Molecular Weight and Subunit Size of Rabbit Mammary-Gland Fatty Acid Synthetase

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1. The molecular weights of fatty acid synthetases isolated from lactating rabbit, rat, cow and goat mammary glands were estimated by sucrose gradient centrifugation and compared by chromatography on Sepharose 6B. 2. The values obtained for all four enzymes were in the same range (0.40×10^6 – 0.55×10^6) as that found for other mammalian and avian fatty acid synthetases. The molecular weight found for the rabbit mammary enzyme therefore differs from published values of approx. 0.9×10^6 . 3. The molecular weights of the subunits of these four synthetases were 225 000–242 000. Again, the value for the rabbit mammary enzyme differs from published values.

The molecular weights of fatty acid synthetase isolated from various sources range from 0.4×10^6 to 2.5×10^6 (Bloch & Vance, 1977). Mammalian fatty acid synthetases are of approximately the same size (0.4×10^6 – 0.53×10^6) whether they are isolated from liver (Burton *et al.*, 1969; Roncari, 1974; Demassieux & Lachange, 1974; Stoops *et al.*, 1975) or from lactating mammary gland (Smith & Abraham, 1970, 1971; Strong & Dils, 1972; Knudsen, 1972; Maitra & Kumar, 1974; Kinsella *et al.*, 1975). The same is true for the enzyme from pigeon liver (Burton *et al.*, 1968; Butterworth *et al.*, 1967), chicken liver (Hsu & Yun, 1970) or goose uropygial gland (Kolattukudy *et al.* 1976; Buckner & Kolattukudy, 1976). The exception is the molecular weight of fatty acid synthetase from lactating rabbit mammary gland, which has been estimated to be 0.91×10^6 (Carey & Dils, 1970) and 0.97×10^6 (Paskin & Mayer, 1976).

Regardless of source and of size, fatty acid synthetases appear to contain either single or multiple copies of multifunctional polypeptide chains which have similar mol.wts., 185 000–250 000 (Bloch & Vance, 1977). Fatty acid synthetase from lactating rabbit mammary gland has also been reported to be different, in having a subunit mol.wt. of about 350 000 (Paskin & Mayer, 1976).

The synthesis of medium-chain fatty acids by rabbit mammary gland is due to the presence in this tissue of a specific acylthioester hydrolase of mol.wt. 29 000 which terminates chain elongation at these acids (Knudsen *et al.*, 1976).

To determine the molar ratio of this hydrolase to fatty acid synthetase in rabbit mammary gland we re-examined the molecular weight and the subunit size of this synthetase, and have compared the results with those obtained for fatty acid synthetases from lactating cow, goat and rat mammary glands.

Both the molecular weights and the subunit sizes of the four enzymes were found to be similar.

Materials and Methods

Materials

New Zealand White rabbits and Wistar rats were both used at 14 days *post partum*. Red Danish dairy cows at 6–7 months *post partum* and goats of mixed breed at 14 days *post partum* were used. Sepharose 6B was from Pharmacia Fine Chemicals, Uppsala, Sweden. Dithiothreitol, phenylmethanesulphonyl fluoride and sodium dodecyl sulphate were from Sigma Chemical Co., St. Louis, MO, U.S.A., and sucrose was from BDH Chemicals, Poole, Dorset, U.K. Acrylamide and bisacrylamide were from Fluka A.G., Buchs, Switzerland. Yeast alcohol dehydrogenase (EC 1.1.1.1) and rabbit muscle phosphorylase *a* (EC 2.4.1.1) were from Boehringer, Mannheim, West Germany. Rabbit muscle myosin was a gift from Dr. I. M. Petersen, Odense University, Odense, Denmark. The β -subunit of RNA polymerase from *Escherichia coli* (EC 2.7.7.6) and α -macroglobulin from horse serum were generous gifts from Dr. F. Engbaek and Dr. L. Sottrup-Jensen, Institute of Molecular Biology, Århus University, Århus, Denmark. All other chemicals were of analytical purity.

Methods

Purification of fatty acid synthetases. Fatty acid synthetase was purified from lactating cow, goat, rabbit and rat mammary gland as described by Knudsen (1972). The specific activity of the purified fatty acid synthetases was 800–1400 nmol of NADPH oxidized/min per mg of protein.

Molecular-weight determination. The sedimentation coefficient ($s_{20,w}$) and molecular weight of purified fatty acid synthetases were estimated by sucrose-density-gradient centrifugation as described by Martin & Ames (1961). To prevent the dissociation during centrifugation of the marker alcohol dehydrogenase, the gradient used was made in 0.1 M-potassium phosphate buffer (pH 7.0) / 1.0 mM-EDTA / 0.5 mM-dithiothreitol. Centrifugation ($160000g_{\max}$, for 20 h) was carried out in an International B-60 ultracentrifuge with the SB-283 swing-out head. The enzymes (about 1.0 mg) were layered on the gradient in 200 μ l of 50 mM-potassium phosphate buffer, pH 7.0, containing 1.0 mM-EDTA and 0.5 mM-dithiothreitol. After centrifugation, the tubes were punctured at the bottom and were emptied by pumping in 50% (w/v) sucrose that contained blue ink. The protein concentration in the gradient was measured continuously by monitoring the A_{280} in the effluent from the tube. Yeast alcohol dehydrogenase (mol.wt. 150000; Hayes & Velick, 1954) was used as a marker. The value of $s_{20,w}$ for yeast alcohol dehydrogenase varies from 6.72 S (Hayes & Velick, 1954) to 7.6 S (Martin & Ames, 1961). We have used both of these values to calculate the $s_{20,w}$ of the fatty acid synthetases.

Sephacrose 6B gel filtration. The synthetases were chromatographed at 4°C on a column (2.5 cm $\times$ 96 cm) of Sepharose 6B equilibrated with 0.25 M- or 0.50 M-potassium phosphate buffer, pH 7.0, which contained 1 mM-EDTA and 1 mM-dithiothreitol. The flow rate was 19 ml/h; V_0 was estimated to be 146 ml with Blue Dextran.

Phenylmethanesulphonyl fluoride treatment of fatty acid synthetase. Fatty acid synthetase (1–3 mg) in 1.0 ml of 0.25 M-potassium phosphate buffer, pH 7.0, was mixed with 40 μ l of 25 mM-phenylmethanesulphonyl fluoride/ml in propan-2-ol and incubated for 1 h at 30°C. The enzyme was then precipitated at 4°C by adding 200 g of solid $(\text{NH}_4)_2\text{SO}_4$ /litre and then dialysed against 0.25 M-potassium phosphate buffer, pH 7.0, containing 1 mM-EDTA and 1 mM-dithiothreitol for 2 h at 4°C.

Subunit molecular weight. The molecular weight of the synthetase subunits was estimated by polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate. Two systems were used.

(a) As described by Laemmli (1970). The gels were formed as slabs (2.7 mm thick, 7.5 cm wide, and about 7.0 cm high), and electrophoresis was carried out in a Pharmacia Electrophoresis Apparatus GE-4. The separation gel was 5% (w/v) acrylamide, 0.4% (w/v) methylenebisacrylamide in 375 mM-Tris/HCl buffer (pH 8.8 at 25°C) and 0.1% (w/v) sodium dodecyl sulphate. No stacking gel was used. The electrode buffer was 25 mM-Tris/HCl (pH 8.3 at 25°C), 192 mM-glycine and 0.1% (w/v) sodium dodecyl sulphate. Fatty acid synthetase (0.5–1.5 mg of protein) in 0.5 ml of 0.25 M-potassium phosphate buffer, pH 7.0,

was dialysed twice at 4°C for 1 h against 1-litre portions of water to remove K^+ ions. The dialysed enzyme was mixed with buffer consisting of 62.5 mM-Tris/HCl, pH 6.8, 5% (w/v) sodium dodecyl sulphate, 10% (v/v) glycerol, 5% (w/v) mercaptoethanol and 0.02% (w/v) Bromophenol Blue to give final concentrations of sodium dodecyl sulphate and of mercaptoethanol of at least 1% (w/v). The samples were incubated at 100°C for 3 min immediately before loading on the gels, and 5–10 μ l portions were applied per slot. A constant voltage of 100 V was maintained until the Bromophenol Blue reached the lower edge of the slab.

The gels were stained for 2 h in 0.25% (w/v) Coomassie Brilliant Blue R-250 in methanol/acetic acid/water (5:1:5, by vol.) (Weber & Osborn, 1969). After staining, excess dye was removed with a constant flow of water. The gels were then destained by continuous diffusion in methanol/acetic acid/water (2:3:35, by vol.) (Gathercole & Klein, 1971).

(b) As described by Weber & Osborn (1969). This was modified to allow the slab-gel system (described above) to be used instead of tubes. The concentration of the potassium phosphate in the buffer solution was decreased by half.

With both systems the markers used were: myosin (mol.wt. 220000; Weber & Osborn, 1969); phosphorylase *a* (mol.wt. 94000; Weber & Osborn, 1969); β -subunit of RNA polymerase (mol.wt. 155000; Burgess, 1969); α -macroglobulin (mol.wt. 185000; Harpel, 1973). Linear plots of R_F values against log molecular weights were obtained.

Results

Sucrose gradient centrifugation

The molecular weights and sedimentation coefficients of fatty acid synthetases from lactating cow, goat, rabbit and rat mammary glands were very similar (Table 1). The highest molecular weight (484000) found for the goat mammary enzyme was only 6.4% higher than the lowest value (453000) found for the rat mammary enzyme. One-way analysis of variance showed that there was a slight but significant difference (P 0.01) between the mean values of the four molecular weights.

When the enzyme from rabbit mammary gland was analysed as a mixture with either cow or goat mammary-gland synthetase, the pairs of enzymes migrated as a single symmetrical peak with a mol.wt. of 506000 ± 19000 or of 486000 ± 6000 (each mean $\pm$ S.D. of two determinations) respectively.

Sephacrose 6B column chromatography

The estimated K_{av} values for fatty acid synthetases from cow, goat, rat and rabbit mammary gland on

Table 1. *Sedimentation coefficients and molecular weights of fatty acid synthetases from lactating rat, rabbit, goat and cow mammary gland*

The values for the molecular weights and for $s_{20,w}$ are means $\pm$ S.D. The numbers of determinations are given in parentheses. Values of $s_{20,w}^1$ and $s_{20,w}^2$ for the synthetases were calculated by using $s_{20,w}$ values for yeast alcohol dehydrogenase of 6.72 and 7.60S respectively.

Source of enzyme	Mol.wt.	$s_{20,w}^1$ (S)	$s_{20,w}^2$ (S)
Rat (6)	453 000 $\pm$ 6000	15.9 $\pm$ 0.14	14.0 $\pm$ 0.12
Rabbit (10)	477 000 $\pm$ 8000	16.4 $\pm$ 0.19	14.5 $\pm$ 0.17
Goat (10)	484 000 $\pm$ 17000	16.6 $\pm$ 0.38	14.7 $\pm$ 0.34
Cow (10)	457 000 $\pm$ 23 000	16.1 $\pm$ 0.59	14.1 $\pm$ 0.47

Table 2. *Sephacrose 6B column chromatography of fatty acid synthetases from lactating rat, rabbit, goat and cow mammary glands*

K_{av} is the mean value $\pm$ half the difference between duplicate determinations.

Source of enzyme	Concn. of potassium phosphate buffer, pH 7.0 (M)	K_{av}
Rat	0.25	0.216 $\pm$ 0.003
Goat	0.25	0.216 $\pm$ 0.004
Cow	0.25	0.209 $\pm$ 0.003
Rabbit	0.25	0.208 $\pm$ 0.002
Rabbit	0.50	0.214 $\pm$ 0.003
Mixture of synthetases from cow, goat, rat and rabbit mammary glands	0.25	0.210 $\pm$ 0.005

Sephacrose 6B chromatography in 0.25M-potassium phosphate buffer, pH 7.6, containing 1mM-EDTA and 1mM-dithiothreitol were similar (Table 2). Increasing the concentration of potassium phosphate in the buffer to 0.5M did not change the value of K_{av} for the rabbit mammary enzyme (Table 2). Furthermore, when a mixture of two or of all four of the enzymes were analysed on Sepharose 6B in 0.25M-potassium phosphate buffer, pH 7.0, containing 1mM-EDTA and 1mM-dithiothreitol they were eluted as a single symmetrical peak which had the same K_{av} value as that shown by the individual enzymes (Table 2). There was no significant loss of fatty acid synthetase activity during the chromatography on Sepharose 6B.

Subunit molecular weight

The four fatty acid synthetases migrated with very similar R_F values in both of the systems of polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate (Figs. 1a and 1b). This strongly indicates that the four enzymes have subunits of almost identical molecular weight. The

molecular weight of the subunits was estimated to be 226 000–242 000 in gel-electrophoresis system (a), when phosphorylase a, α -macroglobulin, the β -subunit of RNA-polymerase and myosin were used as markers (Table 3). Similar results were obtained with the gel-electrophoresis system (b). Treating the fatty acid synthetases with phenylmethanesulphonyl fluoride before polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate did not alter the general pattern of migration.

Discussion

The molecular weights of fatty acid synthetases from lactating cow, goat, rabbit, and rat mammary gland were very similar, but were not statistically identical. The molecular weights of the four enzymes are within the range 0.40×10^6 – 0.55×10^6 found for other mammalian and avian fatty acid synthetases (Bloch & Vance, 1977). The value for the rabbit mammary-gland enzyme is about half those reported (910 000 and 968 000) by Carey & Dils (1970) and by Paskin & Mayer (1976) respectively. The molecular weight of the rat mammary enzyme reported here is 5.3% lower than the value of 478 000 quoted by Smith & Abraham (1970). The value for the cow mammary-gland enzyme agrees with that (450 000) estimated by the same technique by Knudsen (1972), but is 13.8% lower than that obtained by Maitra & Kumar (1974) using analytical ultracentrifugation.

The $s_{20,w}$ value for rat mammary fatty acid synthetase has been estimated by Smith & Abraham (1970) to be 12.9S by analytical ultracentrifugation. This is slightly lower than the 14.0S found in the present study, when 6.72S was used as the value for alcohol dehydrogenase in the calculation.

Maitra & Kumar (1974) have estimated the $s_{20,w}$ value for the cow mammary-gland enzyme to be 13.5S by analytical ultracentrifugation; sucrose-density-gradient centrifugation gave a value of 14.04S. This latter result is similar to the $s_{20,w}$ of 14.14S found in the present study. Knudsen (1972) estimated the $s_{20,w}$ for cow mammary-gland synthetase as 15.4S by sucrose gradient centrifugation.

Fig. 1. Polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate (a) In the system described by Laemmli (1970); (b) in the system described by Weber & Osborn (1969). The lower band is phosphorylase *a* used as a marker. Fatty acid synthetases from lactating (1) cow, (2) goat, (3) rabbit and (4) rat mammary gland.

Table 3. Molecular weights of subunits of fatty acid synthetases from rat, rabbit, goat and cow mammary glands

The values represent mean $\pm$ s.d. The numbers of determinations are given in parentheses.

Source of enzyme	Mol.wt. of subunit
Cow (2)	237 000 $\pm$ 7000
Goat (4)	226 000 $\pm$ 7000
Rabbit (2)	235 000 $\pm$ 6000
Rat (2)	242 000 $\pm$ 3000

Knudsen (1972) also used yeast alcohol dehydrogenase as marker and the $s_{20,w}$ value for this enzyme used in the calculation was 7.4S. If 6.72S is used, the $s_{20,w}$ value for the cow enzyme is 13.98S. The $s_{20,w}$ value obtained here for the cow mammary enzyme therefore agrees with published results for this enzyme.

The $s_{20,w}$ for the rabbit mammary-gland enzyme (14.5S and 16.4S) found here is similar to the value of 16.4S reported by Carey & Dils (1970). According to Schachman (1959) an estimate of molecular weight can be obtained from the formula:

$$\frac{s_{20,w_1}}{s_{20,w_2}} = \left(\frac{M_{w_1}}{M_{w_2}} \right)^{\frac{1}{3}}$$

This formula has been used here to calculate molecular weights from sucrose-gradient-centrifugation data (Martin & Ames, 1961). Mol.wts. of 475 000 and 572 000 have been obtained for the rabbit mammary-gland enzyme by using the values 6.72S and 7.6S for the $s_{20,w}$ of alcohol dehydrogenase and 16.4S for the fatty acid synthetase in the calculation.

The $s_{20,w}$ value and the molecular weight of rabbit mammary-gland enzyme reported by Carey & Dils (1970) therefore disagree. However, when the mole-

cular weight (475 000–572 000) of the rabbit mammary enzyme is calculated from the $s_{20,w}$ value reported by Carey & Dils (1970), it is in the same range as found in the present study. This indicates that a value of about 475 000 represents the approximate molecular weight for this enzyme. This is very similar to the molecular weight (453 000) of rabbit liver fatty acid synthetase (Demassieux & Lachange, 1974).

We could not repeat the results of Paskin & Mayer (1976), which gave a mol.wt. of 968 000 for the rabbit mammary enzyme, even though we used the same technique and experimental conditions. In our hands the four fatty acid synthetases behaved identically on Sepharose 6B chromatography (Table 2). Further support that the four enzymes have similar molecular weights was obtained by using polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate.

The molecular weights of the subunits of the four enzymes were also very similar (Table 3 and Fig. 1), although the value for the goat mammary enzyme was slightly lower than that observed for the other three enzymes. The values obtained with both of the electrophoretic systems (226 000–242 000) are approximately half of those of the intact enzymes, and lie within the range of molecular weight found for the subunits of other mammalian and avian fatty acid synthetases (Bloch & Vance, 1977). The present results disagree with those of Paskin & Mayer (1976), who found the subunit mol.wt. to be 350 000. We cannot explain this difference, but we have analysed a sample of rabbit mammary-gland fatty acid synthetase, kindly supplied by Dr. N. Paskin and Dr. R. J. Mayer, by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. It behaved identically with our preparations and gave a subunit mol.wt. of 235 000.

We conclude that fatty acid synthetase from lactating rabbit mammary gland is similar to other mammalian fatty acid synthetases with regard to the molecular weight of the intact enzyme (about 475000) and of its subunits (about 235000).

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