ELSEVIER

Biochimica et Biophysica Acta 1277 (1996) 266

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

Erratum

Erratum to 'ATP hydrolysis by membrane-bound *Escherichia coli* F_0F_1 causes rotation of the γ subunit relative to the β subunits' [Biochim. Biophys. Acta (1996) 1275, 96–100] [1]

Yuantai Zhou, Thomas M. Duncan, Vladimir V. Bulygin, Marcus L. Hutcheon, Richard L. Cross

The contrast in Fig. 1 of this paper was not sufficient to show the faint band at 86 kDa. Therefore the figure is printed again.

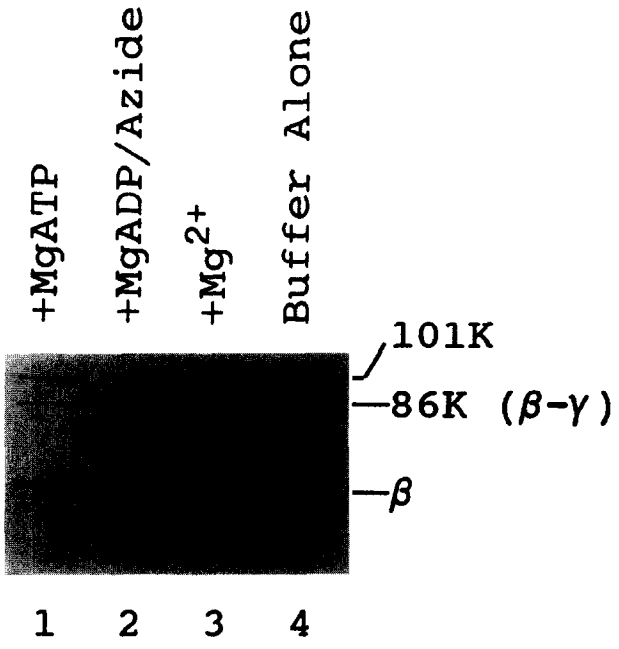

Fig. 1. Testing for rotation of the γ subunit relative to β subunits in membrane-bound F_0F_1. Hybrid F_1 was reconstituted as described [10] from an equimolar mixture of two dissociated F_1 samples: βD380C-F_1 containing a βD380C-γC87 disulfide bond, and β_{flag}D380C/γC87S-F_1. F_1 hybrids were rebound to F_1-depleted membranes and excess F_1 removed as described in Table 1. Membranes were resuspended to $\approx$ 4 mg protein/ml in TM-5 buffer containing 1 μM FCCP, 10 mM glucose, and 1 unit of hexokinase. The ATPase activity of reconstituted membranes (after complete reduction of an aliquot with 20 mM DTT) was 1.6 (μmol min^{-1} (mg protein)$^{-1}$), compared to 1.3 for native (F_1-replete) membranes. Following the specific additions noted below, membrane samples were incubated with 20 mM DTT at 22°C for 30 s, passed through a 1-ml centrifuge column of Sephadex-G50-80 [21] to remove DTT, collected in a tube containing DTNB (0.2 mM final), and incubated at 22°C for 10 min. Specific incubation conditions: lane 1, hexokinase was omitted and ATP was added to 0.5 mM just before DTT; lane 2, ADP and sodium azide (0.5 mM each, final) were added just before DTT; lane 3, no other additions before reduction with DTT; lane 4, Mg^{2+} was omitted from the resuspension buffer. Hexokinase was included in samples 2–4 to scavenge residual ATP, thus preventing any potential ATP hydrolysis by F_0F_1. FCCP was present in all samples to prevent formation of a transmembrane proton gradient. An aliquot of each final sample was added to gel sample buffer containing 2 mM *N*-ethylmaleimide instead of 2-mercaptoethanol. Six μg of membrane protein were loaded in each lane of a 4–15% gradient gel (Bio-Rad Ready gels). Bands containing the Flag epitope were detected colorimetrically by Western blot analysis, using anti-Flag M2 antibody [13] and a secondary antibody–alkaline phosphatase conjugate.

[1] SSDI of original article: 0005-2728(96)00056-4.

0005-2728/96/$15.00
PII S0005-2728(96)00119-3