Dynamic Behavior of Trigger Factor on the Ribosome

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Abstract

Trigger factor (TF) is the only ribosome-associated chaperone in bacteria. It interacts with hydrophobic segments in nascent chain (NCs) as they emerge from the ribosome. TF binds via its N-terminal ribosome-binding domain (RBD) mainly to ribosomal protein uL23 at the tunnel exit on the large ribosomal subunit. Whereas earlier structural data suggested that TF binds as a rigid molecule to the ribosome, recent comparisons of structural data on substrate-bound, ribosome-bound, and TF in solution from different species suggest that this chaperone is a rather flexible molecule. Here, we present two cryo-electron microscopy structures of TF bound to ribosomes translating an mRNA coding for a known TF substrate from *Escherichia coli* of a different length. The structures reveal distinct degrees of flexibility for the different TF domains, a conformational rearrangement of the RBD upon ribosome binding, and an increase in rigidity within TF when the NC is extended. Molecular dynamics simulations agree with these data and offer a molecular basis for these observations.

Introduction

During translation, the nascent polypeptide chain emerges from the ribosomal exit tunnel and is released into the crowded environment of the cytosol where processes such as protein folding and targeting for translocation must occur. These processes are assisted by protein factors such as the signal recognition particle (SRP) and its receptor (SR) in the case of targeting and the different chaperones and chaperonins in the case of protein folding. Whereas in eukaryotes, a whole repertoire of chaperones (such as the ribosome-associated complex (RAC), the nascent polypeptide-associated complex (NAC), and heat shock protein SSB1) interacts with the ribosome near the tunnel exit on the large ribosomal subunit, in bacteria, trigger factor (TF) is the only chaperone, which directly interacts with the ribosome. TF is composed of three domains: an N-terminal ribosome-binding domain (RBD), the peptidyl-prolyl *cis*/*trans*-isomerase (PPIase) domain or head domain, and the C-terminal substrate-binding domain (SBD) located between the RBD and PPIase domain in the TF structure, forming the arms 1 and 2 [1] (see Supplementary Fig. 1). The RBD and the C-terminal domain harbor the main chaperone activity of TF, whereas the PPIase domain is dispensable for the chaperone activity *in vivo* [2,3]. Nevertheless, more recent reports suggest that, at least for some proteins, the PPIase domain provides a second chaperone interaction site for the nascent chain (NC) [4–7]. Most of our knowledge about the involvement of chaperones in protein folding originates from *in vitro* studies, where the refolding of chemically denatured substrate proteins was monitored in the presence of chaperones with various techniques. However, in the recent years, many studies have shown in eukaryotes as well as in prokaryotes, that protein folding already

begins on the ribosome, while the C-terminal part of the polypeptide is still in the process of being extended at the peptidyl-transferase center. Furthermore, several recent studies have shown that the formation of secondary structures, such as α-helices, already begins within the ribosomal exit tunnel [8–15].

To date, three crystal structures of TF RBDs in complex with 50S ribosomal subunits (one heterologous pair of *Escherichia coli* TF and archaeal 50S from *Haloarcula marismortui* and two homologous pairs of TF and 50S from *Deinococcus radiodurans*) have revealed the overall positioning of the RBD on empty ribosomes [1,16,17]. Docking of the crystal structure of a complete TF molecule onto the structure of the 50S-associated RBD indicated that the chaperone is localized in a position so that it "arches" over the ribosomal tunnel exit [1]. A cryo-electron microscopy (cryo-EM) structure of *E. coli* ribosomes carrying a stalled NC of a non-physiological substrate (SH3, which can fold independently of TF) with a TF molecule fixed to the NC via a covalent disulfide bond in close proximity to the tunnel exit confirmed the localization of the chaperone over the ribosomal tunnel exit [18]. Nevertheless, due to the limited resolution of this structure (19 Å), the interaction of TF with the NC was not studied in molecular detail. Therefore, we aimed to use cryo-EM to visualize the ribosome-bound TF when interacting with a physiological substrate emerging from the ribosomal tunnel. For this purpose, we choose the galactitol-1-phosphate dehydrogenase (GatD) as the nascent polypeptide chain because it is known to interact with TF on the ribosome and exhibits two hydrophobic stretches [6,19].

Here, we show that TF interacts with ribosome–NC complexes (RNCs) carrying this substrate with high affinity. The cryo-EM structure of the TF–GatD85–RNC exhibits a sub-nanometer resolution for the ribosome and the N-terminal RBD of TF, whereas the middle domain and the head (PPIase) domain of TF are less well resolved due to increasing flexibility along the molecule. The average resolution of our TF–GatD85–RNC map (7.7 Å) enabled us to visualize the NC within the ribosomal tunnel and even trace it onto the surface of the TF–RBD. We observe the two alpha helices of the RBD undergo a conformational change when bound to the translating ribosome. This rearrangement causes the presentation of a new hydrophobic surface on the RBD, which serves as interaction site for hydrophobic stretches within the emerging NCs. Using microscale thermophoresis (MST), we demonstrate that the affinity of TF for RNCs increases as the NC is elongated, which may reflect an altered conformation of TF. We confirm this finding with a second TF–RNC cryo-EM structure, where TF in complex with RNCs carrying a longer NC of GatD (145 aa total length) gained rigidity partially due to additional interactions of TF with either the NC or the ribosomal surface. Molecular dynamics simulations reveal the molecular interactions between TF and the NC, elucidating how the length of the NC modulates the conformation of RNC-bound TF and the binding/release of TF with RNCs.

Results

We generated RNCs carrying an 85-aa (total length) long GatD nascent polypeptide stalled by the TnaC stalling sequence *in vivo* (Fig. 1a and Supplementary Table 1) [20,21]. In order to enable purification and detection of the RNCs, the used mRNA coded for an N-terminal His-tag followed by an HA tag followed by F61 to C103 of GatD and the stalling sequence of TnaC. The purified GatD85–RNCs showed a homogenous population of ribosomes carrying only peptidyl-tRNAs. Free peptide of the NC was not visible, indicating stably stalled RNCs [22]. Binding of TF to these GatD85–RNCs was shown using microscale thermophoresis (MST; see below). We reconstituted purified GatD85–RNCs with TF and subjected these complexes to cryo-EM and single particle analysis following standard procedures [23]. After several rounds of global and focused sorting of the dataset, a stable population of 100,931 particles was refined to an average resolution of 7.7 Å (FSC 0.5 criterion, see Supplementary Fig. 2a). The resulting structure resembled a programmed 70S ribosome with additional density for the P-site tRNA as expected, as well as the NC and density representing TF at the ribosomal tunnel exit (Fig. 1b–d). This density did not account for the entire TF molecule but rather represented only parts of the RBD of TF. Nevertheless, an analysis of the local resolution revealed that the RBD showed a resolution distribution comparable to that of the ribosome itself, indicating a near stoichiometric occupation of the GatD85–RNCs with TF (Supplementary Fig. 2b). When we low-pass filtered the same density map to a resolution of 16.5 Å, the C-terminal domain with its arms became visible (red mesh in Fig. 1), whereas the PPIase domain remained invisible, indicating a gradual increase in flexibility within TF from the RBD over the C-terminal domain toward the PPIase domain. The crystal structure of full-length TF exhibits two possible pivot points that are likely to explain the observed flexibility. The RBD is connected via an extended unstructured loop (V111 to T133) with the PPIase domain. This loop is packed against another loop connecting the long α-helix forming the back with arm1 (D299 to P303). The second pivot point may be located in the unstructured loops (A149 to K154 and E241 to L248) connecting the PPIase domain with the remaining of the TF molecule (Supplementary Figs. 1 and 5a). These pivot points are in agreement with a normal mode analysis of the TF crystal structure (PDB ID: 1W26) and also with our molecular dynamics (MD) simulations (see below).