

Supplemental Data

Structural and Functional Insights into Dom34,

a Key Component of No-Go mRNA Decay

Hyung Ho Lee, Youn-Sung Kim, Kyoung Hoon Kim, Inha Heo, Sang Kyu Kim, Olesya Kim, Hye Kyung Kim, Ji Young Yoon, Hyoun Sook Kim, Do Jin Kim, Sang Jae Lee, Hye Jin Yoon, Soon Jong Kim, Byung Gil Lee, Hyun Kyu Song, V. Narry Kim, Chung-Mo Park, and Se Won Suh

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Protein Expression, Purification, and Mutagenesis

The Ta Pelota gene (Ta1098) encoding the 339-residue protein was PCR-amplified and cloned into the expression vector pET-21a(+) (Novagen). The recombinant protein fused with a hexahistidine-containing tag at its C-terminus was overexpressed in *E. coli* B834(DE3) cells using Terrific Broth culture medium. Protein expression was induced by 1 mM isopropyl β -D-thiogalactopyranoside and the cells were incubated for additional 48 h at 18 °C following growth to mid-log phase at 37 °C. The cells were lysed by sonication in buffer A (50 mM Tris-HCl at pH 7.9, 500 mM NaCl, and 10% (v/v) glycerol) containing 50 mM imidazole. The crude lysate was centrifuged at \sim 36,000 g for 60 min. The supernatant was applied to an affinity chromatography column of nickel-nitrilotriacetic acid-agarose (Qiagen). The protein was eluted with buffer A containing 500 mM imidazole. The supernatant was applied to a HiLoad XK-16 Superdex 200 prep-grade column (GE Healthcare), which was previously equilibrated with 20 mM Tris-HCl (pH 7.4) and 200 mM NaCl. The elution was applied to a heparin column (GE Healthcare), which was previously equilibrated with 20 mM Tris-HCl (pH 7.4). Upon eluting with a gradient of NaCl in the same buffer, Ta pelota was

eluted at 0.8–1.0 M NaCl concentration. The protein was further purified by gel filtration as described above. The procedure for preparing the selenomethionine (SeMet)-substituted protein was the same except for the presence of 10 mM dithiothreitol in all buffers used during purification steps besides buffer A. When overexpressing the SeMet-substituted protein in *E. coli* B834(DE3) cells, we used the M9 cell culture medium that contained extra amino acids including SeMet. Each of domain 1 (residues 1–129), domain 2 (130–242), and domain 3 (243–339) of Ta Pelota was expressed with a C-terminal fusion tag in *E. coli*, and domain 1 and domain 3 were purified essentially as above. Domain 2 could not be purified due to heavy precipitation. Point mutants of Ta Pelota (E18A, D21A, D22A, E231A, E18A/D21A, E18A/D22A, and D21A/D22A) were purified essentially as above.

Sedimentation Equilibrium Centrifugation

Equilibrium sedimentation studies were performed using a Beckman ProteomeLab XL-A analytical ultracentrifuge in 20 mM Tris-HCl buffer containing 0.2 M NaCl, pH 7.4 at 20 °C. Ta Pelota samples were measured in six-sector cells at two rotor speeds (14,000 rpm and 20,000 rpm) and two protein concentrations: 4.7 μM (0.19 mg ml^{-1}) and 9.4 μM (0.37 mg ml^{-1}) with the loading volume of 130 μl . The time required for the attainment of equilibrium was established by running at the given rotor speed until the scans were invariant for six hours: this was achieved at most by 48 h for the first scan. Five scans were collected and averaged to give the final averaged data for the analysis. The calculated molecular mass of 39,472 Da was used for the data analysis. Partial specific volume of Ta Pelota and the buffer density were calculated using Sednterp (Laue et al., 1992). For the molecular mass analysis, data were fit to an ideal, single component model using partial specific volumes of 0.7313 $\text{cm}^3 \text{g}^{-1}$ and the solution density of 1.00704 g cm^{-3} .

Ribonuclease Activity Assay with a Total RNA Substrate

Total RNA samples were extracted from plant materials using the RNeasy Plant Total RNA Isolation Kit (Qiagen). Prior to use, the RNA samples were extensively pretreated with RNase-free DNaseI to eliminate any contaminating genomic DNA. To test the ribonuclease activity, about 2 μg of total RNA were incubated with each of the full-length Ta Pelota (0.3 mg ml^{-1}), Ta Pelota domain 1 (0.15 mg ml^{-1}), Ta Pelota domain 3 (0.15 mg ml^{-1}), point mutants of Ta Pelota (0.3 mg ml^{-1}), bovine pancreatic ribonuclease A (0.3 mg ml^{-1}), Dom34 domain 1 (0.15 mg ml^{-1}), and Dom34 domain 3 (0.15 mg ml^{-1}), and then were separated by 1.2% (w/v) denaturing formaldehyde-agarose gel electrophoresis. The RNA bands were stained with ethidium bromide for visualization.

Crystallization

Crystals were grown by the hanging-drop vapor diffusion method at 24 °C by mixing equal volumes (2 μl each) of the protein solution (8 mg ml^{-1} concentration in 20 mM Tris-HCl, pH 7.4, and 200 mM NaCl) and the reservoir solution. To grow crystals of the native protein we used a reservoir solution consisting of 100 mM tri-sodium citrate (pH 5.6) and 1.0 M mono-ammonium dihydrogen phosphate. The crystals grew to approximate dimensions of 0.2 mm \times 0.2 mm \times 0.2 mm within a few days. The SeMet-substituted protein was crystallized under crystallization conditions identical to those for the native crystals except for the presence of 10 mM dithiothreitol in the protein solution.

X-ray Data Collection and Structure Determination

A crystal of the SeMet-substituted Ta Pelota was flash-cooled using a cryoprotectant solution containing 30% (v/v) glycerol in the crystallization mother liquor. MAD data were collected at 100 K on a Quantum 210 CCD area detector (Area Detector Systems Corporation, Poway,

California) at the BL-4A experimental station of Pohang Light Source, Pohang, Korea. Two sets were collected, each from a different crystal. For each image, the crystal was rotated by 1° and the crystal-to-detector distance was set to 270 and 250 mm, respectively. The raw data were processed and scaled using the program suite HKL2000 (Otwinowski and Minor, 1997). The first crystal belongs to the space group $P4_32_12$, with unit cell parameters of $a = b = 98.47 \text{ \AA}$, $c = 150.04 \text{ \AA}$. One monomer is in the asymmetric unit, giving the crystal volume per protein mass of $4.61 \text{ \AA}^3 \text{ Da}^{-1}$ and a solvent content of 73.3%. The second crystal belongs to the same space group $P4_32_12$, with unit cell parameters of $a = b = 97.85 \text{ \AA}$, $c = 150.11 \text{ \AA}$. Table 1 summarizes statistics of MAD data collection. Native data were similarly collected on a Quantum 315 CCD area detector (Area Detector Systems Corporation, Poway, California) at the BL-5A experimental station of Photon Factory, Tsukuba, Japan.

All of the five selenium atoms in each monomer of the SeMet-substituted protein were located with the program SOLVE (Terwilliger and Berendzen, 1999). Combination of the two sets of MAD phases derived from different crystals significantly improved the quality of the electron density map. The combined phases were further improved by density modification using the program RESOLVE (Terwilliger, 2000), yielding an interpretable electron density map. Phasing statistics are summarized in Table 1. Model building was done using the program O (Jones et al., 1991). The model was refined with the program CNS (Brünger et al., 1998), and several rounds of model building, simulated annealing, positional refinement, and individual B-factor refinement were performed. Water molecules were added using the program CNS, followed by visual inspection and B-factor refinement.

SUPPLEMENTAL REFERENCES

Brünger, A.T., Adams, P.D., Clore, G.M., DeLano, W.L., Gros, P., Grosse-Kunstleve, R.W., Jiang, J.S., Kuszewski, J., Nilges, M., Pannu, N.S., et al. (1998). Crystallography & NMR system: A new software suite for macromolecular structure determination. *Acta Crystallogr. Sect. D Biol. Crystallogr.* *54*, 905–921.

Jones, T.A., Zou, J.Y., Cowan, S.W., and Kjeldgaard, M. (1991). Improved methods for building protein models in electron density maps and the location of errors in these models. *Acta Crystallogr. Sect. A Found. Crystallogr.* *47*, 110–119.

Laue, T.M., Shah, B., Ridgeway, T.M., and Pelleitier, S.L. (1992). In *Analytical ultracentrifugation in biochemistry and polymer science*, S.E. Harding, ed. (Cambridge, England: Royal Society of Chemistry), pp. 90–125.

Otwinowski, Z., and Minor, W. (1997). Processing of X-ray diffraction data collected in oscillation mode. *Methods Enzymol.* *276*, 307–326.

Terwilliger, T.C. (2000). Maximum-likelihood density modification. *Acta Crystallogr. Sect. D Biol. Crystallogr.* *56*, 965–972.

Terwilliger, T.C., and Berendzen, J. (1999). Automated MAD and MIR structure solution. *Acta Crystallogr. Sect. D Biol. Crystallogr.* *55*, 849–861.

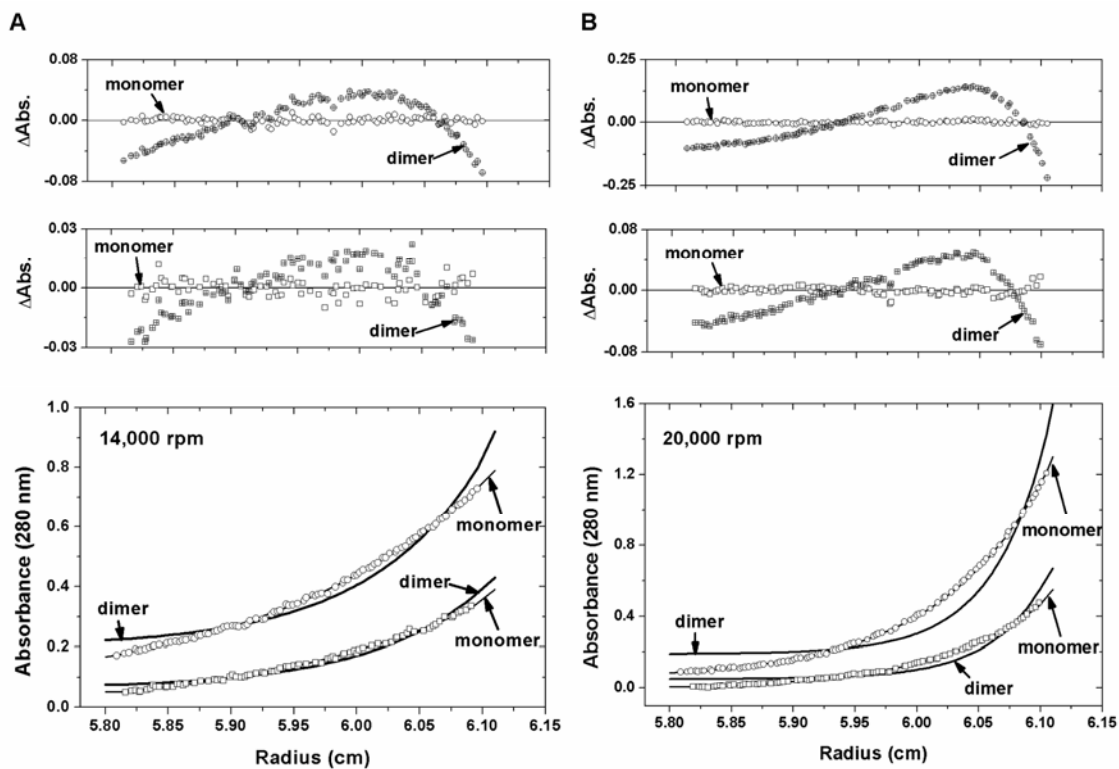


Figure S1. Sedimentation Equilibrium Centrifugation

(A, B) Sedimentation equilibrium of Ta Pelota at 14,000 rpm and 20,000 rpm, respectively. Top panels are distributions of the residuals as a function of radial position at protein concentrations of $4.7 \mu\text{M}$ (squares) and $9.4 \mu\text{M}$ (circles). Bottom panels are distributions of the absorbance at 20°C . The points are experimental data at 280 nm and the fitting lines are for thermodynamically ideal monomers (solid line) and dimers (dotted line), respectively.