First publ. in: Flavins and flavoproteins : proceedings of the 12th International Symposium Calgary (Canada), 30 June - 6 July 1996, pp. 629-632

pH and Substrate Chain Length Dependence of the Activity of "Short-Chain"-, "Medium-Chain"- and "Long-Chain"-Acyl-CoA Dehydrogenase

Sandro Ghisla, Andreas Braunwarth, Petra Vock
Fakultät für Biologie, Universität Konstanz,
Postfach 5560 M644, D-78434 Konstanz, Germany

INTRODUCTION

Acyl-CoA dehydrogenases are a family of flavin dependent mitochondrial enzymes, which dehydrogenate fatty acyl-CoA conjugates of varying chain length. These enzymes have received considerable attention in a recent past due in part also to the recognition of various genetic defects. The latter have been discussed in connection with the "sudden infant death syndrome" (SIDS). The 3D-structure of several of these enzymes has been elucidated (1), and considerable mechanistic information is available. One still unresolved puzzle is the reason for the occurrence of several enzymes, which differ in their specificity for the length of the fatty acid chain, as opposed to a single protein with a broad specificity, acting on all of the substrates. In this context, the function of the single enzymes such as short chain- (SCADH), "medium chain-" (MCADH), "long chain-" (LCADH) and "very long chain acyl-CoA dehydrogenase" (VLCADH) is difficult to assess in extracts from mammalian tissue, due to overlapping activities.

Beinert (2) and later Murfin (3) have reported that the activity of MCADH is pH dependent. During the course of our mechanistic studies we have confirmed this and have found that this reflects apparent pK's around pH 8. We have conjectured, that this might be of physiological relevance due to its potential as a site of activity regulation. In view of this, and in order to better understand and assess the activities of the various acyl-CoA dehydrogenases we have attempted a survey of the effect of pH and chain length on the activities of SCADH, MCADH and LCADH.

RESULTS

The catalytic activity (V_{max}) of pkSCADH, hwtMCADH and hwtLCADH were determined as function of pH with substrates of various chain length using the ferricenium assay (4). This acceptor was selected since it is probably the one, which is closest in properties to electron transferring protein (ETF), the acceptor in the cell. The results are summarized in Table 1, and some of them are depicted in Figs. 1 and 2. Notably, for all substrates the activities at low pH are not zero, but there is an apparent convergence towards $V_{max} \approx 100\text{-}500$. Furthermore, with C14CoA the activity decreases with increasing pH. The apparent pK´s estimated from the profiles such as

TABLE I: **Substrate chain length and pH dependence of catalytic activities of pkSCADH, hwtMCADH and hwtLCADH.**

Substrates XCoA, X =		C4	C6	C7	C8	C10	C12	C14	C16
pkSCADH	V_{max}, pH > pK	1100	1450	2200	< 50	---	---	---	---
	V_{max}, pH < pK	65	50	0	< 50	---	---	---	---
	pK	6.8	7.8	9.0	---	---	---	---	---
	V at pH 8.0	1040	900	20	0	---	---	---	---
hwtMCADH	V_{max}, pH > pK	710[a]	1450	1460	5840	1920	1010	60	---
	V_{max}, pH < pK	5[a]	150	120	430	270	400	490	---
	pK	7.3	7.9	8.2	8.2	8.5	9.2	8.4	---
	V at pH 8.0	590[a]	870	750	2580	650	420	350	---
hwtLCADH	V_{max}, pH > pK	---	---	---	---	710	1740	2690	3360
	V_{max}, pH < pK	---	---	---	---	150	150	270	230
	pK	---	---	---	---	7.5	8.0	8.1	8.6
	V at pH 8.0	---	---	---	---	580	1400	950	830

Activities: ferricenium assay (4). Conditions: [Acyl-CoADH] = 7-20 nM, [Acyl-CoA] = 150-200 µM (saturated conditions), T = 25°C. All buffer systems contain 250 mM KCl: 50 mM acetate/HCl (pH 4.0-5.0), 50 mM Mes/KOH (pH 5.5-6.0), 50 mM Hepes/KOH (pH 6.5-7.0), 50 mM Tricine/KOH (pH 7.5 and 8.5), 50 mM Tris/HCl (pH 8.0 and 9.0), 50 mM glycine/KOH (pH 9.5-11.0). V_{max} values are expressed as turnover numbers Fc • E~FAD^{-1} min^{-1}. V_{max}, pH > or < pK is the turnover number extrapolated to high or low pH values. [a]Km values for hwtMCADH and C4CoA are up to ≈350 µM depending on the pH.

those of Fig. 1 show a linear dependence from the substrate chain lengths as depicted in Fig. 2.

The activity of SCADH is higher with C6- compared to C4-substrates at pH > 8.5 (Table 1). With C7CoA, and to some extent also with C8CoA, there is an apparent decrease of activity at higher pH values (not shown). A similar behavior can be hinted at also with LCADH and C14CoA.

In Fig. 1 the activities of MCADH and LCADH with substrates from C10CoA to C14CoA and at various pH values are compared with special reference to the possible relevance to the conditions in the cell. The dashed region corresponds to the probable pH inside the mitochondrial matrix according to (5). From the Figure it can be deduced that C10CoA is a substrate for both enzymes, however, with a marked preference for MCADH. In contrast, with C12CoA and C14CoA the preference is for LCADH.

DISCUSSION

The absolute and relative activity of acyl-CoA dehydrogenases is an important factor for assessing the function of these enzymes in the cell and in the context of the "energetic metabolism". This holds also for the assessment of the importance of genetic defects

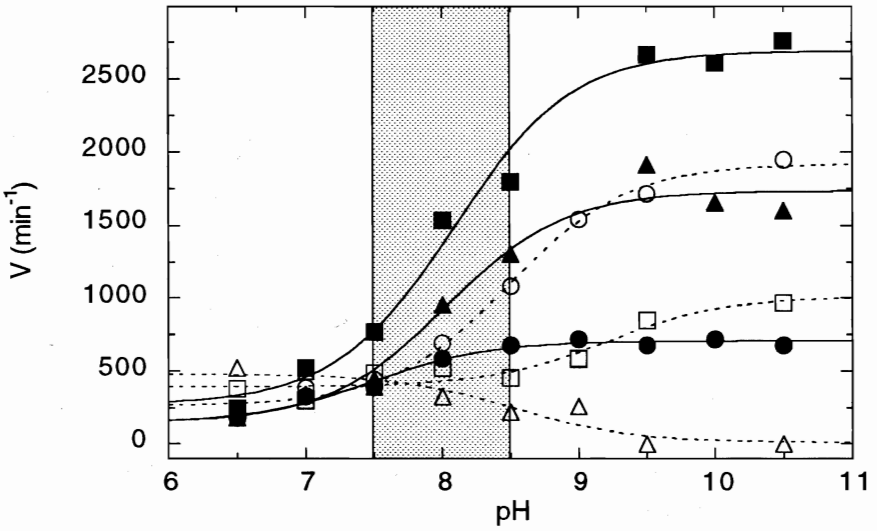

Fig. 1: Comparison of the pH- and substrate chain length dependences of the turnover number for hwtMCADH (open symbols) and hwtLCADH (filled symbols). The substrates used are: C10CoA (●, ○), C12CoA (■, □) and C14CoA (▲, Δ). Conditions as described in Table 1. The lines are the best fits obtained using the pH equation. The dotted field marks the probable physiological intramitochondrial pH (pH 8.0 ± 0.5). The ratios of the activities for hwtLCADH in comparison to hwtMCADH at pH 8.0 are 0.9 for C10CoA, 3.2 for C12CoA and 2.6 for C14CoA.

affecting several of these enzymes. Unfortunately, following the original report by Beinert (2) the pH dependence of the activity of acyl-CoA dehydrogenases has somewhat been overlooked. Thus the activity of acyl-CoA dehydrogenases is generally and routinely measured (only) around pH 7.5 - 8.

We have addressed these aspects and extended the measurements to include the dependence from the chain length of the substrate. The results are quite surprising, in particular with respect to the magnitude of activity changes, and as demonstrated representatively by the plots in Figs 1. In a recent report the pH of the mitochondria matrix has been placed around 8 (5). This is in the range of the observed, apparent pK´s! It is thus quite improbable that these pH dependences are fortuituous. Moreover, we consider it probable that they reflect some mechanism of pH dependent regulation inside the cell. From the data it is also evident, that at high pH there is a preference for the dehydrogenation of long chain substrates and *vice versa.*

The pH dependence of the activities also sheds new light onto the relative selectivity towards substrate chain length specificty of the single enzymes. The substantially higher activities of LCADH at higher pH values and with C12-, C14- and C16CoA stress the importance of this enzyme *in vivo*. These data also support the parallel roles of

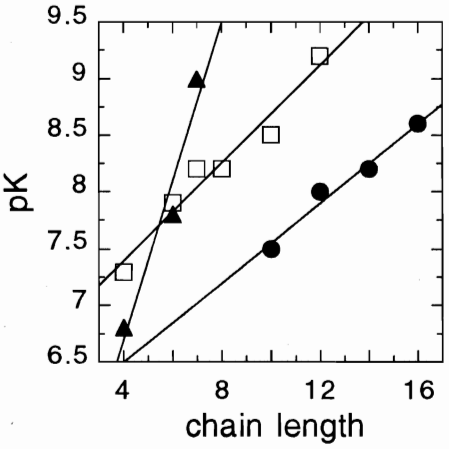

Fig. 2: Dependence of observed, apparent pK´s from the substrate chain length.
The pK´s estimated from the pH-dependences of the turnover for pkSCADH (▲), hwtMCADH (□) and hwtLCADH (●) (Table 1) are plotted *versus* the substrate chain length. Slopes: ≈0.7 for pkSCADH, ≈0.2 for hwtMCADH and ≈0.18 for hwtLCADH.

LCADH, MCADH and SCADH in the dehydrogenation of conjugated fatty acids, and are in agreement with recent conclusions from the group of Strauss (6). From this a reassessment of the relative roles of the different acyl-CoA dehydrogenases should be envisaged.

The functional group(s) which is (are) responsible for the apparent pK´s have not been identified, and this cannot be done from the present data. The linearity of the pK dependence from the chain length is striking and probably has the same origins as the chain length dependences of thioester polarisation reported by Thorpe (7). This effect is thus attributed to the modulation of the hydrophobicity of the active site by bound ligand. This effect is much more pronounced with SCADH suggesting (as to be expected) a smaller volume of the active center cavity.

ACKNOWLEDGEMENT

This work was supported by a grant from the DFG (Gh 2 / 6-2). Mrs. M. Sappelt is acknowledged for her skilled technical assistance.

REFERENCES

1. Kim, J.-J.P., Wang, M. & Paschke, R. 1993. Proc. Natl. Acad. Sci. USA **90**. 7523.
2. Beinert, H. (1963) in *The Enzymes* (Beinert, H. ed.) p. 447.
3. Murfin, W.W. 1974. Thesis, Washington University, St. Louis, MO, USA.
4. Lehman, T.C., Hale, D.E., Bhala, A., Thorpe, C. 1990. Anal. Biochem. **186**, 280.
5. Lemasters, J.J., Chacon, E., Ohata, H., Harper, I.S., Nieminen, A., Tesfai, S.A., Herman, B. 1995. Meth. Enzym. **260**, 428.
6. Hainline, B.E., Kahlenbeck, D.J., Grant, J., Strauss, A.W. 1994. Gene structure and expression **3**, 460.
7. Trievel, R.C., Wang, R., Anderson, V.E. & Thorpe, C. 1995. Biochemistry **34**, 8597.