Supplement

Materials and methods

Observation of rotation

Biotinylated complex was frozen with liquid nitrogen and stored at −80°C before use. The preparation of a flow chamber to observe rotation, blocking the surface by bovine serum albumin, immobilization of the complexes to glass surface through his-tag, and attachment of 40-nm gold beads through biotin-streptavidin were performed as [1,2]. Rotation was initiated by infusion of buffer A (50 mM HEPEPS-KOH, pH 8.0, 50 mM KCl, 5 mM $MgCl_2$) containing 2.5 mM creatine phosphate, 0.2 mg ml^{-1} creatine kinase was infused and rotation was observed at 25°C by laser dark-field microscopy on inverted microscope [1]. Beads images were captured by fast-framing CMOS camera at 8000 frames s^{-1} for 2-8 s. The time-averaged rotation rate was determined from >100 revolutions (2 mM, 200 μM ATP), >30 revolutions (20 μM, 2 μM ATP), or 7 revolutions (200 nM ATP). For rotation assay of $\alpha_3\beta_3\gamma\varepsilon$ at 2 μM (or 200 nM) ATP, 100 nM biotinylated $\alpha_3\beta_3\gamma\varepsilon$ was preincubated for 30 min in S20 μM (or 2 μM) Mg-ATP in buffer B (50 mM 3-(*N*-morpholino)propanesulfonic acid-KOH, pH 7.0, 50 mM KCl) containing 2.5 mM creatine phasphate and 0.2 mg ml^{-1} creatine kinase. The solution was diluted ten-fold with buffer B to adjust $\alpha_3\beta_3\gamma\varepsilon$ at 10 nM and ATP at 2 μM (or 200 nM) and immobilized on the glass surface. For long-time observation at 200 nM ATP, rotation of polystyrene beads of 200-nm diameter attached to ε subunit ($\alpha_3\beta_3\gamma\varepsilon$) or γ subunit ($\alpha_3\beta_3\gamma$) was observed at 25°C by bright-field microscopy and recorded on digital video recorder at 30 frames s^{-1} [3]. For long-time observation at 2 mM ATP, rotation was observed by dark-field microscopy and recorded on fast-framing camera at 150 frames s^{-1}. Preincubation with 2 μM Mg-ATP was performed as described above except that 2.5 mM phosphoenolpyruvate and 0.2 mg ml^{-1} pyruvate kinase were used for ATP-regeneration system. Rotation was initiated by infusion of the buffer A containing ATP-regeneration system consisting of 2.5 mM phosphoenolpyruvate, 0.2 mg ml^{-1} pyruvate kinase. The centroid of beads image was calculated as described [4].

[1] Furuike, S., Hossain, M.D., Maki, Y., Adachi, K., Suzuki, T., Kohori, A., Itoh, H., Yoshida, M. and Kinosita Jr, K., (2008) Axle-less F_1-ATPase rotates in the correct direction. Science. 319, 955-958.

[2] Itoh, H., Takahashi, A., Adachi, K., Noji, H., Yasuda, R., Yoshida, M. and Kinosita Jr, K. (2004) Mechanically driven ATP synthesis by F_1-ATPase. Nature. 427, 465-468.

[3] Nishizaka, T., Oiwa, K., Noji, H., Kimura, S., Muneyuki, E., Yoshida, M. and Kinosita Jr, K. (2004) Chemomechanical coupling in F_1-ATPase revealed by

simultaneous observation of nucleotide kinetics and rotation. Nat. Struct. Mol. Biol. 11, 142-148.

[4] Yasuda, R., Noji, H., Yoshida, M., Kinosita Jr, K., and Itoh, H. (2001) Resolution of distinct rotational substeps by submillisecond kinetic analysis of F_1-ATPase. Nature. 410, 898-904.

Supplement

Legend of Figure S1

Histograms of durations of the dwell at (A-C) 80° and (D, E) 0°. Data were collected from rotation observed for several seconds. Solid lines are the simulated curves with (A-C) constant×{exp(-k_1t)-exp(-k_2t)} and (D, E) constant×exp(-kt). Experimental details are described in Materials and Methods.

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