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Analytical Biochemistry 337 (2005) 55–61
ANALYTICAL BIOCHEMISTRY
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A colorimetric assay for the determination of 4-diphosphocytidyl-2-*C*-methyl-D-erythritol 4-phosphate synthase activity

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Received 21 July 2004

Abstract

A new method for the determination of the activity of 4-diphosphocytidyl-2-*C*-methyl-D-erythritol 4-phosphate synthase, the enzyme catalyzing the third reaction of the 2-*C*-methyl-D-erythritol 4-phosphate pathway for biosynthesis of isoprenoids, is described. This is an end-point assay based on the transformation of inorganic pyrophosphate, one of the products of the reaction, to phosphate by using inorganic pyrophosphatase as auxiliary enzyme. The phosphate formed is reacted then with the dye malachite green to yield a colored product which can be determined spectrophotometrically. The method is easy to perform, sensitive, and robust and can be used in automated high-throughput screening analyses for the search of inhibitors of the enzyme.

Keywords: 4-Diphosphocytidyl-2-*C*-methyl-D-erythritol 4-phosphate synthase; Colorimetric assay; Malachite green; Pyrophosphatase; Isoprenoid biosynthesis; 2-*C*-Methyl-D-erythritol 4-phosphate pathway

Isoprenoids are the most diverse group of natural products with more than 30,000 compounds identified to date [1]. All isoprenoids derive from a common C_5 unit: isopentenyl diphosphate (IPP)[1] and its isomer dimethylallyl diphosphate (DMAPP). Since the previous decade, it was assumed that IPP biosynthesis proceeded in all organisms through the same pathway, the mevalonate pathway. However, an alternative pathway for the synthesis of IPP and DAMPP has recently been identified in eubacteria [2], green algae [3], and plants [4–6], the 2-*C*-methyl-D-erythritol 4-phosphate (MEP) pathway. The first reaction of the MEP pathway yields 1-deoxy-D-xylulose 5-phosphate (DXP) from pyruvate and D-glyceraldehyde 3-phosphate and is catalyzed by the enzyme DXP synthase (DXS) [6–8]. Then, DXP is converted by intramolecular rearrangement and reduction into 2-*C*-methyl-D-erythritol 4-phosphate (MEP) in a reaction catalyzed by DXP reductoisomerase (DXR) [9,10]. This is the first committed step of the pathway and MEP represents the first specific intermediate. In the third step MEP is converted to the CDP derivative 4-diphosphocytidyl-2-*C*-methyl-D-erythritol (CDP-ME) by the enzyme CDP-ME synthase, the protein product of the *Escherichia coli* gene *ygbP* (also called *ispD*) [11–13]. In

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[1] *Abbreviations used:* ADP, adenosine 5′-diphosphate; ATP, adenosine 5′-triphosphate; CTP, cytidine 5′-triphosphate; CDP, cytidine 5′-diphosphate; CDP-ME, 4-diphosphocytidyl-2-*C*-methyl-D-erythritol 4-phosphate; CMP, cytidine 5′-monophosphate; DMAPP, dimethylallyl diphosphate; DXP, 1-deoxy-D-xylulose 5-phosphate; DXR, 1-deoxy-D-xylulose 5-phosphate reductoisomerase; DXS, 1-deoxy-D-xylulose 5-phosphate synthase; DTT, dithiothreitol; HMBPP, hydroxymethylbutenyl 4-diphosphate; HMBR, hydroxymethylbutenyl 4-diphosphate reductase; HMBS, hydroxymethylbutenyl 4-diphosphate synthase; IPP, isopentenyl diphosphate; IPTG, isopropyl β-D-thiogalactoside; MEP, 2-*C*-methyl-D-erythritol 4-phosphate; MEcPP, 2-*C*-methyl-D-erythritol-2,4-cyclodiphosphate; PPi, inorganic pyrophosphate; SDS, sodium dodecyl sulfate.

0003-2697/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.ab.2004.10.011

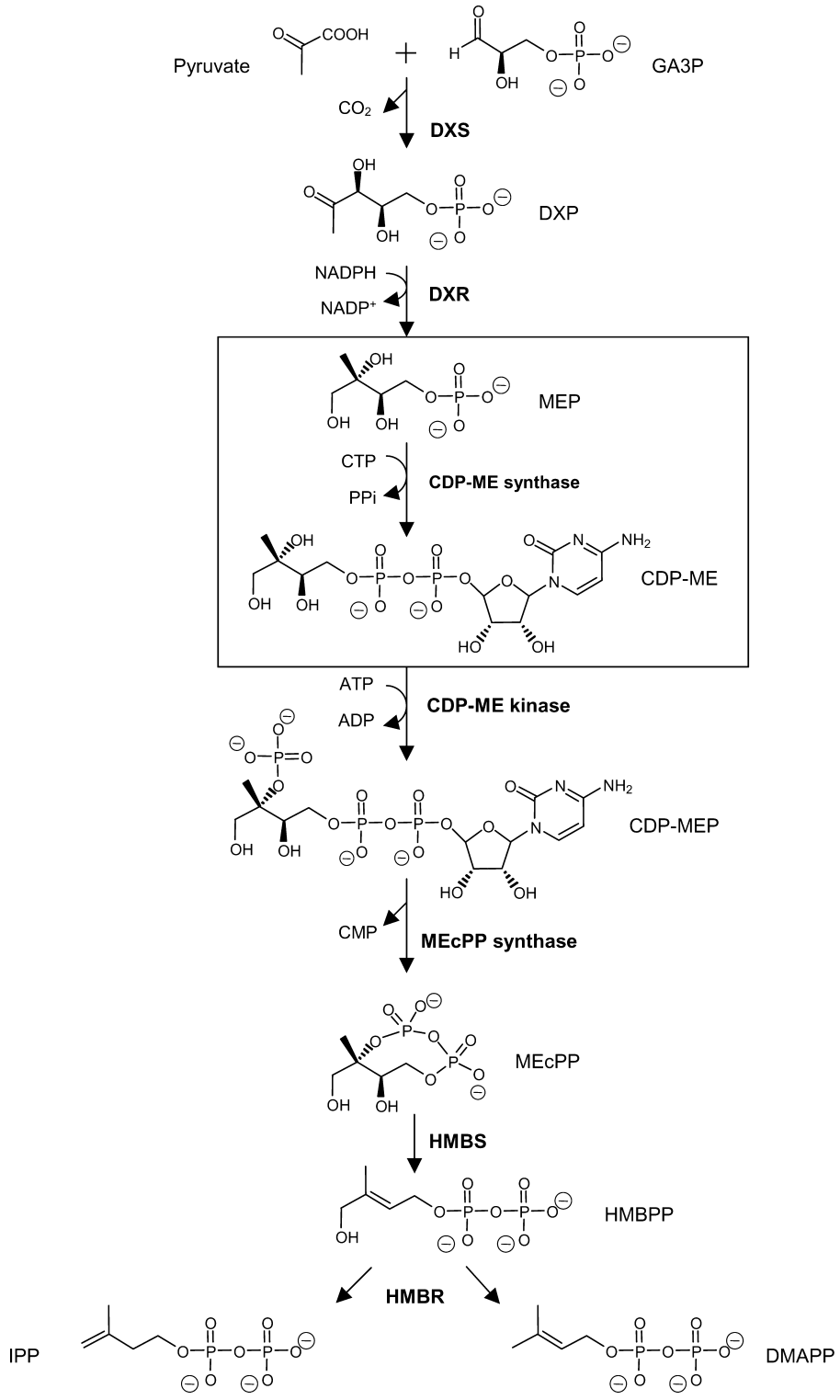

Fig. 1. The MEP pathway is composed of seven reaction steps and starts with pyruvate and D-glyceraldehyde 3-phosphate. The enzyme CDP-ME synthase converts MEP into CDP-ME at the third step of the pathway. DXP, 1-deoxy-D-xylulose 5-phosphate; MEcPP, 2-*C*-methyl-D-erythritol 2,4-cyclodiphosphate; HMBPP, 1-hydroxy-2-methyl-2-(*E*)-butenyl 4-diphosphate; DXS, DXP synthase; DXR, DXP reductoisomerase; HMBS, HMBPP synthase; HMBR, HMBPP reductase.

the following reaction step CDP-ME is phosphorylated in an ATP-dependent reaction by the enzyme CDP-ME kinase to yield 4-diphosphocytidyl-2-*C*-methyl-D-erythritol 2-phosphate (CDP-MEP) [14]. Finally, CDP-MEP is transformed into IPP and DMAPP by three sequential reactions [15–19] (Fig. 1).

Many pathogenic microorganisms, such as *Mycobacterium tuberculosis* [20], *Treponema pallidum* [21], and the