STRUCTURAL BIOLOGY COMMUNICATIONS

Volume 75 (2019)

Supporting information for article:

Functional and structural characterization of IdnL7, an adenylation enzyme involved in incednine biosynthesis

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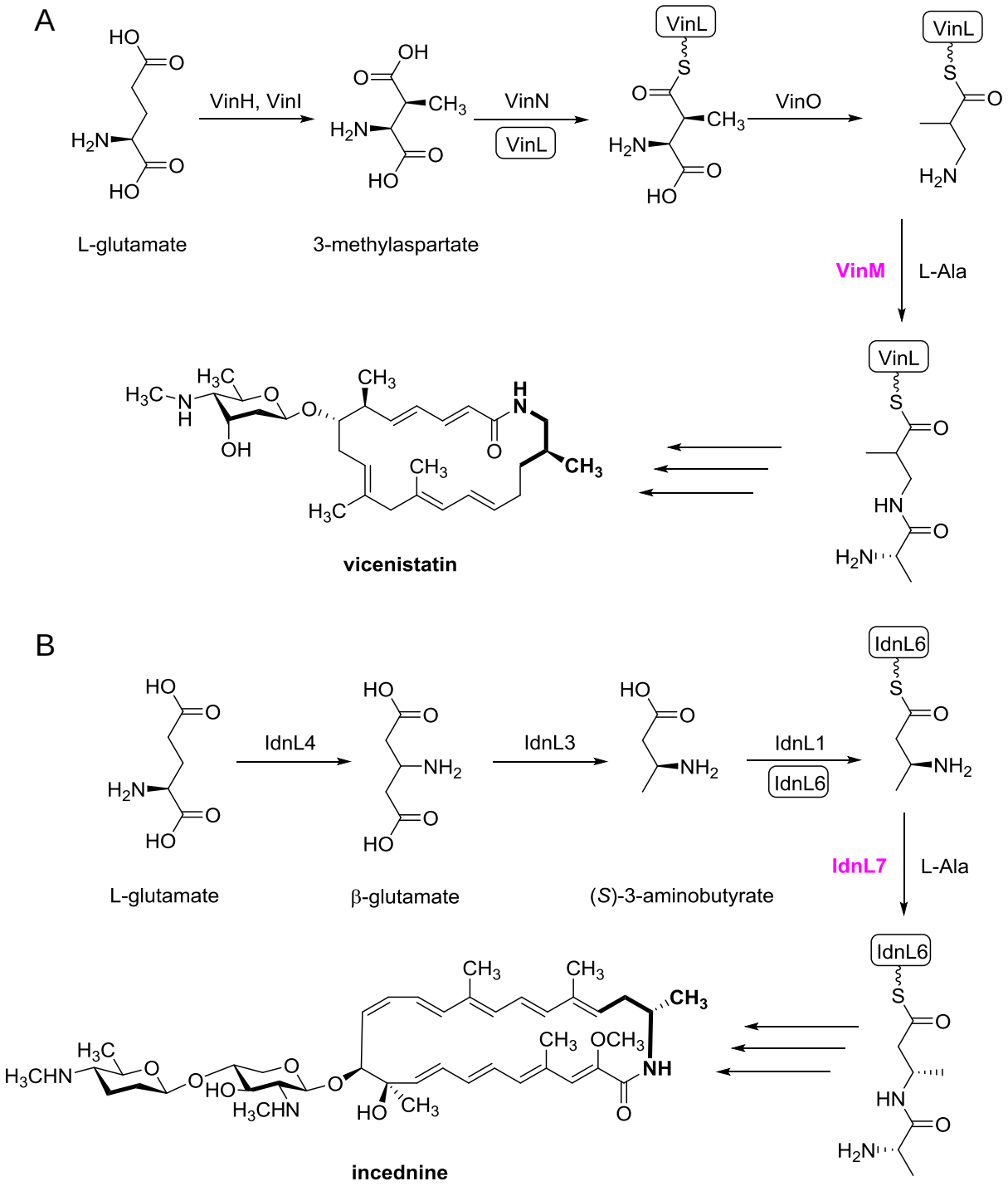

Figure S1 Biosynthetic pathway for vicenistatin (A) and incednine (B) including the reaction of VinM and IdnL7 (pink).

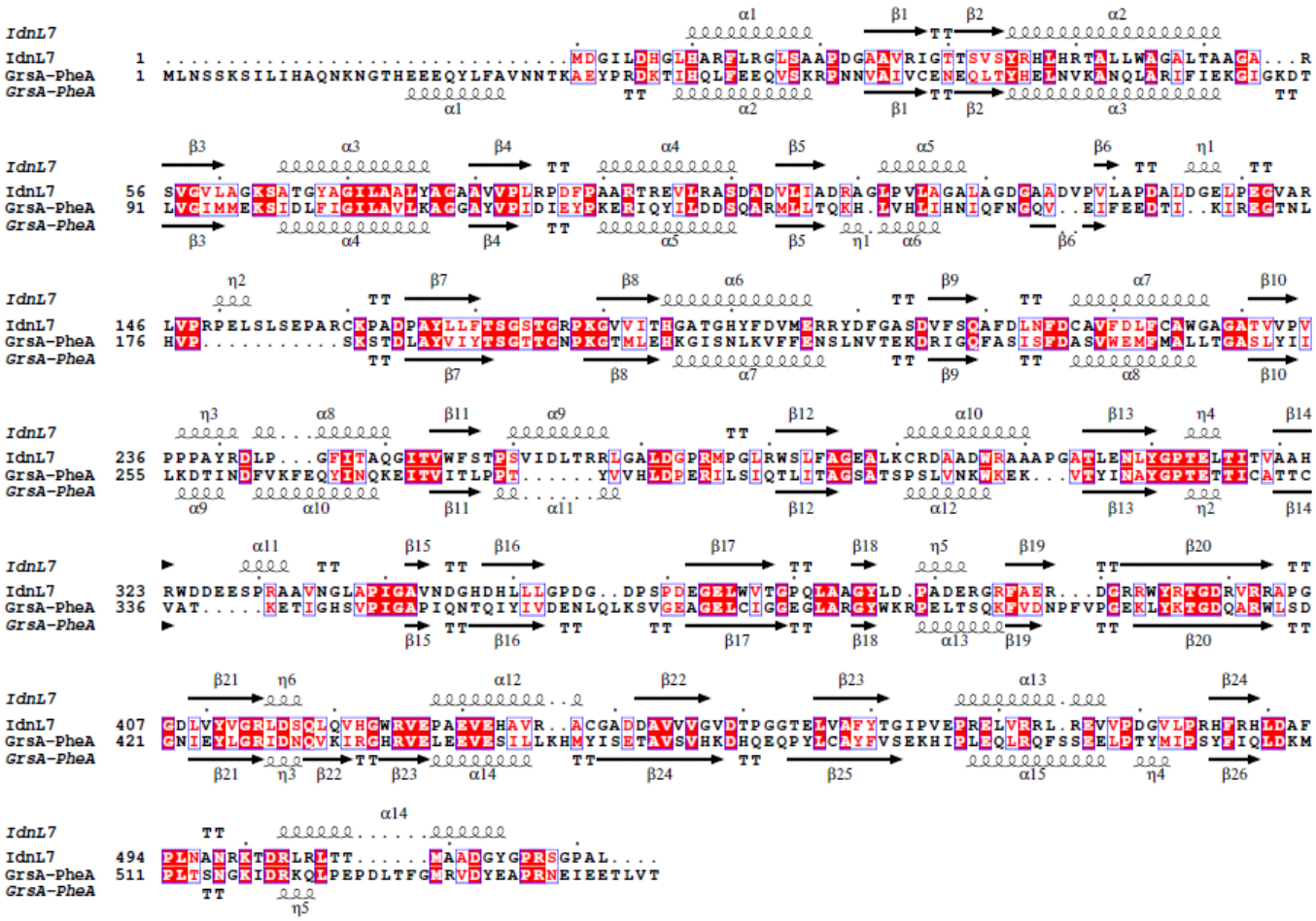

Figure S2 Sequence alignment of IdnL7 and PheA. Secondary structures of IdnL7 and PheA are indicated above and below the sequences, respectively. Conserved residues are boxed with a red background. Sequences was aligned with T-Coffee package (Notredame *et al.* 2000) and analyzed with ESPript (Robert *et al.* 2014).

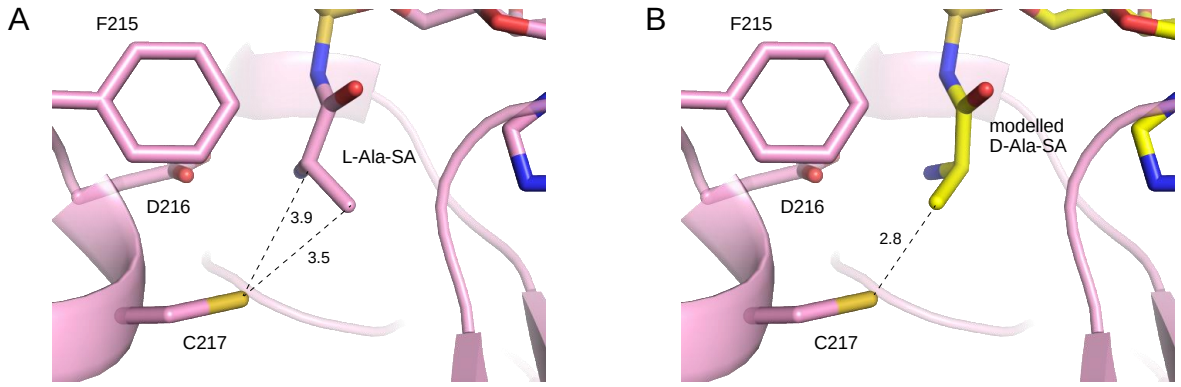

Figure S3 Comparison of the binding mode of L-Ala-SA with that of D-Ala-SA. (A) The crystal structure of IdnL7 in complex with L-Ala-SA. (B) IdnL7 structure modelled with D-Ala-SA. The D-Ala-SA molecule is shown as yellow sticks. The methyl side-chain of D-Ala-SA is very close (2.8 Å) to the S atom of Cys217 side-chain.

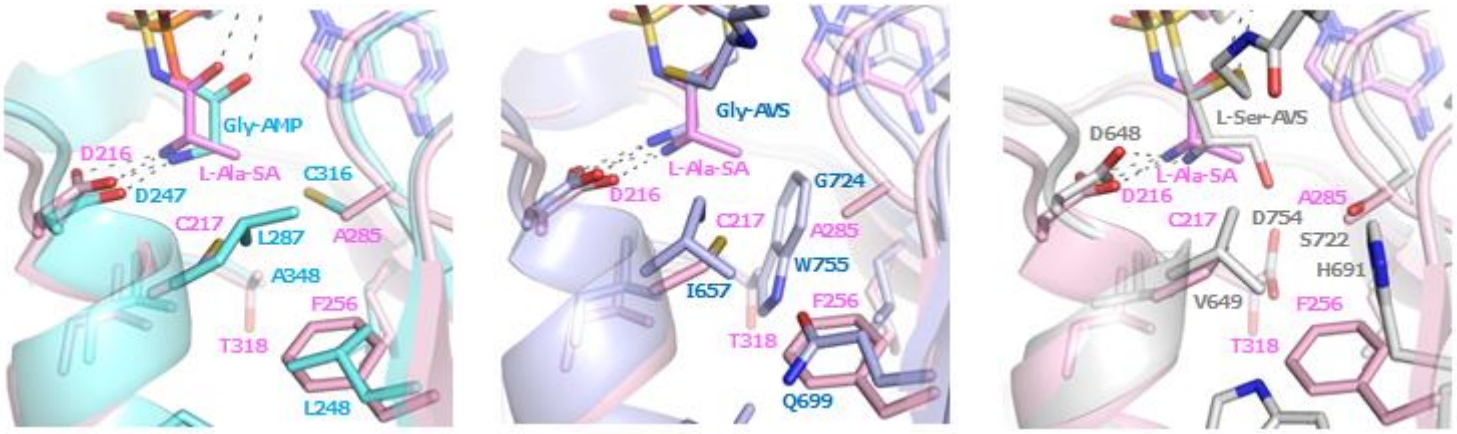

Figure S4 Superimposed substrate binding pocket of IdnL7 (pink) and *Vibrio cholerae* AlmE (cyan) on the left (Henderson *et al.* 2014; PDB code 4OXI), *Bacillus subtilis* DhbF (slate) in the middle (Tarry *et al.* 2017; PDB code 5U89) and *Salmonella typhimurium* EntF (grey) on the right (Miller *et al.* 2016; PDB code 5T3D).

S1. Synthesis of 5'-O-[N-(L-alanyl)sulfamoyl]adenosine (L-Ala-SA)

Synthesis of L-Ala-SA was carried out according to literature procedures (Anderson *et al.*, 1964; May *et al.*, 2005; Van de Vijver *et al.*, 2008).

N-(*tert*-butyloxycarbonyl)-L-alanine (0.33 g, 1.7 mmol) and *N*-hydroxysuccinimide (0.2 g, 1.7 mmol) were dissolved in 1,4-dioxane (8 mL) at 0 °C. Then dicyclohexylcarbodiimide (0.44 g, 2.1 mmol) was added with stirring. Solution was kept at 0 °C for 1 h and the reaction was continued at room temperature for 20 h. Formed urea was separated by filtration and the filtrate evaporated to dryness to give 3.98 g of crude product. Recrystallization from chloroform-hexane yielded the pure *N*-Boc-L-alanyl *N*-hydroxysuccinimide (184 mg, 40%).

N-Boc-L-alanyl *N*-hydroxysuccinimide (43 mg, 0.15 mmol) and DBU (23 mg, 0.15 mmol) was added to 2′,3′-*O*-isopropylidene-5′-*O*-sulfamoyladenosine (60 mg, 0.15 mmol) in DMF (1 mL). After the mixture was stirred for 2 h at room temperature, solution was extracted with ethyl acetate (20 mL) and washed with brine. Crude product was further purified by silica gel column chromatography (ethyl acetate: methanol, 10:1, v/v) to give pure 2′,3′-*O*-isopropylidene-5′-*O*-[*N*-(Boc-L-alanyl)sulfaomyl]adenosine (45 mg, 68%).

A solution of 2′,3′-*O*-isopropylidene-5′-*O*-[*N*-(Boc-L-alanyl)sulfaomyl]adenosine (28 mg, 0.07 mmol) in THF/H_2O (0.4 mL, 5:2, v/v) was stirred for 7 h at room temperature. Next, the volatiles were removed under reduced pressure and the residue was coevaporated three times with ethanol. The residue was purified by Sephadex LH-20 column chromatography (methanol 70% in water). Fractions containing pure product were combined and lyophilized from water to give 5′-*O*-[*N*-(L-alanyl)sulfamoyl]adenosine (12.5 mg, 72%).

^{1}H NMR (400 MHz in D_2O): δ 1.37 (d, 1H, *J* = 7.6 Hz), 3.75 (q, 1H, *J* = 7.2 Hz), 4.34-4.65 (m, 5H), 6.08 (d, 1H, *J* = 4.4 Hz), 8.34 (s, 1H), 8.43 (s, 1H).

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