VALINE AS A SOURCE OF THE BRANCHED SHORT CHAIN PRECURSOR IN THE BIOSYNTHESIS OF ISO-C_{14}, ISO-C_{15}, ISO-C_{16} AND ISO-C_{17} FATTY ACIDS BY BACILLUS SUBTILIS*

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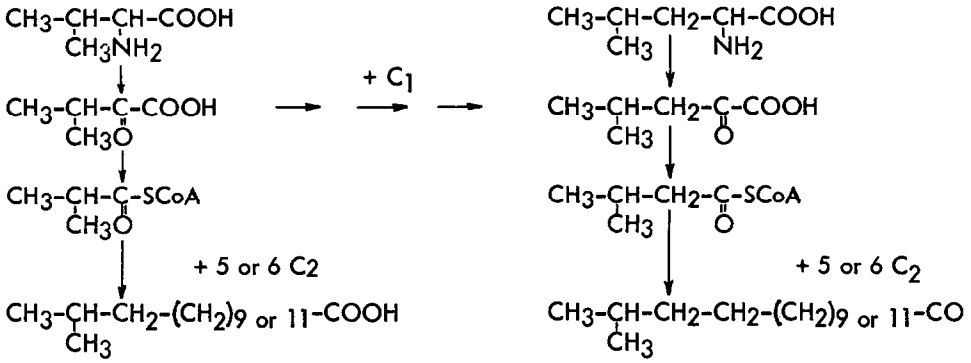
Previous studies on the role of amino acids in the biosynthesis of branched chain fatty acids by growing cells of B. subtilis have shown that U-C^{14}-valine was incorporated into i-C_{15} and i-C_{17} fatty acids+as well as into i-C_{14} and i-C_{16} fatty acids (Kaneda, 1963). The mechanism for incorporation of valine into the even numbered fatty acids was demonstrated on the basis of isotope experiments and enzyme work. However it was not possible at that time to present a mechanism by which the substrate entered the odd numbered fatty acids.

This communication presents evidence to support the following mechanism for the incorporation of valine into both the odd and even numbered fatty acids as an extension of the mechanism previously presented for the incorporation of valine into the even numbered fatty acids.



The microorganism, culture medium, chemicals and experimental procedures used were essentially the same as those previously described (Kaneda, 1963). However, for fractionation

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+ For abbreviations used, see Table I.

of the bacterial fatty acid methyl esters, a succinate polyester column longer than the previous column was used. The column, 14' x 1/4", gave a resolution of 0.65 for iso-anteiso C_{17} fatty acids indicating that complete separation of the two components was possible by use of the following technique. The pure i-C_{17} fatty acid was obtained by collecting the front half of the first peak of the overlapping peaks of the iso-anteiso mixture. Similarly, the pure a-C_{17} fatty acid was obtained by collecting the rear half of the second peak of the overlapping peaks. The purity of the fatty acids collected was confirmed by rechromatography on succinate polyester and Apiezon L columns. Complete separation of iso-anteiso C_{15} fatty acids by a single chromatographi procedure has not been successful since the resolution for iso-anteiso C_{15} fatty acids was found to be 0.50 even though a 24 foot column, which was considered to be the practical upper limit of length, was used.

When U-C^{14}-valine was added to the culture medium, the growing cells of B. subtilis incorporated 30% of the added radioactivity into the bacterial fatty acids. However, when 1-C^{14}-valine was used, only 0.4% of the added activity was found in the bacterial fatty acids. These results indicate that valine is incorporated into the fatty acids after elimination of the carboxyl carbon. The distribution of the radioactivity among the bacterial fatty acids derived from U-C^{14}-valine is shown in Table I. The highest specific activity was found in the i-C_{14} and i-C_{16} fatty acids. Furthermore, considerable activity was found in the i-C_{15} and i-C_{17} fatty acids. Some activity which was found in the a-C_{15} fatty acid could be accounted for by contamination with i-C_{15} fatty acid. These results suggest that the terminal isobutyryl group of i-C_{14} and i-C_{16} fatty acids and similarly the terminal isovaleryl group of i-C_{15} and i-C_{17} fatty acids may be derived from valine. Preferential incorporation of the entire isobutyrate molecule into the i-C_{14} and i-C_{16} fatty acids and of the entire isovalerate molecule into the i-C_{15} and i-C_{17} has been shown previously (Kaneda, 1963).

To investigate further the mechanism of valine incorporation into both the even numbered and odd numbered fatty acids, the radioactive i-C_{16} and i-C_{17} fatty acids isolated from the valine experiment were chemically degraded and the distribution of the radioactivity

TABLE I

Incorporation of U-C^{14}-Valine

into Branched Long Chain Fatty Acids+ by B. subtilis

B. subtilis was incubated at 37C for 16 hours in 50 ml of the standard medium used in the previous work (Kaneda, 1963) containing 0.33 mM U-C^{14}-valine, 14.0×10^5 cpm.

Fraction No.	Fatty Acid	Amount (μmole)	Specific Activity (cpm/μmole $\times 10^{-3}$)
1	i-C_{14}	0.18	189.
2	n-C_{14}	0.01	- °
3-1*	(i+a)-C_{15}	0.21	13.9
3-2*	(a+i)-C_{15}	1.08	1.8
5	i-C_{16}	0.62	181.
6	n-C_{16}	0.20	2.5
7-1	i-C_{17}	0.11	24.3
7-2	a-C_{17}	0.17	0.

+ Abbreviations used are: i-C_{14}, isomyristic; n-C_{14}, myristic; i-C_{15}, isopentadecanoic; a-C_{15}, 12-methyltetradecanoic; i-C_{16}, isopalmitic; n-C_{16}, palmitic; i-C_{17}, isoheptadecanoic; and a-C_{17}, 14-methyl-hexadecanoic acids.

* According to a geometrical adjustment of the chromatogram, 3-1 fraction consisted of i-C_{15} fatty acid (94%)and a-C_{15} fatty acid (6%), while 3-2 fraction consisted of i-C_{15} fatty acid (2%) and a-C_{15} fatty acid (98%).

° Since the amount of n-C_{14} fatty acid synthesized was too small to permit accurate measurement, this value can not be considered as significant.

along the carbon chain of the fatty acids was determined. The terminal methyl carbons of the i-C_{16} fatty acid were found to contain 38% of the total activity (Table II), i.e. each methyl carbon has 19% of the total activity. The tertiary carbon contained 19% of the total activity. No activity was found in the carboxyl carbon. The remaining activity, 43%, should be located

in the 2 to 13 positions of the i-C_{16} fatty acid. Similarly, the terminal methyl carbons of the i-C_{17} fatty acid were found to contain 40% of the total activity, i.e. each methyl carbon has 20% of the total activity. The tertiary carbon contained 19% of the total activity. No activit was found in the carboxyl carbon. The remaining activity, 41%, should be located in the 2 to 14 positions of the i-C_{17} fatty acid. According to the scheme presented, the methylene carbon adjacent to the tertiary carbon of the i-C_{16} and i-C_{17} fatty acids, in both cases, should contai the same activity as those of the methyl and tertiary carbons, i.e. 19% and 20% of the total activity respectively. The remaining activity, about 20%, must be located in the carbons other than those mentioned above indicating that some fragmentation of valine and resynthesis of fatty acids from the fragments occur during the growth of B. subtilis. The experimental results present in the Table I and II are consistent with the scheme shown in the figure.

TABLE II

Distribution of Radioactivity

along the Carbon Chain of i-C_{16} and i-C_{17} Fatty Acids

Derived from U-C^{14}-Valine and U-C^{14}-Leucine in B. subtilis

Substrate	Fatty Acid	CH_3 > CH	CH_2	$(CH_2)_{11 \text{ or } 12}$	COOH+
U-C^{14}-valine	i-C_{16}	134	66	150*	0
	i-C_{17}	64	31	65*	0
U-C^{14}-leucine	i-C_{17}	111	54	160*	0

+ The amount of the radioactivity is expressed in cpm per mmole carbon or carbons x 10^{-2}. Before the degradation was carried out, a carrier of i-C_{16} or i-C_{17} fatty acid respectively (about 43 mg in each case) was added to the radioactive fatty acid sample.

* The radioactivity in this portion is calculated by the difference between the total activity of the fatty acid and that of the carbons measured by the specific degradations.

It is of importance to know which enzyme systems are involved in the formation of the precursor of the isovaleryl group of i-C_{15} and i-C_{17} fatty acids from valine. The possibility of formation of the precursor through direct reduction of α-ketoisovalerate formed from valine to isovalerate is eliminated since 1-C^{14}-valine was not incorporated significantly into i-C_{15} and i-C_{17} fatty acids, although a possibility has been considered in the previous paper on the basis of the evidence that slight but significant activity was found in the mixed fraction of iso-anteiso C_{15} and C_{17} fatty acids when 1-C^{14} pyruvate was used. It is most probable that an enzyme similar to the system involved in formation of α-ketoisocaproate through condensation of α-ketoisovalerate and acetyl-CoA followed by elimination of one carbon as found in microorganisms (Calvo et al., 1962; Jungwirth et al., 1961; Gross et al., 1962; Strassman et al., 1962) may be the actual mechanism of valine incorporation into i-C_{15} and i-C_{17} fatty acids in B. subtilis. Further evidence for participation of α-ketoisocaproate in the biosynthesis of i-C_{15} and i-C_{17} fatty acids by B. subtilis was obtained from isotope experiments with U-C^{14}-leucine and 1-C^{14}-leucine. The growing cells of B. subtilis incorporated 50% of the activity of the added U-C^{14}-leucine into the bacterial fatty acids. The entire activity was restricted to the i-C_{15} and i-C_{17} fatty acids. Degradation of the i-C_{17} fatty acid showed that the specific activities of each of the two terminal methyl carbons and the tertiary carbon are identical and these three carbons contained 50% of the total carbon (Table II). The carboxyl carbon contained no activity. No activity was found in the bacterial fatty acids when 1-C^{14}-leucine was used. These results suggest that leucine is incorporated into i-C_{15} and i-C_{17} fatty acids after oxidative deamination followed by oxidative decarboxylation to yield the precursor of the isovaleryl group, which may be isovaleryl-CoA as shown in the figure.

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