SUPPLEMENTAL DATA

Alternative Epimerization Involved in C_7N-aminocyclitol Biosynthesis is Catalyzed by ValD, a Large Protein ofthe Vicinal Oxygen Chelate Superfamily

Hui Xu, Yirong Zhang, Jongtae Yang, Taifo Mahmud, Linquan Bai, and Zixin Deng

Table S1. Strains used in this study

Strain	Relevant genotype/comments	Source/ref.
Escherichia coli		
DH10B	*F⁻ mcrA Δ(mrr-hsdRMS-mcrBC) ϕ80lacZΔM15 ΔlacX74 recA1 endA1 araD139 Δ(ara, leu)7697 galU galK λ⁻ rspL nupG*	GibcoBRL
ET12567(pUZ8002)	*dam dcm hsdS,* pUZ8002	Paget et al, 1999
BW25113	K12 derivative:*ΔaraBAD*, *ΔrhaBAD*	Gust et al, 2003
S. hygroscopicus		
5008	wild-type producer of validamycin	
ZYR-4	5008 derivative generated by replacement of 1278-bp DNA fragment internal to *valD* with 1.4-kb *aac(3)IV* cassette	This work
ZYR-4/pJTU926	ZYR-4 complemented with pJTU926 harboring full-length *valD*	This work
ZYR-4/pJTU946	ZYR-4 complemented with pJTU946 harboring the recombinant N-terminal half of *valD*	This work
ZYR-4/pJTU947	ZYR-4 complemented with pJTU947 harboring the recombinant C-terminal half of *valD*	This work
ZYR-4/pJTU3258	ZYR-4 complemented with pJTU3258 harboring the H44N mutated *valD*	This work
ZYR-4/pJTU3259	ZYR-4 complemented with pJTU3259 harboring the E183A mutated *valD*	This work
ZYR-4/pJTU3260	ZYR-4 complemented with pJTU3260 harboring the H229N mutated *valD*	This work
ZYR-4/pJTU3261	ZYR-4 complemented with pJTU3261 harboring the E366A mutated *valD*	This work
ZYR-4/pJTU3266	ZYR-4 complemented with pJTU3266 harboring the H44N and H229N double mutated *valD*	This work
ZYR-4/pJTU3267	ZYR-4 complemented with pJTU3267 harboring the E183A and E366A mutated *valD*	This work

Table S2. Plasmids used in this study

Plasmids	Relevant genotype/comments	Source/ref.
pHZ1358	*tsr*, *bla*, *oriT, ori*(pIJ101)	Sun et al, 2002
pHZ2239	pSET152 with the 19kb *Eco*RI fragment from cosmid 20E1	This work
pIJ790	λ-RED(*gam*, *bet*, *exo*), *cat*, *araC*, *rep101*^{ts}	3
pIJ773	*aac(3)IV*, *oriT*	3
pRSET B	P_{T7} RBS 6×His Xpress^{TM} Epitope EK, *bla*	Invitrogen
pMD18 T-vector	pUC18 derivative	TaKaRa
pPM927	*tsr*, *oriT*, *int*, *attP*	Smokvina et al, 1990
pJTU968	pRSET BpRSET B derivative *bla, PermE**	This work
pValD	1.4-kb *valD* fragment cloned in pRSET BpRSET B	This work
pJTU712	7.8-kb BclI fragment from pHZ2239 cloned in BamHI-digested pHZ1358	This work
pJTU713	pJTU712 recombinant with 1384-bp *aac(3)IV* cassette through Redirect Technology	This work
pJTU926	pPM927 carrying *PermE** and *valD*	This work
pJTU942	pRSET B carrying the 603-bp N-terminal half of *valD* (BamHI/EcoRI)	This work
pJTU943	pRSET BpRSET B carrying the 723-bp C-terminal half of *valD* (BamHI/EcoRI)	This work
pJTU946	pPM927 carrying *PermE** and N-terminal half of *valD*	This work
pJTU947	pPM927 carrying *PermE** and C-terminal half of *valD*	This work
pJTU3250	pRSET BpRSET B carrying the 1.4-kb H44N mutated *valD*	This work
pJTU3251	pRSET B carrying the 1.4-kb E183A mutated *valD*	This work
pJTU3252	pRSET B carrying the 1.4-kb H229N mutated *valD*	This work
pJTU3253	pRSET B carrying the 1.4-kb E366A mutated *valD*	This work
pJTU3258	pPM927 carrying *PermE** and H44N mutated *valD*	This work
pJTU3259	pPM927 carrying *PermE** and E183A mutated *valD*	This work
pJTU3260	pPM927 carrying *PermE** and H229N mutated *valD*	This work
pJTU3261	pPM927 carrying *PermE** and E366A mutated *valD*	This work
pJTU3262	pRSET B carrying the H44N/H229N mutated *valD*	This work
pJTU3263	pRSET B carrying the E183A/E366A mutated *valD*	This work
pJTU3266	pPM927 carrying *PermE** and H44N/H229N mutated *valD*	This work
pJTU3267	pPM927 carrying *PermE** and E183A/E366A mutated *valD*	This work
pJTU3289	pRSET B carrying the 1.4-kb E107A mutated *valD*	This work
pJTU3290	pRSET B carrying the 1.4-kb H130N mutated *valD*	This work
pJTU3291	pRSET B carrying the 1.4-kb E291A mutated *valD*	This work
pJTU3292	pRSET B carrying the 1.4-kb H315N mutated *valD*	This work

Table S3. Primers used in this study

Primer	Sequence (5'-3')
ValD-PCR-F	GTGCTCTTCTCCGACCGCCCGAACGCACGTGAAGGAGCC attccggggatccgtcgacc
ValD-PCR-R	TCAGCATGGAGTGCGCGGGTGGTGGTGACAGCCGGTATG tgtaggctggagctgcttc
ValD-Det-F	CCATCACCTAGCCTGAG
ValD-Det-R	CGCCCTAATCTTCGTC
ValD-N-F	AGGGATCCGCATATGCTCTTCTCCGA (BamHI NdeI)
ValD-N-R	GCGAATTCACTCGTACGGCAACACGT (EcoRI)
ValD-C-F	GGGGATCCACATATGCACGGCCTTC (BamHI NdeI)
ValD-C-R	TAGAATTCAGCATGGAGTGCGCGGGT (EcoRI)
H44N-F	GCCCAACGAGTCGAC**A**ATCTGGCGTTCACCGTG
H44N-R	CACGGTGAACGCCAGAT**T**GTCGACTCGTTGGGC
E107A-F	CGACACCAACCTCG**CC**CTGTTCGCCTACAAG
E107A-R	CTTGTAGGCGAACAG**GG**CGAGGTTGGTGTCG
H130N-F	CACCGGGCACGTG**AA**TCTCGCGCTGCAC
H130N-R	GTGCAGCGCGAG**AT**TCACGTGCCCGGTG
E183A-F	GACGCGCCCCTC**GC**ACTGCGCTCGGTAC
E183A-R	GTACCGAGCGCAGT**GC**GAGGGGCGCGTC
H229N-F	GCTCGGTGTCGAC**A**ACTTCGCCAGGACCG
H229N-R	CGGTCCTGGCGAAGT**T**GTCGACACCGAGC
E291A-F	GACCGATACCATC**GCC**CTGGCAAGCTACCG
E291A-R	CGGTAGCTTGCCAG**GG**CGATGGTATCGGTC
H315N-F	GTCGGCGGCAGC**A**ATATCGCCCTGCACG
H315N-R	CGTGCAGGGCGAT**AT**TGCTGCCGCCGAC
E366A-F	CGGCCTCTATGTG**GC**AGTGGTGCGCATGC
E366A-R	GCATGCGCACCAC**T**GCCACATAGAGGCCG

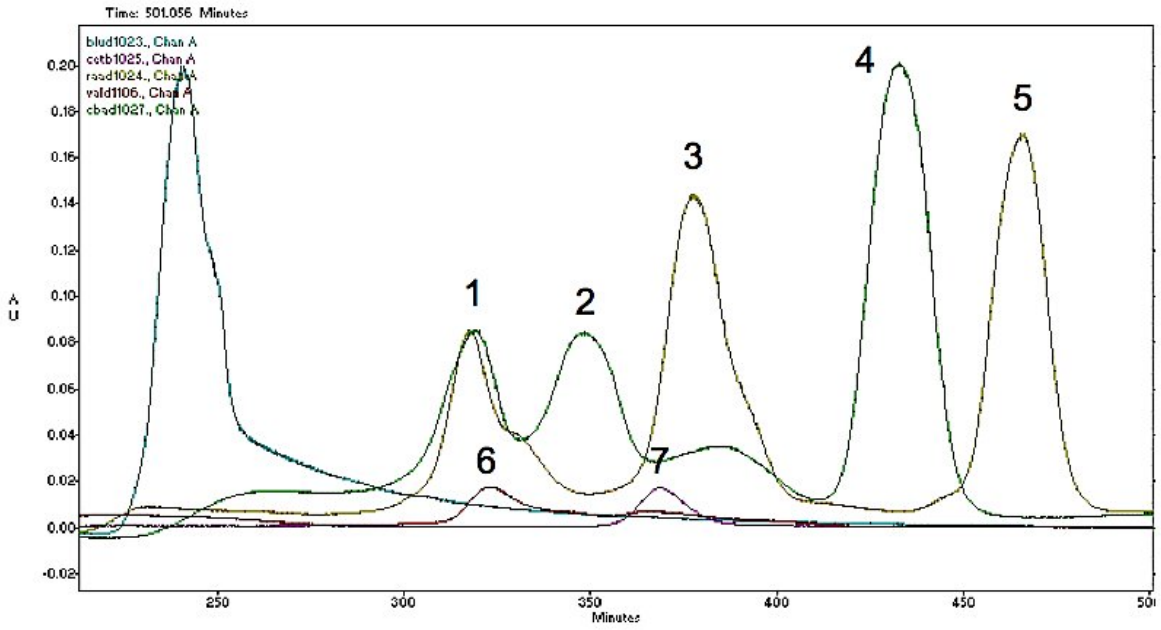

Figure S1. High performance gel filtration chromatography of ValD. 1, alcohol dehydrogenase (150 kDa); 2, BSA (66 kDa); 3, ovalbumin (45 kDa); 4, chymotrypsinogen A (25 kDa); 5, ribonuclease A (13.7 kDa); 6, ValD (dimer); 7, CetB (dimer).

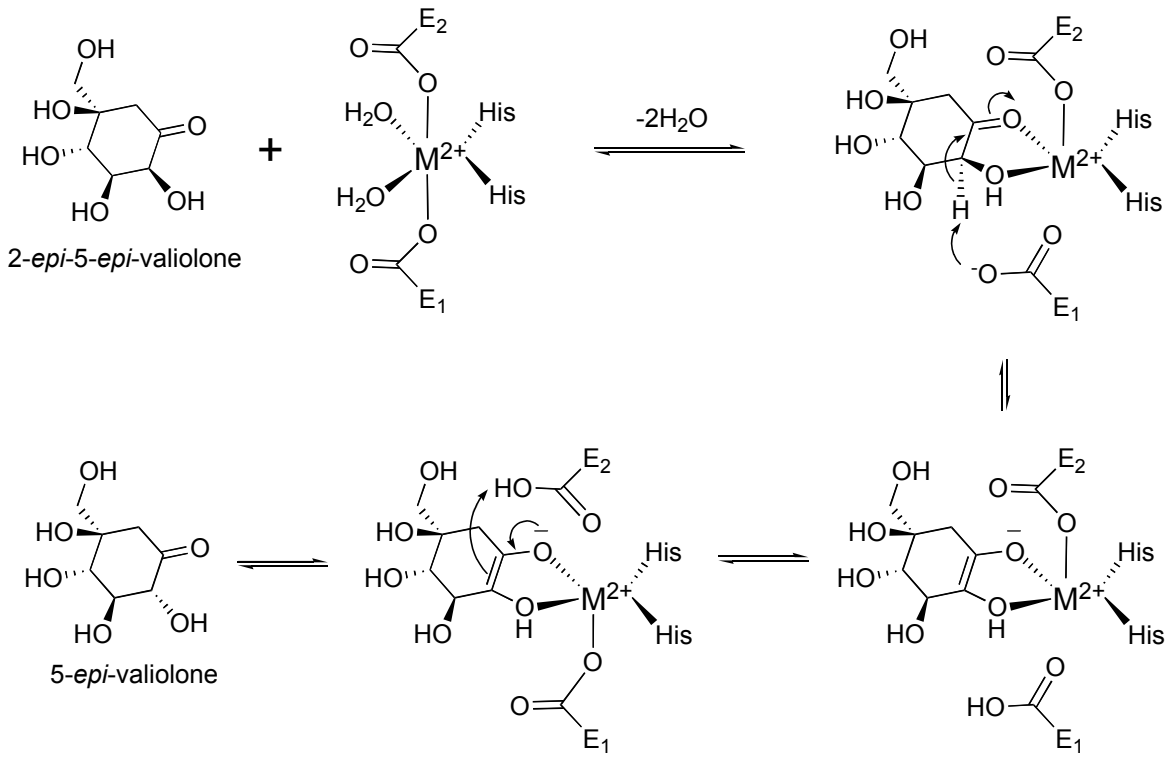

Figure S2. Proposed mechanism for the epimerization of 2-*epi*-5-*epi*-valiolone catalyzed by ValD.