Short Communication

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CONVERSION OF α -HYDROXYPALMITIC ACID TO PENTADECANOIC ACID BY RESTING CELLS OF *ARTHROBACTER SIMPLEX*

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Although recent studies from a number of laboratories revealed the occurrence of α -hydroxy fatty acids in various bacterial species (1–8), α -oxidation pathway of fatty acids has not been established. In an earlier report (9), we demonstrated that ^{14}C -labeled palmitic acid was rapidly converted to α -hydroxypalmitic acid in both growing and resting cells of *Arthrobacter simplex*. The present paper shows that ^{14}C -labeled α -hydroxypalmitic acid is converted further to pentadecanoic acid.

Arthrobacter simplex (*Corynebacterium simplex*), IFO 3530, was grown at 30° on a rotary shaker in a medium containing 1% pentadecane as the sole carbon source with mineral salts described by FUJII and FUKUI (10). After the cells were harvested and washed by centrifugation, they were suspended in 0.1 M phosphate buffer (pH 7.0). Uniformly ^{14}C -labeled DL- α -hydroxypalmitic acid (9 mCi/mmole) was chemically synthesized from the α -bromo compound of palmitic acid [$\text{U-}^{14}\text{C}$] (Radiochemical Centre, Amersham, U.K.) by the method of CASON *et al.* (11), and purified by thin-layer chromatography. The resting cell suspension (approx. 100 mg, dry weight) was incubated with α -hydroxypalmitic acid [$\text{U-}^{14}\text{C}$] (2.5 μCi) in a total volume of 5 ml at 30°. After 5 hr incubation with a shaker, an equal volume of 6 N HCl was added to the mixture. The lipids were then hydrolyzed at a refluxing temperature for 3 hr. The liberated fatty acids were extracted with petroleum ether and methylated with 5% HCl in methanol. The methyl esters of fatty acids were then analyzed by the use of gas-liquid radiochromatography (9). Identification of the major α -hydroxy or branched-chain fatty acids was mainly achieved by mass spectrometry and will be described elsewhere.

Fig. 1. Gas-liquid radiochromatogram of the methyl esters of fatty acids from *A. simplex* after incubation with α -hydroxypalmitic acid [$U\text{-}^{14}\text{C}$] for 2 hr.

The methyl esters of the labeled fatty acids were analyzed by gas-liquid radiochromatography on a Packard model 800 instrument equipped with an argon ionization detector. The coiled glass column packed with 15% ethylene glycol adipate polyester on Chromosorb W (80–100 mesh) was operated at 170° , and simultaneously the radioactivity was recorded. A, mass response of the methyl esters of fatty acids from the cells grown on pentadecane; B, radioactivity response. Peak of the methyl esters of fatty acids: a, $\text{C}_{13}:0$ acid; b, $\text{C}_{15}:0$ acid; c, $t\text{-C}_{16}:0$ plus $\text{C}_{15}:1$ acids; d, $\text{C}_{16}:0$ acid; e, $t\text{-C}_{17}:0$ plus $\text{C}_{16}:1$ acids; f, $\text{C}_{17}:0$ acid; g, $t\text{-C}_{18}:0$ plus $\text{C}_{17}:1$ acids; h, $\text{C}_{18}:0$ acid; i, $t\text{-C}_{19}:0$ plus $\text{C}_{18}:1$ acids; j, $\alpha\text{-OH-C}_{15}:0$ acid; k, $\alpha\text{-OH-iso-C}_{16}:0$

In earlier works (9, 12), cells were grown in a medium containing 1% glucose, 1% peptone, and 0.5% yeast extract. After incubation of the resting cells with α -hydroxypalmitic acid [$U-^{14}C$], a significant amount of radioactivity was found to be incorporated into pentadecanoic acid. In this case, however, a considerable amount of radioactivity was incorporated into many other fatty acids. Subsequently, we found that cells grown on pentadecane as the sole carbon source was better than cells grown on a complex medium since the former cells contained a large amount of endogenous pentadecanoic acid. Such endogenous pentadecanoic acid diluted the radioactive pentadecanoic acid derived from α -hydroxypalmitic acid [$U-^{14}C$], and the incorporation of radioactivity into other fatty acids beyond pentadecanoic acid was greatly reduced.

Figure 1 shows that the major fatty acids of the cells grown on pentadecane consisted of pentadecanoic acid, α -hydroxypentadecanoic acid, and three branched-chain (tuberculostearic acid-type) fatty acids containing 16, 18 and 19 carbon atoms (10). α -Hydroxypalmitic acid [$U-^{14}C$] was incorporated exclusively into pentadecanoic acid. In addition, a small but significant amount of radioactivity was found to be incorporated into α -hydroxypentadecanoic acid. After a 5-hr incubation, about 60% of α -hydroxypalmitic acid was converted to pentadecanoic acid. The data presented here and in earlier paper (9) indicate that fatty acids are degraded to acids with one less carbon via α -hydroxy fatty acids in *A. simplex*.

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acid; 1, α -OH- $C_{16}:0$ acid. The branched-chain fatty acids of peak c, e, g, and i cannot be separated from the corresponding monounsaturated fatty acids with one less carbon. However, argentation thin-layer chromatography (13) showed that the monounsaturated fatty acids accounted for only 10% of the total fatty acids in each peak. *t* and α -OH- refer to tuberculostearic acid-type and α -hydroxy fatty acids, respectively.

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