THE INVOLVEMENT OF BOUND-CoA IN GLYCERIDE BIOSYNTHESIS*

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Recent investigations on the biosynthesis of triglyceride from 2-^{3}H-monopalmitin and palmityl-CoA by hamster intestinal microsomes or a 70 fold purified complex termed triglyceride synthetase, demonstrated the utilization of palmityl-CoA for higher glyceride synthesis but failed to show the release of free CoA (Rao and Johnston, 1966). These observations suggested that during the biosynthetic reaction, CoA remained protein bound. The present paper provides supporting evidence for this postulate.

The synthesis and purity of labeled 1 and 2-monopalmitin, palmityl-CoA, the preparation of microsomes and the purified enzyme complex from hamster intestinal mucosa and the isolation and determination of higher glycerides have been previously described (Rao and Johnston, 1966). The reported data is expressed in terms of mμ moles of labeled monoglyceride incorporated into di- and triglycerides. Incubations were carried out for 10 min. at 37°C using microsomes (3 mg), CoA (1 μmole), Mg^{++} (10 μmoles), KF (25 μmoles), GSH (30 μmoles), and 0.5 ml of 0.5 M Tris Maleate buffer (pH 7.0), in a total volume of 2 ml with 30 μmoles of ATP (Microsome A) or without ATP (Microsome B). A heat inactivated enzyme was prepared by placing the microsomes in a boiling water bath for 15 min. followed by preincubation under the same condition as Microsome A.

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At the end of the preincubation, the microsomes were recovered by centrifugation at 104,000 x g for 30 min., washed, and suspended in 1 ml 0.154 M KCl. Aliquots of the preincubated microsomes (0.5 mg) were utilized to study the higher glyceride synthesis via the monoglyceride pathway both with and without the further addition of fresh microsomes (1 mg). The second incubation was for 30 min. at 37°C and contained 2-^{3}H-monopalmitin (250 mμmoles), palmitic acid (500 mμmoles), ATP (30 μmoles), GSH (30 μmoles), KF (25 μmoles), Mg^{++} (10 μmoles), 0.1 ml of 10% Tween-80 solution, and 0.5 ml of 0.5 M Tris-Maleate buffer (pH 7.0) in a final volume of 2 ml. The results of this experiment are given in Table I.

TABLE I

Formation of Bound-CoA

Contents	mμmoles higher glyceride synthesis	
	without fresh microsomes	with fresh microsomes
Experiment 1		
1. Microsome A	209	235
2. Microsome B	4	47
3. Microsome C	-	33
4. Microsome A minus ATP	14	14
5. Fresh microsomes		9
Experiment 2		
6. Microsome A		136
7. Microsome B		5
8. Heat Inactivated		109
9. TCA Precipitated		68

As can be seen, the preincubation of microsomes with ATP and CoA resulted in a marked stimulation of glyceride synthesis (Flask 1) as compared to preincubations in which the ATP was omitted (Flask 2). The addition of fresh microsomes further amplified this effect. Furthermore, the

binding of CoA or a derivative thereof appears to be enzymatic since heat inactivation of the microsomes prior to preincubation (Microsome C), followed by the addition of fresh microsomes did not show any appreciable synthesis (Flask 3). The requirement of ATP in the second incubation is demonstrated by the results obtained in Flask 4, which was identical to Flask 1 with the exception that ATP was omitted. The contribution of CoA by fresh microsomes is negligible, since in the presence of ATP, 2-monopalmitin, and palmitic acid only a trace amount of higher glycerides are formed (Flask 5).

The availability of CoA following preincubation is shown in the second half of Table I. Bound CoA was prepared under the same conditions as described for Microsome A. The contents of Flask 6 and 7 were the same as Flasks 1 and 2 respectively. A similar quantity of preincubated Microsome A was heat denatured at 100°C for 15 minutes (Flask 8), or precipitated by TCA and washed with buffer (Flask 9). As can be seen, the bound CoA is available for glyceride synthesis following these treatments.

As has been previously mentioned, during the acylation of monoglycerides by palmityl-CoA, the CoA remained protein bound. The question as to whether this CoA was available for the activation of added fatty acid was next investigated. 1-^{14}C-monopalmitin (1 μmole), palmityl-CoA (2 μmoles), KF (50 μmoles), GSH (60 μmoles), 0.2 ml of 10% Tween-80, 3 ml of 0.5 M Tris-Maleate buffer (pH 7.0) and microsomes (6 mg) in a final volume of 6 ml were incubated for 10 min. at 37°. At the end of the incubation an aliquot was removed and the amount of palmityl-CoA utilization for higher glyceride synthesis determined. This gives an index as to the degree of formation of bound CoA. The remaining incubation mixture was cooled to 0° and the microsomes reisolated and suspended in 0.154 M KCl (Microsome D). The presence of bound CoA was tested by determining the amount of 2-^{3}H-monopalmitin incorporation into

higher glyceride via the procedure described above. The results of this experiment are given in Table II.

TABLE II

Formation of Bound-CoA During
Glyceride Synthesis and Its Utilization

Contents	mμmoles higher glyceride synthesis	
	Microsome D	Microsome E
1. + ATP	58	56
2. − ATP	19	14
3. + ATP + fresh microsomes	229	195
4. − ATP + fresh microsomes	19	13

The higher glyceride synthesis was 58 mμmoles when the complete incubation mixture was employed (Flask 1). The omission of ATP resulted in a marked decrease in synthesis (Flask 2). This difference was even more pronounced when the fresh microsomes were added to the incubation vessel (Flasks 3 & 4). No appreciable synthesis (9 mμmoles) occurred when fresh microsomes were incubated in the presence or absence of ATP. If significant amounts of CoA or fatty acid-CoA were present in the fresh microsomes, one would expect an appreciable synthesis of higher glycerides. The most plausible explanation for the stimulation of higher glyceride synthesis by fresh microsomes is the increase in active enzyme concentration. The inactivation of these enzymes might be expected under the conditions of the first incubation, since the biosynthetic enzymes are known to be inactivated by detergents such as Tween-80 and palmityl-CoA (Rao and Johnston, 1966). Almost identical results were obtained when palmitic acid, ATP, CoA, and Mg^{++} were substituted for palmityl-CoA in the first incubation (Microsome E). Although microsomes have been employed as the enzyme source, preliminary experiments suggest a similar binding of CoA by the purified triglyceride synthetase complex. In addition, it has been observed that 2-monopalmitin can be replaced by L-α-glycerophosphate as an acyl acceptor suggesting the possible

general nature of the phenomena described.

Recently, Moyer and Smith (1966) provided direct evidence for the covalent binding of labeled dephospho-CoA to succinic thiokinase. Bar-Tana and Shapiro (1964) suggested the presence of bound-CoA in palmityl-CoA synthetase in an attempt to explain the discrepancy between the hydroxamate formation and ATP generating assay procedures employed in the purification of this enzyme, thus extending the generally accepted reaction sequence for fatty acid activation suggested by Berg (1956).

The reported results provide direct evidence for the formation of enzyme bound CoA, which is necessary for fatty acid utilization. Its formation is energy dependent and is enzymatically catalyzed. A similar bound intermediate is formed during the utilization of palmitate moiety of palmityl-CoA for higher glyceride synthesis. The bound CoA is still available for glyceride synthesis after heat denaturization or protein precipitation.

Recently Majerus et al. (1965) and Pugh and Wakil (1965) have demonstrated the existence of covalently bound 4'-phosphopantetheine as the prosthetic group of a heat stable protein termed "acyl carrier protein" (ACP) which is involved in fatty acid biosynthesis. The reported studies do not differentiate between the binding of intact CoA or a functional portion of the molecule. Therefore, the formation of the active intermediate by the reactions described may be similar to the formation of ACP. Further investigations are necessary to elucidate the chemical nature of this intermediate.

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