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LONG-CHAIN SPECIFIC ENZYME FROM MEDIUM-CHAIN ACYL-COA DEHYDROGENASE

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Introduction

Acyl-CoA dehydrogenases catalyze the α,β-desaturation of acyl-CoA substrates, a reaction initiated by abstraction of the α-hydrogen by an active site base [1]. This functional group has been identified as Glu376 in mature medium chain acyl-CoA dehydrogenase (MCADH, [2,3] see also [1] in this book for a review). A sequence alignment of acyl-CoA dehydrogenases and oxidases [1] shows that a Glu is present at a corresponding position in most enzymes. However, in long-chain acyl-CoA dehydrogenase (LCADH) and isovaleryl-CoA dehydrogenase (IVDH) at the position corresponding to 376, located towards the end of the α-helix K, there is a Gly or Ala residue. This raises the question about the identity of the base which abstracts the proton in the last two enzymes. Crystallographic data from J.-J. Kim and coworkers [4] suggested that in LCADH, and by analogy also in IVDH, a Glu residue might be located at a position corresponding to MCADH-pos. 255 , which is on the α-helix G of the protein. This α-helix is parallel to the K helix and forms part of the active site cavity [4]. The hypothesis has thus been put forward, that Glu 261 in LCADH and Glu 254 in IVDH are the bases having the role of Glu376 in MCADH and the other members of this family.
This is intriguing since the different location of the Glu residues might be involved in determining the chain length specificity of acyl-CoA dehydrogenases. We have thus constructed the Glu376Gly + Thr255Glu-MCADH double mutant and we report on some of the properties of the purified recombinant protein expressed in *E.coli*.

Results

The plasmid used to express the double mutant MCADH (to be called medium-long-chain acyl-CoA dehydrogenase or MLCADH) as well as the purification procedure will be described in detail elsewhere [5]. MLCADH is a stable flavoprotein which is isolated as a tetramer containing 4 FAD, i.e. one per subunit. The enzyme has the typical flavin spectrum shown in Figure 1 and a ε_{450} similar to that of MCADH.

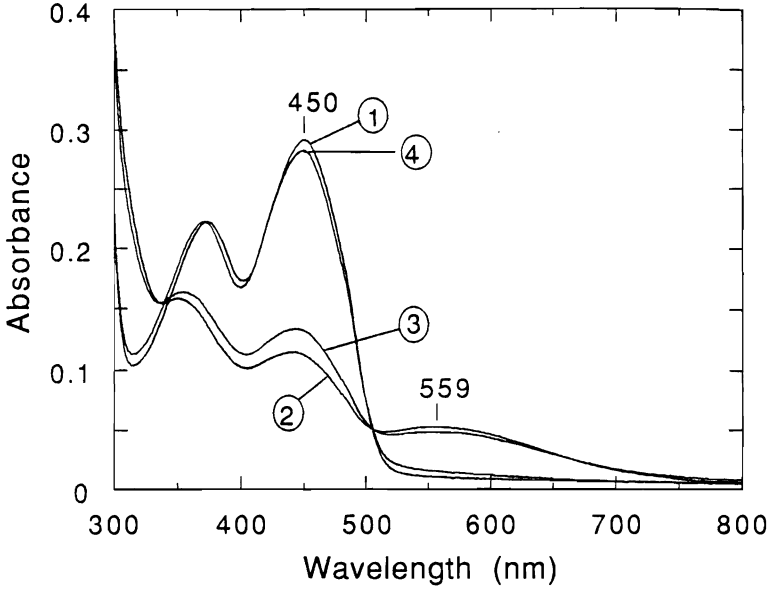

Figure 1. Absorbance spectra of MLCADH. The spectrum of the oxidized enzyme, $\approx 2 \cdot 10^{-5}$M in 0.1 M phosphate buffer pH 7.6 is shown by curve (1). Aerobic addition of 0.1 mM n-dodecanoyl-CoA leads to curve (2) and of 0.1 mM myristoyl-CoA to curve (3) within 1 minute upon mixing. After 30 min all of the substrate has been consumed whereupon the spectrum of (re)oxidized enzyme reappears (curve 4).

Aerobic addition of a 5-fold excess of either n-dodecanoyl-CoA or myristoyl-CoA leads to bleaching of the oxidized flavin spectrum and to formation of a long wavelength band (Figure 1). Within some 10 minutes, however, the spectrum of oxidized enzyme is reformed. This is attributed to turnover of substrate and indicates some oxidase activity of MLCADH.

Most interesting is the chain length dependance of the activity of MLCADH, which is depicted in Figure 2. From this comparison it is apparent that MLCADH has a specificity intermediate between that of MCADH and of LCADH with a maximum at C12. Noteworthy is also the relatively narrow activity profile, which indicates that MLCADH is more specific to its best substrates as compared to both MCADH or LCADH. Note that MCADH has previously been called "general" acyl-CoA dehydrogenase due to its broad chain length specificity [6]. The specific activity of MLCADH with dodecanoyl-CoA and myristoyl-CoA, while smaller than that of MCADH, is indeed higher than that of LCADH. While the values for human LCADH still need to be determined accurately, it is clear that the specific activities of LCADH and MLCADH are similar.

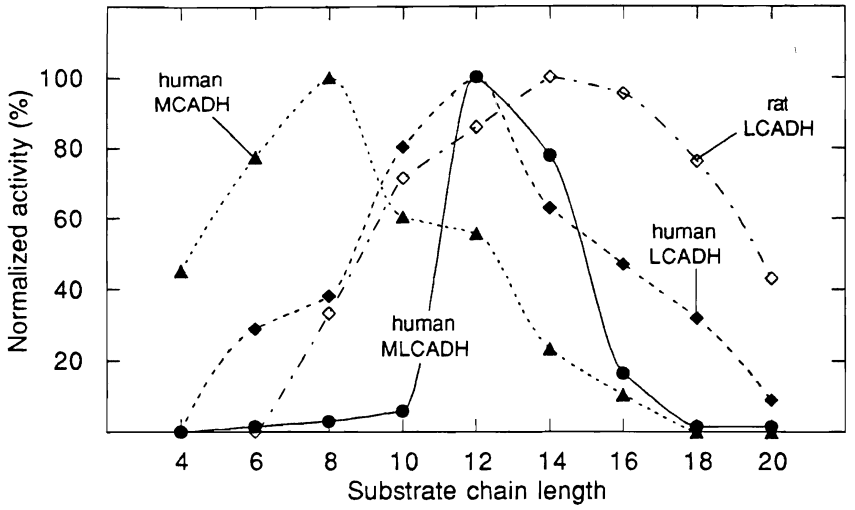

Figure 2. Chain length dependance of the activity of MCADH, MLCADH, LCADH (all human), and rat LCADH. The activity of rat LCADH was measured with the DCPIP [8], all others with the ferricenium assay [7]. For specific activities see Table 1.

The K_m values for the C12 and C14 substrates are ≈ 4μM and ≈ 5 μM, respectively, and thus essentially the same as for MCADH (≈4μM for C12); values for LCADH are not yet available. Isovaleryl-CoA is not a substrate for MLCADH.

Table 1. Comparison of chain length dependance of activities of "human MLCADH", human MCADH, human LCADH expressed in *E.coli*, and rat liver LCADH.

Acyl-CoA Substrate	MLCADH Human[a] (min^{-1})	MCADH Human[a] (min^{-1})	LCADH Human[a] (min^{-1})	LCADH Rat[b] (U/mg)
C4-CoA	0	500	0	0
C6-CoA	3	851	43	0
C8-CoA	6	1101	56	0.7
C10-CoA	12	665	119	1.5
C12-CoA	203	613	148	1.8
C14-CoA	158	259	93	2.1
C16-CoA	34	116	69	2.0
C18-CoA	3	0	47	1.6
C20-CoA	3	0	13	0.9

a) Ferricenium assay in 0.1M phosphate buffer pH 7.6 [7]
b) Data from [8], PMS/DCPIP assay.

Human MCADH and the corresponding enzymes from pig or beef have a characteristic pH dependence of their activity, which reflects pK_a values between 7 and 8.5 depending on the substrate [1]. The pH dependence of the activity of MLCADH determined using dodecanoyl-CoA shows a $pK_a \approx 8$, which is clearly in the same range as that found with MCADH ($pK \approx 8.2$, [1]). This indicates that the factors at the enzyme active site, which influence basicity of functional groups, have not been altered significantly by the double mutation.

Conclusions

The preliminary results presented in this report clearly demonstrate that the hypothesis according to which in LCADH and IVDH Glu261 and Glu254 respectively have the same catalytic role as Glu376 in MCADH is tenable. The similar velocity of turnover of MLCADH as compared with LCADH indicate that the orientation of Glu255 with respect to the substrate and the flavin cofactor must be very similar to that assumed to be present at the active center of LCADH [4]. The finding of K_m values comparable to those of the other MCADH indicates that substrate binding also must be very similar. The most interesting, and perhaps unexpected, finding is the chain length dependence of the activity, which is surprisingly narrow, but which is clearly closer to that of LCADH than that of human MCADH (Figure 2). Therefore we conclude that the position of the proton abstracting base affects the interaction of acyl-CoA dehydrogenases with substrates i.e., the chain length specificity. Crystallographic studies currently carried out in collaboration with Dr. J.J. Kim might help clarify this point.

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