Biochimie 94 (2012) 1621–1634

Contents lists available at SciVerse ScienceDirect

Biochimie

journal homepage: www.elsevier.com/locate/biochi

Review

Isopentenyl diphosphate isomerase: A checkpoint to isoprenoid biosynthesis

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ARTICLE INFO

Article history:
Received 20 February 2012
Accepted 27 March 2012
Available online 4 April 2012

Keywords:
Isopentenyl diphosphate isomerase
IPP
DMAPP
Isoprenoid synthesis
Terpens

ABSTRACT

Even if the isopentenyl diphosphate (IPP) isomerases have been discovered in the 50s, it is only in the last decade that the genetical, enzymatical, structural richness and cellular importance of this large family of crucial enzymes has been uncovered. Present in all living kingdoms, they can be classified in two subfamilies: type 1 and type 2 IPP isomerases, which show clearly distinct characteristics. They all perform the regulatory isomerization of isopentenyl diphosphate into dimethylallyl diphosphate, a key rate-limiting step of the terpenoid biosynthesis, *via* a protonation/deprotonation mechanism. Due to their importance in the isoprenoid metabolism and the increasing interest of industry devoted to terpenoid production, it is foreseen that the biotechnological development of such enzymes should be under intense scrutiny in the near future.

1. Introduction

More than 65,000 isoprenoid compounds (a.k.a terpenes) are produced by Nature [1,2]. These molecules represent the oldest and the largest class of small biomolecules, constituting the so-called "terpenome". They perform a large variety of functions and properties in all living organisms, making them an incredible resource of natural products (for review see [3]). Therefore, terpenes can be classified according to their basic structure and function as shown in Fig. 1. They are widely represented in plants, where they act as insecticides, pesticides, pollinator attractors, hormones, flavors, pigments, etc… Their features make isoprenoids very attractive for applications in food, and cosmetics (for example as essential oils), and chemical, pharmaceutical and rubber industries.

Their common characteristic, postulated first by Otto Wallach as soon as 1887 [4], is their construction from an identical building block. The successive addition of C_5 units derived from isoprene (C_5H_8), allows polymerizations into various structures ranging from the linear to polycyclic (Fig. 1A). These polymers are of different sizes, ranging from the C_5 basic units (hemiterpenes) to high molar mass polymers such as natural rubber made of thousands C_5 units. In Nature, terpenoids are constructed from four basic coupling reactions: chain elongation, cyclopropanation, branching and cyclobutanation [5,6]. The most common reaction occurring in all organisms is chain elongation, which is usually a 1′-4 condensation of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). This reaction is mainly produced by sequential head-to-tail additions and pyrophosphate (PPi) eliminations with the help of prenyl transferases [7]. Non-head-to-tail joining (irregular) produced by the three other reactions can also be observed in less common terpenes [5,8,9]. Elongation *via* 1′-4 prenyl addition, results successively in geranyl diphosphate (GPP, C_{10}), then in farnesyl diphosphate (FPP, C_{15}) and finally geranylgeranyl diphosphate (GGPP, C_{20}). These three basic compounds may then generate most of the terpenoid families (Fig. 1A).

Indeed both diphosphorylated C_5 units, IPP and DMAPP, are essential and may be differently produced by their host, following different metabolic pathways. But the enzyme controlling the availability and isomerization of both universal units is the isopentenyl diphosphate isomerase, also called IDI. IPP isomerase (EC 5.3.3.2) catalyzes the crucial conversion of IPP into DMAPP, in an Mg-dependent and reversible isomerization process presented in Fig. 1B, which is the first step controlling the overall biosynthesis of all terpenoids. This enzyme has been first characterized in the baker's yeast by Feodor Lynen in 1959 [10], but has been found ever since in most living systems. F. Lynen and K. Bloch won the joint 1964 Nobel Prize for their discoveries on the mechanism of regulation of cholesterol metabolism, in which IDI plays a central role.

Abbreviations: IDI, isopentenyl diphosphate isomerase; PP, diphosphate; IPP, isopentenyl diphosphate; DMAPP, dimethylallyl diphosphate; GPP, geranyl diphosphate; FPP, farnesyl diphosphate; GGPP, geranylgeranyl diphosphate; *MEP*, 2-*C*-methyl-D-erythritol-4-phosphate; MVA, mevalonic acic; NUDIX, NUcleoside DIphosphate linked to another moiety (X); TIM, Triose phosphate IsoMerase; FMN, flavin mono nucleotide; NADPH, reduced nicotinamide adenine dinucleotide phosphate.

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doi:10.1016/j.biochi.2012.03.021

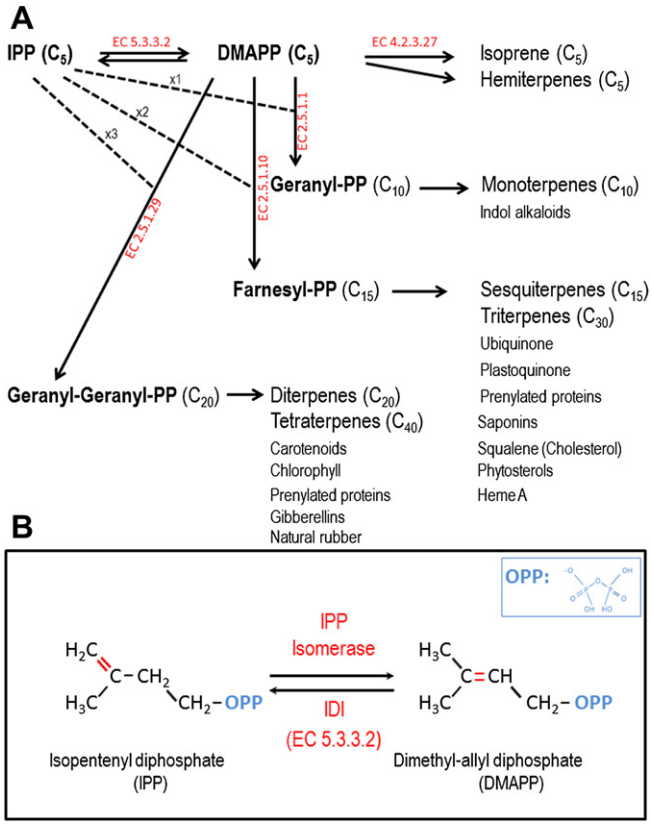


Fig. 1. IPP isomerase as a key enzyme in terpenomic diversity. A) Overview of isoprenoid biosynthesis from IPP and DMAPP. B) Reaction catalyzed by IPP isomerases in presence of Mg^{2+}.

2. Biosynthesis & compartmentation of IPP and DMAPP basic structures

At least two pathways have been well characterized so far producing IPP/DMAPP: the mevalonic acid (MVA) pathway and the non-mevalonate pathway also called methyl-erythritol-phosphate (MEP) pathway. They directly derive from the sugar metabolism, based on C3 components from the glycolysis cycle: pyruvate, acetyl-CoA and glyceraldehyde-3-phosphate (G3P). Plant cells have the particularity to possess both pathways (as certain strains of *Streptomyces* and algae), but compartmentalized, one in the cytosol and the other in plastids (Fig. 2A). For decades, it was believed that the MVA pathway was a unique pathway as it was identified in diverse organisms, particularly eukaryotes (Fig. 2B). This pathway converts 3 acetyl-CoA to IPP, *via* HMG-CoA and mevalonic acid intermediates (Fig. 2A). In 1996, Michel Rohmer discovered the first step of the alternate *MEP* pathway [11,12]. This pathway mostly found in prokaryotes and plastid (but not present in humans), is initiated by the condensation of pyruvate and G3P, produces 1-deoxy-D-xylulose-5-phosphate, which is then reduced to 2-*C*-methyl-D-erythritol-4-phosphate before converting simultaneously to both IPP and DMAPP. This "branching" of the *MEP* pathway is allowed by the last enzyme of the pathway, an hydroxymethylbutenyl diphosphate reductase also called isopentenyl diphosphate:dimethylallyl diphosphate synthase (IDS, Fig. 2A), which produces IPP/DMAPP in an 85:15 ratio [13]. In this case, the role of plastidial IPP isomerase may be questioned, as IDI does not seem essential for organisms producing *MEP*-derived isoprenoids. We may suppose that production of IPP and DMAPP could be sometimes different and probably adapted to species or compartmentation. IPP and DMAPP are the end-products in both pathways, and considering the potential toxicity of prenyl diphosphates [14–16], the presence of IDI is doubtlessly regulating the IPP/DMAPP pool and the flux in the terpene biosynthesis pathways [14,15]. In isoprene emitting plants, the detoxification or regulation of DMAPP pool by IDIs is also correlated to isoprene emission with the help of an isoprene synthase (EC 4.2.3.27; Fig. 1A) [17]. In addition, cytochrome P450 enzymes present in the plant endoplasmic reticulum (ER) may also be able to oxidize and modify primary terpenes [18–20] (Fig. 2A).

3. Phylogenetic analysis

In the last decade, a great number of IDI genes have been cloned and identified. Interestingly, if we perform a homology search and a phylogenetic analysis on those DNA sequences, two distinct families of IDI emerge as IDI1 and IDI2 (Fig. 3). Additionally, both types may be found in living organisms expressing either *MEP* and/or MVA pathways [21].

Type I IDI (IDI1), the first discovered in the 50s [10] has been largely studied. IDI1s possesses a wide phylogenetic repartition and are found in a large variety of organisms, from bacteria to human (Fig. 3A). The IDI1 family in plants is tremendous and much conserved. It has been very often identified two isoforms or more by plant species: a long one usually localized in plastids and a shorter one present in cytosols. In addition, IPP is also present in other organelles such as mitochondria and endoplasmic reticulum [22,23], while IDIs have been localized in mitochondria [24–26] and peroxisomes [27–29]. It is believed that IPP could diffuse from the cytoplasm into some organelles through permeable membranes [22,23,30] or a putative IPP transporter as suggested by reference [31].

Type II IDI (IDI2) was discovered more recently by Haruo Seto et al. [32]. IDI2s correspond to flavoenzymes sharing a significant degree of sequence similarity, but are very different from type I enzymes. They are mainly found in Gram positive bacteria (*Firmicutes*, *Actinobacteria*), some few Archaea, proteobacteria, and cyanobacteria, with an intriguing prevalence of thermophilic or hyperthermophilic species as shown Fig. 3B. Many of them can grow in extreme conditions (extremophiles): anaerobic, methanophilic, halophilic, oligotrophic, antibiotic or acidophilic.

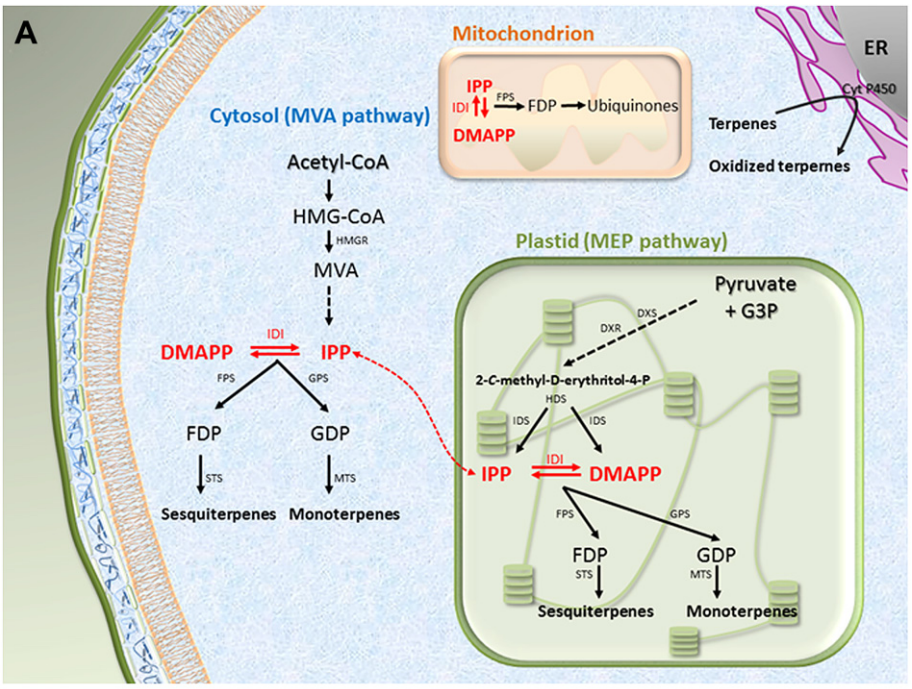
In addition, a preceding analysis on 283 genomes have reported that only a very few bacterial species apparently lack both types of IDIs (*eg. Mycoplasma*, *Spiroplasma* and *Nanoarchaeum equitans*) while some few others may present both forms (*Halobacterium* sp. NRC1, *Mycobacterium marinum*, *Photorhabdus luminescence*) [21,33]. The current analysis also shows that *Streptomyces griseoflavus* presents both types of IDIs. Indeed, *Streptomyces* bacteria are known to have the largest bacterial genome and an unusual metabolic diversity.

Based on the assumption that IDI1 was not an essential gene at least in plastid and *Escherichia coli* [13,34], but could be essential in some other species, as for example yeast or *Caenorhabditis elegans* [35–37], it seems therefore plausible that several ways may generate DMAPP crucial for terpenoid synthesis. In addition, IDI genes have been shown to be duplicated in mammals, many plants and algae [38].

4. Two types of isomerases with different features

4.1. DNA sequence analysis

For the resolution of the present phylogenetic analysis, we searched homologous genes with the BLASTP program. Then we retrieved and analyzed their sequence after alignment with the CLUSTALW program to compare them in terms of sequence similarity and conservation. The genetic conservation of 9 sequences



B

MVA pathway	MEP pathway
Eukaryotes	Higher plants *(Plastid)*
Animals	Algae[b]
Higher plants (*Cytoplasmic*)	*Plasmodium* spp.
Algae[a]	Enterobacteriaceae
Fungi / yeast	Mycobacteria
Archae	Gram negative bacteria
Gram negative bacteria	*Neisseria meningitis, gonorrhoea*
Haemophilus influenza, Borrelia burgdorferi	*Vibrio cholerae*
Myxococcus fulvus, Chloropseudomonas ethylica	*Helicobacter pylori*
Coxiella burnetti	*Camphylobacter jejuni*
Gram positive bacteria	Gram positive bacteria
Streptomyces spp.	*Bacillus subtilis, anthracis*
Staphylococcus spp, *Streptococcus* spp.,	*Streptomyces* spp., *Listeria monocytogenes*
Enterococcus faecalis	*Clostridium botulinum, tetani*
Low-GC cocci	

Fig. 2. Localisation of IPP/DMAPP isomerizations. A) Schematic of mevalonic acid (MVA) and 2-*C*-methyl-D-erythritol-4-phosphate (*MEP*) pathways in a plant cell for the biosynthesis of IPP/DMAPP (modified from [18,96]). B) Lists of organisms performing each pathway for the biosynthesis of terpenoids (adapted from [53,97]). [a] Including species such as *Scenedesmus obliquus*, *Chlorella fusca*, and *Chlamydomonas reinhardtii*. [b] Including species such as *Euglena gracilis*. IDI, isopentenyl diphosphate isomerase; IPP, isopentenyl diphosphate; DMADP, dimethylallyl diphosphate; FDP, farnesyl diphosphate; GDP, geranyl diphosphate; G3P, glyceraldehyde-3-phosphate; HMG-CoA, hydroxymethylglutaryl-CoA; MVA, mevalonic acid; MEP, methyl-erythritol-phosphate; Cyt P450, cytochrome P450 hydroxylase; FPS, FDP synthase; GPS, GDP synthase; HMGR, HMG-CoA reductase; MTS, monoterpene synthase; STS, sesquiterpene synthase. ER, endoplasmic reticulum; HMGR, 3-hydroxy-3-methylglutaryl coenzyme A reductase; DXS, 1-deoxy-D-xylulose-5-phosphate synthase; DXR, 1-deoxy-D-xylulose-5-phosphate reductoisomerase; HDS, hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate synthase; IDS, isopentenyl diphosphate:dimethylallyl diphosphate synthase; Dashed arrows indicate more than one step.

from IDI1 and IDI2 enzymes are presented in Figs. 4 and 5 respectively. IDI1 and IDI2 families differ greatly, while in each type highly conserved motives or sequences are observed, corresponding to the catalytic site of the enzyme.

4.2. Type 1 isomerase: IDI1

Type I IDI sequence is shorter, in particular in the catalytic site, which remains quite conserved as seen on the black and gray alignments (Fig. 4B). Conserved motives are N*x*x*C*x*H*P, E*x*E and many G-rich sequences [32,34]. *E.coli* IDI1 is one of the shortest IDIs with only 182 amino-acids [34], but this short enzyme is also very efficient, acting as a monomer (Table 1). For this reason, it became a good and attractive model for structural studies. In diverse protein databases, IDI1s are associated members of the NUDIX superfamily. These NUDIX enzymes classically catalyze the hydrolysis of NUcleoside DIphosphate linked to another moiety (X) by nucleophilic substitution in presence of a divalent cation [39]. IDI1 may be found as isoforms with various localization signals, particularly plastid or mitochondrion targeting peptides at the N-ter, or a PST1 motif for peroxisome targeting at the C-ter (Fig. 4A).

4.3. Type 2 isomerase: IDI2

Type 2 IDIs present larger sequences (more than 300 amino-acid) with no localization signal as expected for prokaryotes.

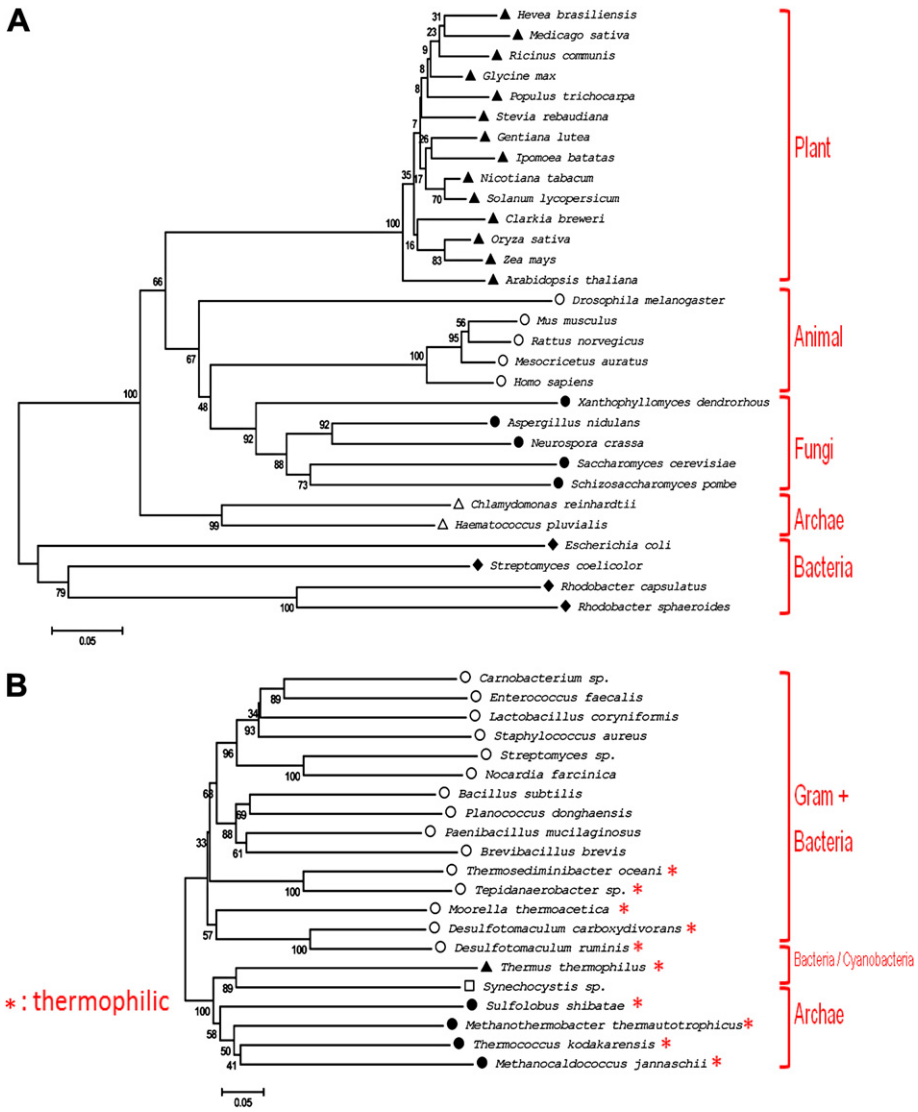


Fig. 3. Phylogenetic analysis of IPP isomerases type I (A) and type II (B) from a variety of organisms. The GenBank, Swiss-Prot or NCBI accession numbers of each 30 aligned sequences were as follows: *Arabidopsis thaliana* AAL57687, *Aspergilus nidulans* CBF89196, *Bacillus subtilis* BAB32625, *Brevibacillus brevis* YP_002771925, *Carnobacterium* sp. YP_004375520, *Chlamydomonas reinhardtii* AAC32601, *Clarkia breweri* AAB67743, *Desulfotomaculum carboxydivorans* YP_004498178, *Desulfotomaculum ruminis* YP_004546909, *Drosophila melanogaster* AAM50284, *Enterococcus faecalis* ZP_07556461, *Escherichia coli* Q46822, *Gentiana lutea* BAE92732, *Glycine max* ACU18155, *Haematococcus pluvialis* AAC32209, *Hevea brasiliensis* AAD41765, *Homo sapiens* Q13907, *Ipomea batatas* AAZ94730, *Lactobacillus coryniformis* ZP_08573676, *Medicago sativa* AEC13301, *Mesocricetus auratus* O35586, *Methanocaldococcus jannaschii* NP_247857, *Methanothermobacter thermautotrophicus* O26154, *Moorella thermoacetica* YP_430184, *Mus musculus* P58044, *Neurospora crassa* XP_961969, *Nicotiana tabacum* BAB40974, *Nocardia farcinica* YP_118421, *Oryza sativa* AAF29978, *Paenibacillus mucilaginosus* YP_004641193, *Planococcus donghaensis* ZP_08094905, *Populus trichocarpa* XP_002325469, *Rattus norvegicus* O35760, *Rhodobacter capsulatus* P26173, *Rhodobacter sphaeroides* YP_353350, *Ricinus communis* XP_002514848, *Saccharomyces cerevisiae* AAA34708, *Schizosaccharomyces pombe* AAA80596, *Solanum lycopersicum* ABX55779, *Staphylococcus aureus* P58052, *Stevia rebaudiana* ABJ96279, *Streptomyces sp.* strain CL190 Q9KWG2, *Streptomyces coelicolor* NP_630823, *Sulfolobus shibatae* BAC82424, *Synechocystis sp.* strain P74287, *Tepidanaerobacter* sp. YP_004460794, *Thermococcus kodakarensis* Q76CZ1, *Thermus thermophilus* BAD71906, *Xanthophyllomyces dendrorhous* BAA33979, *Zea mays* AAQ14869. Evolutionary analyses were conducted in MEGA5 [98] using the Neighbor-Joining method, the bootstrap test (1000 replicates) and the p-distance method. All positions containing gaps and missing data were eliminated.

They are usually found as native and enzymatically active tetramers. Amino-acids engaged in the catalysis are also quite conserved (Fig. 5B). The catalytic site encompasses almost all the protein length. They are flavoproteins (using Flavin Mono-Nucleotide and NADPH as cofactors) from the TIM-barrel protein family, named after the protein Triose phosphate IsoMerase, or TIM [40].

5. Assays for IDI activity & enzymatic characteristics

IDI activity has been detected since 1959 using ^{14}C-IPP as substrate, and ^{14}C-DMAPP formed was usually detected after specific hydrolysis of the tethered diphosphate group (by treatment with HCl 6 N for example) to yield ^{14}C-DMAOH [10]. IPP is resistant to such an HCl treatment. With the acid-labile treatment DMAOH may also rearrange as methylvinylcarbanol also termed 2-methyl-3-buten-2-ol [41]. Additionally, acidic phosphatase was also often used to generate respective alcohols that were subsequently detected [41]. In both cases, the alcohols formed are subsequently extracted with an organic solvent, such as toluene, diethyl ether, petroleum ether, chloroform or alkane (heptane, hexane, pentane). Radioactivity counting coupled to chromatography methods (GC, GC–MS and HPLC) were usually used to identify and quantify the products.

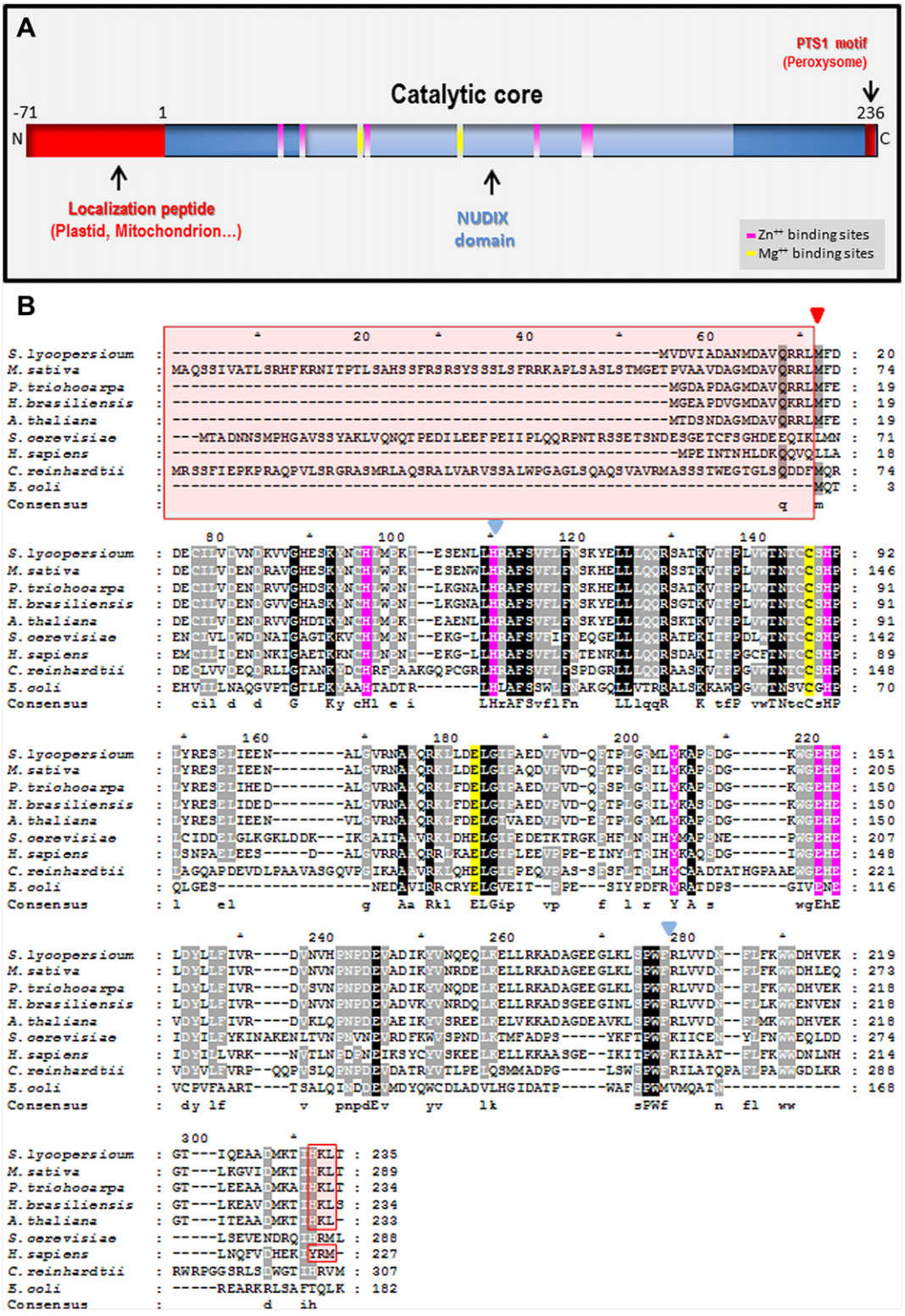


Fig. 4. Conservation through IPP isomerases of type I. A) Schematic representation of an IDI1. B) Amino acid sequence alignment of IPP isomerases from various IDI1 families. The 9 sequences originating from Genbank accession numbers (see Fig. 3 caption) were aligned by using CLUSTALW (http://www.genome.jp/tools/clustalw/program) and shaded by using GENEDOC 2.7.0 (http://www.nrbsc.org/downloads/). Black, rose and pink shadings indicate conserved motives with total consensus on the alignment, and gray shading with only partial consensus (>70%). Red box represents the non-catalytic part of the enzyme used for localization. Red arrow shows where the functional core of the enzyme is starting, and blue arrows delimitate the NUDIX domains.

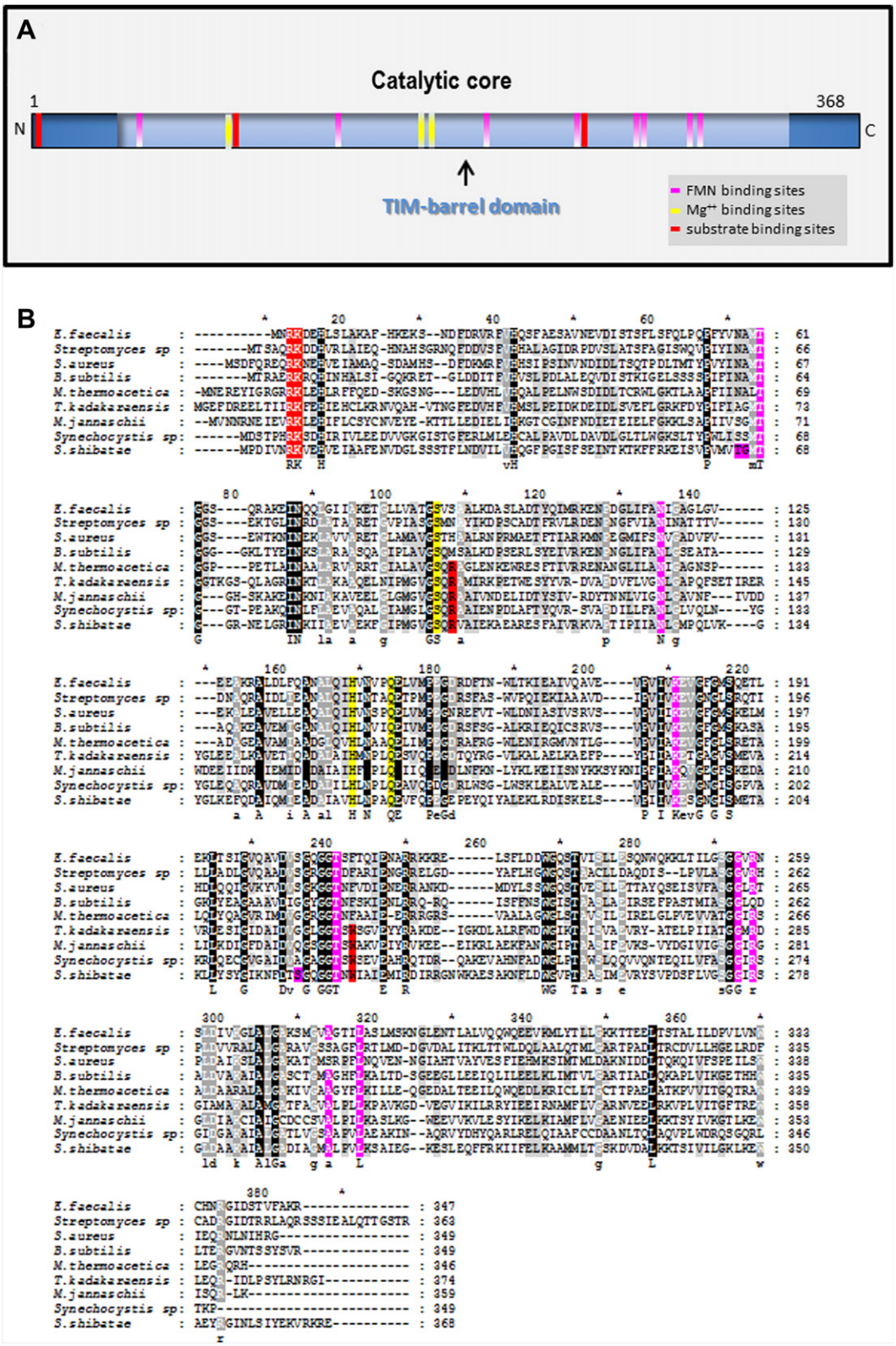


Fig. 5. Conservation through IPP isomerases of type II. A) Schematic representation of an IDI2. B) Amino acid sequence alignment of 9 different IDI2 originating from Genbank accession numbers (see Fig. 3 caption). Sequences were aligned by using CLUSTALW (http://www.genome.jp/tools/clustalw/program) and shaded by using GENEDOC 2.7.0 (http://www.nrbsc.org/downloads/). Black, rose and pink shadings indicate conserved motives with total consensus on the alignment, and gray shading with only partial consensus (>70%).

Table 1
List and characteristics of isopentenyl diphosphate isomerases studied enzymatically.

Proteins	Amino acids	MW (kDa)	pI	Km (μM)	Cofactors	pH opt	S.a. (μmol/mg/min)	GeneBank accession No;	Origin; Name; Protein Specificity	References
Type I, IDI1 :										
From plants										
HbIPI				71	Mg^{2+}, Mn^{2+}, Zn^{2+}	7–8			*Hevea Brasiliensis*, latex (CS, BF, WRP[a])	[99,100]
	234	26.7	4.7		Mg^{2+}, Mn^{2+}			AAD41765-6	HbIPI2, latex	[101,102]
	234	26.9	5.2					BAF98287	HbIPI1; isoform	[b]
	306	34.3	5.5					BAF98286	HbIPI II; delta-isomerase, transit peptide	[b]
SlIPI	294	34.0	5.3	5.7	Mg^{2+}, Mn^{2+}	8.2	0.008	ACS34993	*Solanum lycopersicum*, tomato plastid, SlIPI2	[61,88]
		34.0	5.7		Mg^{2+}, Mn^{2+}	7.5	0.004		chromoplast/plastid	[103]
	235	27.2	5.1					ABX55779	SlIPI1; cytoplasm	[88]
	235	27.3	5.2					ADE88149	SlIPI1; isoform	[c]
AtIPI	233	27.1	4.9	20	Mg^{2+}, Mn^{2+}, Zn^{2+}	7	~1	AAC49932	*Arabidopsis thaliana*, AtIPI1; plastid	[25,104]
	284	32.6	6.3	16	Mg^{2+}, Mn^{2+}, Zn^{2+}	7	~0.3	AAC49920	AtIPI2; mitochondria	[25,104]
									Cytosol with shorter transcript	[104]
CpIDI				22/45	Mg^{2+}	7			*Cucurbita pepo*, pumpkin	[105,106]
IbIDI	296	33.8		5.75				AAZ94730	*Ipomoea batatas*, sweet patato	[87]
CaIDI		33.5		6	Mg^{2+}	7–8	6.2		*Capsicum annuum*, plastid	[107]
NpIDI		32/28		7.6	Mn^{2+}	6–8	0.006		*Narcissus pseudonarcissus*, daffodil	[108]
GhIDI		98		73.6	Mg^{2+}	7.4–8	6.2		*Gossipium hirsutum*; cotton root	[109]
CrIDI		29/34	5.4	1/5.1	Mg^{2+}, Mn^{2+}	7.2–7.5	~0.05		*Cinchona robusta*	[58]
QrIDI				0.7/39.5	Mg^{2+}	6.8–7.2	0.130		*Quercus robus*, biphasic activity in leaves	[17]
From eukaryotes										
HsIDI	284	32.5	8.1					NP_004499	*Homo sapiens*; HsIDI1	[110]
	227	26.3	5.9	33	Mg^{2+}, Mn^{2+}	7	3.6	Q13907	HsIDI1; **PDB: 2DHO**, **2ICJ**, **2I6K**	[68,111]
	227	26.7	6.0	22.80	Mg^{2+}, Mn^{2+}, Zn^{2+}	7–8	0.12	NP_150286	HsIDI2; **PDB : 2PNY**	[27]
PigIDI		82.5	6–6.2	2.7	Mg^{2+}, Mn^{2+}	6.3	6.7		*Sus scrofa*, pig liver	[44]
		22.0	6.2	8.2	Mg^{2+}	4–8.3	0.86			[47,52]
				4	Mn^{2+}, Mg^{2+}	6	29			[60,112]
GgIDI		22.0	6.7	11.2	Mg^{2+}				*Gallus gallus*, chicken liver	[52]
			6.3	1.3–2.5	Mn^{2+}, Mg^{2+}	6.6	73			[45,46]
BmIDI	251	30–35	6.4	20	Mg^{2+}, Mn^{2+}	7–8		NP_001040323	*Bombyx mori*; silkworm	[113,114]
HpIPI	293	32.5	5.9		Mg^{2+}, Mn^{2+}	7.5–8		AAC32209	*Haematococcus pluvialis* ; IPIHp2; cytoplasm	[55,91]
	305	34.0	6.0						IPIHp1; chloroplast; increased carotenoid level	[55,115]
From Archaea										
HbIDI	213	24.6	4.6	2930	Co^{2+}, Mg^{2+}	6	8.12	NP_280550	*Halobacterium* sp. NRC-1	[33]
From fungi										
CpIDI		35	6.2	5.4	Mg^{2+}, Mn^{2+}	6–8.5	3000		*Claviceps purpurea* (sp. SD58)	[52]
		35		2.4	Mg^{2+}, Mn^{2+}	7–8	3.6			[116]
PcIDI		20–50	5.3	6.7	Mg^{2+}, Mn^{2+}	6	50		*Penicillium cyclopium*	[117]
ScIDI	288	39	4.5	43	Mg^{2+}		12.3	J05090	*Saccharomyces cerevisiae*; baker's yeast	[62,118]
		41		35	Mg^{2+}	6–9	2.65		Post-translational modifications (33 kDa)	[119,120]
				36	Mg^{2+}	5.5–9.3	0.11		Autolysat	[10,48]
SpIDI	227	26.8	5.5		Mg^{2+}		idem	AAA80596	*Schizosaccharomyces pombe* Shorter but activity similar ScIDI	[121]
From prokaryotes										
EcIDI				5	Mg^{2+}, Mn^{2+}	7.5–8.5			*Escherichia coli*; basic catalytic core	[63]
	182	24.5	5.9	7.9/9.5	Mg^{2+}, Mn^{2+}	7.4	0.97	Q46822	antarafacial rearrangement	[34,70]
	182	21.6		10	Mg^{2+}, Mn^{2+}	7.4			**PDB**: **1HZT**, **1NFS**, **1I9A**	[65,66]
	182				Mg^{2+}, Mn^{2+}				α/β domain metalloprotein	[64,72]
					Mg^{2+}, Mn^{2+}, Zn^{2+}	7	0.39			[42,43]
RcIDI	176	20		5.5	Mg^{2+}	7	0.14	P26173	*Rhodobacter capsulatus*	[122]
Type II, IDI2:										
Bacteria Gram +										
SaIDI2	349	39.0	5.2	16.8/19	Mg^{2+}, FMN, NADPH	7	270	P58052	*Staphylococcus aureus*	[32,123]
BsIDI2	349	40/45	6.2	670	Mg^{2+}, FMN, NADPH	6.5–7	400	BAB32625	*Bacillus subtilis*; **PDB**: **1PON**, **1POK**	[74,124]
		38.5			Ca^{2+}, FMN, NADPH	8	0.63		aerobic and anaerobic	[21]
SspIDI2	363	41.0	5.4	450	Mg^{2+}, FMN, NADPH	7	490	Q9KWG2	*Streptomyces* sp. strain CL190	[32]
Bacteria Gram - and cyanobacteria										
TtIDI2	332	36.0	6.6	5.6	Mg^{2+}, FMN, NADPH	7.6		BAD71906	*Thermus thermophilus*; **PDB**: **1VCF**, **3DH7**	[125,126]
SynIDI2	349	37.5	5.0	52	Mg^{2+}, FMN, NADPH	7		P74287	*Synechocystis* sp. PCC 6803	[127]
Archaea										
MtIDI2	349	48.0	4.7	64	Mg^{2+}, FMN, NADPH	7	0.476	O26154	*Methanothermobacter thermautotrophicus*	[128]
SsIDI2	368	40.4	6.9	63/84	Mg^{2+}, FMN, NADPH	6		BAC82424	*Sulfolobus shibatae*; **PDB**: **2 ZR**(**U-Z**)	[76,81,129]
TkIDI2	374	41.5	5.5	84/211	Mg^{2+}, FMN, NADPH	8		Q76CZ1	*Thermococcus kodakaraensis*	[130,131]
MjIDI2	359	39.9	5.1	15.3	Mg^{2+}, FMN, NADPH	7–7.2		NP_247857	*Methanocaldococcus jannaschii*	[132]

Isomerase protein in bold are abbreviated according to their host of origin, by the first letters of their Latin name. Table adapted from [26,53].

[a] CS : C-Serum; BF : Bottom Fraction; WRP : Washed Rubber Particles.

[b] Sando et al., 2008, unpublished data released in Pubmed/Genbank.

[c] Lee et al., 2010, unpublished data released in Pubmed/Genbank.

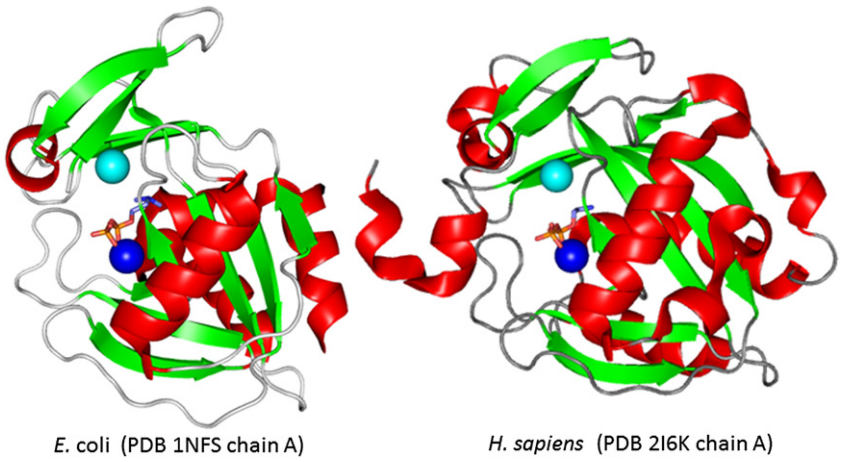


Fig. 6. Overall structures of type I IPP isomerases. Type I isomerases are found as compact globular proteins that belong to the class of α/β proteins. IDIs are presented bound with substrate analogues (*E. coli*: *N,N*-dimethyl-2-amino-1-ethyl diphosphate; *H. sapiens*: 2-aminoethyl diphosphate) represented as atom colored sticks in the catalytic site in presence of metal ions. α-Helices and β-strands are indicated as ribbons in red and green respectively. Mg^{2+} is shown as a dark blue sphere, and Mn^{2+}, Zn^{2+} as cyan spheres. All structures were produced using PyMOL 1.3 (PyMOL molecular Graphics system, Schrödinger, LLC) with referenced Protein database Bank (PDB) numbers.

Fifteen years ago, a comprehensive review on biochemical and enzymatic properties of IDIs was made [26]. We compiled in Table 1 an up-to date overview of 31 different IDIs, including now the type 2 enzymes. The Km of these relatively acidic enzymes were usually in the μM order, whereas the activity could range from 0.004 to 3000 μmol/mg/min. Enzymatic activities were optimal at pH around 6–8 (usually in Tris/HCl buffer) and mesophilic temperatures (37 °C), but certain type 2 IDIs could be more specific of

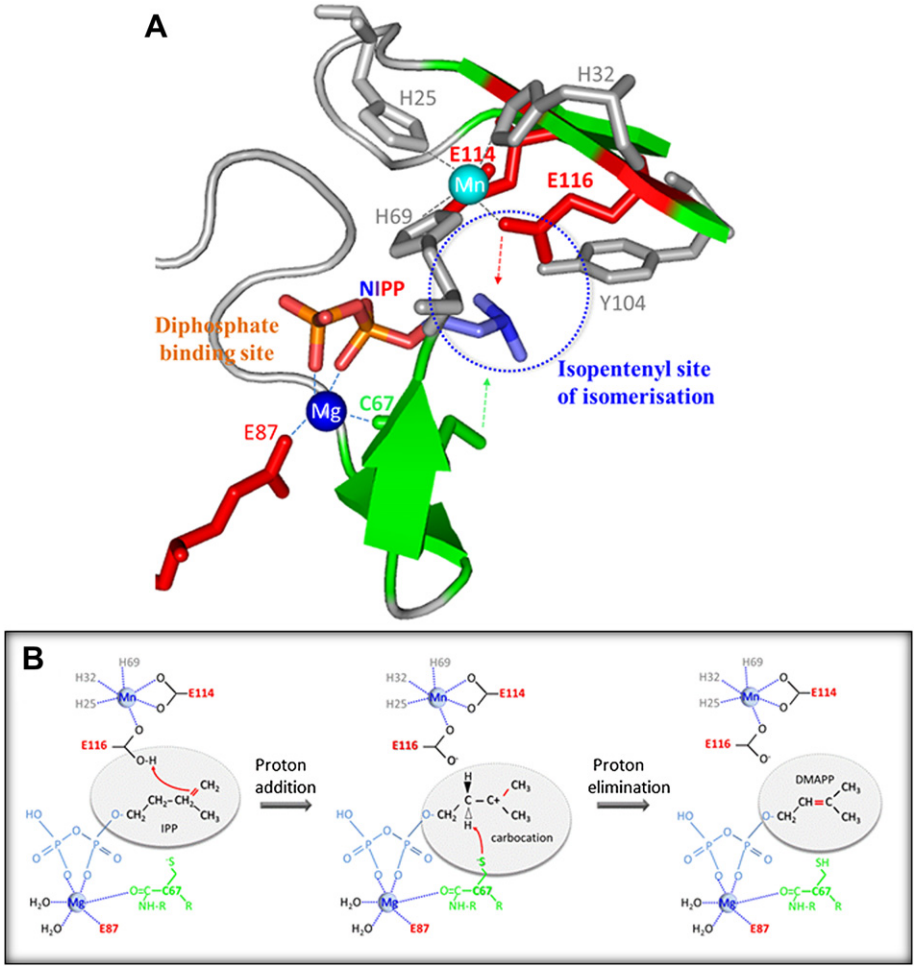


Fig. 7. Hypothetical mechanism of isomerization by type I isomerase. A) Catalytic site of *E. coli* IDI1 based on the PDB 1NFS chain A (modified from [69]). The site is presented with the substrate analogue NIPP (*N,N*-Dimethyl-2-amino-1-ethyl diphosphate). B) Mechanistic proposal of isomerization by type I IPP isomerase (adapted from [72]).

thermophilic or hyperthermophylic conditions. All reactions were performed with at least Mg^{2+} as catalyst, but FMN and NADPH were a requisite for IDI2 activity (Table 1). In addition to Mg^{++}, Mn^{2+} were also very often used in the assays, but it appears that IDI1 may also be stimulated by Co^{2+}, Ni^{2+}, Cd^{2+} and Zn^{2+} as cofactors [42]. *E. coli* IDI has been shown to contain one essential atom of zinc in addition to Mg^{2+} [43] and *Halobacterium* sp. NRC-1 IDI utilizes specifically Co^{2+} for catalysis [33]. Other compounds susceptible to stabilize the enzyme such DTT, β-mercaptoethanol, BSA, leupeptin, L-cystein or glutathione are currently used in the assays [44–47]. KF or NaF were also sometimes added in assays with crude protein extracts to inhibit phosphatases and sodium azide could be used without a loss of activity [45,48]. Many inhibitors of the isomerases have been identified: in particular inorganic pyrophosphates, monophosphate esters, bisphosphonates, GPP, synthetic substrate analogs and other products from the terpene pathway [44,49–53]. Reductive agents such as iodoacetamide or metal chelating agents as EDTA also inhibit the reaction [10,33,45]. IDIs typically have a rather narrow selectivity, isomerizing either IPP or DMAPP.

In addition, *in vivo* environmental factors may stimulate the isomerase activity. In tabacco, high-light and high-salt stress conditions stimulate IDI mRNA synthesis, but the direct stimulation of IDI activity was not demonstrated in this study [54]. Oppositely, *Haematococcus pluvialis*, *Aspergillus giganteus* and *Zea mays* IDI activities were shown to be directly stimulated by light [55–57]. Fungal elicitation may also be a positive stimulation [58].

It is now established that the IPP isomerase activity is a rate-limiting step in terpenoid synthesis [57] even if it was not always obvious [59]. The reversibility of the isomerase process may regulate the terpenoid flux, but *in vivo* the enzymes in the upstream pathway seem to be quite efficient using both IPP and DMAPP. F. Lynen was first to describe the yeast IDI1 activity as reversible *in vitro* [10]. However, reversibility is weak *in vitro* and it exists an equilibrium in favor of DMAPP, were K_{eq} = [DMAPP]/[IPP] = 9 [47,48,60]. Indeed, using DMAPP as substrate, Km were determined for *E.coli* IDI (14.3 μM) and *Cinchona robusta* IDI (17 μM) [34,58]. But it seems that DMAPP is a competitive inhibitor of the isomerization reaction [34,61]. In addition, the reversibility of type 2 isomerase from *Bacillus subtilis* was also established *in vitro*, with a similar equilibrium ratio to IDI1 [21].

6. Structures of IDIs & mechanisms of isomerization

Fortunately, IPP isomerases can be well-expressed in *E. coli*, and subsequently purified as recombinant and functional enzyme [62], using either native or synthetic genes. This was very helpful for the resolution of the crystallographic structures of many of them, in both type I and type 2 IDIs.

First to be crystallized was the *E.coli* IDI1. Enzymatically identified since 1986 [63], it is only in 1999 that Dale Poulter and his colleagues isolated its 546 base pair-long gene [34]. Because of its small size, this enzyme is believed to represent the basic minimal catalytic core of type 1 IDIs. The crystals revealed that EcIDI1 is a compact globular protein belonging to the class of single α/β proteins, and possessing a conserved motif characteristic of the NUDIX family [64–66]. In 2007, two structures of human IDI1 from liver and brain were simultaneously solved with high similarity to

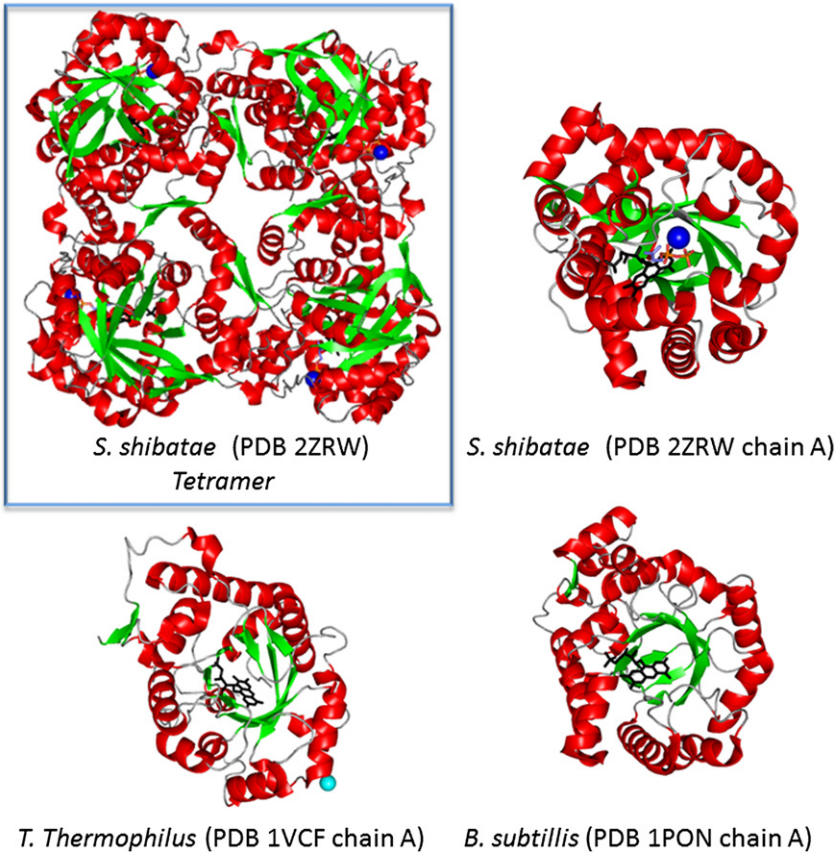


Fig. 8. Structure of type II isomerases. Type II isomerases are found as tetramers in which monomers (chain A) fold as a TIM-barrel with a bound flavin cofactor (black sticks), NADPH (not shown) and an Mg^{2+} cation. Monomer of *S. shibatae* is also presented with an IPP molecule in the central binding site. α-Helices and β-strands are indicated as ribbons in red and green respectively. Mg^{2+} is shown as a dark blue sphere and Cd^{2+} as a cyan sphere. All structures were produced using PyMOL 1.3 (PyMOL molecular Graphics system, Schrödinger, LLC) with referenced Protein database Bank (PDB) numbers.

the EcIDI1 structure (Fig. 6). The main differences concern the presence of a well-defined N-ter α-helix in human and probably of different substrate entrances [67,68]. Then only two type 1 enzymes are described, but EcIDI received further investigations (mostly by Johan Wouters laboratory), showing that IDI is a bimetallic enzyme with binding sites for Mg^{2+} but also for one molecule of Zn^{2+} [43,69]. A closer image of the catalytic site bound to a substrate analog is presented in Fig. 7A. Mg^{2+} is crucial for catalysis and appears engaged in interactions with the diphosphate moiety through bindings with the cysteine C67 and the glutamic acid E87. This cation certainly allows the recruitment of IPP in the cavity. Zn^{2+} plays a role in catalysis but also is involved in the proper folding of the enzyme [43,69]. The Zn binding site involves 3 histidines (H25, H32 and H69) and 2 glutamic acids (E114 and E116). Tyrosine Y104 is also engaged but has been shown useful for the catalytic folding and stability of the protein [70]. The proposed mechanism of isomerization of IPP to DMAPP (Fig. 7B) occurs as a step-wise "antarafacial" mechanism by an addition/elimination of protons [71,72]. This happens through the protonation of IPP insaturation with E116 (proton donor), which then forms an intermediate tertiary carbocation. This carbocation enzymatic state was initially proposed by Toteva and Richard [73]. A proton is released on the thiol function of C67 (proton acceptor) and DMAPP is formed.

Secondly, type II isomerase was originally described in *Streptomyces* sp. as flavoenzyme requiring a divalent metal, an FMN and NADPH for catalysis [32], but the first crystals were obtained from *B. subtilis* IDI2 [74]. Other crystals of IDI2 from *Thermus thermophiles* [75], *Sulfolobus shibatae* [76] and *Methanocaldococcus jannaschii* [77] revealed that the protein was a tetramer/octamer forming a *D4* symmetrical open cage-like structure. In addition, the monomers displayed a classical TIM-barrel fold, which is usually composed of 8 parallel β-strands inside the protein, surrounded by eight α-helices (Fig. 8). The (β/α)8 or TIM-barrel scaffold in enzyme is the most common fold among protein catalyst, conferring a high catalytic versatility, which is present in numerous flavin-dependent enzymes [40]. The catalytic site of type 2 IDIs bound to one molecule of FMN is shown in Fig. 9A, and seems localized on the top face of the β-barrel, surrounded by α-helices. Having a closer look, the diphosphate is coordinated to the Mg^{2+}, while the isopentenyl moiety is engaged with the reduced FMN (Fig. 9B). In *S. shibatae*, several amino acids (Ser195, His155, Gln160 and Trp225) are susceptible to form a box surrounding the isoprenoid part [78]. The affinity of IDI2 for FMN is relatively low, and FMN can be completely lost during purification [32,74]. The supposed mechanism of isomerization of IPP to DMAPP (Fig. 9B) occurs through reduced FMN, *via* the same kind of protonation/deprotonation process than the one observed in IDI1 [79,80]. Upon incubation with NADPH, the FMN complexed to the enzyme is rapidly reduced, and the $FMNH_2$ formed may then act as a proton donor [81]. A radical mechanism has also been suggested but not demonstrated [53,81]. In anaerobic conditions, it has been shown the BsIDI2 did not require NADPH for isomerization [21]. Several mechanisms have been proposed and are still under investigation. Protonation mechanisms are conceivable in particular an N1/O4 catalyzed concerted mechanism [76,82], however lately Heaps and Poulter, suggested that the isomerization was not a concerted process [83]. Several other mechanisms are possible based on the N5 catalyzed carbocation mechanism [76,78,84]. Indeed, the reduced FMN plays the role of an acid/base catalyst and favor a carbocation in C3 of IPP (Fig. 9B),

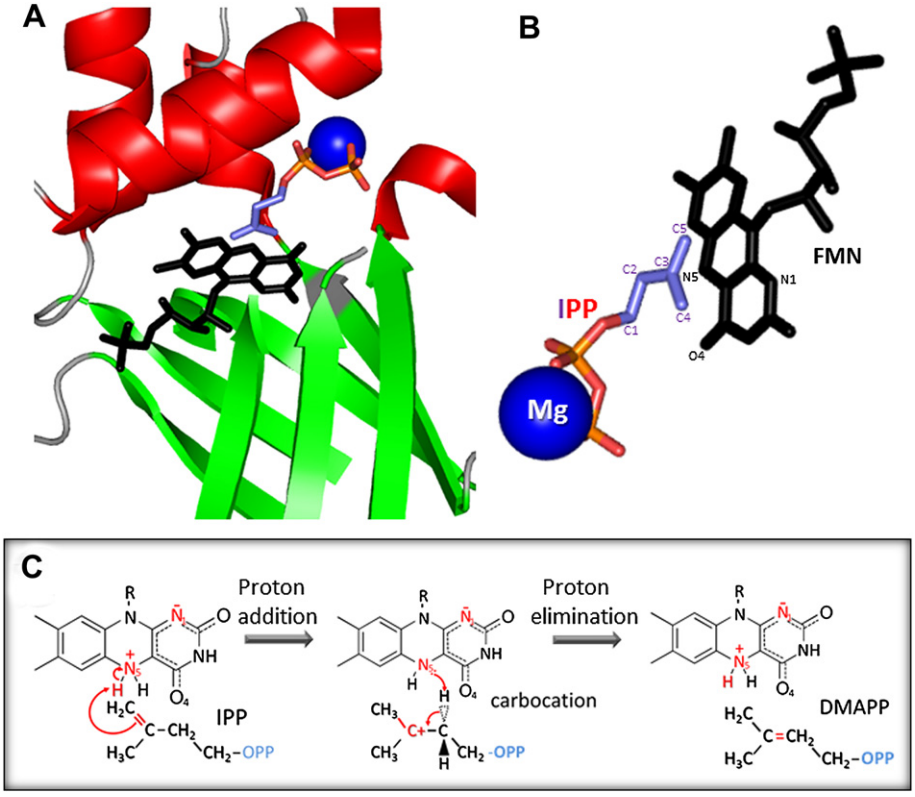


Fig. 9. Hypothetical mechanism of isomerization by type II isomerases. A) Catalytic site of *Sulfolobus shibatae* IDI2 based on the PDB 2ZRW chain A. The site is presented with the substrate IPP (atom colored sticks), FMN cofactor (black sticks) and the Mg^{2+} cation (blue sphere). B) Potential interaction points between the 3 partners. C) Mechanistic proposal of isomerization by type II IPP isomerase, based on the presence of a zwitterionic form of reduced FMN. In this scheme an N5- catalyzed carbocation mechanism is proposed (adapted from [76,78,84]).

stabilized by the C4a of FMN before the deprotonation at C2 of IPP by N5 [78,84]. A simplified possible mechanism is proposed in Fig. 9C based on these recent findings. As IDI2 is present in several human pathogens, but is absent in humans, it is thus a potential interesting target for new antimicrobial agents. We can expect in the near future the development of more inhibitors that could help to fully elucidate the exact mechanism of isomerization of type 2 enzymes.

7. Control of the isoprenoid synthesis by IDI overexpression

In 1993, Ladeveze et al. first showed that an increase of yeast IDI activity was correlated to the accumulation of ergosterol, the main terpene in yeast [59]. But since 1994, engineered non-carotenogenic *E. coli* in which the β-carotene biosynthetic pathway is reconstructed (known as "carotenoid color-reporting system") is now currently used to assay IDI functional activity on Petri dishes or liquid cultures [85–90]. This system is mainly based on a co-transformation with a whole cluster of carotenoid genes from *Erwinia uredovora*, *Erwinia herbicola* or *Agrobacterium aurantiacum* [86,91,92]. This cluster is usually constituted of GGPP synthase (*crtE*), phytoene synthase (*crtB*) and phytoene desaturase (*crtI*), and additionally lycopene cyclase (*ctrY*) and β-carotene hydroxylase (*crtZ*).

Heterologous overexpression in *E. coli* of different exogenous IDIs was demonstrated to enhance β-carotene, lycopene, astaxanthin and zeaxanthin production [57,88,89,91,93]. Recently, IDI was shown to increase β-carotene in a "plasmid-free" *E. coli* strain where the astaxanthin cluster from *Pantoea anantis* was stably integrated in the chromosome [94].

8. Concluding remarks

Even if it was sometimes believed that IPP isomerase is not a rate-limiting enzyme in the pathway [59,95], it seems now clearly established that the IDI activity is a crucial rate-limiting step in terpenoid synthesis [55,57]. We can therefore play on this particular step to overproduce some isoprenoids of interest. Two kinds of IDIs have been largely described genetically, enzymatically and structurally. They are present in all living kingdoms and two families are defined (type 1 and type 2 IPP isomerases) with clearly distinct characteristics. Their mechanism of isomerization starts to be quite well investigated and now their future seems involved in the biotechnological development for the isoprenoid bio-industry. Type 1 IDI is more prone to further investigations, as it is the smallest and simplest protein with no need of additional cofactors. As it appears that all IDIs do not display the same enzymatic activity, it could be of particular interest for biotechnological application, to identify or generate high efficiency IPP isomerases, which could specifically enhance the flux in terpenoid pathway. Such enzymes could be used in *E. coli* to modulate *in vivo* the pathway, but also in other "transformed" hosts such as yeast, fungi or plants. Terpenoids are widely used resource for traditional and modern human exploitation, and it is foreseen that the near future will probably see the development of "cell-free" system or complete *in vitro*-reconstituted system for the production and the valorization of isoprenoids of all sorts.

9. Authors' contributions

KB conceived and designed the study, analyzed the data and wrote the paper. YE helped with the phylogenetic analyses. YE, AD and FP analyzed data and critically read the manuscript before submission.

Conflict of interest

The authors have no conflict of interest to declare.

Acknowledgments

We thank Dr Roger C. Hiorns for proofreading the manuscript. KB and YE acknowledge the "Agence Nationale pour la Recherche" for their Post-Doctoral Researcher Fellowship (ANR Polyterp; ANR-10-CD2I-08).

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