THE ROLE OF ISOLEUCINE IN THE BIOSYNTHESIS OF BRANCHED-CHAIN FATTY ACIDS BY MICROCOCCUS LYSODEIKTICUS

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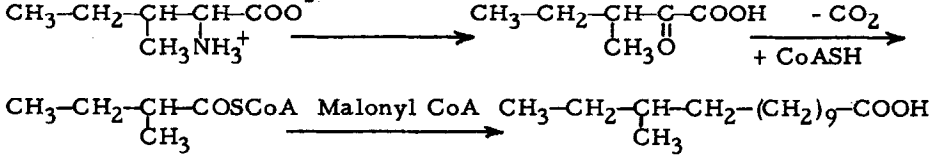
The presence of C_{15} and C_{17} branched-chain fatty acids in bacteria has recently been reported (Akashi and Saito, 1960; Saito, 1960; Macfarlane, 1961). The C_{15} compounds found consist of two types: the anteiso isomer, 12-methyltetradecanoic acid, and the iso isomer, 13-methyltetradecanoic acid. The occurrence of both the anteiso and iso branched-chain acids and the resemblance between the branched-chain portion of these acids and the side chains of isoleucine and leucine suggested that these amino acids might serve as precursors of the fatty acids. Verbeke et al. (1959) had similar views, but were unable to confirm them in experiments with perfused cow udders. The possible role of branched-chain amino acids in the biosynthesis of branched-chain fatty acids was also suggested by Horning et al. (1960) in connection with their observation that short branched-chain CoA esters, including α-methylbutyryl CoA, can condense with malonyl CoA to form branched-chain fatty acids in the presence of an adipose tissue enzyme. Furthermore, Allison and Bryant (1961) have shown that Ruminococcus flavefaciens utilizes isovaleric acid in the biosynthesis of a C_{15} branched-chain acid, while Wegner and Foster (1961) have demonstrated incorporation of labeled isobutyrate into uncharacterized fatty acids by Bacteroides succinogenes.

The following scheme can be envisioned for the synthesis of 12-methyltetradecanoic acid:



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The present study was carried out with M. lysodeikticus, A. T. C. C. 4698, grown in a defined medium (Grula et al., 1961). Macfarlane (1961) has reported that 12-methyltetradecanoic acid is the predominant isomer in the C_{15} branched-chain acid fraction obtained from M. lysodeikticus. Shown in Table I, column 1, is the percentage composition of the total fatty acids of M. lysodeikticus. Acids of chain length greater than C_{17} are present only in trace quantities. The C_{15} branched-chain ester and authentic 12-methyltetradecanoate have identical retention times, while the 13-methyl isomer has a slightly lower retention time. Mass spectrographic analysis by Dr. R. Ryhage of the Karolinska Institute demonstrated conclusively that the purified C_{15} acid from M. lysodeikticus is 12-methyltetradecanoic acid. The fact that the peak due to the C_{15} branched-chain acid isolated from M. lysodeikticus grown in the defined, isoleucine-free medium contains a slight shoulder in the region where 13-methyltetradecanoate occurs suggests that the C_{15} fraction contains approximately 10-20% of the 13-methyl isomer (see below).

In order to test the scheme postulated above, the incorporation of U-C^{14}-isoleucine, as well as U-C^{14}-α-methylbutyric, was studied. Cells grown in the presence of U-C^{14}-isoleucine incorporated ca. 10% of the added radioactivity into the total fatty acids. The percentage distribution of the radioactivity and the relative specific activities of the individual fatty acids are given in Table I, columns 2 and 3. It is evident that the relative specific activities of the C_{15} acid and the acid presumed to be the homologous C_{17} acid are at least twenty times greater than that of any of the straight-chain acids. Decarboxylation of the labeled C_{15} acid demonstrated that C-1 contained no radioactivity. If the acid were randomly labeled, 6.6% of the C^{14} would be present in C-1. Kuhn-Roth oxidation afforded a 45-55% recovery of the radioactivity in acetic acid derived from the two C-methyl groups. The value expected on the basis of the scheme shown above is 80%; however, control oxidations of U-C^{14}-isoleucine gave values that were also low. These results show that the incorporation of U-C^{14}-isoleucine into the branched-chain acid occurs in a specific manner, without significant breakdown to C_2 units followed by resynthesis.

According to the postulated scheme, the carboxyl group of isoleucine should not be incorporated into the branched-chain acids. When M. lysodeikticus was grown in the presence of 1-C^{14}-isoleucine only 0.1% of the added

TABLE I*

Fatty Acids Isolated from M. lysodeikticus
Grown in the Presence of U-C^{14}-Isoleucine

Cells were grown in 200 ml of medium containing 2.8×10^6 cpm (2.79 mg) of U-C^{14}-isoleucine, and harvested in late log phase. The washed cells were saponified and the crude fatty acids isolated. After chromatography on silicic acid to remove carotenoids and polar fatty acids, 4.6 mg (231,000 cpm) of fatty acid was obtained. The methyl esters were analyzed by vapor phase chromatography.

Fatty Acid[a]	% of wt.	% of C^{14}	RSA[b]
C_{14}	6.7	0.1	0.
C_{15br}	42.	83.8	400.
?	3.7	0.4	< 22.
C_{16}	23.	0.	0.
C'_{16}	19.	0.1	1.
C_{17br}	4.9	10.7	430.
?	1.	3.8	"1000."[c]

*Abbreviations: RSA = relative specific activity; C_{14} = tetradecanoate; C_{15br} = 12 (and 13)-methyltetradecanoate; C_{16} = palmitate; C'_{16} = hexadecanoate; C_{17br} = 15-methylhexadecanoate.

[a]Fatty acids identified by their relative retention times before and after separation into saturated and unsaturated fractions by the mercuric acetate method (Goldfine and Bloch, 1961).

[b]Relative specific activities of the fatty acids obtained by measuring the areas of peaks and the radioactivity in each peak; the values are estimated to be accurate within ±15% (Lennarz et al., 1961).

[c]An approximation since this uncharacterized, unsaturated acid is present in trace amounts.

radioactivity was found in the total fatty acids. Furthermore, the specific activity of these acids was reduced to 1/300th of that obtained in experiments where an equal quantity of U-C^{14}-isoleucine was added.

Finally, U-C^{14}-α-methylbutyric acid was tested as a precursor of 12-methyltetradecanoic acid. Cells grown in the presence of this compound incorporated 63% of the added radioactivity into the total fatty acids. Relative specific activity determinations (Table II) indicated that the α-methyl-

butyric acid was incorporated specifically into the C_{15} and C_{17} acids. No radioactivity was found in the carboxyl group of the C_{15} acid.

TABLE II

Fatty Acids Isolated from M. lysodeikticus
Grown in the Presence of U-C^{14}-α-Methylbutyric Acid

Cells were grown in 200 ml of defined medium containing 600,000 cpm of α-methylbutyric acid. The fatty acids, (6.3 mg, 380,000 cpm), were isolated and analyzed as indicated in Table I.

Fatty Acid	RSA
C_{14}	0.
C_{15br}	220.
?	20.
C_{16}	0.
C'_{16}	14.
C_{17br}	240.

The addition of isoleucine (0.5 mg/ml) and α-methylbutyric acid (0.5 mg/ml) to the growth medium caused a pronounced increase (40 - 90%) in the proportion of C_{15} branched-chain acid. Interestingly, both compounds inhibited growth to some extent. Whether this inhibition is due to an "unbalanced" fatty acid composition in the cell or to some other factor is not known.

The supposition that the C_{15} acid fraction also contains a small amount of 13-methyltetradecanoate was strengthened by the finding that, when M. lysodeikticus was grown in the presence of U-C^{14}-leucine, radioactivity was found in the C_{15} acid fraction and was localized in the "shoulder" region which corresponds to 13-methyltetradecanoate. Detailed studies on this apparently analogous synthesis of the branched-chain portion of the 13-methyl isomer from leucine have not been carried out.

Isoleucine and leucine are often used as "marker" amino acids in studies on protein synthesis. The results reported here indicate that, when such studies are carried out with organisms which synthesize branched-chain fatty acids, the possibility that the amino acid markers may also be incorporated in lipids must be considered.

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