although it has a domain similar to the EF-Tu part, it has no domain similar to the tRNA. The similarity distance between eRF3 and EF-Tu-tRNA is 59.5, indicating little similarity between the two shapes. Within this range, we can readily map the shape similarities of related molecules.

When domain 1 of RRF overlapped the anticodon arm of tRNA, RRF appeared as a perfect tRNA mimic (Fig. 11B). The similarity distance between the RRF and the tRNA in this classical orientation was 15.5. However, we noticed that the shape of the RRF domain 2 and the anticodon arm of tRNA were also similar (Fig. 11C). The shape similarity distance in this second orientation was only 9.5, even closer than in the classical orientation. The structures of EF-G and the EF-Tu-tRNA complex are highly similar, and it has been observed that both bind to the ribosome similarly. In our optimized alignment (Fig. 11D), their similarity distance was 18.3.

Next, we compared the shape similarities between conformational changes of the eRF1 and RF2. The most similar were the eRF1 observed in our MD simulation and the RF2 fitted from cryo-EM experiments (Fig. 11F). The two conformations were very similar, particularly eRF1 domains 1 and 2 and domains 2–4 of RF2 (Fig. 11F). Domain 3 of eRF1 and domain 1 of RF2 have different shapes. However, their dimensions are still similar. The overall similarity distance between the eRF1 and RF2 was 21, slightly larger than that between EF-G and the EF-Tu-tRNA complex. When we compared only domains 1 and 2 of eRF1 and domains 2–4 of RF2, the shape similarity was only 10.9, comparable with the similarity between the RRF and tRNA in the new orientation. The twisted arms of domains 1 and 2 of eRF1 may have a shape like RRF. However, the similarity measure yielded a rather large distance of 33.

We further calculated the similarity between the eRF1 and EF-Tu-tRNA as well as the similarity between the RF2 and EF-Tu-tRNA. As may be seen in Table II, the shape similarities for the release factors and the elongation factors were pretty low.

**DISCUSSION**

Conformational changes in proteins can be affected by intramolecular physical forces, solvation, and their interacting partners (like RNA). Often, the intramolecular physical forces (hydrogen bonding, electrostatic interactions, and the hydrophobic effect) are strongly coupled with solvation effects. The situation is even more complex when the shift of balance of the intramolecular forces is coupled with the (local) folding/unfolding of the protein structure. In the case of eRF1, the kinked helix can lock the domain motion. For the RF2, unfolding of a large portion of the protein is needed to achieve its ribosome binding conformation. Here, our studies point to an important role of histidine protonation on the protein conformational dynamics.
In their functional forms, the protonation states of histidines in eRF1 and RF2 could be sensitive to the ribosome environment. Due to the overall high negative charge of both eRF1 and RF2, the histidines might already be partially protonated in the free forms in solution. The overall negative RNA backbone charges will enhance the histidine protonation. From solution to RNA backbone, there should be an electrostatic gradient, depending on the three-dimensional structure of the RNA and the ionic strength surrounding the RNA. As the release factor approaches the RNA, the chance of histidine protonation increases, and its conformation shifts to its RNA binding state. Regardless of the initial histidine protonation state, binding the stop codon should drive the histidine protonation dramatically. The histidine protonation may then drive the protein toward its final functional conformation. Thus, it appears to be a remarkable cooperative event of protein conformational change and RNA recognition. This scheme is consistent with the suggestion by Rawat et al. (7) that the unfolding of RF2 may be only signaled by the binding of RF2 to the stop codon.

Histidine protonation has been shown to be important for protein-nucleic acid interactions (28–30). Histidine protonation also induces the G protein conformational changes and drives membrane fusion (31). Our results indicate that release factors eRF1 and RF2 act like molecular machines, most likely fueled by histidine protonation. We further speculate that histidine protonation may prove a general control mechanism in flexible proteins associated with the regulation of DNA/RNA expression. An observed conformational change coupled with histidine conservation in an environment with a high percentage of charged residues may point to a protonated/neutral histidine involvement. In agreement with this proposition, we have observed that the loop 1 of the p53 core domain flips away in the
DNA binding conformation with neutral histidine residues. However, remarkably, histidine protonation restores the loop 1 of p53 core domain to its DNA binding position.2

It is known that aminocyl-tRNA and EF-tu require a carefully adjusted affinity to function effectively in translation (13, 14). Similarly, the binding of the release factors to the ribosome also requires an adequate thermodynamic control. Histidine protonation/deprotonation may provide a convenient, reversible ribosome-binding mechanism for the Class I release factors.

The role of RRF as a tRNA mimic has been questioned recently (33), since the matching of the RRF and the tRNA in the classical orientation does not agree with the radical cleavage assay. We find that the shapes of the RRF and the tRNA match well in a different orientation, which might be more consistent with the biochemical evidence. Nevertheless, the biological significance of the shape similarity in the new orientation is unclear. Most likely, as many have realized, the correspondence between function and shape similarity might be weak (2, 8, 9). A weak correspondence may also be inferred by our comparison of the RRF and domains 1 and 2 of eRF1. RRF strongly competes with RF1 and RF2 with mutually exclusive and perhaps overlapping binding sites on the ribosome (32, 34, 35). The shape similarity might explain the binding competition. However, although the RRF and the two arms of domains 1 and 2 from the twisted eRF1 have a similar L shape, our measure of similarity distance indicated that they are quite dissimilar.

The conformations of the eRF1 and RF2 are more similar in their p-His forms as compared with their n-His forms. The closed (p-His) form of eRF1 confirms the suggestion that RRF and eRF1 may have comparable structures when bound to their respective ribosomes (6). However, our simulations do not reproduce the cryo-EM pictures of the unfolding domain 3 upon dissociation from the core domain in RF2 (6, 7). There are several possible reasons. First, room temperature MD simulations (17–21) usually cannot unfold the protein structure, thus limiting the scope of structural exploration. Second, as suggested by Rawat et al. (7), the unfolding may be only signaled by the binding of RF2 to the stop codon. Nevertheless, our observation of the unified conformational control mechanism of eRF1 and RF2 provides support to the proposition that termination of protein synthesis involves similar mechanisms across species.

Instead of static structural similarity, our studies point to the crucial role of the dynamic behavior of proteins within certain geometrical and functional restrictions. The ribosome, the master molecular machine, can never be static. Accordingly, other machinery in the system must also be dynamically functional. The dynamic convergence of the functional distances between key domains in the eRF1 and RF2 is much more significant than the static picture of their structural similarity.

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