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

F. A. Sattar, D. J. Darley, F. Politano, T. J. Woodman, M. D. Threadgill and M. D. Lloyd

Medicinal Chemistry, Department of Pharmacy & Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY, United Kingdom; M.D.Lloyd@bath.ac.uk

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 2S- and 2R-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 2H2O resulted in proton exchange, as judged by 1H-NMR. The reaction was reversible, as shown by deuterium loss from 2,2'-[2H2]-decanoyl-CoA when incubated with AMACR in 1H2O-containing buffer. Incubation of 2-[13C1]-decanoyl-CoA with AMACR in the presence of 2H2O showed only one proton was exchanged, as judged by the conversion of the substrate 13C-singlet to a triplet in the NMR spectrum. Unexpectedly, chiral derivatization of incubation products followed by 1H NMR analysis showed that the 2S-proton was exchanged much more frequently than the 2R-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.

219 words

MTG2011/0001041