Volume 220, number 2, 323-326 FEB 05022 August 1987

Transacylase activity of lactating bovine mammary fatty acid synthase

Gregory J. Anderson† and Soma Kumar

Department of Chemistry, Georgetown University, Washington, DC 20057, USA

Received 16 June 1987

An assay for the transacylation reaction catalyzed by fatty acid synthase was developed which does not require model substrates or labelled acyl-derivatives of CoA. It involves the transfer of the acyl group from unlabelled CoA to [^{3}H]CoA. This assay shows the occurrence of transacylation at a relatively high rate with a variety of substrates that the enzyme is able to utilize. The activity is unaffected by dissociation of the enzyme or modification by iodoacetamide or 2-chloroacetyl-CoA.

Transacylase; Fatty acid synthase; Acyl exchange; Substrate specificity

1. INTRODUCTION

Transfer of the acyl groups from acetyl-CoA and of malonyl-CoA to a specific thiol group of the fatty acid synthase are the first two reactions catalyzed by the multi-functional enzyme complex, fatty acid synthase, during the synthesis of fatty acids. In the enzyme from the animal tissues the same transferase is believed to transacylate both acyl groups [1]. Lactating bovine mammary synthase lacks strict substrate specificity on account of which it is able to reduce acetoacetyl-CoA and crotonyl-CoA to produce butanoyl-CoA and to be able to utilize each of these compounds for further chain elongation [2,3]. The transacylase reaction has been assayed generally by either determining the rate of acylation of the enzyme [4] or from the rate of exchange of acyl group between CoA and pantetheine, a model acyl acceptor [5,6]. Both of these assays require radiolabelled acyl esters of CoA. We developed an assay involving the transfer of unlabelled acyl group from CoA to free tritiated CoA [2]. This assay has been improved in order to follow the transacylation using a wider group of substrates.

Correspondence address: S. Kumar, Department of Chemistry, Georgetown University, Washington, DC 20057, USA

† Present address: Department of Medicine, Oregon Health Science University, Portland, OR 97201, USA

2. MATERIALS AND METHODS

2.1. *Materials*

Lactating bovine mammary fatty acid synthase was purified as in [2]. Goose uropygial synthase was a generous gift from Dr P.E. Kolattukudy.

2.2. *Enzyme activity and chromatography*

Fatty acid synthetase activity was measured spectrophotometrically as in [2]. Transacylase reaction was carried out using dithiothreitol-treated [G-^{3}H]CoA after gel-filtration to remove the mercaptan. 32 nmol [^{3}H]CoA and 165 nmol acyl-CoA in 0.1 M potassium phosphate buffer, pH 7.0, containing 10^{-3} M EDTA were preincubated at 0°C. Dithiothreitol-free synthase (0.8 μg) was added to obtain a final volume of 100 μl. The reaction was terminated, unless stated otherwise, in 60 s by the addition of 10 μl of 1 M HCl. It was then neutralized with KOH and the free CoA remaining oxidized by the addition of a 2-fold excess of dithionitrobenzoic acid and allow-

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

ing to stand for 10 min at room temperature. The sample was then gel-filtered through Sephadex G-10 to remove salts, lyophilized, dissolved in 20 μl of H_2O and chromatographed on a polyethylenemine-cellulose TLC plate. The plate was developed with 1 M acetic acid/0.6 M LiCl and the spots were detected under a UV-lamp. R_f values of the acyl derivatives of CoA and oxidized CoA are around 0.5 and 0.08, respectively. R_f of malonyl-CoA was 0.35. The area corresponding to the CoA esters was scraped, transferred to a scintillation vial and eluted with 1.0 ml of 1.5 M LiCl/0.05 M acetic acid for 30 min. 10 ml of the scintillation cocktail was then added and counted for radioactivity. Appropriate blanks were run which were subtracted.

2.3. *Preparation of 2-chloroacetyl-CoA*

2-Chloroacetyl-CoA was prepared by the procedure of Kawaguchi et al. [7] and purified by DEAE-cellulose chromatography.

3. RESULTS AND DISCUSSION

The time course of the reaction with acetoacetyl-CoA is shown in fig.1A. A slight deviation from linearity is seen with approach towards equilibrium. A linear curve is obtained even under these conditions (or longer periods of incubation) if the results are plotted according to the rate law derived for the following equation [8,9]:

$$\text{Acyl-CoA} + [^3\text{H}]\text{CoA} + \text{Enz} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{acyl-}[^3\text{H}]\text{CoA} + \text{CoA} + \text{Enz}$$

$$\text{Enzyme } R \text{ (forward rate)} = \frac{AB}{E(A+B)t} \ln(1-F) \quad (1)$$

where A, B, E and t represent nmol of acyl-CoA, [^{3}H]CoA, the quantity of enzyme and time, respectively, and F the fractional attainment of isotope equilibrium.

$$F = \frac{(\text{net acyl-}[^3\text{H}]\text{CoA formed})\ (A+B)}{AB}$$

Plotting of $\ln(1-F)$ t (fig.1B) gives a more accurate forward rate even when the reaction is fast because the relationship is still valid when equilibrium is approached to within 50 – 75%.

Table 1 shows the specificity of the reaction. The noteworthy features are: (i) Relatively high rates

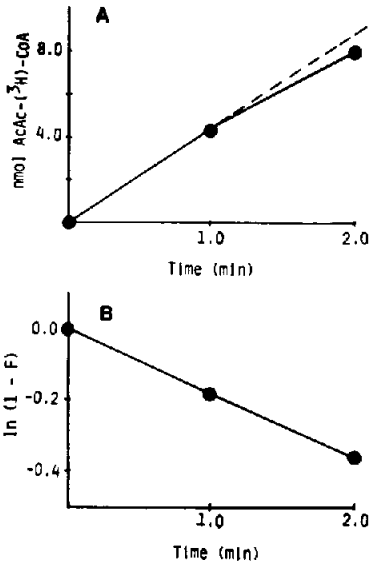

Fig.1. Linearity of the transacylase reaction. (A) Net formation of AcAc-[^{3}H]CoA; dashed line represents the expected course had the reaction been linear. (B) Same data plotted as $\ln(1-F)$ versus time (see text). Duplicates were within the circles representing the data points.

were obtained for the first six substrates with mammary synthase. These have been established to be good substrates for the different activities of the enzyme [2,10]. The rates for acetyl and malonyl transfer are comparable to those for rat mammary synthase [6]. (ii) The rate constant for acetylation is estimated to be 29 s^{-1} which compares with 43 s^{-1} for chicken liver enzyme at 23°C [4]. (iii) D-(−)-β-Hydroxybutanoyl-CoA, 2- and 3-methylcrotonyl-CoA and methylmalonyl-CoA were relatively poor substrates for transacylation as they were for further reaction [11]. (iv) Similarly, acetoacetyl-CoA was a poor substrate for the uropygial enzyme with respect to transacylation as it was for its reduction [2]. (v) Methylmalonyl-CoA, a substrate this enzyme utilizes for the synthesis of branched chain acids, was a much better one.

The data presented in table 2 show that neither the modification of the cysteinyl SH by iodoacetamide or pantetheinyl SH by chloroacetyl-CoA [4], nor the dissociation of the enzyme into its monomeric form [2] has any effect on the net rate of transacylation. There are three acylation sites on the enzyme [4]. Blocking of the two SH sites ap-

Table 1

Specificity of the transacylase reaction

Substrate	Net acyl-[^{3}H]CoA formed[a] (nmol · min^{-1} · mg^{-1})	Forward rate (R)[b] (nmol · min^{-1} · mg^{-1})
A		
1 Acetyl-CoA	7732	8791
2 Malonyl-CoA	5133	5572
3 Butynoyl-CoA	8584	9917
4 Acetoacetyl-CoA	4785	5163
5 Hexanoyl-CoA	4144	4924
6 *trans*-Crotonyl-CoA	1880	1935
7 D-(−)-β-Hydroxybutanoyl-CoA	550	555
8 2-Methylcrotonyl-CoA	569	574
9 3-Methylcrotonyl-CoA	344	346
10 Methylmalonyl-CoA	0	0
B		
1 Acetyl-CoA	1851	
2 Malonyl-CoA	3278	
3 Methylmalonyl-CoA	304	
4 Acetoacetyl-CoA	68	

[a] Variation between duplicates was $\leq$15–20%

[b] According to eqn 1

Activities are for the bovine mammary (A) and goose uropygial (B) fatty acid synthase. All values are the means of two or more replicates. The overall synthetase activities of the enzymes, under their respective optimum conditions, were 820 and 900 nmol NADPH oxidized · min^{-1} · mg protein^{-1} at 37°C

Table 2

Effect of modification of synthase on the transacylase activity

Modification	Substrate	Transacylase activity/ net acyl-[^{3}H]CoA formed (nmol · min^{-1} · mg^{-1})	
		Control	Modified
1 Iodoacetamide treated enzyme	acetyl-CoA	7329	6678
	acetoacetyl-CoA	2397	2285
2 Chloroacetyl-CoA treated enzyme	acetyl-CoA	8211	6723
3 Dissociated enzyme	acetyl-CoA	7502	8072
	malonyl-CoA	5833	6664
	butanoyl-CoA	8078	7029

77 μg of the gel-filtered enzyme was treated with 2 mM iodoacetamide in 220 μl of buffer for 45 min at 0°C. The reaction was terminated by the addition of dithiothreitol to obtain 10 mM concentration. It was again gel-filtered before assay. Reaction with 2-chloroacetyl-CoA involved a 56 molar excess of the reagent over the enzyme for 5 min. Controls were treated with the same concentration of KCl and HCl present in the reagent. Dissociation of the enzyme was attained as in [2]. Modification by iodoacetamide, chloroacetyl-CoA and dissociation resulted in the inhibition of the overall fatty acid synthetase activity by 81%, 98% and 96%, respectively

pears to leave the OH site free to sustain the transacylase activity.

The results presented in this paper establish that the transacylase component of the synthase does discriminate between various acyl groups and that the enzymes from at least the two different sources studied show significant differences in substrate specificity.

ACKNOWLEDGEMENT

This work was supported in part by National Institutes of Health research grant AM, 16086.

REFERENCES

[1] McCarthy, A.D., Aitken, A., Hardie, D.G., Santikarn, S. and Williams, D.H. (1983) FEBS Lett. 160, 296–300.

[2] Dodds, P.F., Guzman, M.G.F., Chalberg, S.C., Anderson, G.J. and Kumar, S. (1981) J. Biol. Chem. 256, 6282–6290.

[3] Abdinejad, A., Fisher, A.M. and Kumar, S. (1981) Arch. Biochem. Biophys. 208, 135–145.

[4] Yuan, Z. and Hammes, G.G. (1985) J. Biol. Chem. 260, 13532–13538.

[5] Lynen, F. (1969) Methods Enzymol. 14, 607–617.

[6] Smith, S., Agradi, E., Libertini, L. and Dileepan, K.N. (1976) Proc. Natl. Acad. Sci. USA 73, 1184–1188.

[7] Kawaguchi, A., Yoshimura, T. and Okuda, S. (1981) J. Biochem. 89, 337–339.

[8] Purich, D.L. and Allison, R.D. (1980) Methods Enzymol. 64, 3–46.

[9] Hersh, L.B. (1982) J. Biol. Chem. 257, 12820–12825.

[10] Hansen, J.K. and Knudsen, J. (1980) Biochem. J. 186, 287–294.

[11] Strom, K.A., Galeos, W.L., Davidson, L.A. and Kumar, S. (1979) J. Biol. Chem. 254, 8153–8158.