

FIGURE LEGENDS TO SUPPORTING DATA

Figure S1. The partitioning of FtsH into various fractions obtained by the digitonin treatment of thylakoids. *A*, a diagram showing the steps of differential centrifugation to separate the grana and stroma thylakoids after the treatment of the thylakoids with 0.5% digitonin and Triton X-100 (Triton:chlorophyll ratio was set at 15:1 (w/w)). Red bars indicate the fractions containing FtsH, whereas yellow bars represent the fractions not containing FtsH or containing only a small amount of FtsH. “Sup” and “Ppt” represent supernatant and precipitate, respectively, after differential centrifugation steps. These fractions are marked A, B, C, G and S where G and S represent the fractions containing the grana and stroma thylakoids, respectively. *B*, Western blot analysis of the fractions obtained by digitonin treatment of thylakoids and subsequent centrifugation. An antibody against VAR2 was used. The sample loaded into each lane contained 1.25 µg of chlorophyll. The samples used are shown at the top of the gel and the marks attached to the samples were the same as those described in *A*. Grana membranes obtained by digitonin and Triton treatment contained only a small amount of FtsH proteases (see Figs. 1 and 2), because approximately one half of the FtsH proteases was solubilized by digitonin and partitioned into the supernatant by centrifugation, while the other half was left in the pellet and was not used. The FtsH proteases in the supernatant of the first centrifugation step were mostly partitioned into the stroma thylakoids (S) in the subsequent centrifugation steps. FtsH was barely detected in the fractions representing the grana (G).

Figure S2. The effects of heat stress and light stress on oxygen-evolving activity and the D1 protein in thylakoids. *A, B*, oxygen-evolving activity under moderate heat stress (*A*) and light stress (*B*) *C, D*, fluorograms of Western blot analysis showing degradation of the D1 protein under moderate heat stress (*C*) and light stress (*D*). Under moderate heat stress, the samples were incubated for a given time at 40 °C in the dark. Under light stress, thylakoids were illuminated for a given time with strong light having intensity of 1,000 µmol photons m⁻² s⁻¹ at 20 °C. For Western blot analysis, the antibody against the DE-loop of the D1 protein was used.

Figure S3. Association of FtsH hexamers with LHCII during the treatment of PSII membranes with n-heptyl-β-D-thioglucoside. *A*, Western blot analysis of PSII membranes, PSII core complexes and the supernatant after HTG treatment of the PSII membranes showed the coexistence of FtsH and LHCII. The membrane samples were first subjected to SDS/urea-PAGE and then immunoblotting with antibodies against anti-VAR2 (top gel) and anti-LHCb1 (bottom gel). The sample loaded into each lane contained 1 µg of chlorophyll. To obtain the PSII core complexes, PSII membranes were treated with 2.0% HTG to remove the LHCII. “PSII”, “PSII core” and “HTG-sup” represent PSII membranes, PSII core complexes and the supernatant after HTG treatment of PSII membranes, respectively. *B*, CN-PAGE and western blot analysis showing the presence of the hexameric FtsH protease in the supernatant of HTG treated PSII membranes. Molecular markers are shown on the left-hand side of the gel and the position of hexameric FtsH is indicated on the right-hand side of the gel. Other conditions are the same as those described in *A*.

