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RAT LIVER MICROSOMAL PALMITOYL-CoA SYNTHETASE: SUBUNIT STRUCTURE

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Summary

Long chain fatty acyl-CoA synthetase (acid:CoA ligase (AMP-forming), EC 6.2.1.3) from rat liver microsomes was shown to dissociate completely into one polypeptide chain in 75% 2-chloroethanol in water. The presence of one amino and one carboxy terminal was established. The molecular weight of the subunit as deduced from sedimentation equilibrium as well as quantitative carboxy terminal analysis agrees with the value of 28 000 ± 1000 as reported previously (Bar-Tana, J. and Rose, G. (1973) Biochem. J. 131, 443—449). Hence, the catalytic unit of 168 000 daltons appears to be composed of identical subunits.

The structural properties of long-chain fatty acyl-CoA synthetase (acid: CoA ligase (AMP-forming), EC 6.2.1.3) purified from rat liver microsomes have been described recently [1]. By active site titration a molecular weight of 168 000 was assigned to the catalytic unit. The native protein was shown to undergo extensive self-aggregation into multiples of molecular weight 170 000, but the specific activity was not affected by the extent of aggregation of the catalytic units. The elucidation of the subunit structure of the 170 000-dalton catalytic unit was complicated because of the inadequacy of conventional denaturing agents in dissociating membrane proteins completely into their polypeptide chains [2,3]. Using SDS polyacrylamide gel electrophoresis or sedimentation equilibrium in the presence of 6 M guanidinium·HCl, it was shown that the protein dissociated into 27 000—, 54 000-, 85 000- and 170 000-dalton species. The claim for homogeneity of the purified protein thus had to wait until complete dissociation of the

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enzyme protein into one molecular weight species could be achieved.

In the present paper, the isolation of one polypeptide chain has been described and the carboxy and amino terminals analyzed. These findings, together with those published previously, provide conclusive evidence that the concerned purified protein is indeed homogeneous.

Rat liver microsomal palmitoyl-CoA synthetase was isolated and purified as described previously [4]. 2-Chloroethanol (B.D.H., Poole, England) was redistilled (124—128°C), stored at 4°C and used within one month. Purity was confirmed by density and refractive index measurements. Sedimentation coefficients were measured in a Beckman model E ultracentrifuge using 12-mm double sector Kel-F cells and Schlieren optics. Sedimentation equilibrium was measured in the analytical ultracentrifuge using spectrophotometric scanning. Following the equilibrium run, the background absorbance was determined by sedimenting the sample at 52 000 rev./min for 5 h as described by Chervenka [5]. Carboxy terminal residues were determined by hydrolysis according to the method of Fraenkel-Conrat [6] and by treatment with Carboxypeptidase A according to the method of Ambler [7]. Amino terminal residues were determined according to the method of Gray and Hartley [8] and the dansylated amino acids identified by two-dimensional polyamide thin-layer chromatography as described by Woods and Wang [9].

Since conventional denaturing agents like guanidinium·HCl and sodium dodecyl sulfate had not proved powerful enough to lead to complete dissociation of the catalytic unit [1], dissociation by acidic 2-chloroethanol/water mixtures was attempted as described by Zahler et al. [10]. As shown in Table I, complete dissociation of the protein into a homogeneous subunit in the ultracentrifuge was accomplished by dissolving the protein in 75% 2-chloroethanol, containing 0.5% mercaptoethanol. Extrapolation to zero concentration gave an $s^0_{20,w}$ value of 0.87 for the dissociated protein. Dissociation in the presence of 8 M urea always yielded a fast moving band comprising of non-dissociated protein.

TABLE I

SEDIMENTATION COEFFICIENTS FOR FATTY ACYL-CoA SYNTHETASE IN UREA AND 2-CHLOROETHANOL

C, 0.75 *C*, 0.40 *C*: relative protein concentrations used for sedimentation velocity. 2-Chloro ethanol/water mixture (2-CE) contained 0.5% of 2-mercaptoethanol.

	Slow component		Fast component	
	Urea (8 M)	2-CE (75%)	Urea (8 M)	2-CE (75%)
C	0.621	0.392	2.951	No fast
0.75 *C*	0.725	0.306	3.080	component
0.40 *C*	0.854	0.274	3.365	
s^0	1.08	0.18	3.63	
$s^0_{20,w}$	2.58	0.87	8.67	

Sedimentation equilibrium in 75% 2-chloroethanol proved the weight homogeneity of the dissociated form of the enzyme (Fig. 1). The molecular weight of the subunit as calculated from these data is 35 000 ± 1000. Due to preferential solvent binding in 2-chloroethanol/water mixtures, molecular weights determined under these conditions are expected to be positively biased [11,12]. The molecular weight of bovine serum albumin in 60% 2-chloroethanol yields an apparent value which is 134% of the expected molecular weight. By taking into account the solvation effect, the observed molecular weight is reduced to the expected value of 70 000 [12]. Hence, the molecular weight 35 000 reported here for the protein subunit agrees with the minimum value of 27 000 which was found previously using SDS polyacrylamide gel electrophoresis or sedimentation equilibrium in 6 M guanidinium·HCl.

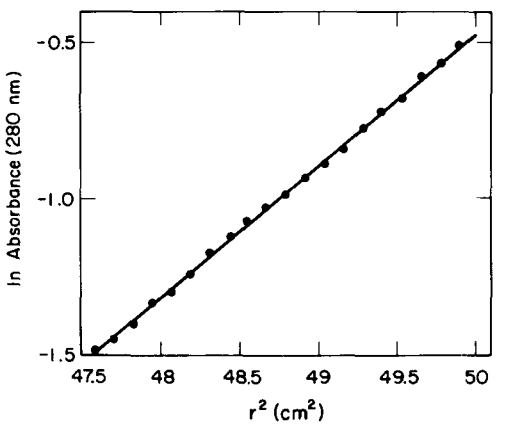

Fig. 1. Sedimentation equilibrium of fatty acyl-CoA synthetase in 75% 2-chloroethanol. The sample was overspun for 3 h at 24 000 rev./min and then equilibrated for 22 h at 18 000 rev./min (20° C). 75% 2-chloroethanol/0.5% 2-mercaptoethanol in water.

The subunit composition was further analyzed for the amino and carboxy terminal residues. A single dansyl amino-terminal residue was detected, but it was impossible to ascertain whether it was aspartic or glutamic acid. Hydrazinolysis showed glycine as the only carboxy terminal residue. The reaction, performed on 0.29 μmol of protein (mol.wt. 27 000), yielded 0.28 μmol of glycine. Recovery has been normalized by analyzing concomitantly bovine serum albumin for its carboxy terminus.

Results reported here together with previously reported studies now give the following subunit structure for the enzyme under study. The catalytic unit of 168 000-daltons appears to be composed of six identical subunits of molecular weight 27 000 ± 1000. The binding forces which seem to be responsible for the aggregation of catalytic units into superstructures appear to be weaker than those which are responsible for the association of protomeric polypeptide chains into catalytic units. The only denaturing agent which proved efficient in dissociating the catalytic unit into polypeptide chains was 2-chloroethanol: a finding of relevance to future handling of membrane-bound enzymes. The lipid content of the enzyme is probably

involved in the association-aggregation phenomena observed. About eight moles of phospholipid were shown to be bound per mole of enzyme protein, consisting of phosphatidylinositol (52%), phosphatidylcholine (26%) and phosphatidylethanolamine (21%) [1].

Elucidation of the structural properties of this protein is of interest in the light of its tight binding to the microsomal membrane. The intrinsic structure may reflect interactions similar to those which are responsible for the assembly of membrane-bound enzymes. The structure-function relationship of this enzyme awaits further investigation in the light of a possible sixth of the sites reactivity implied by the observation of one catalytic site being composed of six seemingly identical subunits.

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