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Type II Isopentenyl Diphosphate Isomerase from *Synechocystis* sp. Strain PCC 6803

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Open reading frame *sll1556* in the cyanobacterium *Synechocystis* sp. strain 6803 encodes a putative type II isopentenyl diphosphate (IPP) isomerase. The His_6-tagged protein was produced in *Escherichia coli* and purified by Ni^{2+} chromatography. The homotetrameric enzyme required NADPH, flavin mononucleotide, and Mg^{2+} for activity; K_m^{IPP} was 52 μM, and k_{cat}^{IPP} was 0.23 s^{-1}.

The isomerization of isopentenyl diphosphate (IPP) to dimethylallyl diphosphate (DMAPP) is an essential reaction in the mevalonate (MVA) pathway to isoprenoids (13) and, although not essential, probably serves to balance the IPP and DMAPP pools in the methylerythritol phosphate (MEP) route (9). Two types of IPP isomerase are known. The type I enzyme, found in *Eucarya* and some of the *Bacteria*, is well characterized (16). Type II IPP isomerase, found in *Archaea* and some of the *Bacteria*, was discovered much more recently (10), and only a few studies of the protein have been reported. Analysis of the genome of *Synechocystis* sp. strain PCC 6803 indicated that the cyanobacterium synthesizes isoprenoid compounds by the MEP route. Although it was reported elsewhere that the bacterium does not have detectable IPP isomerase activity under phototrophic growth conditions (6, 7), open reading frame (ORF) *sll1556* encodes a protein that is substantially similar to other type II IPP isomerases. We now report that purified ORF *sll1556* protein is an active type II IPP isomerase.

Evidence that *Synechocystis* sp. strain PCC 6803 ORF *sll1556* encodes a functional type II IPP isomerase was obtained by complementation studies with *Salmonella enterica* serovar Typhimurium strain RMC29 (Table 1) (2). The chromosomal copy of *idi* (IPP isomerase) in RMC29 was disrupted with a chloramphenicol (CAM) marker, and *dxs* (deoxyxylulose synthase) was disrupted with a minioperon that includes the yeast genes required for biosynthesis of IPP from MVA and an ampicillin (AMP) marker. Thus, RMC29 is viable when supplemented with methylerythritol (ME) but does not grow on MVA unless *idi* activity is restored. RMC29 was transformed with plasmid pJMSB02278-8 bearing a copy of ORF *sll1556* from *Synechocystis* sp. strain PCC 6803 and the expression plasmid without ORF *sll1556*. Both strains were resistant to AMP and expressed the plasmid-carried genes when induced with arabinose. As shown in Fig. 1, strains JMSB02278-8, JMSB0354, and RMC29 grew on LB broth-CAM-ME, demonstrating that they can utilize the MEP pathway to synthesize IPP and DMAPP without a functional IPP isomerase. Strains JMSB02278-8 and JMSB0354 contain plasmids conferring resistance to AMP and grew on LB-AMP-CAM-ME, while RMC29 did not. Only JMSB02278-8 grew on LB-AMP-CAM-MEV-arabinose by utilizing the IPP isomerase encoded by ORF *sll1556* from *Synechocystis* sp. strain PCC 6803 to convert IPP synthesized from MVA to DMAPP.

Nickel-nitrilotriacetic acid affinity chromatography of the supernatant from a cell extract of *Escherichia coli* strain JMSB0373a yielded a protein that gave a single band upon sodium dodecyl sulfate-polyacrylamide gel electrophoresis with an apparent molecular mass of ~50 kDa. This band was isolated, and an in-gel tryptic digestion was performed. Analysis of the matrix-assisted laser desorption ionization mass spectrometry of the tryptic peptides revealed 69% coverage of the amino acids from the expressed sequence of ORF *sll1556* from *Synechocystis* sp. strain PCC 6803 (data not shown). The state of aggregation of the protein was examined by sedimentation equilibrium. The data were best described by a tetramer-octamer equilibrium, with K_d being 0.2 μM (Fig. 2). The protein exists in a homotetrameric state at the concentrations used

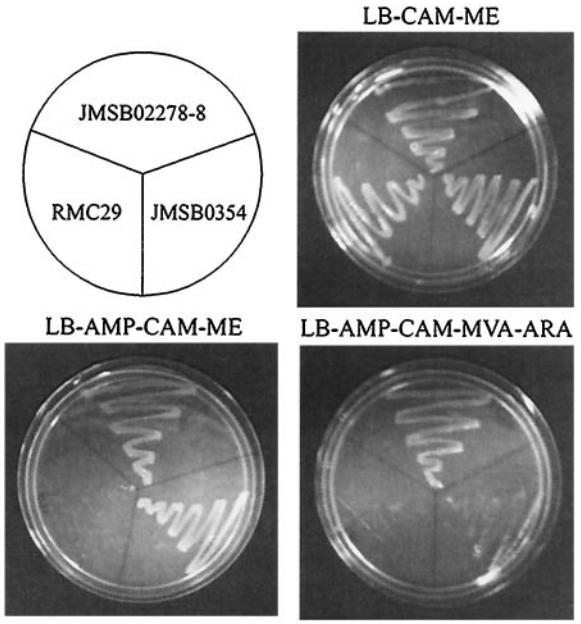

FIG. 1. Complementation of an *idi*::MVA knockout in *S. enterica* serovar Typhimurium with ORF *sll1556* from *Synechocystis* sp. strain 6803. Strains are RMC29 (*dxs*::MVA operon; *idi*::CAM), JMSB0354 (RMC29; Amp^r), and JMSB02278-8 (RMC29; Amp^r; ORF *sll1556*). AR, arabinose.

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TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Description	Source or reference
E. coli		
M15 pREP$_4$	Host for pQE-30 overexpression vectors; Kan^4	Qiagen
JMSB0373a	M15 pREP$_4$ containing pJMSB0373Sa	This work
S. enterica serovar Typhimurium		
RMC29	*dxs*::mevalonate operon (*araC Pbad erg8 erg12 erg19* Kan^r) and *idi*::Cam^r	2
JMSB02278-8	RMC29 containing pJMSB02278-8	This work
JMSB0354	RMC29 containing pBAD Myc/HisC	This work
Plasmids		
pBAD Myc/HisC	*E. coli* overexpression vector; Amp^r	Invitrogen
pQE-30	Expression vector with an N-terminal His$_6$ tag; Amp^r	Qiagen
pJMSB02278-8	pBADA Myc/HisC with a 1.1-kb XhoI-KpnI insert corresponding to ORF *sll1556*	This work
pJMSB0373Sa	pQE-30 with a 1.1-kb BamHI-KpnI insert corresponding to ORF *sll1556*	This work

for assays (29 nM). Isomerase activity was measured by the acid lability technique (2). The optimal pH and concentrations for Mg^{2+}, flavin mononucleotide, and NADPH were determined, and under these conditions (37°C, 50 mM HEPES buffer [pH 7.0], 20 μM flavin mononucleotide, 10 mM NADPH, 20 mM $MgCl_2$, 50 μM dithiothreitol), K_m^{IPP} was 52 μM, k_{cat}^{IPP} was 0.23 s^{-1}, and k_{cat}/K_m was 4.4×10^3 M^{-1} s^{-1}.

Isoprenoid metabolites play major roles during photosynthesis (8). Carotenoids are essential for the normal development of photosynthetic membranes, for dissipation of light energy, and for protecting plants from excess light (3–5, 15). Carotenoids quench singlet oxygen, and their absence is lethal in plants and oxygenic photosynthetic bacteria such as *Synechocystis* sp. strain PCC 6803. Chlorophyll is anchored to membranes by a hydrophobic phytyl chain. DMAPP is the essential allylic diphosphate primer for the biosynthesis of geranylgeranyl diphosphate required for carotenoids and phytol. In the MEP pathway DMAPP and IPP are synthesized simultaneously from hydroxydimethylallyl diphosphate to give a nonequilibrium (~6:1) mixture favoring IPP (1). The isomerization of IPP and DMAPP results in a ~1:2.5 ratio of IPP to DMAPP (14). In those organisms containing the MEP pathway and a type II IPP isomerase, such as cyanobacteria, the ratio of DMAPP relative to IPP can be increased in order to balance the pools of IPP and DMAPP during periods of high carotenoid biosynthesis where only three molecules of IPP are required for each DMAPP in the chain elongation reaction to produce geranylgeranyl diphosphate.

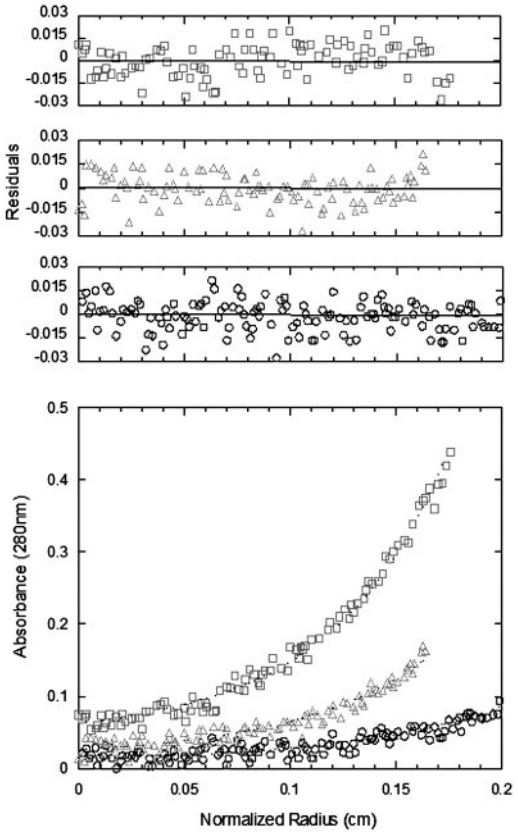

FIG. 2. Sedimentation equilibrium data for *Synechocystis* sp. strain 6803 type II IPP isomerase. The lower panel shows experimental data points for three different loading concentrations (□, 2.8 μM; △, 1.4 μM; ○, 0.7 μM) of the protein with the corresponding calculated fit (dotted line). The upper panels show the residuals for these fits; all are small and random, indicating a good fit corresponding to a K_d of 0.2 μM.

Prior to the discovery of type II IPP isomerase, Gannt and Ershov reported that the chromosome of *Synechocystis* sp. strain PCC 6803 did not appear to encode an IPP isomerase and that a cell extract from the bacterium did not have IPP isomerase activity (6). While this paper was under review, Poliquin and coworkers reported that the *Synechocystis* ORF *sll1556* protein was inactive in a type II IPP isomerase assay, although the *Streptomyces* type II enzyme was active under the same conditions (12). This is in marked contrast to our findings for the ORF *sll1556* protein, which has IPP isomerase activity comparable to that of other type II enzymes (Table 2). Given

TABLE 2. Kinetic constants for type II IPP isomerases[a]

Organism	K_M^{IPP} (μM)	k_{cat} (s^{-1})	k_{cat}/K_M (M^{-1} s^{-1})
Synechocystis sp. strain 6803	52	0.23	4.4×10^3
Bacillus subtilis		0.39	
Methanobacter thermoauto trophicus	64	1.6	2.5×10^4
Staphylococcus aureus	19	1.3	6.8×10^4
Streptomyces sp. strain C1190	450	0.70	1.6×10^3

[a] Kinetic constants for *Bacillus subtilis* (11), *Methanobacter thermoautotrophicus* (2), *Staphylococcus aureus* (10), and *Streptomyces* sp. strain CL190 (10) are compared to those for *Synechocystis* sp. strain 6803.

the similarities between the constructs and protocols used to obtain purified protein and the procedures used to assay for activity, we cannot explain the difference between our results and theirs.

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