Supplementary information

An alternative role of F_oF_1-ATP synthase in *Escherichia coli*: synthesis of thiamine triphosphate

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Supplementary Materials and Methods

Purification of F_1 subunit and ThTP hydrolysis

The *E. coli* C41[1] was used to overproduce the *E. coli* $F_1\Delta\delta$-ATPase ($EcF_1\Delta\delta$, $\alpha_3\beta_3\gamma\varepsilon$). The *uncAGDC* operon was amplified and subcloned to a pTrc99a vector to form plasmid pTrcEcF1. His_6 tags were attached at the N-terminus of beta subunits. Cells were grown in 2xYT medium in the presence of 100 µg/ml ampicillin at 37°C with shaking at 225 rpm and induced with 1 mM isopropyl *β*-D-thiogalactopyranoside (IPTG) at an OD_{600} 0.5 for 16 hours at 25°C. Cells were harvested by centrifugation at 5, 300 x g for 20 min at 4°C, washed with pre-cooled buffer (100 mM Tris-HCl, pH 7.4, 5 mM $MgCl_2$, 10% glycerol) and resuspended in the Ni column buffer (50 mM Tris-HCl, pH 7.4, 5 mM $MgCl_2$, 10% glycerol, 150 mM NaCl, 20 mM imidazole). Protease inhibitors (Complete EDTA-free Protease Inhibitor Cocktail, 1 tablet/50 ml, Roche) and DNAase I (1 mg/50 ml) were added and the cells were disrupted by two passages through Constant Cell Disruption Systems (Constant System Ltd) at 30, 000 psi (4°C). Lysate was cleared by centrifugation at 214, 000 x g for 45 min at 4°C and then filtered through a 0.45 µm filter (Sartorius Stedim, Epsom, UK). The filtrate was passed through a 5-ml HisTrap HP column (GE healthcare) previously washed with water and equilibrated with pre-filtered Ni column buffer. The column was washed with Ni buffer containing 40 mM imidazole, and $EcF_1\Delta\delta$ was eluted with 250 mM imidazole in the same buffer. After analysis by SDS-PAGE, the ATPase-containing fractions were pooled and dialyzed against Q column buffer (20 mM BisTris-HCl, pH 6.5, 5 mM $MgSO_4$, 10% glycerol, TCEP 1 mM). Proteins were applied to a 5-ml HiTrap Q HP column (GE healthcare), which was equilibrated with Q buffer. Bound proteins were eluted with twelve column volumes of a linear gradient of 0-400 mM NaCl in the same buffer. The collected fractions were pooled and dialysed through a dialysis cassette (Slide-A-Lyzer® Dialysis Cassette, molecular weight cutoff (MWCO) 10, 000 Da, Thermo Scientific) to remove NaCl. The sample was then concentrated with a Vivaspin centrifugal concentrator (MWCO, 100, 000 Da, Sartorius Stedim).
For enzymatic assays, F_1 was incubated in the presence of Mg^{2+} (5 mM) and ThTP or ATP at 37 °C.

1. Miroux, B. & Walker, J. E. Over-production of proteins in *Escherichia coli*: mutant hosts that allow synthesis of some membrane proteins and globular proteins at high levels. *J. Mol. Biol.* **260**, 289-298 (1996).

Supplementary Tables

Table S1

Genotypes of the *E. coli* strains used.

Name	Genotype
CV2	Hfr(PO2A), *fhuA22*, *ΔphoA8*, *adk-2*(ts), *ompF627*(T2R), *fadL701*(T2R), *relA*, *glpR2(glp^c)*, *glpD3*, *pitA10*, *spoT1*, *rrnB-2*, *mcrB1*, *creC510*
AN120	F-, *glnV44(AS)?, galK2(Oc), λ$^-$, rpsL704(strR), xylA5, mtl-1, atpA401, argE3(Oc), thi-1, tfr-3*?
AN382	F-, *glnV44*(AS)?, *galK2*(Oc), *λ$^-$*, *rpsL704*(strR), *xylA5*, *mtl-1*, *atpB402*, *argE3*(Oc), *thi-1*, *tfr-3*?
AN718	F-, *entA403, glnV44(AS), λ$^-$, rpsL109(strR), pyrE41, atpA401, ΔargH1*
JW0110	F-, *Δ(araD-araB)567*, *ΔaceE732::kan*, *ΔlacZ4787*(::rrnB-3), *λ$^-$*, *rph-1*, *Δ(rhaD-rhaB)568*, *hsdR514*
JW0111	F-, *Δ(araD-araB)567*, *ΔaceF733::kan*, *ΔlacZ4787*(::rrnB-3), *λ$^-$*, *rph-1*, *Δ(rhaD-rhaB)568*, *hsdR514*
JW0112	F-, *Δ(araD-araB)567*, *Δlpd-734::kan*, *ΔlacZ4787*(::rrnB-3), *λ$^-$*, *rph-1*, *Δ(rhaD-rhaB)568*, *hsdR514*
CF5802	MG1655,*DppkDppx::kan (Km^r)*
DK8	*bglR thi-1 rel-1* Hfr PO1 *ilv:* :Tnl0 *A(uncB-uncC)*

The source for the genotype was the *E. coli* Genetic Resource Center (Yale University, New Haven, CT, U.S.A.), except for CF5802[38] and DK8[39].

Table S2

Effect of valinomycin and nigericin on the ThTP content of *E. coli* cells permeabilized by EDTA treatment[a]

	ThTP (pmol/mg of protein)
0 mM K^+	
Glucose (10 mM)	78 ± 5
Glucose + valinomycin (50 µM)	76 ± 9
Glucose + nigericin (50 µM)	60 ± 5
22 mM K^+ (M9 medium)	
Glucose (10 mM)	316 ± 17
Glucose + valinomycin (50 µM)	34 ± 7
Glucose + nigericin (50 µM)	150 ± 17
64 mM K^+	
Glucose (10 mM)	307 ± 20
Glucose + valinomycin (50 µM)	14 ± 3
Glucose + nigericin (50 µM)	208 ± 12

[a] The protocol was as described[23,24]. An overnight culture of the BL21 strain was diluted in LB medium to a A_{600} of 0.4. After doubling, the cells were centrifuged (12,000 x g, 10 min) at room temperature. The pellet was washed twice in Tris-HCl buffer (120 mM, pH 8.0) and resuspended in 50 ml of the same buffer. The culture was incubated for 1 min at 37 °C before addition of 1 mM EDTA. Incubation was continued for 20 min (37 °C) under gentle stirring. Then, the cell suspension was diluted 5 times in Tris-HCl buffer (120 mM, pH 8.0) and centrifuged (5000 x g, 5 min) at room temperature. The pellet was washed once with the same buffer and suspended in M9 medium ($[K^+]$ = 22 mM), M9 medium with all K^+ replaced by Na^+ ($[K^+]$ = 0) or M9 medium with all Na^+ replaced by K^+ ($[K^+]$ = 64 mM). Then, the samples were incubated (20 min at 37 °C) in the presence of valinomycin or nigericin and thiamine derivatives were determined by HPLC. Stock solutions of the two ionophores were prepared in dimethyl sulfoxide. The final dimethyl sulfoxide concentration under experimental conditions was 1% and control experiments were made at the same dimethyl sulfoxide concentration.

Table S3

Effect of oligomycin on the ThTP content of intact *E. coli*.

	ThTP (pmol/mg of protein)
Glucose (10 mM)	134 ± 30
+ oligomycin 10 µM	59 ± 11
+ oligomycin 50 µM	16 ± 7
L-Lactate (10 mM)	158 ± 6
+ oligomycin 10 µM	64 ± 20
+ oligomycin 50 µM	31 ± 8

The bacteria (BL21 strain) were grown overnight in LB medium, transferred to minimal M9 medium and incubated 20 min at 37 °C in the presence of either D-glucose or L-lactate with or without oligomycin. Stock solutions of oligomycin were prepared in dimethyl sulfoxide (final concentration 1%). Mean ± SD, n = 3

Supplementary Figure

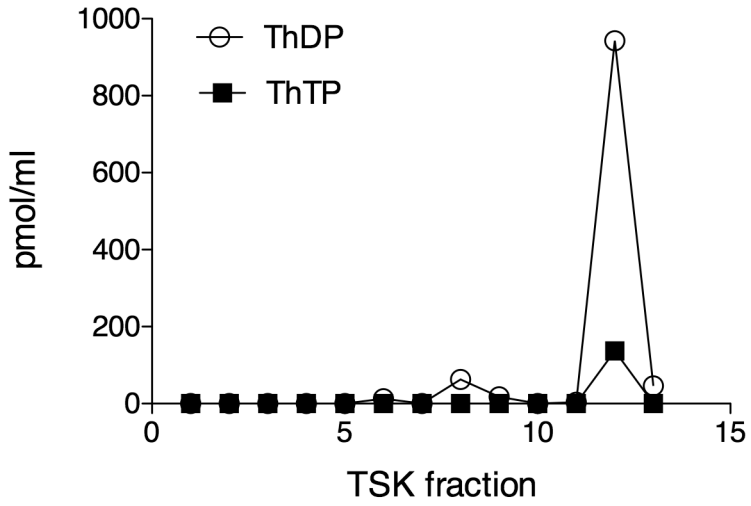

Figure S1. BL21 bacteria grown overnight in LB medium were transferred to M9 medium containing 10 mM glucose. After incubation for 1 h (37 °C, 250 rpm), the samples were sonicated (100 kHz, 3 x 30 s with 1 min intervals) on ice and centrifuged (5 min, 10,000 x g, 4 °C). The supernatant (100 µl) was injected on a TSK-Gel® HPLC column ($G3000SW_{XL}$, 30 x 0.75 cm, 10 µm, Tosoh, Bioscience GmbH, 70567, Stuttgart, Germany) equilibrated in Na acetate buffer (25 mM, pH 7.2) at a flow rate of 0.5 ml/min. Fractions of 1 ml were collected and thiamine derivatives were determined after treatment with trichloroacetic acid.