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PURIFICATION AND SOME PROPERTIES OF FIVE DISTINCT ACYL-COA DEHYDROGENASES FROM BOVINE LIVER

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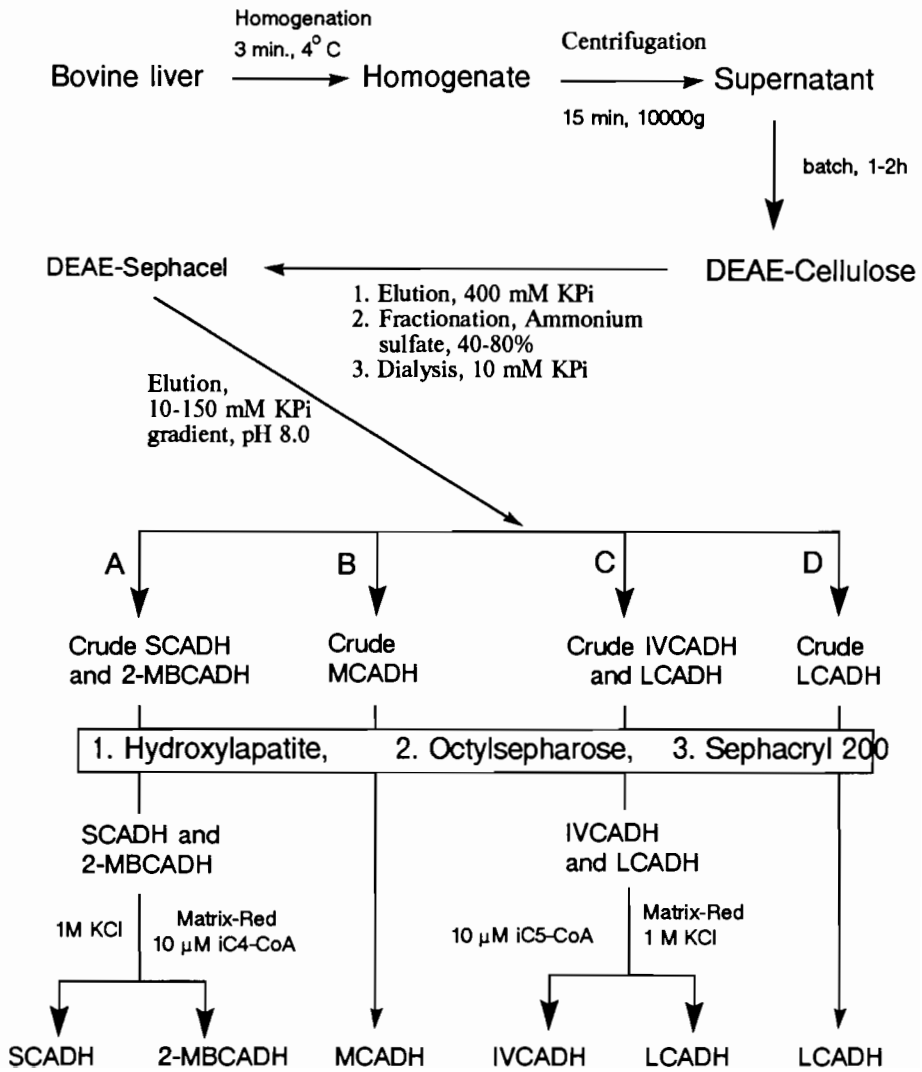
Introduction:

Acyl-CoA dehydrogenases have gained considerable interest in the past few years. This was due to a large extent to the discovery of genetic defects in the ß-oxidation cycle and their connection with the "sudden infant death syndrome" (SIDS) [1]. Up to date, several cases of genetic defects involving a-ß-dehydrogenation and in particular the enzyme medium chain acyl-CoA dehydrogenase have been reported [2,3].The occurrence of such defects involving the short chain- and long chain acyl-CoA dehydrogenases is more limited. Thus, the availability of each of the five enzymes involved in a-ß-dehydrogenation is of importance in view of mechanistic studies and also for obtaining antibodies. Several procedures have been described in the last decade describing the purification of the one or other of the enzymes in question [4-6]. However, only Ikeda et al [4] have reported a method for the simultaneous separation of all five acyl-CoA dehydrogenases using rat-liver as enzyme source. While we do not question the validity of this method, it is inappropriate when large quantities of the enzymes are required, e.g. for mechanistic studies. We have therefore attempted the simultaneous isolation of these enzymes from material which is better available.

Results and Discussion:

The procedure for the simultaneous isolation of the five acyl-CoA dehydrogenases is outlined in scheme. Up to the three chromatographic steps with hydroxylapatite, octyl-sepharose and sepharose 200 the procedure follows those published by others for the purification of the single enzymes [5,6]. The separation of SCADH and 2-MBCADH, as well as that of IVCADH and LCADH was achieved over Matrix Red where all four enzymes bind tightly. The single enzymes were eluted using the substrates for which they are specific. The substrate interacts strongly with the active center reducing the FAD cofactor, thus lowering the affinity for the dye with which it probably shares the binding site. The enzymes are eluted in the reduced state as exemplified by the case of isovaleryl-CoA dehydrogenase in figure 1.

Scheme: Procedure for the isolation of five acyl-CoA dehydrogenases from bovine liver. Abbreviations used in the scheme: SCADH: short-chain, MCADH: medium chain LCADH: long-chain, IVCADH: isovaleryl, 2-MBCADH: 2-methylbutyryl-CoA dehydrogenase.



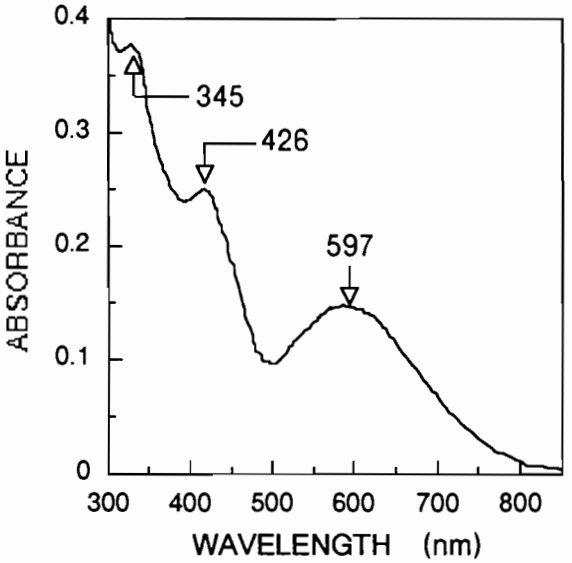


Figure 1: Spectrum of IVCADH after matrix red column chromatography. The enzyme was eluted in its reduced form using 10 ml 10 mM isovaleryl-CoA. The enzyme reoxidizes slowly upon standing.

Using this procedure isobutyryl-CoA- and isovaleryl-CoA- dehydrogenase have been isolated for the first time from beef liver in yields of ~5 and ~10 mg /kg fresh material. The relative yields are thus similar to those obtained by Ikeda et al from rat liver, however, our procedure allows the purification of larger amounts of enzymes. The properties of long chain- medium chain- and shortchain-Acyl-CoA dehydrogenases are similar to those of the enzymes purified by different procedures or from other sources. Isobutyryl-CoA dehydrogenase is an enzyme which, to date, has been little studied, it is characterized by a very week binding of FAD as was found also for the rat liver enzyme. This leads to absorption spectra in which the ratio of absorption at 280 and 450 nm is relatively high (~13). Isovaleryl-CoA- dehydrogenase binds FAD tighter and is comparatively more stable. The kinetic properties of these two latter enzymes have been determined and will be reported elsewhere.One catalytic property, however, stands out: The two enzymes do practically not show cross reactivity with each other's substrates.

Table:

Comparison of enzymatic activities measured at varying stages during the purification of "Short chain"-(SCADH), measured with butyryl-CoA as substrate; "2-Methylbutyryl-"-(2-MBCADH; Isobutyryl-CoA); "Isovaleryl"- (IVCADH; Isovaleryl-CoA); "Medium chain"-(MCADH; octanoyl-CoA and butyryl-CoA); and "Long chain"-acyl-CoA-dehydrogenase (LCADH; octanoyl-CoA and butyryl-CoA). Note that both LCADH and MCADH are active with octanoyl-CoA, LCADH is differentiated from MCADH based on its lack of activity with butyryl-CoA. The column "Prep" refers to the purification sequences identified as A,B,C, and D in the scheme.

		Activity with the corresponding CoA derivatives			
Step	"Prep"	Isovaleryl-	Isobutyryl-	butyryl-	octanoyl-
DEAE-Sephacel	A	0	38	415	0
	B	0	0	416	626
	C	72	0	0	63
	D	0	0	0	395
Hydroxyl-apatite	A	0	22	306	0
	B	0	0	317	463
	C	38	0	0	26
	D	0	0	0	201
Octyl-sepharose	A	0	8	260	0
	B	0	0;	220;	308
	C	26	0	0	34
	D	0	0	0	115
Sephacel S 200	A	0	6	240	0
	B	0	0	155	290
	C	16	0	0	22
	D	0	0	0	90

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