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 $^{2}H_2O$ resulted in proton exchange, as judged by ^{1}H-NMR. The reaction was reversible, as shown by deuterium loss from 2,2'-[$^{2}H_2$]-decanoyl-CoA when incubated with AMACR in $^{1}H_2O$-containing buffer. Incubation of 2-[$^{13}C_1$]-decanoyl-CoA with AMACR in the presence of $^{2}H_2O$ 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 ^{1}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_{cat}/K_m values (114 *vs.* 37 M^{-1} 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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