Supplemental information for

α-Methyl Acyl CoA Racemase Provides Mycobacterium tuberculosis Catabolic Access to Cholesterol Esters

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Materials and Methods

Materials, strains, media, and general methods. Ferricenium hexafluorophosphate and ATP were purchased from Sigma-Aldrich (St. Louis, MO). Coenzyme A was purchased from MP Biomedicals (Solon, Ohio). HEPES and TAPS were purchased from Fisher Scientific (Pittsburgh, PA). MALDI mass spectra were acquired on a Bruker Autoflex II TOF/TOF. UV-visible spectra were acquired on a Shimadzu UV2550 UV/visible light spectrophotometer. (25*R*, 25*S*)-3-OCS-CoA was synthesized as previously described.[1] MCR[2] and ChsE4-ChsE5[1] were obtained as purified proteins as previously described.

MCR activity assay. (25*R*, 25*S*)-3-OCS-CoA was used as a 1:1 25*R*:25*S* mixture. The MCR activity was monitored in a continuous coupled assay in which the dehydrogenation reaction catalyzed by ChsE4-ChsE5 was followed at 300 nm. 3-OCS-CoA (2.5 - 60 μM), 250 μM ferricenium hexafluorophosphate and 1 μM ChsE4-ChsE5 were incubated in 100 mM TAPS buffer (pH 8.5) at 25 °C. 22 nM MCR was added when the 300 nm absorption was stable. Initial velocities were obtained for the first 10-15% of the reaction. The rates of product formation were fit to the Michaelis-Menten equation to determine K_M and k_{cat}. Controls were run without MCR or without substrate, and both showed negligible decreases in absorbance at 300 nm.

Synthesis of (25S) Δ^7-dafachronyl CoA and ChsE4-ChsE5 stereochemistry assignment. (25*S*)-Δ^7-dafachronic acid (12.5 μg, AdipoGen AG-CN2-0014 Lot No. A00075, contaminated with ~30% (25*R*)-Δ^7-dafachronic acid) was dissolved in 30 μL 25% (w/v) 2-hydroxypropyl-β-cyclodextrin aqueous solution to obtain a stock solution of 1 mM acid. Thioesterification of (25*S*) Δ^7-dafachronic acid (0.25 mM) was performed in 100 mM HEPES buffer (100 μL, pH = 8.0) with 1.5 mM ATP, 1 mM CoA, 10 mM $MgCl_2$, and 2 μM FadD19 at 30 °C for 1 h. Formation of the reaction product (25*S*)-Δ^7-dafachronyl-CoA was confirmed by MALDI-TOF spectroscopy. After confirming the product was formed, 20 μL of the reaction mixture was mixed with 20 μL DDI water, 5 μL 1M HEPES pH = 8.0, 3 μL 10 mM ferrocenium hexafluorophosphate and 2 μL 52 μM ChsE4-ChsE5. The reaction mixture was analyzed by MALDI-TOF after 1, 2 and 24 h. After 24 h, 20 μL of the dehydrogenation reaction mixture was mixed with 1 μL 3.6 μM MCR to test the racemase activity. The MCR reaction mixture was also analyzed by MALDI-TOF spectroscopy. Substrate and product ratios were calculated based on the integrations of respective m/z peaks.

1. Yang, M., Lu, R., Guja, K. E., Wipperman, M. F., St Clair, J. R., Bonds, A. C., Garcia-Diaz, M., and Sampson, N. S. (2015) Unraveling Cholesterol Catabolism in : ChsE4-ChsE5 $\alpha_2\beta_2$ Acyl-CoA Dehydrogenase Initiates beta-Oxidation of 3-Oxo-cholest-4-en-26-oyl CoA. *ACS Infect. Dis. 1*, 110-125.

2. Bhaumik, P., Kursula, P., Ratas, V., Conzelmann, E., Hiltunen, J. K., Schmitz, W., and Wierenga, R. K. (2003) Crystallization and preliminary X-ray diffraction studies of an α-methylacyl-CoA racemase from *Mycobacterium tuberculosis*. *Acta. Cryst. D 59*, 353-355.