"SOLUBILIZATION" OF THE STEAROYL-CoA DESATURASE OF RAT LIVER MICROSOMES

M.I.GURR, K.W.DAVEY and A.T.JAMES
Unilever Research Laboratory, Colworth House, Sharnbrook, Bedford

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1. Introduction

Nearly all the many fatty acid desaturating enzymes which have so far been investigated are firmly bound to cellular particles or membrane systems [1–3]. As far as we are aware, the stearoyl-ACP desaturase of *Euglena gracilis* [4] is the only well documented example of a "soluble" desaturase. A short report in 1964 [5] indicated that it might be possible to obtain the rat liver microsomal stearoyl-CoA desaturase in a soluble, partially purified form.

We are interested in studying the mechanism by which the two hydrogen atoms are removed, in a highly specific way, from a saturated fatty acid chain during the enzymic formation of a double bond. Studies of this kind demand a soluble highly purified enzyme and in this paper we shall describe some initial steps towards this objective.

2. Experimental procedure

2.1. *Preparation of the "soluble" extract*

For each preparation, livers (6–7 g each) were taken from two eight-week-old rats fed on a normal laboratory diet. The tissue was homogenized in 0.3 M sucrose and fractionated by standard methods and the microsomal membranes were disrupted by the technique of Boyd [6], with several modifications. The microsomal pellet was suspended in a few ml of 0.3 M sucrose and rapidly frozen to −40°, as a very thin film on the side of a large wide-necked flask, in an atmosphere of N_2. The material was freeze-dried as rapidly as possible (about 1–2 hr at a pressure of 0.03 mm Hg). The freeze-dried powder was then extracted by gentle homogenization in 4 ml 1 M phosphate buffer, pH 7.4 at 0°. This suspension was centrifuged at 100 000 × *g* for 1 hr and the supernatant fraction dialysed against 2 l distilled water overnight at 0°. The final volume of the dialysed preparation was usually about 6 ml and contained on average 5 mg protein per g liver. Its appearance varied considerably from preparation to preparation but it was usually reddish and slightly opalescent.

2.2. *Determination of desaturase activity*

A standard incubation mixture contained: phosphate buffer, pH 7.6 (100 mM), NADH (140 μM), [1-^{14}C]stearoyl-CoA (60 μM, 5μC/μmole) and protein (2–6 mg) in a volume of 1 ml. The open tubes were shaken for 5 min at 37°, and the reaction products saponified by refluxing with 1 ml 10% KOH in MeOH. Carrier acids were added, the mixture acidified with HCl, and after extraction of the fatty acids with petrol, methyl esters were prepared by refluxing in MeOH-benzene-H_2SO_4 (20:10:1, by vol) for ½ hr. The amount of [1-^{14}C]oleic acid formed was measured, either by gas-liquid radiochromatography [7] or by argentation TLC [8]. In the latter case, the absorbent was silica gel H and the developing solvent, 5% diethyl ether in petrol was run twice. Saturated and monounsaturated ester bands were located with dichlorofluorescein and the appropriate areas of silicic acid scraped directly into scintillation vials for assay of radioactivity.

2.3. *Determination of lipid labelling*

For measurement of lipid labelling, the reaction was stopped with an equal volume of methanol and lipids extracted by shaking with 5 ml $CHCl_3$-MeOH

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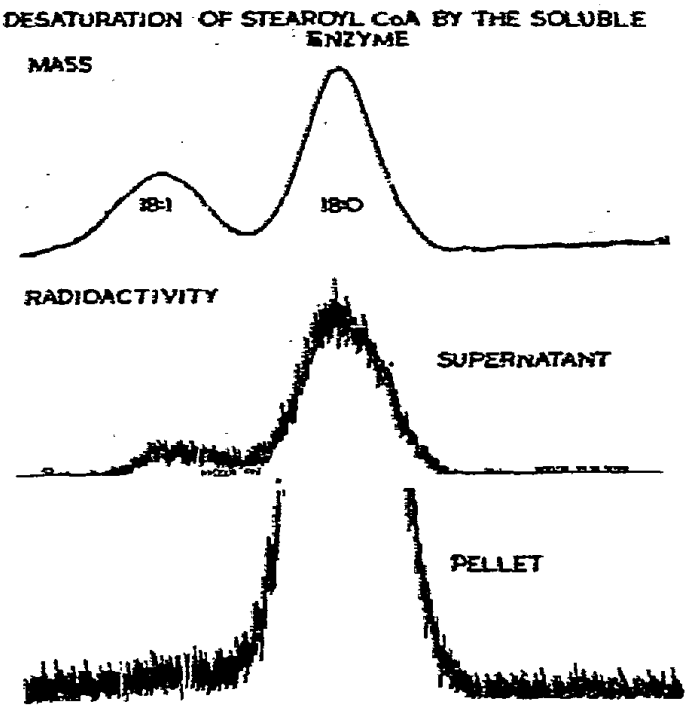

Fig. 1. Desaturation of stearoyl-CoA of the "Soluble" enzyme. Gas-liquid radiochromatography of the methyl esters on DEGA at 200°.

(2:1, v/v). Water soluble radioactivity was washed out with 0.7% saline and the $CHCl_3$ extract taken to a small volume. Lipids were separated by TLC on silica gel H with $CHCl_3$-MeOH-acetic acid-H_2O (85:15:10:3, by vol.) as developing solvent. Labeled lipids were located by scanning with a Panax radioactivity scanner.

2.4. *Analytical methods and reagents*

Protein was determined by a biuret method [9]. [1-^{14}C]stearic acid was purchased from the Radiochemical Centre, Amersham and the CoA ester prepared by a chemical method [10]. The content of -S-acyl groups in the substrate was determined by the hydroxamate method [10]. All cofactors were products of Sigma London Limited.

3. Results and discussion

When microsomes are freeze-dried, extracted with 1 M phosphate buffer and centrifuged at 100000 × *g* for 1 hr, most of the stearoyl-CoA desaturase activity is found in the supernatant fraction, very little in the pellet (fig. 1). Lower ionic strength phosphate is much less effective. Although some activity is sedimented after a further 3 hr centrifugation, the specific activity of the enzyme remaining in the supernatant is enhanced (table 1). NADH is more effective than NADPH at all concentrations (fig. 2) in agreement with other data for the firmly-bound microsomal enzyme [11]. The K_m value for NADH is 4.8×10^{-5} M, but in all cases it was not possible to obtain a reliable Lineweaver-Burke plot when NADPH was the electron donor. The reaction is extremely rapid and maximum conversion occurs after 5 min incubation at 37°. Oshino et al. [11] attributed this rapid fall-off in activity in the case of the firmly-bound microsomal enzyme to the utilization of the substrate in alternative reactions such as deacylation or transacylation to lipids. The "soluble" extract described here also contains an enzyme capable of transferring fatty acids to lipids. This transacylation is extremely rapid and might explain both the rapid fall-off in desaturase activity and the non-classical form of substrate concentration curve. As fig. 2 shows, the reaction rate is maximal when the concentration of substrate is 50 μM, but the curve is S-shaped. A Lineweaver-Burke plot based on the 'classical' part of the curve gives a K_m value of 2×10^{-5} M in close agreement with other data [11]. However, it should be borne in mind that the acyl-CoA is present as a micellar, not a true solution and

Table 1
Cofactor requirements of the soluble enzyme. Details of incubation conditions given in text.

Enzyme	Addition	Specific activity (μμ moles/mg protein/min)
Microsomes	$C_{18:0}$-CoA, NADH, air	209
Cell supernatant	$C_{18:0}$-CoA, NADH, air	32
Soluble enzyme 1 h supernatant	$C_{18:0}$-CoA, NADH, air	327
Soluble enzyme 4 h supernatant	$C_{18:0}$-CoA, NADH, air	641
Soluble enzyme	$-O_2$ $+N_2$	26
Soluble enzyme	-NADH	25
Soluble enzyme	-NADH, +NADPH	208

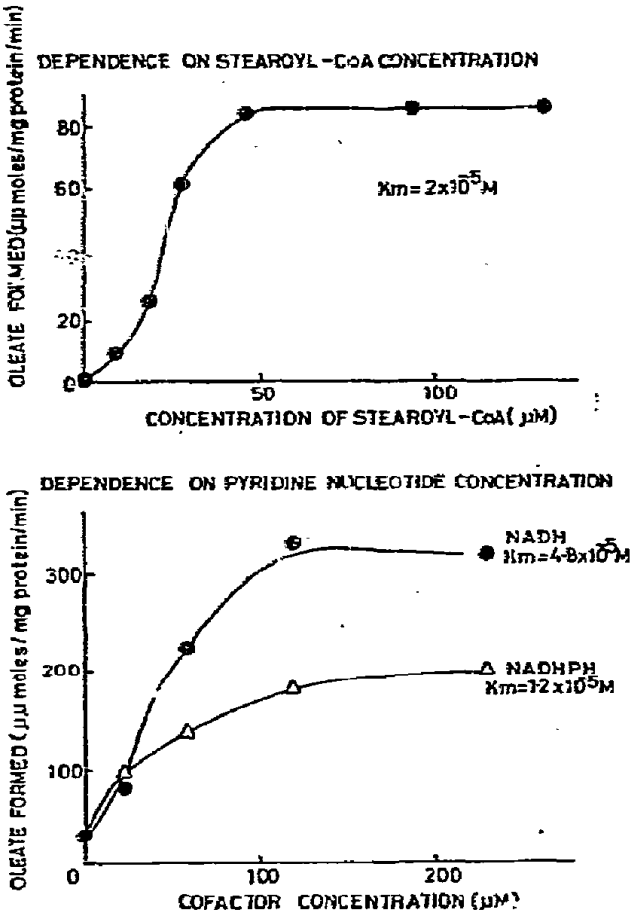

Fig. 2. Dependence of oleate formation on concentration of substrate and cofactors. Details of incubations given in text.

some doubts may thus be expressed as to whether a K_m is meaningful in such circumstances.

The rate of the reaction is linearly dependent on the amount of protein up to 6 mg/ml and there is a definite though not very sharp pH optimum at 7.8. In all these respects, the enzyme appears to be identical to the firmly bound microsomal enzyme.

We would like to emphasize that the word "soluble" is not an absolute term but a definition based on the techniques of centrifugation. The enzyme is doubtless still bound to fairly large aggregates, each containing at least those enzymes participating in the electron transport chains associated with fatty acid desaturation and with steroid hydroxylation [6]. Nevertheless the specific activity of the enzyme is increased several-fold over that of the microsomes and does not sediment after overnight dialysis or centrifuging several hours. In future experiments we hope to determine how much of the remaining structure may be stripped away without less desaturase activity and thereby shed more light on the mechanism of oxidative desaturation.

Acknowledgement

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