THE CONDENSATION OF MALONYL COA WITH ACETYL OR BUTYRYL COA*

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Previous reports have presented evidence that malonyl CoA (Brady, 1958; Wakil, 1958; Formica and Brady, 1959; Wakil and Ganguly, 1959) and butyryl CoA (Long and Porter, 1959) are intermediates in the biosynthesis of palmitic acid in a soluble, pigeon liver enzyme system (Wakil, Porter and Gibson, 1957). Evidence is now presented that the same, or a more purified enzyme fraction, condenses malonyl CoA with either acetyl or butyryl CoA. A similar or identical condensation of malonyl CoA and butyryl CoA is effected by a carrot root plastid system. Evidence is also presented that TPNH is oxidized in the presence of the condensation product and a pigeon liver enzyme fraction with the formation of a second compound. The latter is separated from the condensation product via paper chromatography.

Biosynthesis of the condensation products may be achieved either through generation of malonyl and butyryl CoA in the presence of the R_2 enzyme fraction (Long and Porter, 1959) or by incubation of malonyl CoA with either acetyl or butyryl CoA and a more purified R_2 enzyme fraction. The latter enzyme fraction is purified through adsorption on calcium phosphate gel and subsequent chromatography on DEAE cellulose. The condensation product is separated from the parent thioester compounds, following incubation and removal of proteins and lipids, through ascending chromatography in a modified ammonia-isobutyric

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acid-EDTA system of pH 4.5. After the first chromatographic separation the condensation product is eluted and then separated from a colored impurity by adsorption of the latter on charcoal. The supernatant solution is then lyophilized and the dry residue is washed with ethyl ether and ethanol. A second chromatographic separation yields a sharply defined band of the condensation product, Table I and Figure 1.

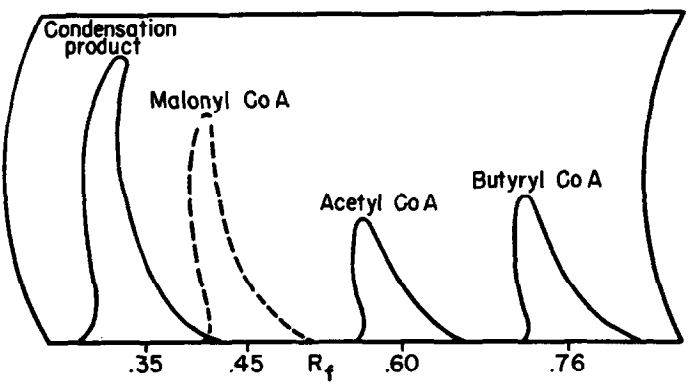
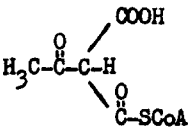


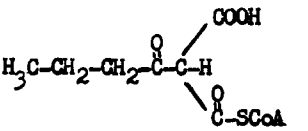
Figure 1. Separation of the Condensation Product from the Parent Thioester Compounds. The figure shown was obtained by superimposition of the curves of each of the chromatographically purified compounds. All curves of radioactivity were obtained with a Nuclear-Chicago Chromatogram Scanner.

The values given in Table I are evidence that the same or a similar radioactive product is obtained in the incubation of malonyl CoA with either acetyl or butyryl CoA when either of the condensing partners is labeled with carbon-14. A similar result was obtained with malonyl CoA and butyryl CoA in the carrot system. Variation in R_f values of the carrot system condensation products from those of the pigeon liver system are a reflection of variation in chromatograms. Further chromatography of each of these compounds on the same paper yielded values of 0.31 and 0.35 for the condensation products of the carrot and pigeon liver systems, respectively.

Structures of the condensation products are unknown. However, it seems

reasonable to assume (from the R_f values as compared to that of malonyl CoA) that the following are the probable structures.





Other structures are, of course, possible.

Hydroxamate derivatives of the condensation products were prepared and chromatographed in a water saturated butanol system. R_f values for these compounds and the hydroxamates of acetate, malonate and butyrate are given in Table I.

TABLE I

The Condensation of Malonyl CoA with Acetyl or Butyryl CoA

System	Substrate	Acyl CoA* R_f	Hydroxamates** R_f
Pigeon Liver Enzyme	C^{14}malonyl CoA + butyryl CoA	.37	.32
	C^{14}butyryl CoA + malonyl CoA	.38	.32
	C^{14}malonyl CoA + acetyl CoA	.37	.33
	C^{14}acetyl CoA + malonyl CoA	.36	.33
Carrot Root Plastids	C^{14}malonyl CoA + butyryl CoA	.25	.26
	C^{14}butyryl CoA + malonyl CoA	.27	.26
Controls	C^{14}acetyl CoA	.60	.50
	C^{14}butyryl CoA	.76	.74
	C^{14}malonyl CoA	.45	.36

The complete incubation mixture contained 50 µmoles of phosphate-bicarbonate buffer, pH 7.2, 8 µmoles of cysteine, 1 µmole of Mn^{++}, 2.5 µmoles of ATP, 50-100 mµmoles of acyl CoA, enzyme (1.0 mg. pigeon liver enzyme, or plastids from 10 gm. of carrots) and water to a volume of 1.0 ml., pigeon liver system, or 5.0 ml., carrot plastid system. Enzyme, buffer and cysteine were preincubated for 30 minutes at 38°. The subsequent incubation was made for one hour under an atmosphere of nitrogen.

* Ammonia-isobutyric acid-EDTA system.

** Water saturated butanol system.

One of the protein fractions recovered after fractionation of the R_2 gel-treated fraction on DEAE cellulose oxidizes TPNH in the presence of the condensation product. The resultant reduction product (R_f 0.26) is separated from the condensation product via chromatography in the ammonia-isobutyric acid - EDTA system. Hydroxamate derivatives of the reduction product (R_f 0.26) and condensation product (R_f 0.32) are separated on chromatography in a water saturated butanol system.

TABLE II

Incorporation of Condensation and Reduction Products into Palmitic Acid

Crystallization	Condensation product			Reduction product		
	Total c/min.	Crystals c/min./mg.	Supernate	Total c/min.	Crystals c/min./mg	Supernate
1	3360	69	240	9190	125	1650
2	2250	56	116	6150	95	352
3	2010	68	64	3950	82	183
4	1500	61	55	2670	61	109
5	1265	63	60	1740	60	75
6	986	61	—	1200	62	62

Cofactors and incubation conditions were the same as those reported for the pigeon liver system, Table I. Radioactivity was determined in a Packard Tri-Carb liquid scintillation spectrometer.

Both the condensation product and the reduction product were converted to palmitic acid on incubation with the R_2 gel-treated enzyme fraction, ATP, Mn^{++}, cysteine, TPNH and non-radioactive acetyl CoA. The fatty acid fraction was removed from the incubation mixture with petroleum ether, as previously reported (Long and Porter, 1959). Separation of C^{14}-labeled palmitic acid was achieved through chromatography in the kerosene-85% acetic acid system (Kaufmann and Nitsche, 1954). Crystallization to constant specific radioactivity was made from petroleum ether after the addition of 50 mgs. of non-radioactive palmitic acid, Table II.

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