REQUIREMENT FOR A MALONYL CoA-CO_2 EXCHANGE REACTION IN LONG CHAIN BUT NOT SHORT CHAIN FATTY ACID SYNTHESIS IN CLOSTRIDIUM KLUYVERI

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Long chain fatty acid biosynthesis in a number of experimental systems (Wakil 1959, Lynen 1960, Brady 1960, Martin, Horning, and Vagelos 1961) procedes through a reaction between malonyl-CoA and acetyl-CoA. A mechanism of condensation with concomitant decarboxylation has been proposed (Vagelos 1959, Lynen 1960). Subsequent sequential condensations with decarboxylation of malonyl-CoA then occur until, by successive two carbon atom additions, a chain length of approximately sixteen carbon atoms is achieved. A malonyl-CoA-CO_2 exchange reaction ($R\text{-}COSCoA + {}^*COOHCH_2COSCoA \rightleftharpoons {}^*CO_2 + RCOCH_2COSCoA + CoASH$) demonstrated in Clostridium kluyveri (Vagelos and Alberts 1960) may represent the condensation-decarboxylation necessary for long chain fatty acid synthesis. This possibility is suggested by the identification of a malonyl-CoA-CO_2 exchange reaction in the fatty acid synthesizing systems of rat adipose tissue (Martin, Horning, and Vagelos 1961) and yeast (Lynen 1961). However, the dependence of fatty acid synthesis on the enzymes catalyzing the exchange reaction has not been established.

This report describes an enzyme system in C. kluyveri that synthesizes long chain fatty acids from malonyl-CoA and acetyl-CoA. The preparation, in contrast to that of avian liver (Wakil 1961) and yeast (Lynen 1961), does not sediment when centrifuged at 140,000 x g for five hours. The system has been separated into three protein fractions, two of which are necessary for the malonyl-CoA-CO_2 exchange

reaction and all three of which are necessary for the biosynthesis of long chain fatty acids. In addition, data are presented which show that malonyl-CoA is not a required intermediate for the synthesis of butyrate which is synthesized by a mechanism consistent with the direct condensation of acetyl-CoA units as originally proposed (Barker 1951, Stadtman 1954).

Methods for the synthesis of malonyl-CoA and acetyl-CoA and for the separation and identification of butyrate have been listed previously (Martin and Vagelos 1961). C^{14} long chain fatty acids were extracted into isooctane (Dole 1956) and the isooctane washed three times with acidified ethanol and water to remove short chain radioactive precursors and side products. Long chain acids were dissolved in a solution of 2,5-diphenyl-oxazole in toluene (5 mg/ml) and the radioactivity assayed in a Packard Tricarb Liquid Scintillation spectrometer.

An enzyme preparation that catalyzes the synthesis of long chain fatty acids is precipitated from crude extracts of C. kluyveri at an ammonium sulfate saturation between 65 and 95%. By calcium phosphate gel adsorption and elution the system is resolved into two protein fractions: a fraction containing the enzymes necessary for the malonyl-CoA-CO_2 exchange reaction (I and II) and fraction III. Fraction I (a heat-labile protein) is separated from fraction II (a heat-stable protein) by paper electrophoresis as previously described (Alberts and Vagelos 1961). The fraction II used in the experiments below had been boiled for 10 minutes. The radioactive long chain fatty acids synthesized under the conditions of Table I were analyzed by gas-liquid chromatography and found to be palmitate, stearate and arachidate.

The dependence of long chain fatty acid synthesis on fractions I, II, and III and the dependence of the exchange reaction on fractions I and II is shown in Table I. Incorporation of malonyl-2-C^{14}-CoA into long chain fatty acids is about eight times as high when the three fractions are combined as it is in the sum of the three fractions.

Table I

Enzyme fraction	C.p.m. incorporated into product		Exchange enzyme activity, c.p.m.[t]
	Long chain fatty acids	[a]Butyrate	
Fraction I	140		0
Fraction II	0		0
Fractions I and II	200	10,600	3,243
Fraction III	390	18,300	198
Fractions I, II and III	4,520	27,700	4,235

Incubations were anaerobic in a volume of 1.0 ml for 60 minutes at 30°C. Reaction mixtures contained 50 μmoles of triethanolamine-HCl buffer at pH 7.5, 10 μmoles of 2-mercaptoethanol, boiled cell extract from 10 mg of C. kluyveri, 0.02 μmoles of TPNH and a TPNH generating system (4 μmoles of glucose 6-phosphate and 0.13 K. units of glucose-6-phosphate dehydrogenase). The following additional substrates were then added depending on whether long or short chain fatty acid synthesis was to be studied. For long chain fatty acids: 0.1 μmole of malonyl-2-C^{14}-CoA (100,000 c.p.m.) and 0.03 μmole of acetyl-CoA. For short chain fatty acid synthesis: 0.1 μmole of acetyl-1-C^{14}-CoA (100,000 c.p.m.).

[t]Exchange enzyme activity was assayed by the amount of $C^{14}O_2$ fixed in malonyl-CoA (Vagelos and Alberts 1960).

[a]Butyrate synthesis was so active that only 1% of the enzymes used for long chain fatty acid synthesis were used when butyrate synthesis was studied.

Furthermore, other studies have shown that long chain fatty acid synthesis exhibited a range of linear dependence on the concentration of each of the three fractions in the presence of saturating amounts of the other two fractions.

Short chain fatty acids are derived from acetyl-CoA even in the presence of malonyl-CoA. This can be seen in Table II where acetyl-1-C^{14}-CoA is incorporated into butyrate in the presence of malonyl-CoA whereas little malonyl-2-C^{14}-CoA is incorporated into butyrate in the presence of acetyl-CoA. Thus malonyl-CoA is not an obligatory intermediate in the condensation of acetyl-CoA units to form butyrate.

Table II

Substrates	Synthesis of butyrate (c.p.m.)
0.1 μmole of acetyl-1-C^{14}-CoA (100,000 c.p.m.) 0.1 μmole of malonyl-CoA	10,600
0.1 μmole of acetyl-CoA 0.1 μmole of malonyl-2-C^{14}-CoA (100,000 c.p.m.)	440

The system was as described in Table I except that the additional substrates were as shown above and there were 5 μmoles each of Mn^{++} and Mg^{++}. The enzyme was obtained from an ammonium sulfate fractionation between 65% and 95% saturation.

Although the small amount of malonyl-2-C^{14}-CoA incorporated into butyrate can be explained by the decarboxylation of malonyl-2-C^{14}-CoA to form acetyl-2-C^{14}-CoA, a direct conversion by a mechanism similar to that for long chain synthesis can not be excluded. A further distinction between long and short chain fatty acid synthesis is shown in Table I. It is seen that short chain fatty acid synthesis occurs in both of the fractions necessary for long chain synthesis and that the combination of the fractions gives only an additive effect on synthesis.

In the long chain fatty acid system DPNH could not substitute for TPNH. Confirming the recent findings in yeast (Lynen 1961) there is complete dependence on a flavin cofactor. FMN is more effective than FAD. Boiled cell extract supplies the needed flavin coenzyme and in addition contains a stimulatory factor not yet identified which survives ignition for thirty minutes (see Table III).

Requirements for long chain fatty acid synthesis in a soluble enzyme system from C. kluyveri have been described. The system utilizes malonyl-CoA and is dependent on the enzymes that catalyze the malonyl-CoA-CO_2 exchange reaction. In these respects it differs from the

synthesis of butyrate from acetyl-CoA in this organism in which malonyl-CoA is shown not to be an intermediate.

Table III

Additions	Synthesis of long chain fatty acids (c.p.m.)
None	16
Boiled cell extract	907
FMN (10^{-4}M)	330
FAD (10^{-4}M)	225
FMN (10^{-4}M) + boiled cell extract	801
FMN (10^{-4}M) + ignited boiled cell extract	600
Ignited boiled cell extract	22

The system was the same as that described in Table I for long chain fatty acid synthesis except that boiled cell extract was omitted except as shown above. Boiled cell extract or its derivatives were from 10 mg of dried C. kluyveri.

REFERENCES

Alberts, A. W., and Vagelos, P. R., Federation Proc. 20, 273 (1961).

Barker, H. A., in Phosphorous Metabolism, Vol. I, McElroy, W. D., and Glass, B., (Editors). The Johns Hopkins Press, Baltimore, 1951, p. 204.

Brady, R. O., J. Biol. Chem., 235, 3099 (1960).

Dole, V. P., J. Clin. Investigation, 35, 150 (1956).

Lynen, F., Kessel, T., and Eggerer, H., S. B. Bayer. Akad. Wiss. Math-Wiss Sitzung Vom 4 Marz, 1, (1960).

Lynen, F., Federation Proc. Symposium on Lipid Metabolism (1961).

Martin, D. B., Horning, M. G., and Vagelos, P. R., J. Biol. Chem., 236, 663 (1961).

Martin, D. B., and Vagelos, P. R., Biochem. Biophys. Res. Comm. 5, 16 (1961).

Stadtman, E. R., Record of Chem. Progress 15, 1 (1954).

Vagelos, P. R., J. Am. Chem. Soc., 81, 4119 (1959).

Vagelos, P. R., and Alberts, A. W., J. Biol. Chem. 235, 2786 (1960).

Wakil, S. J., J. Am. Chem. Soc., 81, 2597 (1959).

Wakil, S. J., J. Lipid Research, 2, 1 (1961).