Supplemental Data

Formation of an Aminoacyl-*S*-Enzyme Intermediate is a Key Step in the Biosynthesis of Chloramphenicol

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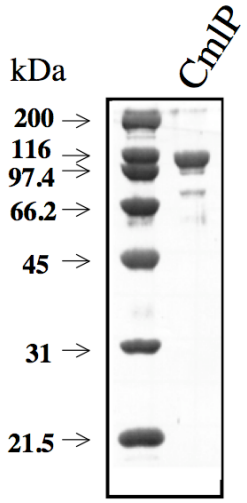
Cloning *of* cmlP The gene encoding CmlP was PCR amplified from *S. venezuelae* genomic DNA using the following primers: *CmlP4/Nde*I (5'- CAA CAT ATG GAA CTC CCT GAC GTG TTG- 3') and *CmlP*/*Hin*dIII (5'- CAT GGT AAG CTT CGG CTC CGT TCC TGT CAG CGC- 3'). The amplicon was subsequently digested with *Nde*I and *Hin*dIII and cloned into the corresponding sites of pET16b (Novagen). The cloned gene was sequenced to ensure no PCR-induced mutations occurred.

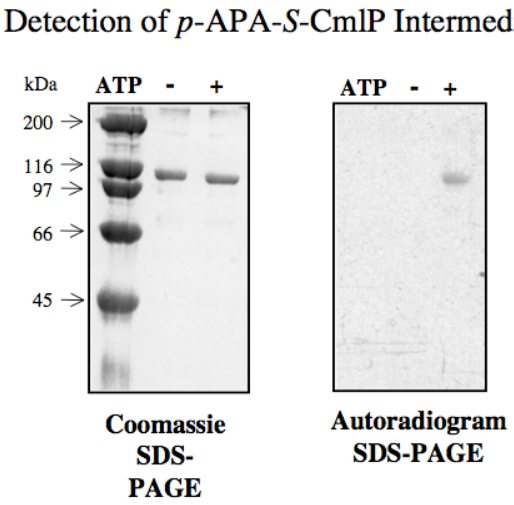
Heterologous Overproduction of CmlP The expression construct was transformed into *E. coli* BL21(DE3) to produce the *apo*-protein with an *N*-terminal, deca-histidine affinity tag. To produce the corresponding *holo*-protein, *E. coli* BL21 (DE3) was co-transformed with pH10-CmlP and pSU20-Sfp,which harbors the gene encoding Sfp, the phosphopanthetheinyl transferase from *B. subtilis*. Cells were grown were grown in LB media with ampicillin (and chloramphenicol for pSU20-Sfp selection) at 20°C for 36 h. Cells were harvested by centrifugation.

Purification of CmlP The harvested cells were resuspended in 25 mM Tris-HCl (pH 8.0), 400 mM NaCl, 2 mM imidazole, 10 mM $MgCl_2$, and 1 mM tris-(2-carboxyethyl)phosphine hydrochloride. The resuspended cells were broken using a French Press and the cell debris was removed by centrifugation. The supernatant was incubated with 1 ml of Ni^{2+}-NTA Superflow Resin (Qiagen) for 4 hr at 4°C. After recovery and washing of the resin, the protein was eluted in this buffer with a step gradient of increasing imidazole concentration (5, 20, 40, 60 and 200 mM). CmlP eluted at 20 mM imidazole as determined by SDS-PAGE with Coomassie staining. The eluted protein was then dialyzed against 50 mM Tris-HCl, pH 8.0, 50 mM NaCl, 10% glycerol. The protein was then subjected to ion exchange chromatography using a 5 ml Q-Sepharose Hi-Trap column (Amersham Biosciences). The protein was eluted in this buffer using a linear gradient of increasing NaCl concentration from 50 mM to 500 mM. An coommassie-stained SDS-PAGE gel showing purified CmlP is shown in Supplemental Data Figure 1. The protein concentration was determined from its calculated molar extinction coefficient.



Supplemental Data Figure 1. 12% acrylamide SDS-PAGE gel of purified CmlP

Autoaminoacylation of holo-CmlP. To assay autoaminoacylation, *holo*-CmlP was incubated with [^{3}H]-L-*p*-aminophenylalanine and with or without ATP. The enzyme's incorporation of the radiolabel was measured by liquid scintillation counting and by autoradiography. An autoradiogram of such a reaction is shown in Supplemental Data Figure 2.



Supplemental Data Figure 2. (Left) Coomassie-stained SDS-PAGE gel of purified holo-CmlP incubated with [^{3}H]-L-*p*-aminophenylalanine with and without ATP. (Right) Autoradiogram of the same gel.