



Citation for published version:

Sattar, FA, Darley, DJ, Politano, F, Woodman, TJ, Threadgill, MD & Lloyd, MD 2012, 'Acyl-CoA -proton exchange by -methylacyl-CoA racemase' Biochemical Society Conference, 2012, Cambridge, UK United Kingdom, 1/01/12, .

Publication date:
2012

Document Version
Early version, also known as pre-print

[Link to publication](#)

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Unexpected stereoselective exchange of straight-chain fatty acyl-CoA α -protons by human α -methylacyl-CoA racemase 1A (P504S)

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α -Methylacyl-CoA racemase (AMACR; P504S) enzyme levels are increased ~10-fold in prostate and other cancers. The enzyme catalyses chiral inversion of 2-methylacyl-CoA substrates by removal of the C-2 proton followed by non-stereoselective reprotonation resulting in a ~1:1 epimeric mixture of 2*S*- and 2*R*-methyl products. AMACR is localized in peroxisomes and mitochondria. Both organelles contain straight-chain acyl-CoA esters, which are potential substrates for AMACR.

The present study investigates whether straight-chain acyl-CoA esters are substrates for the enzyme. Incubation of decanoyl-CoA with AMACR in the presence of $^2\text{H}_2\text{O}$ resulted in proton exchange, as judged by $^1\text{H-NMR}$. The reaction was reversible, as shown by deuterium loss from 2,2'-[$^2\text{H}_2$]-decanoyl-CoA when incubated with AMACR in $^1\text{H}_2\text{O}$ -containing buffer. Incubation of 2-[$^{13}\text{C}_1$]-decanoyl-CoA with AMACR in the presence of $^2\text{H}_2\text{O}$ showed only one proton was exchanged, as judged by the conversion of the substrate ^{13}C -singlet to a triplet in the NMR spectrum. Unexpectedly, chiral derivatization of incubation products followed by ^1H NMR analysis showed that the 2*S*-proton was exchanged much more frequently than the 2*R*-proton. Steady-state kinetic analysis of decanoyl-CoA and *S*-2-methyldecanoyl-CoA esters showed that straight-chain acyl-CoA esters are poor substrates for AMACR, as judged by k_{cat}/K_m values (114 vs. 37 $\text{M}^{-1} \text{s}^{-1}$).

Straight-chain acyl-CoA esters are AMACR substrates *in vitro*, but these results suggest that AMACR does not substantially catalyse proton-exchange of straight-chain acyl-CoA substrates *in vivo*.

219 words

MTG2011/0001041