Inhibition of the Fe(4)S(4)-Cluster-Containing Protein IspH (LytB): Electron Paramagnetic Resonance, Metallacycles, and Mechanisms.

Wang K, Wang W, No JH, Zhang Y, Zhang Y, Oldfield E

J Am Chem Soc 2010 Apr 28 [abstract on PubMed] [citations on Google Scholar] [related articles] [full text] Discover UIUC full text linking

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Electron paramagnetic resonance (EPR) and electron nuclear double resonance (ENDOR) spectroscopies provide the first strong evidence for a mechanism of the IspH (LytB)-catalyzed reaction, involving a pi/sigma metallacycle intermediate formed with the unique Fe of the Fe4S4 cluster. The knowledge of the mechanism allowed for the discovery of novel acetylenes that bind to IspH and act as inhibitors.

IspH (LytB) is the last enzyme in the nonmevalonate pathway for formation of isoprenoids in bacteria, in which it catalyzes the 2H+/2e- reduction of E-4-hydroxy-3-methyl-but-2-enyl diphosphate (HMBPP) to isopentenyl diphosphate (IPP) or dimethylallyl diphosphate (DMAPP) in a five-to-one ratio. This enzyme is a potential drug target since it is essential in many pathogenic bacteria and Plasmodium falciparum, yet it is not found in humans. The enzyme contains an essential Fe4S4 cluster, and the stoichiometry of the reaction indicates a requirement for reducing equivalents. A number of mechanisms have been proposed for the action of this enzyme, most involving the essential Fe4S4 cluster, yet there has been a dearth of spectroscopic data that would elevate any particular mechanism above the others. Given that the reaction involves loss of a molecule of water, several of these proposals have suggested coordination of the hydroxyl group of HMBPP to the unique site of the Fe4S4 cluster, as occurs in aconitase. This paper by Wang et al. demonstrates, using EPR and ENDOR spectroscopy, that the substrate most likely binds via its double bond to the unique iron of the Fe/S cluster, forming a pi/sigma metallacycle intermediate, similar to the binding of ethylene and allyl alcohol to the nitrogenase FeMo protein cofactor {1-3}. Consistent with this proposal, they find that similarly structured alkynes act as good inhibitors of the protein, with propargyl diphosphate displaying a Ki of 0.97 μM. Using an X-ray crystal structure as a guide, they performed steady-state kinetic analyses on variants of three conserved active site residues (H42, H124, and E126). The significantly decreased Vmax, yet unchanged KM compared to WT, obtained for H124A suggests a role for this residue in catalysis, while the H42A variant is proposed to

be involved in substrate binding due to the higher KM. Importantly, the significantly lower Vmax for the E126A variant suggests that it is also involved in catalysis, specifically to protonate the 4-OH group to allow dehydration to occur. There are similar changes to the EPR spectra of the WT enzyme in the presence of substrate, HMBPP, or product, IPP, compared to enzyme alone, suggesting that the spectrum is that of product bound to the cluster. The addition of substrate to the E126A variant produces a different spectrum than WT, suggested to be a reaction intermediate bound to the cluster in the variant rather than product. The g-values for this spectrum are similar to those for ethylene and allyl alcohol bound to a mutant of nitrogenase FeMo cofactor protein, suggesting the possible formation of a pi/sigma metallacycle intermediate in IspH {1,3}. This was confirmed by ENDOR spectroscopy of 2H and 13C isotopically labeled HMBPPs bound to reduced E126A IspH, which also showed formation of the pi/sigma metallacycle intermediate. These data propose and confirm a novel mechanism for IspH and a method of inhibition, which can allow for the development of future inhibitors of IspH and similar enzymes.

References: {1} Lee et al. J Am Chem Soc 2004, 126:9563-9 [PMID:15291559]. {2} Pelmenschikov et al. Inorg Chem 2008, 47:6162-72 [PMID:18578487]. {3} Lee et al. J Am Chem Soc 2005, 127:15880-90 [PMID:16277531].

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