First publ. in: Flavins and flavoproteins 1990 : proceedings of the tenth International Symposium, Como, Italy, July 15 - 20, 1990, pp. 325-328

ON THE ROLE OF GLU^{376} IN CATALYSIS OF ACYL-CoA DEHYDROGENASES

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Introduction:

Acyl-CoA dehydrogenases are flavoproteins involved in the degradation of fatty acids and of branched chain amino acids. Their reaction mechanism is assumed to involve a concerted α,ß-elimination, starting with abstraction of the α-proton [1]. Incubation of medium and short chain acyl-CoA dehydrogenases (MCADH and SCADH) with 2-octynoyl-CoA leads to covalent modification of the enzyme active site [2,3]. The amino acid involved is Glu^{376}, which has been proposed to be the base abstracting the hydrogen as an α-proton [4]. Since Glu^{376} is not conserved in all acyl-CoA dehydrogenases (Isovaleryl-CoA and LCADH have Gly at position 376) [5], the above mentioned role of Glu^{376} can be questioned. In order to investigate the role of Glu^{376}, we studied the reactivity with 2-octynoyl-CoA of two enzymes lacking Glu^{376}. These are long chain acyl-CoA dehydrogenase (LCADH) which has a Gly^{376} [5], and the Glu^{376}-Gln mutant of human MCADH [6].

Results and Discussion:

Reaction of 2-octynoyl-CoA with LCADH: The incubation of LCADH with 2-octynoyl-CoA leads to changes of the oxidized flavin spectrum which are similar to those reported by Freund et al.[3] using MCADH. A difference is the complete

disappearance of the charge-transfer complex absorption in our case (see Figure 1). Importantly, however, in the present case the enzyme activity could be partially restored (~50%) after ultrafiltration. This indicates that 2-octynoyl-CoA does not lead to covalent modification of the active site, although it is being acted on by the enzyme. The product acts as a strong competitive inhibitor, thus anaerobic incubation of 6 equivalents of octanoyl-CoA with enzyme which had been inactivated with 2-octynoyl-CoA and then ultrafiltered leads to complete reduction of the flavin spectrum within a few minutes (data not shown).

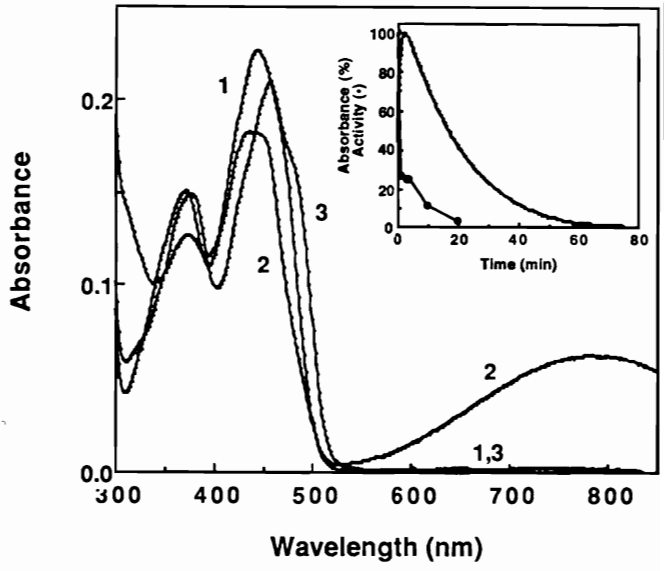

Figure 1:Spectral changes observed upon incubation of LCADH with 2.4 equivalents of 2-octynoyl-CoA in 100 mM phosphate, pH 8, 25°C. Curves 1-3: spectra recorded at 0 min. (1), 3 min. (2), and 75 min. (3). The inset shows the formation and decay of the charge transfer complex absorption at 800 nm and the activity of the enzyme at the times shown. Note that ~50 % activity could be regained after ultrafiltration.

Reaction of 2-octynoyl-CoA with human Glu^{376}-Gln MCADH mutant: Incubation of this human MCADH mutant with 3 equivalents of 2-octynoyl-CoA leads to spectral changes which are significantly different to those observed upon incubation with MCADH or LCADH.

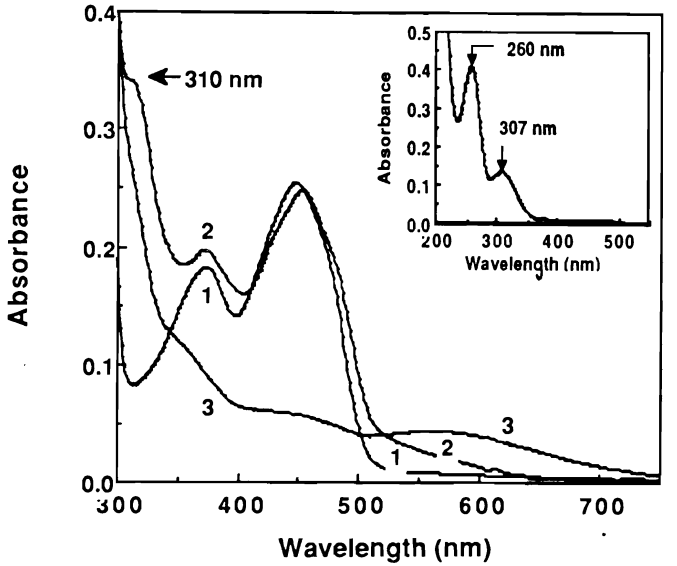

Figure 2: Spectral properties of the Glu^{376}-Gln mutant of human MCADH before (1) and after 17h of aerobic incubation with 3 equivalents of 2-octynoyl-CoA (2). Spectrum (3) represents the reduced enzyme (2) after 20h of anaerobic incubation with 6.7 equivalents of octanoyl-CoA. The inset shows the spectral properties of the product of human Glu^{376}-Gln MCADH mutant incubation with 2-octynoyl-CoA after ultrafiltration in 100 mM phosphate, 25 mM $MgCl_2$, pH 7. At pH 5 and 9 the spectrum is unchanged (not shown).

Immediately after addition, the red-shift of the 450 nm band typical for binding of CoA-derivatives to the enzyme occurs (not shown). After 20h a distinct shoulder at 310 nm and an absorption at around 550 nm has developped as shown in figure 2. This absorption is probably due to formation of a charge

transfer complex and is similar to what is observed upon incubation of human MCADH mutant with 3-oxoacyl-CoA derivatives (data not shown). The absorbance at 310 nm was decreased by ultrafiltration. The spectrum of the product, present in the filtrate, is compatible with a CoA-thioester (λ_{max} 260 nm). However, since changes in the pH (pH 5,7 and 9) have only little effect on the spectrum, it is unlikely that it is a 3-oxoacyl-CoA ester. The enzyme recovers the ability to be reduced by octanoyl-CoA.

Conclusions:

Our results, together with those described elsewhere in this volume for the Glu^{376}-Gln mutant [7], strongly suggest that this Glu is an important, but not necessary residue for catalysis of α,β-dehydrogenation in some acyl-CoA dehydrogenases. If its role is taken over by another residue in LCADH, then this residue obviously is incapable of reacting with the products arising from octynoyl-CoA to form a covalent adduct.

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