Characterization of 5-Chloro-5-Deoxy-D-Ribose 1-Dehydrogenase in Chloroethylmalonyl Coenzyme A Biosynthesis

SUBSTRATE AND REACTION PROFILING

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Andrew J. Kale‡, Ryan P. McGlinchey‡, and Bradley S. Moore‡§

From the ‡Department of Chemistry, ‡Center of Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, and ‡Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California at San Diego, La Jolla, California 92093

SalM is a short-chain dehydrogenase/reductase enzyme from the marine actinomycete Salinispora tropica that is involved in the biosynthesis of chloroethylmalonyl-CoA, a novel halogenated polyketide synthase extender unit of the protease inhibitor salinosporamide A. SalM was heterologously overexpressed in Escherichia coli and characterized in vitro for its substrate specificity, kinetics, and reaction profile. A sensitive real-time $^{13}$C NMR assay was developed to visualize the oxidation of 5-chloro-5-deoxy-D-ribose to 5-chloro-5-deoxy-D-ribono-$\gamma$-lactone in an NAD$^+$-dependent reaction, followed by spontaneous lactone hydrolysis to 5-chloro-5-deoxy-D-ribonate. Although short-chain dehydrogenase/reductase enzymes are widely regarded as metal-independent, a strong divalent metal cation dependence for Mg$^{2+}$, Ca$^{2+}$, or Mn$^{2+}$ was observed with SalM. Oxidative activity was also measured with the alternative substrates D-erythrose and D-ribose, making SalM the first reported stereospecific non-phosphorylative ribose 1-dehydrogenase.

The marine actinomycete Salinispora tropica produces a suite of $\gamma$-lactam/$\beta$-lactone natural products identified as potent 20 S proteasome inhibitors (1). Exploration into the biosynthesis of the most bioactive family member, salinosporamide A, resulted in the characterization of a pathway for the biosynthesis of chloroethylmalonyl-CoA, a novel polyketide synthase substrate (Fig. 1) (2). Previous gene replacement of salM, which encodes a short-chain dehydrogenase/reductase (SDR)$^2$ enzyme, dramatically and selectively reduced the production of salinosporamide A by $\sim 98\%$ relative to the wild-type organism, whereas production of the nonchlorinated salinosporamide B remained unchanged. As salinosporamide B is alternatively produced from ethylmalonyl-CoA, the specific role of SalM in the biosynthesis of chloroethylmalonyl-CoA was established (2). Furthermore, the in vivo substrate of SalM was identified in this mutant strain as 5-chloro-5-deoxy-D-ribose (5-CIR) by detection of the accumulated fermentation product (2). Chemical complementation with 5-CIR to a separate upstream mutation in the chloroethylmalonyl-CoA pathway via the chlorinase SalM restored salinosporamide A production (2). Thus, on the basis of our understanding of the chloroethylmalonyl-CoA pathway, we predicted that SalM would oxidize 5-CIR at the anomeric carbon by acting as a pentose 1-dehydrogenase.

It is intuitive to presume that SalM evolved from a primary metabolic ribose 1-dehydrogenase to oxidize a halogenated sugar derivative. However, despite the ubiquitous nature of ribose in biology, non-phosphorylative ribose 1-dehydrogenases (EC 1.1.1.115) have not been well characterized. Instead, pentose catabolism utilizes phosphorylated intermediates in the pentose phosphate pathway, nucleotide metabolism, and pentose-glucuronate conversion. Phosphorylated pentoses are also used in anabolic pathways such as the Calvin-Benson cycle and in the generation of nucleosides. The only previously reported “ribose 1-dehydrogenase” was isolated from pig liver and oxidized both D-ribose and D-xylose with approximately equal activity (3). Oxidative enzyme activity for ribose has been reported as an alternative substrate for other sugar oxidoreductase enzymes with broad substrate specificity (4–8); however, a non-phosphorylative pentose 1-dehydrogenase specific to the stereochemistry of ribose has yet to be reported.

Potentially related pentose 1-dehydrogenases such as L-arabinose 1-dehydrogenase and D-xylose 1-dehydrogenase have been shown to oxidize a cyclical hemiacetal substrate to the corresponding lactone (5, 6, 9, 10). Glucose 1-dehydrogenase has also been reported to possess “gluconolactonase” activity, catalyzing both the oxidation of glucose to gluconolactone and the subsequent hydrolysis to gluconate (6). The next anticipated enzyme in the chloroethylmalonyl-CoA biosynthetic pathway, SalH, is a dihydroxy-acid dehydratase and expected to accept 5-chloro-5-deoxyribonate as its substrate. Because the salinosporamide biosynthetic gene cluster (sal) does not encode a putative lactonase enzyme (2), we were compelled to determine whether a lactone intermediate exists and, if so, to decipher the fate of this pathway product. We thus set out to explore whether SalM produces a lactone or an acid or possesses bifunctional dehydrogenase/lactonase activity.

Traditional analysis of oxidoreductase enzymes such as SalM utilizes changes in optical absorption corresponding to...
the conversion of a cofactor such as NAD(P)(H) or FAD(H).

Although this method provides a simple non-invasive way to
monitor redox kinetics, it fails to identify the structure of the
enzymatic product. Subsequent cofactor-independent
reactions such as hydrolysis are thus not observed. Therefore, real-
time visualization of product structures is imperative when
transient intermediates are formed. A sensitive time-arrayed
NMR approach was consequently developed to monitor the
progress of the SalM reaction and to identify structures of inter-
mediates and products. Here, we report a real-time
$^{13}$C NMR-based characterization of SalM, a novel 5-chloro-5-deoxy-
d-ribose 1-dehydrogenase.

**EXPERIMENTAL PROCEDURES**

**Chemicals**—All purchased chemicals were reagent-grade
from Sigma-Aldrich unless noted otherwise. Isopropyl $\beta$-$d$-
thiogalactopyranoside was obtained from Denville Scientific,
$\alpha$-erythrose from Alfa Aesar as a 70% (w/v) syrup, [U-$^{13}$C]
ribose (98% $^{13}$C) from Cambridge Isotope Laboratories,
and nickel-nitrilotriacetic acid (Ni-NTA) from Qiagen. The puta-
tive SalM substrate and products 5-ClR (11), 5-chloro-5-deoxy-
d-ribose (5-ClRI) (2) were all synthesized according to literature
procedures.

**Expression and Purification of Recombinant SalM—**

Ge-\n
dnomic DNA was obtained from cultures of *S. tropica* CNB-440
as described previously and used as a template for PCR (13).
The 768-bp *salM* gene (Stro_1027) was PCR-amplified from
genomic DNA using *Pfu* polymerase (Stratagene, La Jolla, CA)
with forward primer 5'-CGTGTGCCATGCGACGCGGC-3' and reverse primer 5'-GCTGAGTTACAGTGGTGC-3'. The PCR product was digested with Ncol and HindIII (the intro-
duced restriction sites are underlined) and ligated into NcoI/
HindIII-digested pHIS8, and its sequence was verified
in a Superdex 200 column (GE Healthcare) and resuspended in 50 mM
sodium phosphate buffer adjusted to pH 8.0, 500 mM NaCl, and 2 mM
dithiothreitol.

**Construction of C-terminal Mutants—** SalM C-terminal mu-
tants were PCR-amplified from genomic DNA with forward
primer 5'-GCATACCATAGATTGAGACGCGGCGCCTAT-3' and the following reverse primer for the
specified mutant: Q255E, 5'-GCTGAGATTACAGTGGTGCACGCGGCCGCAATCTC-3'; Q255N, 5'-ATTGAGAGCTGCGGGCGCTCAGTT-
GCGAGGTTACACTCCTCGTA-3'; Q255V, 5'-ATTGAGAGCTGCGGGCGCTCAGGACGCGGGCGCTCAGTT-
GCGAGGTTACACTCCTCGTA-3'; A254-Q255 deletion, 5'-ATTGAGAGCTGCGGGCGCTCAGTT-
GCGAGGTTACACTCCTCGTA-3'; and Q255 deletion, 5'-ATTGAGAGCTGCGGGCGCTCAGTT-
GCGAGGTTACACTCCTCGTA-3'.

All protein purification steps took place at 4°C. Protein puri-
ification buffers contained 300 mM NaCl, 50 mM sodium phos-
phate adjusted to pH 8.0, and increasing concentrations of
imidazole. Buffers A (lysis), B (wash), and C (elution) contained
10, 20, and 250 mM imidazole, respectively. Cells were pelleted
at 6300 × g for 45 min, resuspended in buffer A, and lysed with
six 30-s bursts of probe sonication with resting periods of 30 s.
The lysate was centrifuged for 30 min at 10,000 × g. Soluble
protein was collected and purified on an Ni-NTA column by
washing with several volumes of buffer B and eluting with 2.5
ml of buffer C. Eluant was desalted using a PD-10 desalting
column (GE Healthcare) and resuspended in 50 mM
sodium phosphate buffer adjusted to pH 8.0. Desalted protein was con-
centrated on a Vivaspin 6 10-kDa membrane centrifuge con-
centrator (Sartorius Stedim Biotech S.A., Aubagne Cedex,
France) and then subjected to size exclusion chromatography
on a Superdex 200 column (GE Healthcare) with 100 mM Tris-
HCl adjusted to pH 8.0, 500 mM NaCl, and 2 mM disothio-
purification was carried out in a manner analogous to that described for wild-type SalM.

**Enzyme Assays—**In vitro enzyme assays were performed in a Greiner 96-well half-area microtiter plate. Conversion of NAD$^+$ to NADH was monitored at a wavelength of 340 nm using a SpectraMax M2 spectrometer (Molecular Devices, Sunnyvale, CA). All microplate assays were performed at 30 °C in a 50-μl volume with 100 mM Tris-HCl (pH 7.5) unless noted otherwise.

**Divalent Cation Analysis—**To identify suitable metal cofactors, SalM was assayed for activity with 0.5 mM 5-CIR, 0.5 mM NAD$^+$, and 2.4 μg (0.048 mg/ml) SalM added. These assays were performed in triplicate and averaged. The maximum velocity under steady-state conditions for each concentration was fitted with a linear line using SigmaPlot 11.0 (Systat Software, Inc., Chicago, IL).

**Comparative Substrate Assay—**Ten sugars were assayed for activity with SalM: d-ribose, 2-deoxy-d-ribose, d-ribose 5-phosphate, 5-CIR, d-erythrose, d-alloose, d-glucose, d-xylene, d-arabinose, and l-arabinose. A final concentration of 2 mM carbohydrate was used for all substrates with excess NAD$^+$ cofactor at 2.5 mM. SalM (1.6 μg, 0.032 mg/ml) was added to each 50-μl reaction buffered with 100 mM Tris-HCl (pH 7.5) containing 2 mM MgCl$_2$. After enzyme addition, absorbance measurements were recorded every minute for 3 h.

**Kinetics Assays—**Kinetic data were determined for d-ribose, d-erythrose, and 5-CIR. All reactions contained 2.4 μg SalM (0.048 mg/ml), presoaked in 10 mM MgCl$_2$, and 4 mM NAD$^+$ cofactor. Substrate concentrations versus enzyme velocities were plotted on SigmaPlot 11.0 and fit with a nonlinear Michaelis-Menten curve. Concentrations of d-ribose ranged from 0.5 to 200 mM, representing a range of 0.3–10.8 $K_m$; whereas concentrations of d-erythrose ranged from 0.10 to 40 mM, representing a range of 0.4–16 $K_m$. However, the $K_m$ for 5-CIR was at the lower limit of detection for NADH absorbance. Therefore, kinetic assays with 5-CIR were repeated on a 100-μl scale to increase the absorbance path length. The concentration of SalM was reduced to 1.2 μg/reaction (0.012 mg/ml). Concentrations tested for 5-CIR ranged from 10 μM to 10 mM, representing a range of 0.6–600 $K_m$.

**Lactone Opening Assay—**A colorimetric assay for the detection of functionalized carboxylic acids was used to monitor the hydrolysis of 5-CIRL to 5-CIRI. 10 mM synthetically prepared 5-CIRL was dissolved in 100 mM Tris-HCl (pH 7.5), and 3 ml was aliquoted into two identical tubes. Active or denatured (boiled for 10 min) SalM (0.008 mg/ml), both presoaked with 10 mM MgCl$_2$, was added to the 5-CIRL solution. Two 200-μl aliquots were removed from each tube at regular intervals and subjected to derivatization and colorimetric analysis as described previously (15). The experiment was repeated with 0.5 mM NAD$^+$ and 0.5 mM NADH present in the buffer. Absorbance measurements were converted to 5-CIRL concentration by reference to a standard curve generated at the time of the assay. To determine the hydrolysis rate constants, a linear line was fit to the plot of the natural logarithm of lactone concentration versus time using SigmaPlot 11.0.

**NMR-based Assays—**Carbon-detected NMR experiments were measured on a Varian VX500 spectrometer equipped with an Xsens TM Cold Probe. All assays were performed with the sample chamber set at a constant temperature of 30 °C. Carbon-free 60 mM sodium phosphate buffer at pH 7.5 was used instead of Tris-HCl. The final reaction volume was 250 μl in a 3-mm diameter NMR tube.

**Uniformly 13C-Labeled Ribose—**A 3 mM solution of [U-13C]ribose, 3.5 mM NAD$^+$, and 2 mM MgCl$_2$ in 200 μl of 62.5 mM sodium phosphate buffer (pH 7.5) with $\sim$30% deuterium oxide was placed in the NMR tube. To the reaction was added 35 μg of SalM (in a 50-μl volume of 50 mM sodium phosphate buffer at pH 7.5) for a final enzyme concentration of 0.140 mg/ml. One-dimensional 13C NMR spectra were measured using 256 scans with a 1-s $T_1$ relaxation time. A spectrum was taken before enzyme addition, and then following enzyme addition, spectra were recorded every 10–15 min for 4 h. Additional spectra were taken 21 and 72 h after enzyme addition during which the sample was exposed to ambient temperature.

**Unlabeled 5-CIR Distortionless Enhancement of Polarization Transfer (DEPT) NMR Assay—**4 mM unlabeled 5-CIR, 3.5 mM NAD$^+$, and 2 mM MgCl$_2$ were dissolved into 200 μl of 62.5 mM sodium phosphate buffer (pH 7.5). To the reaction was added 24 μg of SalM (in a 50-μl volume of 50 mM sodium phosphate buffer at pH 7.5) for a final enzyme concentration of 0.120 mg/ml. The final deuterium oxide concentration was $\sim$50%. A 2048-scan DEPT135 spectrum with a $T_1$ of 1 s was recorded before enzyme addition and then repeatedly following enzyme addition for the first four spectra. Each acquisition required $\sim$68 min. A final spectrum was started 8 h after enzyme addition.

**RESULTS**

**Bioinformatics Analysis—**Amino acid sequence similarity to SalM was used to identify potential enzymatic homologs. BLAST analysis of the 255-amino acid sequence of SalM indicated a classical SDR enzyme (16). On the basis of previously reported phylogenetic analyses of the SDR superfamily, it was expected that SalM should perform a simple ketone/alcohol redox reaction and would not participate in any additional chemistry such as epimerization, decarboxylation, or dehydration (16, 17). The highest scoring sequence was an uncharacterized 67% identical protein (accession number YP_638874) from several terrestrial *Mycobacterium* species (strains KMS, MCS, and JCS). SalM shows 40% sequence similarity to annotated glucose 1-dehydrogenases from *Listeria grayi* DSM 20601 and *Brevibacillus brevis* NBRC 100599 (accession numbers ZP_04443055 and YP_002770578, respectively).

**Enzyme Purification and Cofactor Identification—**Recombinant SalM was expressed in *E. coli* BL21(DE3) for in vitro characterization. The N-terminal His$_4$-tagged enzyme was purified by Ni-NTA affinity chromatography and afforded $\sim$20 mg/liter recombinant protein at $\geq$90% purity. All enzyme activity assays utilized the tagged protein without further purification because
A 5-Chloro-5-deoxy-D-ribose 1-Dehydrogenase

Substrate Specificity and Kinetics—To identify enzyme substrate specificity, 10 different sugars were assayed. Only 5-CIR, D-ribose, and D-erythrose showed activity, with 5-CIR being the preferred substrate. Sugars tested and found inactive (<2% activity relative to 5-CIR) included 2-deoxy-D-ribose, D-ribose 5-phosphate, D-xlyose, D-arabinose, L-arabinose, D-allose, and D-glucose. The $K_m$ differed significantly among the three substrates, with 5-CIR binding to SalM being 2 orders of magnitude greater than D-erythrose and 3 orders of magnitude greater than D-ribose (Table 1). $V_{max}$ values were comparable.
Upon the addition of SalM, the consumption of ribose was observed as the four $^{13}$C doublets between 93 and 102 ppm representing the anomeric C1 positions of the ribose congeners decreased in intensity over time. With the oxidation of C1, a new doublet of weak intensity likewise emerged at 178.9 ppm. Unfortunately, because the chemical shifts of the C1 carbonyls of ribono-$\gamma$-lactone and ribonate standards are nearly identical, we turned our attention to other more diagnostic signals for analysis. Significantly, two clear signals emerged characteristic of ribono-$\gamma$-lactone: a doublet of doublets centered at 87.4 ppm and a doublet centered at 61.1 ppm corresponding to C4 and C5, respectively. No peaks corresponding to ribono-$\delta$-lactone were observed, suggesting that the less abundant furanose is the preferred enzyme substrate. As the reaction progressed further, these characteristic lactone peaks decreased in intensity with the concomitant emergence of a new cluster of signals at 72–74 ppm corresponding to C2–C4 of ribonate. It is evident that the initial product of SalM was a five-membered lactone, which was then hydrolyzed to an acid. However, the role of SalM in lactone hydrolysis remained unclear.

We next explored the putative natural substrate 5-ClR using a complementary NMR spectroscopic strategy. Because we instead used unlabeled material, we utilized the coherence transfer spectroscopic technique DEPT, which resulted in enhanced 4-fold sensitivity (23). The DEPT135 experiment allowed the visualization of all protonated carbons with differential phasing of methylene versus methyl and methine carbons.

Although the use of 5-ClR simplified the NMR spectrum by eliminating the carbon signals pertaining to the two pyranose anomers, the increased scan time of this assay from 9 to 68 min complicated the analysis by allowing the two ribose moieties per NAD(H) cofactor molecule to be equally visible. This scenario posed a challenge to differentiate the product profile from that of the cofactor. To simplify this dilemma, we identified a diagnostic set of signals to monitor throughout the enzymatic reaction pertaining to the C5 ribose methylene carbons. C5 of the chlorinated sugar substrate was significantly upfield-shifted in relation to the phosphate-attached cofactor riboses. C5 of the chlorinated sugar substrate was significantly upfield-shifted in relation to the phosphate-attached cofactor riboses. This was true as well in the potential products 5-ClRL and 5-ClRI standing in relation to the phosphate-attached cofactor riboses. The DEPT135 experiment allowed the visualization of all protonated carbons with differential phasing of methylene versus methyl and methine carbons.

for all three substrates, indicating that the $K_m$ is the driver of differential activity among the three preferred substrates. $k_{cat}$ values were not calculated due to enzyme aggregation, which led to an unknown fraction of the total SalM enzyme being inactive.

**Carbon NMR Assays of SalM**—To explore the product structure(s) of SalM, we first assayed activity with [U-$^{13}$C]ribose in an arrayed NMR experiment (Fig. 3). A standard $^1$H-decoupled $^{13}$C NMR spectrum of 256 scans was recorded of the reaction mixture immediately prior to the addition of SalM. After enzyme addition, equivalent scans were repeated at selected time points extending up to 72 h. The short scan time of $\sim$9 min allowed only the labeled ribose carbon signals to be readily detected in the assay mixture that also contained NAD$^+$ and its two ribose residues. However, as ribose adopts four cyclical anomeric forms in solution, its NMR spectrum is rather complex for a five-carbon molecule. The six-membered $\alpha$- and $\beta$-pyranoses account for $\sim$21.5 and 58.5%, respectively, of the total sugar at a temperature of 30 °C, whereas the five-membered $\alpha$- and $\beta$-furanoses account for the remaining 6.5 and 13.5%, respectively (22). On the other hand, the open-chain aldehyde is only a transient intermediate and thus not observed by NMR analysis. Upon oxidation of the anomeric C1, we anticipated that the spectrum would significantly simplify as the reaction progressed to give a single product.
appeared at 43.2 ppm correlating to C5 of 5-ClRL, which eventually gave way to a second product peak at 48.0 ppm relating to C5 of 5-ClRI, confirming the result of the labeled ribose experiment.

**Lactone Opening Assay**—The NMR assays established that the SalM reaction involves the enzymatic oxidation of a furanose hemiacetal to a lactone. To identify the role of SalM in the subsequent hydrolysis of the lactone to the corresponding carboxylic acid, a colorimetric assay was employed. Active and boiled SalM enzymes were separately added to solutions of 10 mM 5-ClRL in 100 mM Tris (pH 7.5) and analyzed colorimetrically for lactone concentration at periodic time points. At all time points, the concentration of lactone was approximately equal regardless of whether the active or boiled control enzyme was added (supplemental Tables S1 and S2), indicating that SalM does not actively participate in the hydrolysis of the lactone.

The hydrolysis rate of lactones in aqueous solution is known to follow second-order kinetics, dependent on both lactone concentration and hydroxide concentration (pH) (24). Lactone hydrolysis may liberate a proton in basic solutions, which alters the pH. However, if a sufficiently strong buffer is used, this effect is minimum, converting the hydrolysis rate to a pseudo first-order equation. The hydrolysis rates of 5-ClRL in the presence of active SalM and in the absence of SalM were \( 0.0035 \pm 0.0001 \) /min and \( 0.0036 \pm 0.0002 \) /min, respectively (supplemental Fig. S1).

**DISCUSSION**

**Substrate Specificity and Kinetic Analysis**—In this study, we have shown that SalM accepts 5-ClR, d-ribose, and d-erythrose as substrates with varying activity (Fig. 5). In addition to 5-ClR, the 5-fluoro and 5-bromo analogs are presumed as substrates.
A 5-Chloro-5-deoxy-D-ribose 1-Dehydrogenase

**FIGURE 5. SalM-mediated transformation of select furanoses.** SalM oxidizes C1 of furanose carbohydrates with stereochemistry of D-ribose at C2 and C3 to the corresponding γ-lactone. The four-carbon D-erythrose was accepted, whereas the six-carbon D-allose was not, thereby indicating a limit to the size of the C4 furanose substituent. Lactone hydrolysis was found not to be mediated by SalM.

Metal Dependence and Lactonase Activity—Convergent evolution has produced multiple strategies for catalyzing the oxidation of hydroxyls to carbonyls. Two of the most prominent families of such enzymes are the SDRs and the medium-chain dehydrogenases/reductases. Although the reactions catalyzed may be similar, their mechanisms are distinct. Metal dependence is synonymous within the medium-chain dehydrogenase/reductase family, with zinc acting as a catalytic component to activate a coordinated water molecule for abstraction of the hydroxyl proton of the substrate (26). Glucose-1-dehydrogenase from the medium-chain dehydrogenase/reductase family has been reported to oxidize glucose to gluconolactone, followed by “lactonase” activity to hydrolyze the lactone (7, 27, 28). However, no mechanism has been reported for catalysis of this additional functionality.

Unlike the medium-chain dehydrogenase/reductase family, the metal-independent SDRs are typically catalyzed by a lysine-activated tyrosine (17). Because the mechanism of classical SDRs is well established to be metal-independent and because SalM possesses the highly conserved YXXK catalytic group, it is likely that the metal ion is not contributing to substrate oxidation (17). Our initial speculation as to the atypical metal dependence of SalM included the possibility of additional lactonase activity. Lactonase enzymes such as Drp35 from *Staphylococcus aureus* bind a catalytic zinc cation to activate water for hydrolysis of lactones (29). This enzyme was also shown to exhibit lactonase activity when bound to Mg$^{2+}$ or Mn$^{2+}$. However, when SalM was assayed without Mg$^{2+}$, 5-CIR was not oxidized to 5-CIRL, indicating that the metal ion is required for the first step of the reaction and not the latter. As SalM does possess a C-terminal glutamine, as in the case of R-specific alcohol dehydrogenase from *L. brevis*, we anticipate the divalent metal cation to play a similar structural role. This hypothesis is supported by the total loss of solubility for all C-terminal mutants of SalM.

Having established that SalM does not participate in lactone hydrolysis, we explored the possibility of a missing chloroethylmalonyl-CoA biosynthetic enzyme. In metabolic pathways that require lactone hydrolysis, a lactonase is often employed to facilitate the reaction (5, 10, 30, 31). Although the salinosporamide gene cluster does not contain a lactonase, a search of the total genome sequence of *S. tropica* CNB-440 (32) resulted in one annotated gluconolactonase. This gene (Str_0658) is located ~400 open reading frames from the sal locus. Although it is not known if this enzyme participates in the lactone opening of 5-CIRL, it seems unlikely to be specialized for this reaction because *Salinispora arenicola* CNS-205, the closest sequenced relative of *S. tropica*, contains a 92% similar gluconolactonase yet does not contain the salinosporamide gene cluster (33). It is therefore possible that the biosynthesis of chloroethylmalonyl-CoA depends on the spontaneous hydrolysis of 5-CIRL. This may result in a spe-
cific bottleneck in salinosporamide A production, suggesting that fermentation yields of this prospective drug candidate may be increased by engineering a lactonase into S. tropica.

Evolution of SalM and the Chloroethylmalonyl-CoA Pathway—Previously characterized pentose dehydrogenases for \(\beta\)-arabinose (EC 1.1.1.117), \(\alpha\)-arabinose (EC 1.1.1.46), and \(\beta\)-xylose (EC 1.1.1.179) have been linked to non-phosphorylative pentose catabolism (5). In such pathways, the pentose is oxidized to a sugar lactone, followed by lactonase-mediated hydrolysis to the pentonic acid. A pentonic-acid dehydratase then converts the 5-chloro-5-deoxy-\(\beta\)-ribose 1-dehydrogenase.

It is tempting to envision this portion of chloroethylmalonyl-CoA biosynthesis as being recruited from non-phosphorylative pentose oxidation. SalM has been shown here to act as a fura

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