SYNTHESIS OF UNSATURATED FATTY ACIDS IN THE CELLULAR SLIME MOLD

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The cellular slime mold D. discoideum contains a high percentage of mono- and polyunsaturated fatty acids, some of which are of unique structure (Davidoff and Korn, 1962). It was therefore of interest to investigate the pathway of biosynthesis of these compounds. The preliminary results indicate that octanoic and decanoic acids are incorporated into the long chain unsaturated fatty acids by a mechanism of chain elongation not previously described.

RESULTS AND DISCUSSION

The distribution of labelled fatty acids derived from labelled octanoic and decanoic acids was identical (Table I). The distribution of the radioactivity derived from labelled stearic acid, however, was quite different. This is most clearly seen by comparing the radioactivity in the 18:2 and 18:1 fractions[1] in the three experiments.

In order to determine the manner of incorporation of the precursors, fractions 18:1 and 18:2 were isolated, oxidatively degraded, and the resulting fragments analyzed for radioactivity. There was no randomization of the label in any of the experiments as indicated by the absence of radioactivity in the heptanoic and pelargonic acids (Tables II and III).

[1]The first number refers to the number of carbon atoms in the fatty acid chain, the second to the number of double bonds in the molecule, and the number in parenthesis to the position of the double bonds, counting from the carboxyl end.

TABLE I

Comparison of Octanoic, Decanoic, Stearic, and Oleic Acids as Precursors of Unsaturated Fatty Acids in D. discoideum

An aggregateless mutant of D. discoideum was grown in submersion culture with aeration, on lipid-free, autoclaved E. coli suspended in 1.5 l of 0.04 M phosphate buffer, pH 6.0, containing 60 mg of penicillin/l. After 18-24 hrs. growth, 10 microcuries of each radioactive precursor (specific activity 0.7 to 2.5 microcurie/micromole) was added to separate batches of cells. The amebae were harvested 24 hours after the addition of isotope, washed three times by centrifugation at 60 x g, and the total lipids extracted by blending in 20 vol. of $CHCl_3$-CH_3OH, 2:1. The lipids were transesterified in anhydrous H_2SO_4-methanol overnight at 65°. Methyl esters of the fatty acids were separated by gas-liquid chromatography (GLC) on 10% Apiezon M on Chromosorb W at 220° with an argon pressure of 30 lbs/sq. in. The effluent stream was split, one portion going to the detector, the other to a collecting outlet. Fractions were collected in U-tubes immersed in liquid N_2. Effluent was collected and counted between peaks as control samples. Recoveries ranged from 50-70% of the injected material. Fractions were washed into vials with 10 ml. of toluene containing 0.04% diphenyloxazole and radioactivity determined in a scintillation spectrometer.

Precursor	Total Incorporation %	Fatty Acid Fraction % of total radioactivity incorporated					
		16:0	16:1	16:2	18:0	18:1	18:2
1-C^{14}-octanoic	6	3	3	6	2	34	51
1-C^{14}-decanoic	6	3	3	8	2	28	50
1-C^{14}-stearic	60	0.3	0.12	0.08	6	15	73
1-C^{14}-oleic	34	0	0	0	0	10	90

The preponderant 18:1 acid synthesized from both octanoic and decanoic acids was 18:1(11); i.e. undecanedioic acid had a much higher specific activity than azelaic acid, (Table II). The difference is even greater than indicated in Table II, since the quantity of 18:1(11) was four times that of 18:1(9), (Davidoff and Korn, 1962). In marked contrast to this, the labelled stearic acid was incorporated predominantly into 18:1(9), (Table II). Thus although stearic acid can be converted to some extent to 18:1(11), it does not appear to be an intermediate in the synthesis of 18:1(11) from octanoic and decanoic acids. However, it is not possible from these data to decide whether or not stearic acid is an intermediate in the conversion of the shorter chain fatty acids to 18:1(9).

TABLE II

Distribution of Radioactivity in the Products of Oxidative Degradation of the Monounsaturated Fatty Acids

The 18:1 fractions, isolated as described in Table I, were oxidatively degraded by the procedure of von Rudloff (1956). The methyl esters of the products were separated by GLC on 20% SE-30 silicone rubber gum, on Gaschrom P, with an argon pressure of 15 lbs/sq. in., and the temperature programmed from 82°. Acids were identified by comparison to known standards. Individual fractions were collected and counted as described in Table I, and relative specific activities calculated for the compounds within each chromatographic run from areas of the peaks. A radium foil argon-ionization detector was used

Precursor acid	Monocarboxylic acid		Dicarboxylic acid	
	C 7	C 9	C 11	C 9
	Relative specific activity			
1-C^{14}-octanoic	0	0	44	8
1-C^{14}-decanoic	0	0	30	7
1-C^{14}-stearic	0	0	16	375

Essentially all of the radioactivity in the 18:2 fatty acids derived from 1-C^{14}-octanoic acid and 1-C^{14}-decanoic acid was found in the glutaric acid fragment produced by oxidative degradation (Table III). From the established structure of the major 18:2 acid, 5,11-octadecadienoic acid (Davidoff and Korn, 1962), the glutaric acid must have been derived from the carboxyl end of that molecule. When a portion of the 18:2 fraction from the 1-C^{14}-octanoic acid experiment was decarboxylated by the procedure of Brady (1961), most of the radioactivity was recovered in the CO_2, indicating that the label was in the carboxyl group of the polyunsaturated acid.

These results must mean that octanoic and decanoic acids were elongated by addition to the methyl-terminal end of the molecules. The results of oxidative degradation of the 18:1 fractions from the octanoic and decanoic acid experiments also support this hypothesis. The label in 18:1(9) derived from 1-C^{14}-octanoic acid was found in azelaic acid. If chain elongation had occurred at the carboxyl end of the 1-C^{14}-octanoic acid, the radioactivity

would have been only in pelargonic acid. Furthermore, 1-C^{14}-decanoic acid was incorporated into 18:1(11), a conversion that would not be expected to occur if this acid were synthesized by the chain-elongation mechanism found by Bloch (1961) to occur in other microorganisms.

TABLE III

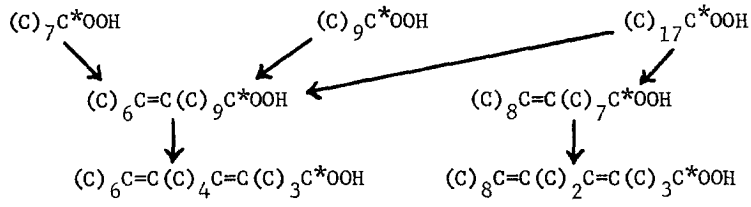
Distribution of Radioactivity in the Products of Oxidative Degradation of the Diunsaturated Fatty Acids

The 18:2 fractions were prepared as described in Table I, and oxidatively degraded and the products analyzed as described in Table II

Precursor acid	Monocarboxylic acid	Dicarboxylic acid	
	C 7	C 6	C 5
	Relative specific activity		
1-C^{14}-octanoic	0	3	20
1-C^{14}-decanoic	0	5	35
1-C^{14}-stearic	0	7	326

Finally, it should be noted that added 1-C^{14}-oleic acid, 18:1(9), was almost completely transformed into the 18:2 acid fraction (Table I). Since stearic acid was rapidly converted to 18:1(9), (Tables I and II), it is reasonable to assume that the structure of the labelled 18:2 acid in these two experiments was 5,9-octadecadienoic acid.

All of the above observations appear consistent with the following scheme for the biosynthesis of 18-carbon unsaturated fatty acids in D. discoideum:



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