Characterization of YbjG, a pyrophosphate phosphatase from *E. coli* involved in the lipid carrier undecaprenyl phosphate metabolism

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I. Introduction

Undecaprenyl phosphate (C_{55}-P) is an essential lipid carrier molecule involved in the biosynthesis of cell surface carbohydrate polymers such as the peptidoglycan, a vital component of the bacterial cell wall. C_{55}-P is synthesized *de novo* or recycled from a previous use but in either case it is the result of the dephosphorylation of the undecaprenyl pyrophosphate (C_{55}-PP) by specific phosphatases. In *Escherichia coli* this dephosphorylation can be performed by four integral membrane proteins, BacA, and three members of the type 2 phosphatidic acid phosphatase family (PAP2), PgpB, YbjG, and LpxT (1; 2). Complementation of a temperature-sensitive triple mutant harbouring deletions in *bacA*, *ybjG* and *pgpB* can be achieved with an intact chromosomal copy of only one of the *bacA*, *ybjG* and *pgpB* genes (2).

PgpB was first discovered for its implication in the metabolism of the phosphatidylglycerol (PG), one of the three main phospholipid in *E. coli* membranes where it dephosporylates the phosphatidylglycerol-phosphate to form the PG (3). PgpA and PgpC are two other soluble proteins also displaying such activity. LpxT was shown to transfer the distal phosphate from C_{55}-PP to the lipid A, none of the three other C_{55}-PP phosphatases are involved in the lipid A metabolism (4).

Here we present the purification and characterization of YbjG, the third *E. coli* PAP2 enzyme with C_{55}-PP phosphatase activity.

II. Experimental procedures

Production and purification of YbjG

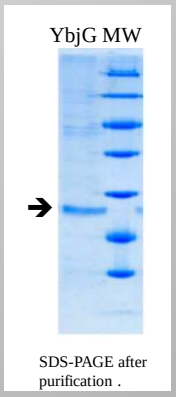
YbjG is fused to a N-terminal H6-tag using the pET28MHL plasmid. C43 *E. coli* cells are grown in Terreific Broth medium and the induction is initiated with 1mM IPTG and performed at 20°C for 24h. The purification is performed with a IMAC nickel resin. YbjG is eluted at a 500 mM imidazole concentration.

C_{55}-PP phosphatase activity

Standard C_{55}-PP phosphatase assays were performed in a 10 µl reaction mixture containing 30 mM Tris-HCl, pH 7,0, 400 mM NaCl, 2 mM DTT, 10 % glycerol, 0,05 % DDM, and 50 µM [^{14}C]C_{55}-PP. Pure YbjG protein was added to initiate the reaction, and after incubation at a specific temperature the reaction was stopped by quick freezing of the enzyme in liquid nitrogen. The samples were analyzed by TLC.

Other phosphatase activity

Phosphatase assays were performed in a 100 µl reaction mixture containing the same buffer as C_{55}-PP phosphatase assays with a substrate concentration of 100 µM. YbjG protein was added to initiate the reaction at a specific temperature. The reaction was stopped and the amount of free Pi was quantified by adding a malachite green reagent before measuring the absorbance at 620 nm.



SDS-PAGE after purification .

III. Results

In vitro

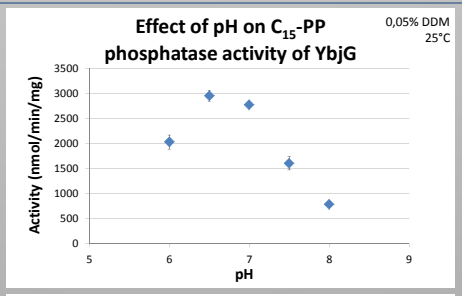
The initial rate of hydrolysis of YbjG on C_{55}-PP is 20 nmol liberated Pi /min /mg of protein at 37 °C but it increases twice when the experiment is done at 25 °C (39 ± 9 nmol/min/mg). Moreover optimal DDM concentration is around 0,5%.

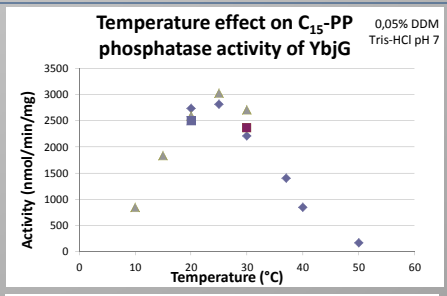
At 37°C, the C_{15}-PP phosphatase activity of YbjG (1.500 nmol/min/mg) is 200 times lower than that of PgpB (300±5µmol Pi /min /mg of protein) with the same amount of C_{15}-PP (100µM). The best C_{15}-PP phosphatase activity (3.000 nmol/min/mg) is mesured at pH 6,5, at a temperature of 25°C, and at DDM concentrations between 0,03% and 0,1%. This DDM concentration is significantly lower than the one measured with C_{55}-PP (~0,5%).

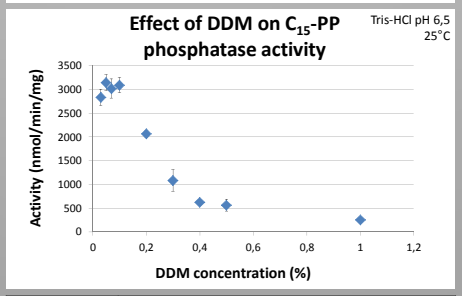
YbjG also efficiently dephosphorylates diacylglycerol pyrophosphate (DGPP) (807 ± 57 nmol/min/mg), but no activity could be detected on inorganic pyrophosphate (PPi), phosphatidic acid (PA), C_5-PP, Glucose 6-phosphate (G6-P) & p-nitrophenyl phosphate (PNPP).

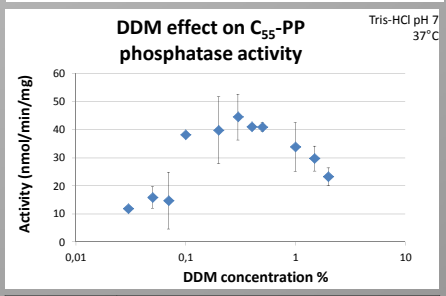
In vivo

Complementation of a temperature-sensitive triple mutant harbouring deletions in *pgpA*, *pgpB* and *pgpC* could not be achieved with a plasmid copy of *ybjG*, whatever the induction level of the gene.









Substrat	Phosphatase activity at 37 °C (nmol/min/mg)
C_{15}-PP	1.500
C_{55}-PP	20
DGPP	807 ± 57
PPi	N.D.

Substrat	Phosphatase activity at 37 °C (nmol/min/mg)
PA	N.D.
C_5-PP	N.D.
G6P	N.D.
PNPP	N.D.

IV. Conclusion

For the first time, YbjG has been purified and we showed its ability to dephosphorylate C_{15}-PP, DGPP and C_{55}-PP *in vitro* with respectively decreasing efficiency. No activity has been detected on five other potential substrats (PPi, PA, C_5-PP, G6P & PNPP).

On C_{15}-PP, the best phosphatase conditions are obtained at a pH of 6,5, at a temperature of 25 °C, and at DDM concentrations between 0,03% and 0,1%. C_{55}-PP phosphatase activity is optimum at DDM concentrations around 0,5 %.

In vivo, YbjG is involved in the C_{55}-PP metabolism as shown by its complementation of a temperature-sensitive mutant Δ*bacA*, Δ*pgpB*, Δ*ybjG* (2). However no implication of YbjG is observed in the phosphatidylglycerol synthesis (unlike PgpB) and in the lipid A metabolism (4). Consequently, YbjG seems to be the only C_{55}-PP specific PAP2 phosphatase of *E. coli*.

V. References

1. El Ghachi *et al*, J Biol Chem. 2004; 279(29):30106-13
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3. Icho & Raetz, J Bacteriol. 1983; 153(2):722-30
4. Touzé *et al*, Mol Microbiol. 2008; 67(2):264-77

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