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Medium Chain Acyl-CoA Dehydrogenase Genetic Defects: Identification and Partial Characterisation of Two New Patient Mutants

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Introduction

Acyl-CoA dehydrogenases catalyze the α,β-dehydrogenation of various acyl-CoA substrates. There are four known members of this flavoprotein family, which convert straight chain substrates. They can be subdivided according to the specificity for the chain length of the latter. The best studied member of the this family is the homotetrameric medium-chain acyl-CoA dehydrogenase (MCADH), which has a rather broad substrate chain length specificity centered around octanoyl-CoA (C_8-CoA).

Deficiency of this enzyme is the most common known genetic disorder in mitochondrial β-oxidation in humans (1). About 90% of the reported cases involve a substitution of lysine to glutamic acid at position 304 of the mature enzyme (K304E-MCADH), that is positioned at the interface between the subunits. The mutant protein shows a specific activity similar to that of wildtype MCADH, but is only present at decreased levels in patient cells. This is due to impaired folding and tetramer assembly, and results in a premature degradation of the enzyme. In addition to K304E-MCADH, new mutations have been found (2). Two of these are point mutations in the amino acid code of the mature protein resulting in the replacement of a cysteine at position 91 to a glycine or a tyrosine (Figure 1).

Results

We have purified and partially characterized C91G-MCADH and C91Y-MCADH and compared these to hwt-MCADH. Both mutants are unstable. The activity of both mutants is decreased with the best substrates, compared to human, wild type MCADH (hwt-MCADH). Furthermore, the chain length/activity profile of C91G-MCADH is shifted towards long chain substrates. The instability is due to or accompanied by loss of FAD during purification.

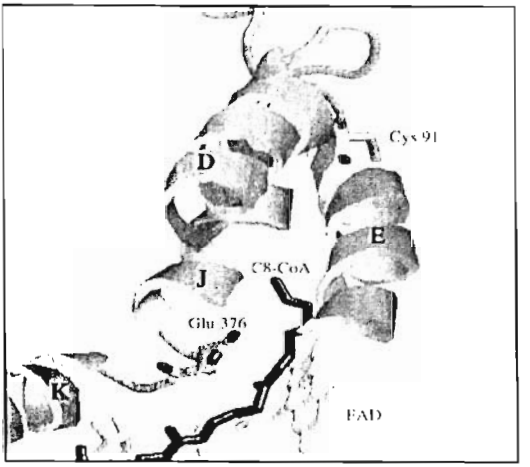

Figure 1: Three dimensional structure of the active center of hwt-MCADH. C91 lies between the loop connecting Helices D and E behind the bottom of the active center cavity. The substrate is positioned between Helices J and K and above the plane of the isoalloxazine ring (bottom right).

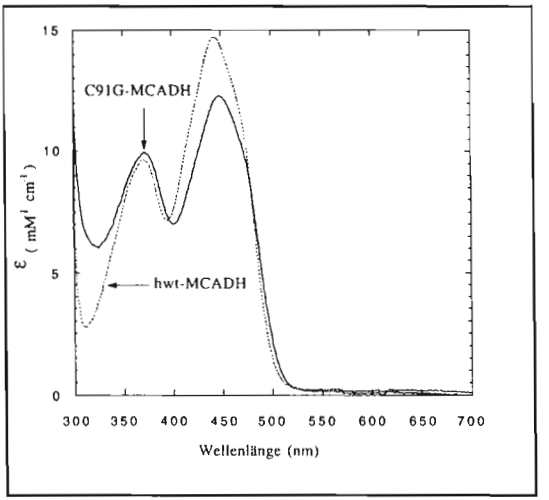

Figure 2. Comparison of the absorption spectra of hwt-hMCADH and C91G-MCADH. Conditions: 20 mM sodium phosphate buffer, pH 7.5.

The UV/VIS-spectra of oxidized C91G-MCADH and C91Y-MCADH-mutants are identical and similar to the spectrum of hwt-MCDAH (Figure 2). A minor difference exists and corresponds to a 2 nm shift from 448 nm (wild type) to 450 nm (mutants) of the flavin visible band.

C91G-MCADH has about 30% the activity of hwt-MCADH using the ferricenium-assay (3) and reaches its maximum with C14-CoA and C16-CoA. This corresponds to the activity profile of VLCADH. C91Y-MCADH has ~50% the activity of hwt-MCADH with its best substrate C8-CoA and a similar activity profile (Figure 3).

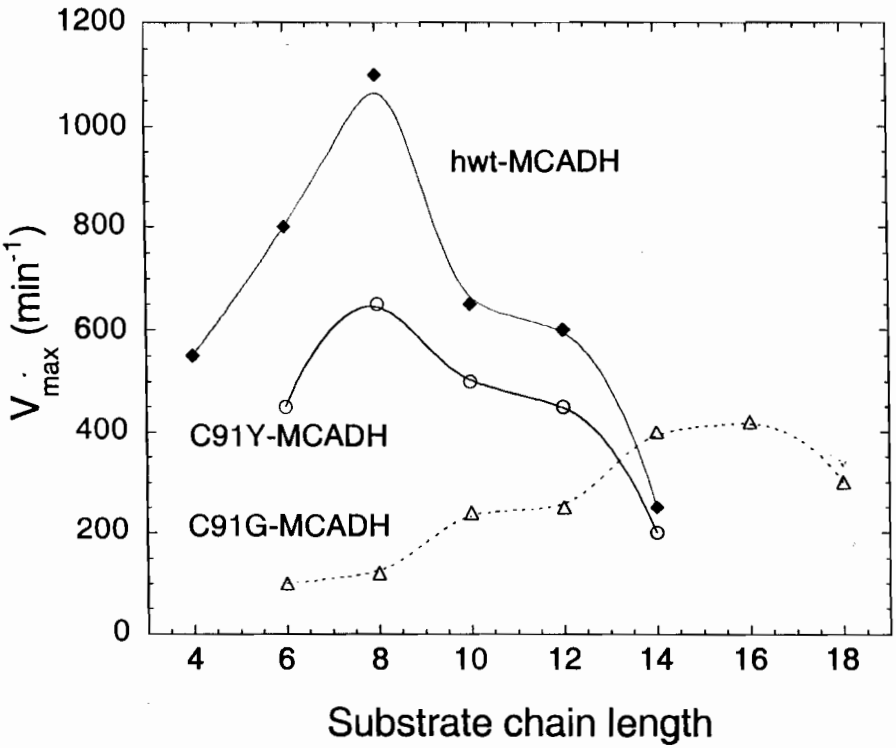

Figure 3: Dependence of the activity of C91Y- and C91G-MCADH from the chain length and comparison with hwt-MCADH. The activity was measured with the Ferricenium assay (3).

Discussion

The loss of FAD during the purification of the mutants correlates with the instability of the mutants. This is similar to the effects found in other patient mutants such as K304E-MCADH and T168A-MCADH (4,5) and would appear to be an important factor leading to the phenotypical symptoms in these patients.

It is interesting to note, that the activity profile of C91Y-MCADH is similar to that of hwt-MCADH, This contrasts with the profile of the C91G-MCADH mutant, which is shifted substantially to longer chain substrates, and has a "VLCADH-character". This suggests, that the removal of the Cys-SH and Tyr-OH groups, together with a substantial enlargement of the active site cavity, concur in generating the effect. The substantial loss of the ability to "oxidize" medium chain substrates such as C_6-CoA and C_8-CoA could be crucial in inducing the efects observed with the patients.

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