Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic Chemistry 32 (2004) 483–493
BIOORGANIC CHEMISTRY
www.elsevier.com/locate/bioorg

Mini-Review

1-Deoxy-D-xylulose 5-phosphate reductoisomerase: an overview

Philip J. Proteau*

Department of Pharmaceutical Sciences, College of Pharmacy, Pharmacy Building, Room 203, Oregon State University, Corvallis, OR 97331-3507, USA

Received 18 August 2004
Available online 8 October 2004

Abstract

The methylerythritol phosphate pathway to isoprenoids, an alternate biosynthetic route present in many bacteria, algae, plants, and the malarial parasite *Plasmodium falciparum*, has become an attractive target for the development of new antimalarial and antibacterial compounds. The second enzyme in this pathway, 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR; EC 1.1.1.267), has been shown to be the molecular target for fosmidomycin, a promising antimalarial drug. This enzyme converts 1-deoxy-D-xylulose 5-phosphate (DXP) into the branched compound 2-*C*-methyl-D-erythritol 4-phosphate (MEP). The transformation of DXP into MEP requires an isomerization, followed by a NADPH-dependent reduction. The discovery of DXR, its subsequent characterization, and the identification of inhibitors will be presented.

Keywords: Deoxyxylulose 5-phosphate reductoisomerase; Methylerythritol 4-phosphate; MEP pathway; Isoprenoids; Non-mevalonate pathway; MEP synthase; Isomeroreductase

1. Introduction

The methylerythritol phosphate (MEP) pathway to isoprenoids has stimulated widespread interest since its discovery in the early 1990s [1–3]. This biosynthetic pathway, comprised of seven enzymatic steps, begins with the condensation of pyru-

* Fax: +1 541 737 3999.
E-mail address: phil.proteau@oregonstate.edu.

0045-2068/$ - see front matter
doi:10.1016/j.bioorg.2004.08.004

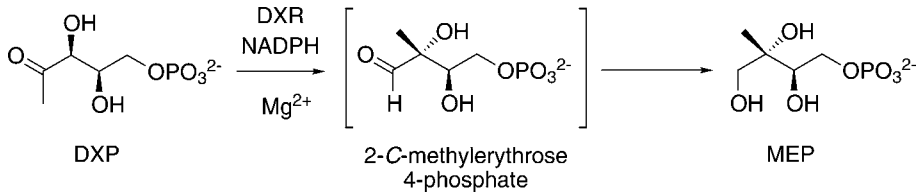


Fig. 1. Conversion of DXP to MEP mediated by DXR.

vate and glyceraldehyde-3-phosphate to form 1-deoxy-D-xylulose 5-phosphate (DXP) and ends with the formation of the isoprenoid precursors isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) from the common intermediate 1-hydroxy-2-methyl-2-*E*-butenyl 4-diphosphate [4,5]. The second step in the pathway is the conversion of DXP into 2-*C*-methyl-D-erythritol 4-phosphate (MEP) mediated by the enzyme 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR; EC 1.1.1.267; Fig. 1). The discovery of DXR and its subsequent characterization are the focus of this review.

2. DXR characterization

The *Escherichia coli dxr* (*yaeM*) gene and the successful expression of the recombinant protein were first reported in 1998 [6,7]. This discovery was aided by an earlier biosynthetic study that established that 2-*C*-methyl-D-erythritol, but not the enantiomer 2-*C*-methyl-L-erythritol, was incorporated into *E. coli* isoprenoids [8]. In order to identify the gene(s) necessary to convert DXP into the putative intermediate, 2-*C*-methyl-D-erythritol 4-phosphate, *E. coli* mutants were generated and screened for those that could survive only in the presence of added 2-*C*-methylerythritol. Complementation studies with the surviving mutants using a genomic library from *E. coli* resulted in the identification of the *yaeM* gene as the single gene that allowed growth of the mutants. This gene was overexpressed in *E. coli* and incubation of the recombinant enzyme with DXP resulted in a product that was identified as 2-*C*-methyl-D-erythritol 4-phosphate, revealing that a single enzyme promoted isomerization and reduction steps [6]. An enzyme involved in branched chain amino acid biosynthesis, ketol acid reductoisomerase (EC 1.1.1.86), also combines rearrangement and reduction steps, so the name DXP reductoisomerase[1] was proposed for this new enzyme. The corresponding gene was renamed *dxr*.

[1] Several names have been used for this enzyme and its corresponding gene. The original *yae*M designation for the *E. coli* gene was revised to *dxr* upon determination of the function of the corresponding enzyme [6]. Later, *isp*C was introduced [45], as *dxr* precedes the final five genes in the MEP pathway, which are now designated *isp*D–*isp*H. The originally proposed name, 1-deoxy-D-xylulose-5-phosphate reductoisomerase has been adopted as the official Enzyme Commission name. The "reductoisomerase" name places priority on the oxidation/reduction action of the enzyme, the approach used in formal enzyme nomenclature. The isomerization action of the enzyme, even though it occurs prior to the reduction, does not take precedence in the naming of the enzyme. Other synonymous names for DXR include: DOXP reductoisomerase, DXP isomeroreductase, MEP synthase, IspC, and DOXPR.

The general characteristics of the DXR enzymes are that they are homodimers with monomer molecular weights of 42–45 kDa. The cofactor NADPH and a divalent cation (Mg^{2+}, Mn^{2+}, or Co^{2+}) are required for activity. The enzymes typically have a pH optimum in the range 7–8, with a maximum rate at 50–60 °C. In plants, the gene product typically has a plastidial targeting sequence that is subsequently cleaved to provide the active enzyme [9,10], although there are examples of the full-length recombinant enzyme being active [9,11].

In addition to the gene from *E. coli*, the *dxr* genes from a variety of sources have been cloned: *Plasmodium falciparum* [12], *Arabidopsis thaliana* [13], *Mentha × piperita* [9], *Zymomonas mobilis* [14], *Synechococcus leopoliensis* [15], *Catharanthus roseus* [16], *Pseudomonas aeruginosa* [17], *Streptomyces coelicolor* [18], *Lycopersicon esculentum* [11], *Synechocystis* sp. PCC6803 [19], *Mycobacterium tuberculosis* [20], and *Zea mays* [21]. The DXR enzymes for which kinetic parameters have been determined are listed in Table 1. As can be seen from the table, the $K_{m(DXP)}$ values vary from 42 to 720 μM depending on the enzyme source, while the $K_{m(NADPH)}$ varies from 0.5 to 190 μM, with most values being <20 μM. The ranges for the *E. coli* DXR parameters are due to reports from several laboratories. These values likely reflect slightly different N-terminal histidine modifications and minor variations in the assay conditions. Although several plant enzymes have been cloned, Michaelis constants have not been reported, so comparisons are not possible at this time. Both native and N-terminal His-tagged enzymes have been characterized. The differences noted for the two forms of the *Synechocystis* DXR were relatively small, whereas larger differences are noted for parameters for the *E. coli* enzymes, especially the much lower $K_{m(NADPH)}$ for the native enzyme and lower k_{cat} for the His-tagged forms. The difference in k_{cat} may be due to instability of the *E. coli* DXR, and possibly other DXRs, at low protein concentrations [22]. Addition of bovine serum albumin to the assay mixture can help to stabilize the enzyme [22]. The higher $K_{m(NADPH)}$ for the His-tagged *E. coli* DXR may be due to variations in the enzyme preparations and assay conditions,

Table 1
Comparison of kinetic parameters for DXR from several sources

DXR	K_M (μM)[a]		k_{cat} (s^{-1})[b]
	DXP	NADPH	
Escherichia coli (native) [18,22]	115–720	0.5	22–116
Escherichia coli (His-tagged) [24,37,44]	97–99	18	8
Zymomonas mobilis (His-tagged) [14]	300	5	14
Streptomyces coelicolor (native) [18]	190	190	19.2
Synechocystis PCC 6803 (native) [19]	134	5.0	5
Synechocystis PCC 6803 (His-tagged) [19]	170	4.6	7
Mycobacterium tuberculosis (native) [20]	42	5.0	2.1

[a] Because Mg^{2+} is reported to be the relevant metal ion in vivo, the kinetic parameters used for comparisons were those determined with Mg^{2+} as the divalent cation (except *Z. mobilis*, 2 mM Mn^{2+}). The parameters for the *E. coli* His-tagged DXR have also been determined by several groups using Mn^{2+} as the metal ion ($K_{m(DXP)}$ = 73–250 μM, $K_{m(NADPH)}$ = 7.4–25 μM, k_{cat} = 8–13 s^{-1}) [24,37,44].

[b] k_{cat} values were calculated from the reported V_{max} values when required.

although a direct comparison of native and His-tagged enzymes under the same conditions would be necessary to rule out a role for the N-terminal extension.

2.1. Cofactor requirements

The metal ion requirement for DXR was established in initial studies to be Mg^{2+}, Mn^{2+}, or Co^{2+}. Numerous other divalent metal ions were assayed, but all of the others resulted in minimal activity or no activity at all [7,14,19,20]. The K_m values for the metal ions have been determined for the *Synechocystis* and *Mycobacterium* enzymes [19,20]. DXR has higher affinity for Co^{2+} (*Synechocystis* $K_{m(Co)} = 10\ \mu M$; *M. tuberculosis*, 1.2 μM) and Mn^{2+} ($K_{m(Mn)} = 15\ \mu M$; 21 μM) ions, than for Mg^{2+} ($K_{m(Mg)} = 2400\ \mu M$; 1200 μM). Although the greatest k_{cat}s are often achieved with Mn^{2+}, the lowest $K_{m(DXP)}$s are seen with Co^{2+}, and the affinity of DXR for Mg^{2+} is comparatively low, it is likely that Mg^{2+} is the relevant divalent cation in vivo [19,20,22]. This is due to the more abundant levels of Mg^{2+} ions available relative to Mn^{2+} or Co^{2+} in vivo.

As mentioned above, DXR utilizes NADPH as a cofactor, in preference to NADH. The *E. coli* DXR was reported to use NADH to an extent of 1% relative to NADPH [7], while no use of NADH was reported for the *Z. mobilis* [14] and *Synechocystis* [19] enzymes. Slightly less discrimination was observed with partially purified *S. leopoliensis* DXR, where a drop in activity to 16% was found [15]. Recent experiments with the *M. tuberculosis* DXR have provided more detailed information on the utilization of NADH as a cofactor [20]. With this enzyme, similar k_{cat} values were obtained with both cofactors, but with NADH, the $K_{m(NADH)}$ was 80–120-fold higher than the corresponding $K_{m(NADPH)}$ depending on the metal ion present. For example, when Mg^{2+} was the divalent ion, $K_{m(NADPH)}$ was 5 μM, while $K_{m(NADH)}$ was 410 μM and the $K_{m(DXP)}$ increased from 42 to 210 μM with the change to NADH [20]. These results suggest that the lower activity with NADH observed with DXR from other sources was likely due to insufficient concentrations of NADH and DXP. Even though NADH can be utilized as a cofactor in vitro, the higher k_{cat}/K_m values with NADPH support NADPH as the relevant in vivo cofactor.

2.2. 2-*C*-Methyl-D-erythrose 4-phosphate as an intermediate

The compound 2-*C*-methyl-D-erythrose 4-phosphate, which was first proposed as a potential intermediate in the MEP pathway [23], was subsequently proposed as an intermediate in the DXR reaction (Fig. 1) [7]. Attempts to identify this intermediate from a standard reaction mixture were unsuccessful [7], as were attempts to detect the intermediate by NMR while using dihydro-NADPH, an unreactive NADPH analog [22,24]. Finally, 2-*C*-methyl-D-erythrose 4-phosphate was synthesized and demonstrated to be kinetically competent for the reduction step, supporting its role as an intermediate for the enzymatic reaction [24]. Not only was the conversion of 2-*C*-methyl-D-erythrose 4-phosphate into MEP demonstrated, but when DXR was incubated with $NADP^+$ and 2-*C*-methyl-D-erythrose 4-phosphate, a low level conversion to DXP was detected. These experiments strongly suggest that 2-*C*-methyl-

D-erythrose 4-phosphate is an intermediate in the enzymatic transformation even though efforts to directly detect its formation have failed.

2.3. Stereochemistry

The specific formation of the 2-*C*-methyl-D-erythritol 4-phosphate enantiomer from DXP established the overall stereochemical outcome of the DXR reaction, but further stereochemical issues have also been addressed. The stereospecific delivery of hydride from NADPH has been studied with the cyanobacterial (*Synechocystis* PCC6803) [25], *E. coli* [26], and *M. tuberculosis* [20] enzymes. These studies concluded that the enzyme is a class B dehydrogenase, delivering the pro*S* hydride from NADPH (Fig. 2). Additional studies with the recombinant *Synechocystis* DXR [25] and on the in vivo formation of 2-*C*-methylerythritol in the plant *Liriodendron tulipfera* [27] established that the C3 hydrogen of DXP becomes the pro*S* hydrogen at C1 of MEP, with the C1 pro*R* hydrogen deriving from NADPH. Based on these results, it was established that the *re* face of the intermediate aldehyde is attacked by the hydride.

2.4. Mechanism for the rearrangement of DXP to 2-C-methyl-D-erythrose 4-phosphate

Three general mechanisms have been proposed for the rearrangement of DXP to the methylerythrose intermediate (Fig. 3) : (1) an α-ketol rearrangement [24], (2) a retro-aldol/aldol reaction sequence [24], and (3) a hydride/methyl shift mechanism [20]. There is precedence for the retro-aldol/aldol-type sequence in the mechanism for the enzyme L-ribulose 5-phosphate 4-epimerase [28]. If the retro-aldol reaction occurs, an enolate of hydroxyacetone and glycolaldehyde phosphate would be generated. Attempts have been made to mimic the aldol condensation part of this mechanism by incubating DXR with hydroxyacetone and glycolaldehyde phosphate, but no production of MEP was observed [24,29]. The mechanism was further probed with 3*S*-hydroxypentan-2-one 5-phosphate, which could potentially undergo the rearrangement by the α-ketol mechanism, but would be incapable of participating in the retro-aldol/aldol mechanism. This compound, however, acts as an inhibitor of the enzyme and is not an alternate substrate [24]. The hydride/methyl shift mech-

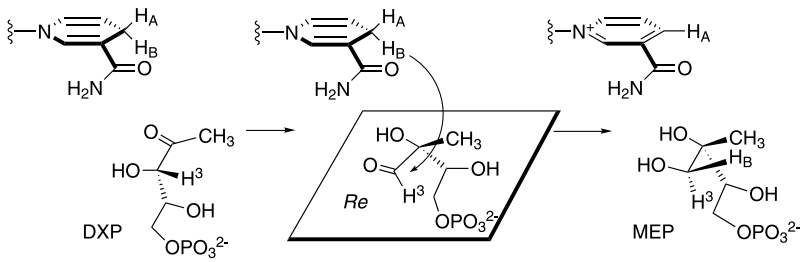


Fig. 2. Summary of the stereochemical features of the DXR reaction. The pro*S* hydride of NADPH is delivered to the *Re* face of the intermediate aldehyde. The C3 hydrogen of DXP becomes the pro*S* hydrogen at C1 of MEP.

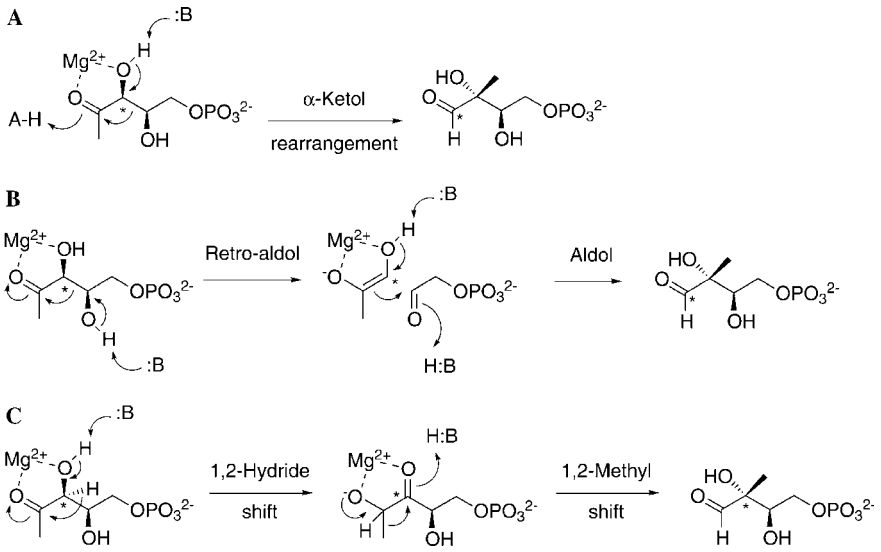


Fig. 3. Proposed mechanisms for the rearrangement of DXP to the 2-*C*-methylerythrose 4-phosphate intermediate: (A) α-ketol rearrangement, (B) sequential retro-aldol/aldol reactions, (C) sequential 1,2-hydride and 1,2-methyl shifts. The asterisk marks the position of the carbon originally at C3 of DXP.

anism is not consistent with labeling studies [30,31] and can be ruled out. At the current time, either of the first two mechanisms is still possible for DXR.

2.5. Reversibility of the DXR reaction

Beyond the general characterization of DXR from several sources, in-depth examination of the *E. coli* and *M. tuberculosis* enzymes has occurred [20,22,24]. Both enzymes are reversible, with an equilibrium that lies heavily in favor of the formation of MEP. The $K_{m(MEP)}$ for *E. coli* DXR is slightly higher than $K_{m(DXP)}$ [22,24], while the $K_{m(MEP)}$ and $K_{m(DXP)}$ for the *M. tuberculosis* DXR are equivalent [20]. The $K_{m(NADP^+)}$ values were 50-fold (*E. coli* [22]) and 34-fold (*M. tuberculosis* [20]) higher than the $K_{m(NADPH)}$ values. Studies with the *E. coli* enzyme indicated that it operates by an ordered sequential mechanism, with NADPH binding first, followed by DXP [22]. The experiments with the *M. tuberculosis* enzyme suggested a steady-state random mechanism, although the ordered mechanism could not be ruled out [20]. Based on the work with the *E. coli* enzyme and the insights from the crystal structures (see below) which suggest an ordering of the active site upon NADPH binding, it seems likely that the ordered mechanism operates for most DXR enzymes.

2.6. DXR crystal structures

Three research groups have published crystal structures of the *E. coli* DXR [32–34] and a structure of the *Z. mobilis* DXR [35] has also been reported. Each of the *E. coli* structures has provided important new information about DXR.

The first structure published was the apoenzyme [32]. This structure provided an initial look at the general fold of the protein. The enzyme is a homodimer with three distinct domains: an N-terminal NADPH binding domain, a central connecting domain, and a C-terminal helical domain. The central domain contains most of the active site residues, as well as a flexible loop region that appears to function as a lid over the active site. In this structure, the flexible loop region was not well defined in two of the three protein molecules present in the asymmetric unit. The second structure was of the selenomethionine-substituted enzyme with both NADPH and a sulfate ion bound [33]. The binding of NADPH helps to stabilize the structure allowing for a more ordered flexible loop region. The sulfate ion appeared to bind in the putative phosphate binding pocket, a conclusion which has been supported by a subsequent structure. The third article reported three structures of DXR: the apoenzyme, DXR with Mn^{2+} bound, and DXR with Mn^{2+} and fosmidomycin bound [34]. The Mn^{2+}-containing structures were obtained by soaking solutions of the apoenzyme with Mn^{2+} alone or Mn^{2+} and fosmidomycin. The NADPH binding site in this structure is blocked by the C-terminus of an adjacent monomer in the crystal, preventing access by NADPH. The *Z. mobilis* DXR structures, one of which has NADPH bound, are quite similar to the *E. coli* structures, although there are differences in the residues that interact with the adenine ring of NADPH [35].

Each of the *E. coli* structures utilized slightly different N-terminal His-tagged versions of the recombinant DXR, although these N-terminal extensions were disordered in all of the structures. A common feature in the preparation of the crystals was the use of mildly acidic conditions (ranging from pH 5.1 to 5.8), which differs from the normal functional pHs of these enzymes. Because of the different pH values, the protonation state of residues at the active site may not be the same as in the functional enzyme. A recent study on the metal ion affinity with the *Mycobacterium* DXR indicates that the Mn^{2+} ion affinity decreases at lower pH due to a proposed change in protonation state of a carboxylate ligand(s) [20]. The relevance for the crystal structures is that the hydrogen bonding networks observed in the structures could be slightly different from those present at pH 7–8.

A NMR solution structure of the *E. coli* DXR in complex with NADPH was published about the same time as the first X-ray structure [36]. The structure revealed the importance of several methionine and isoleucine residues in the binding of NADPH and the general active site, although the identification of the specific methionine residues was not possible.

2.7. Mutagenesis studies

Prior to the publication of the crystal structures, four mutants of the *E. coli* DXR were evaluated which demonstrated the importance of conserved histidine and glutamate residues for activity [37]. A summary of the results is presented in Table 2. The E231K mutant had relatively minor increases in $K_{m(DXP)}$ and $K_{m(NADPH)}$, but a greater than 400-fold decrease in k_{cat}. Because Glu231 acts as a ligand to Mn^{2+} in the crystal structure, replacement with a lysine residue would be expected to destabilize the octahedral coordination sphere, resulting in reduced

Table 2
Comparison of *E. coli* wild-type and mutant DXRs [37]

Enzyme[a]	K_m		Relative k_{cat} (%)
	DXP (μM)	NADPH (μM)	
Wild-type	250	7.4	100
E231K	270	18	0.24
H153Q	880	7.0	10
H209Q	1900	18	0.15
H257Q	4800	160	0.08

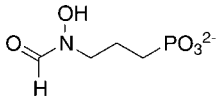
[a] Enzymes were characterized in the presence of 1 mM Mn^{2+}.

catalytic efficiency [34]. Three histidine to glutamine mutants were made: H153Q, H209Q, and H257Q. The H257Q mutation had the most drastic effect on the enzyme, with significant increases in K_m values for both DXP and NADPH, as well as a 1300-fold drop in k_{cat}. The increase in $K_{m(NADPH)}$ is somewhat surprising considering that H257 is distant from the NADPH binding site. It has been proposed that H257 and W212 participate in a stabilizing interaction along with the nicotinamide ring of NADPH that is important for full activity of the enzyme [34]. The H153Q mutation resulted in a 3.5-fold increase in $K_{m(DXP)}$, with no effect on the $K_{m(NADPH)}$ and a 10-fold decrease in k_{cat}. This histidine residue has been proposed to play mainly a structural role because it is not in direct contact with the substrate, but rather is surrounded by conserved Asp and Glu residues [33]. Based on crystal structures, H209 is positioned near the phosphate group binding site and possibly is involved in H-bonding to the phosphate group of DXP, helping to position the molecule for optimal catalysis. Mutation of this residue to a glutamine increased the $K_{m(DXP)}$ eight-fold, with a minor increase in the $K_{m(NADPH)}$, accompanied by an almost 700-fold decrease in k_{cat}. Alteration of the putative H-bonding interaction with the phosphate group of the substrate has a strong negative impact on the rate of catalysis.

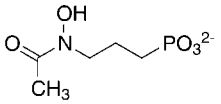
3. Inhibitors of DXR

Shortly after the discovery of DXR, it was reported that the natural product fosmidomycin was a potent inhibitor for this enzyme [38]. Fosmidomycin, an antibacterial compound, was determined to be a mixed inhibitor of DXR with a K_i of 38 nM. When fosmidomycin was tested as an inhibitor of the *Z. mobilis* DXR, it was determined to be a competitive inhibitor with a K_i of 600 nM [14]. Recent studies have more accurately defined fosmidomycin as a slow, tight-binding inhibitor that displays two inhibition modes, an initial step competitive with DXP and another non-competitive with DXP [22]. A related natural product, FR900098, is also known as an effective inhibitor of DXR and has shown greater antimalarial activity than fosmidomycin in a mouse model system [12]. Although fosmidomycin was initially proposed to inhibit DXR by binding in a similar fashion to the intermediate aldehyde, the crystal structure of *E. coli* DXR with Mn^{2+} and fosmidomycin bound provided evidence that

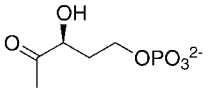
fosmidomycin binds in a fashion more similar to the substrate DXP [34]. Several carboxylic acid [39] and hydroxyurea [40] analogs of fosmidomycin have been synthesized, but their characterization as DXR inhibitors has not been reported.



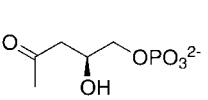
Fosmidomycin



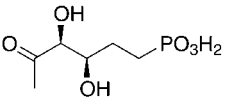
FR900098



3*S*-Hydroxypentan-2-one 5-phosphate



4*S*-Hydroxypentan-2-one 5-phosphate



3R,4*S*-Dihydroxy-5-oxohexyl phosphonic acid

Two DXP analogs lacking a hydroxyl group (3*S*-hydroxypentan-2-one 5-phosphate and 4*S*-hydroxypentan-2-one 5-phosphate) have also been found to be inhibitors of *E. coli* DXR, albeit at much higher concentrations than fosmidomycin [24]. The 4-deoxy analog was 6.7-fold more potent than the 3-deoxy analog and both had K_i values greater than the K_m for DXP. Because neither of these compounds was a substrate for DXR, the critical roles for both hydroxyls have been established.

An additional approach to inhibitors has focused on the NADPH binding site [41]. A series of adenosine derivatives that have moderate antimalarial activity was examined for DXR inhibition. Although some of these compounds showed inhibition of DXR, it is unclear how much DXR inhibition contributes to the biological activity. A potential complicating factor is that the DXR inhibition studies utilized *E. coli* DXR, while the in vivo target would be the *Plasmodium* DXR.

A phosphonate derivative of DXP, 3*R*, 4*S*-dihydroxy-5-oxohexylphosphonic acid, has been characterized as an alternate substrate for DXR [42]. This compound has a K_m approximately four-fold higher than DXP and a k_{cat} 10-fold lower. The difference between the phosphate and phosphonate group not only affects the binding, but the efficiency of turnover of this compound is reduced. Erythrose 4-phosphate (E4P) has been tested as a substrate for DXR, with no turnover observed with the His-tagged enzyme [24] and slow turnover with the native enzyme [22]. Deoxyxylulose itself, at concentrations up to 2 mM, is not a substrate for DXR [7].

4. Conclusion

Extensive investigation of DXR has provided a deeper understanding of how this unique enzyme accomplishes the transformation of DXP to MEP. Several inhibitors of DXR have been identified, with fosmidomycin and its close analog being the most

potent to date. Because fosmidomycin has important antimalarial activity and the MEP pathway is an attractive target for new antibacterials [43], efforts are underway to identify other inhibitors of DXR for their antimalarial and antibacterial potential. The X-ray crystal structures of DXR and the continuing research to fully elucidate the mechanism should be of great value in these studies.

Acknowledgment

A grant from the National Institutes of Health (RO1 AI42558) provided partial support for preparation of this review.

References

[1] M. Rohmer, M. Knani, P. Simonin, B. Sutter, H. Sahm, Biochem. J. 295 (1993) 517–524.
[2] S.T.J. Broers, Ph.D. Dissertation, Eidgenössischen Technische Hochschule, Zürich, 1994.
[3] M.K. Schwarz, Ph.D. Dissertation, Eidgenössischen Technischen Hochschule, Zürich, 1994.
[4] W. Eisenreich, A. Bacher, D. Arigoni, F. Rohdich, Cell. Mol. Life Sci. 61 (2004) 1401–1426.
[5] M. Rodriguez-Concepcion, Curr. Pharm. Des. 10 (2004) 2391–2400.
[6] T. Kuzuyama, S. Takahashi, H. Watanabe, H. Seto, Tetrahedron Lett. 39 (1998) 4509–4512.
[7] S. Takahashi, T. Kuzuyama, H. Watanabe, H. Seto, Proc. Natl. Acad. Sci. USA 95 (1998) 9879–9884.
[8] T. Duvold, P. Cali, J.M. Bravo, M. Rohmer, Tetrahedron Lett. 38 (1997) 6181–6184.
[9] B.M. Lange, R. Croteau, Arch. Biochem. Biophys. 365 (1999) 170–174.
[10] L. Carretero-Paulet, I. Ahumada, N. Cunillera, M. Rodriguez-Concepcion, A. Ferrer, A. Boronat, N. Campos, Plant Physiol. 129 (2002) 1581–1591.
[11] M. Rodriguez-Concepcion, I. Ahumada, E. Diez-Juez, S. Sauret-Gueto, L.M. Lois, F. Gallego, L. Carretero-Paulet, N. Campos, A. Boronat, Plant J. 27 (2001) 213–222.
[12] H. Jomaa, J. Wiesner, S. Sanderbrand, B. Altincicek, C. Weidemeyer, M. Hintz, I. Türbachova, M. Eberl, J. Zeidler, H.K. Lichtenthaler, D. Soldati, E. Beck, Science 285 (1999) 1573–1576.
[13] J. Schwender, C. Müller, J. Zeidler, H.K. Lichtenthaler, FEBS Lett. 455 (1999) 140–144.
[14] S. Grolle, S. Bringer-Meyer, H. Sahm, FEMS Microbiol. Lett. 191 (2000) 131–137.
[15] B. Miller, T. Heuser, W. Zimmer, FEBS Lett. 481 (2000) 221–226.
[16] B. Veau, M. Courtois, A. Oudin, J.C. Chenieux, M. Rideau, M. Clastre, Biochim. Biophys. Acta 1517 (2000) 159–163.
[17] B. Altincicek, M. Hintz, S. Sanderbrand, J. Wiesner, E. Beck, H. Jomaa, FEMS Microbiol. Lett. 190 (2000) 329–333.
[18] D.E. Cane, C. Chow, A. Lillo, I. Kang, Bioorg. Med. Chem. 9 (2001) 1467–1477.
[19] X. Yin, P.J. Proteau, Biochim. Biophys. Acta 1652 (2003) 75–81.
[20] A. Argyrou, J.S. Blanchard, Biochemistry 43 (2004) 4375–4384.
[21] J. Hans, B. Hause, D. Strack, M.H. Walter, Plant Physiol. 134 (2004) 614–624.
[22] A.T. Koppisch, D.T. Fox, B.S.J. Blagg, C.D. Poulter, Biochemistry 41 (2002) 236–243.
[23] T. Duvold, J.M. Bravo, C. PaleGrosdemange, M. Rohmer, Tetrahedron Lett. 38 (1997) 4769–4772.
[24] J.F. Hoeffler, D. Tritsch, C. Grosdemange-Billiard, M. Rohmer, Eur. J. Biochem. 269 (2002) 4446–4457.
[25] P.J. Proteau, Y.-H. Woo, R.T. Williamson, C. Phaosiri, Org. Lett. 1 (1999) 921–923.
[26] T. Radykewicz, F. Rohdich, J. Wungsintaweekul, S. Herz, K. Kis, W. Eisenreich, A. Bacher, M.H. Zenk, D. Arigoni, FEBS Lett. 465 (2000) 157–160.
[27] D. Arigoni, J.-L. Giner, S. Sagner, J. Wungsintaweekul, M.H. Zenk, K. Kis, A. Bacher, W. Eisenreich, Chem. Commun. (1999) 1127–1128.
[28] A.E. Johnson, M.E. Tanner, Biochemistry 37 (1998) 5746–5754.

[29] C. Phaosiri, PhD Dissertation, Oregon State University, Corvallis, 2004.
[30] M. Rohmer, M. Seemann, S. Horbach, S. Bringer-Meyer, H. Sahm, J. Am. Chem. Soc. 118 (1996) 2564–2566.
[31] D. Arigoni, S. Sagner, C. Latzel, W. Eisenreich, A. Bacher, M.H. Zenk, Proc. Natl. Acad. Sci. USA 94 (1997) 10600–10605.
[32] K. Reuter, S. Sanderbrand, H. Jomaa, J. Wiesner, I. Steinbrecher, E. Beck, M. Hintz, G. Klebe, M.T. Stubbs, J. Biol. Chem. 277 (2002) 5378–5384.
[33] S. Yajima, T. Nonaka, T. Kuzuyama, H. Seto, K. Ohsawa, J. Biochem. 131 (2002) 313–317.
[34] S. Steinbacher, J. Kaiser, W. Eisenreich, R. Huber, A. Bacher, F. Rohdich, J. Biol. Chem. 278 (2003) 18401–18407.
[35] S. Ricagno, S. Grolle, S. Bringer-Meyer, H. Sahm, Y. Lindqvist, G. Schneider, Biochim. Biophys. Acta 1698 (2004) 37–44.
[36] M. Pellecchia, D. Meininger, Q. Dong, E. Chang, R. Jack, D.S. Sem, J. Biomol. NMR 22 (2002) 165–173.
[37] T. Kuzuyama, S. Takahashi, M. Takagi, H. Seto, J. Biol. Chem. 275 (2000) 19928–19932.
[38] T. Kuzuyama, T. Shimizu, S. Takahashi, H. Seto, Tetrahedron Lett. 39 (1998) 7913–7916.
[39] T. Kurz, D. Geffken, C. Wackendorff, Z Naturforsch. 58b (2003) 457–461.
[40] T. Kurz, D. Geffken, C. Wackendorff, Z. Naturforsch. 58b (2003) 106–110.
[41] C. Herforth, J. Wiesner, P. Heidler, S. Sanderbrand, S. Van Calenbergh, H. Jomaa, A. Link, Bioorg. Med. Chem. 12 (2004) 755–762.
[42] O. Meyer, C. Grosdemange-Billiard, D. Tritsch, M. Rohmer, Org. Biomol. Chem. 1 (2003) 4367–4372.
[43] C.A. Testa, M.J. Brown, Curr. Pharm. Biotechnol. 4 (2003) 248–259.
[44] S. Hecht, J. Wungsintaweekul, F. Rohdich, K. Kis, T. Radykewicz, C.A. Schuhr, W. Eisenreich, G. Richter, A. Bacher, J. Org. Chem. 66 (2001) 7770–7775.
[45] R. Meganathan, in: G. Litwack (Ed.), Vitamins and Hormones, vol. 61, Academic Press, San Diego, 2001, pp. 199–201.