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

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$\alpha$ -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  $^{13}\text{C}$ -singlet to a triplet in the NMR spectrum. Unexpectedly, chiral derivatization of incubation products followed by  $^1\text{H}$  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  $k_{\text{cat}}/K_{\text{m}}$  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*.

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