Supporting Information

Production of ribosome-released nascent proteins with optimal physical properties

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Abstract

This supporting information provides evidence, via a puromycin sensitivity assay, that ribosomes do not lose catalytic activity in the presence of hydroxylamine. This section also provides descriptions of the SDS-PAGE and RNA chip experimental conditions used in this study, as well as a detailed explanation of the principles and procedures used to estimate the expected $\tau_{c,g}$ of apoHmpH and apoMb (a reference protein) in dilute solution.

Supporting Text

Puromycin sensitivity assays. In order to further examine the effect of hydroxylamine and sodium hydroxide on RNCs, separate ribosome-release reactions triggered by either hydroxylamine (final concentration 1.5 M, final pH 7.8) and sodium hydroxide (final concentration 75 mM, final pH 9.5) were allowed to proceed for a short time (15 min) so that some intact peptidyl-tRNA remained. Samples were then analyzed by Tris•MES SDS-PAGE before and after treatment with puromycin (final concentration 1 mM) for 15 min at 37°C (Fig. S-1). The mechanism by which puromycin, a Tyr-tRNA^{Tyr} structural analog (Fig. S-2), releases nascent proteins requires full 23S rRNA peptidyl transferase activity. Puromycin sensitivity can thus be regarded as a reporter of ribosomal integrity[1-3]. While RNCs incompletely reacted with hydroxylamine were sensitive to puromycin-induced nascent protein release, RNCs incompletely reacted with sodium hydroxide were not. This result suggests that ribosomes remain catalytically active in the presence of hydroxylamine.

SDS-PAGE analysis of ribosome-released nascent chains. The Tris•MES gels consisted of a 4.5 cm resolving layer (13.3% T, 3.3% C, 6% glycerol), a 1 cm spacer layer (8.1% T, 3.3% C), and a 1 cm stacking layer (3.2% T, 3.3% C). T and C denote acrylamide plus bis-acrylamide and bis-acrylamide alone, respectively. All three layers were prepared in aqueous 1 M Tris•HCl (pH 6.8) and 3.5 mM SDS. Polymerization was initiated by addition of 1, 1, and 3 mM ammonium persulfate (APS) and 5, 5, and 15 mM N,N,N',N'-tetramethylethylenediamine (TEMED) to the resolving, spacer, and stacking layers,

respectively. Samples were mixed with loading buffer (100 mM Tris•HCl at pH 6.8, 139 mM SDS, 20% glycerol) and heated at 100°C for 3 min before loading onto the gel. Gels were run at constant voltage (55 V) for 150 min at room temperature in 100 mM Tris•MES (pH 6.8) and 3.5 mM SDS. The gels were analyzed by fluoroimaging (UC 4x4 scanner from Genomic Solutions, λ_{ex} 488 nm, emission channel bandpass filter centered at 512 nm) to detect the nascent proteins.

RNA microchip electrophoresis. The RNA chip assay provides a small-volume, high-resolution denaturing gel analysis of RNA. RNA in the sample is separated electrophoretically under denaturing conditions and scanned for fluorescence response to an intercalating molecule. All chips were primed and loaded with samples according to the manufacturer's specifications. Data were analyzed with the Agilent 2100 Expert software. The *E. coli* ribosome is composed of large 50S (1.6 MDa) and small 30S (0.9 MDa) subunits. The large subunit includes 23S (2900 nt) and 5S (120 nt) rRNA chains and 34 proteins, while the small subunit includes a 16S (1540 nt) rRNA chain and 21 proteins. rRNA signal reduction and the presence of degradation fragments were correlated with ribosomal degradation.

Estimation of the expected $\tau_{c,g}$ of monomeric apoHmpH in dilute solution. The expected $\tau_{c,g}$ of monomeric apoHmpH at 25 °C in dilute solution was estimated as follows, considering that the overall structure of this protein is well approximated by a rigid oblate ellipsoid of revolution. The rotational diffusion of an ellipsoid of revolution is described by a tensor with parallel and perpendicular components $D_{\|}$ and $D_{\perp}$ components defined as

$$D_{\|} = D[\frac{3\rho(\rho - S)}{2(\rho^2 - 1)}] \qquad \text{(S-1)}$$

and

$$D_{\perp} = D[\frac{3\rho[(2\rho^2 - 1)S - \rho]}{2(\rho^4 - 1)}] \quad , \qquad \text{(S-2)}$$

where ρ is the ellipsoid axial ratio

$$\rho = \frac{a}{b} \qquad \text{(S-3)}$$

and, for an oblate ellipsoid, S is

$$S = (1-\rho^2)^{-1/2} \arctan \frac{(1-\rho^2)^{1/2}}{\rho} \quad . \tag{S-4}$$

For apoHmpH, a = 25 Å and b = 44 Å, which coincide with the axial dimensions of myoglobin[4], as the two proteins have superimposable structures[5]. D is the diffusion coefficient of a hypothetical hydrated sphere with molecular mass M identical to that of apoHmpH, in a medium of viscosity η, according to the Stokes-Einstein-Debye relation

$$D = \frac{RT}{6\eta V} = \frac{RT}{6\eta M(\bar{v} + \delta h)} \quad , \tag{S-5}$$

where R = 8,314 g m^2 s^{-2} K^{-1} mol^{-1}, T = 298 K, $\eta = 0.89$ cP (gm^{-1} s^{-1}), M = 15,719.9 g mol^{-1}, the specific volume of the protein, $\bar{v}$, is equal to 7.43x10^{-7} $m^3_{protein}/g_{protein}$, the specific volume of water, δ, is equal to 1.0035x10^{-6} m^3_{H2O} g^{-1}_{H20}, and the specific protein hydration, h, is equal to 0.50 g_{H2O} $g^{-1}_{protein}$. In general, the fluorescence anisotropy decay $r(t)$ of an ellipsoid of revolution is properly described by a sum of three exponentials[6, 7, 8] according to

$$r(t) = r_1 e^{-t/\tau_{c1}} + r_2 e^{-t/\tau_{c2}} + r_3 e^{-t/\tau_{c3}} \quad , \tag{S-6}$$

where r_1, r_2 and r_3, and τ_{c1}, τ_{c2} and τ_{c3} are the amplitudes and the rotational constants for each component of the anisotropy decay. The decay amplitudes are defined as

$$r_1 = 0.3 \sin 2\beta_A \sin 2\beta_E \cos \xi \quad , \tag{S-7}$$

$$r_2 = 0.3 \sin^2 \beta_A \sin^2 \beta_E \cos 2\xi \quad , \text{and} \tag{S-8}$$

$$r_3 = 0.1(3\cos^2 \beta_A - 1)(3\cos^2 \beta_E - 1) \quad , \tag{S-9}$$

where the β and ξ angles are defined as in Figure S-3, $\xi = 13°$[9] and the rotational constants are defined as

$$\tau_{c_1} = (D_{\parallel} + 5D_{\perp})^{-1} \quad , \tag{S-10}$$

$$\tau_{c_2} = (4D_{\parallel} + 2D_{\perp})^{-1} \quad , \text{and} \tag{S-11}$$

$$\tau_{c_3} = (6D_{\perp})^{-1} \quad . \tag{S-12}$$

While three discrete anisotropy decay components are expected, it is well known that, for flattened ellipsoids such as the oblate ellipsoids of revolution, the three rotational constants are approximately equal[10]. Therefore, only one anisotropy decay due to macromolecular tumbling is observed experimentally. This value corresponds to the averaged rotational correlation time for global tumbling $<\tau_{c,g}>$, equivalent to $\tau_{c,g}$ in relations (1) and (2), according to

$$\langle \tau_{c,g} \rangle = \sum_{i=1}^{3} \frac{r_i}{r_0} \tau_{c_i} \quad , \tag{S-13}$$

with r_0 equivalent to

$$r_0 = r_1 + r_2 + r_3 \quad . \tag{S-14}$$

Relation (S-13) and corresponding input from equations (S-1) - (S-12) were used to estimate the expected rotational correlation time for apoHmpH in dilute solution. To carry out the computations, absorption and emission dipoles were assumed to be colinear. The two limiting cases of absorption and emission dipoles perpendicular ($\beta_A = \beta_E = 90°$) or parallel ($\beta_A = \beta_E = 0°$) to the oblate ellipsoid short axis were considered. The resulting calculated rotational correlation time is 8.28 ns for $\beta_A = \beta_E = 90°$, and 7.51 ns for $\beta_A = \beta_E = 0°$.

Supporting Figures and Legends

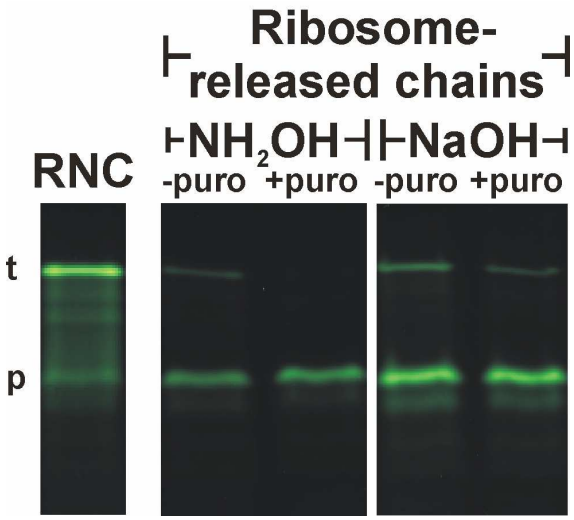

Figure S-1. Integrity of unreacted RNCs following partial nascent chain release from the ribosome. The gel shows ribosome-bound peptidyl-tRNA before (-puro) and after (+puro) the addition of puromycin to samples that were reacted with either hydroxylamine (1.5 M) or sodium hydroxide (75 mM) for 15 min. Samples transition from peptidyl-tRNA (**t**) to the released peptide species (**p**). Unreleased RNCs remain completely sensitive to puromycin in the presence of hydroxylamine, demonstrating that the reagent does not destroy the catalytic activity of the ribosome. In contrast, unreleased RNCs are no longer sensitive to puromycin after reaction with sodium hydroxide, indicating that the ribosome is no longer catalytically active.

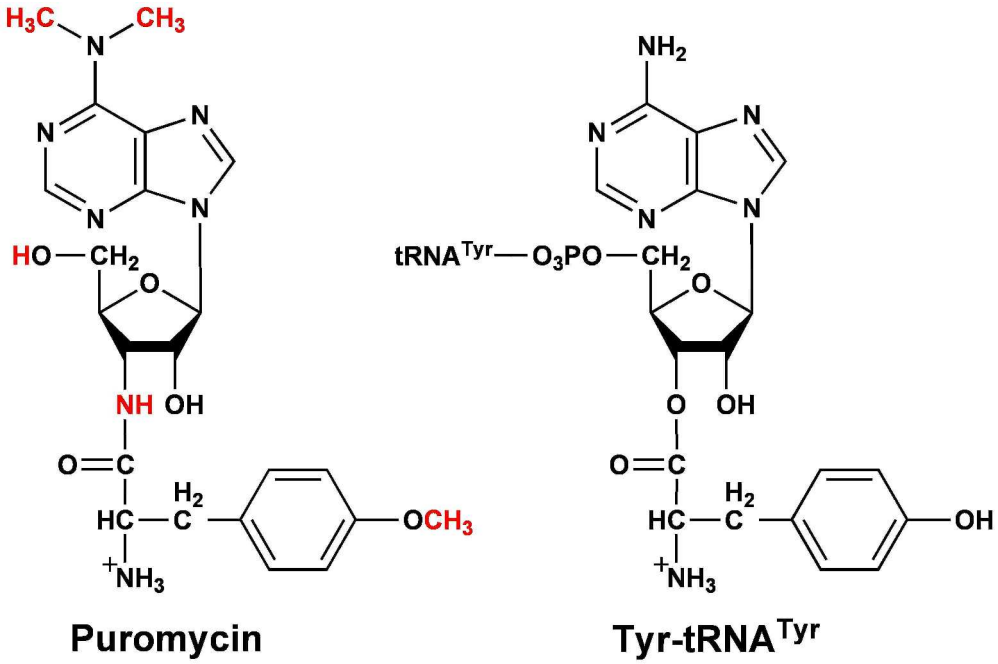

Figure S-2. Structural comparison of puromycin and Tyr-tRNA^{Tyr}. Puromycin's deviations from the structure of Tyr-tRNA^{Tyr} are shown in red.

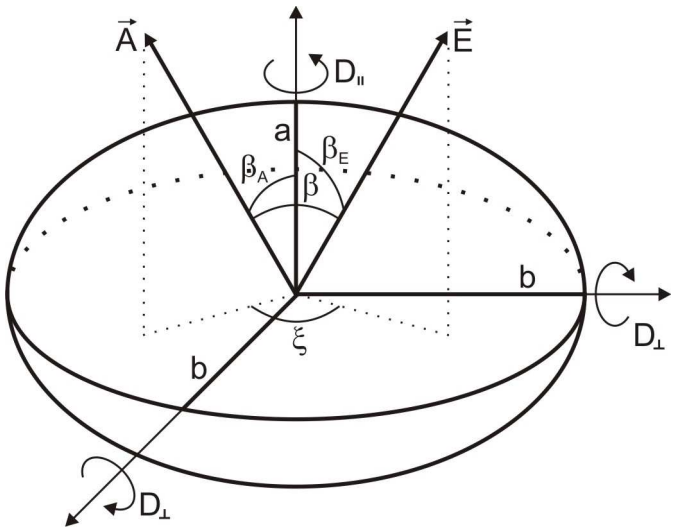

Figure S-3. Cartoon illustrating the main geometrical features of a macromolecule well represented by a rigid oblate ellipsoid with semiaxes a and b in relation to the fluorescence absorption and emission dipoles, $\vec{A}$ and $\vec{E}$, respectively, of a relevant macromolecule-bound fluorophore.

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