

The stereochemical reaction course for the two C-3 hydrogens of leucine to produce a characteristic isoprenoidal lipid in halophilic archaea was observed using incubation experiments with whole cell *Halobacterium salinarum*. Deuterium-labeled (3*R*)- and (3*S*)-[3-²H]leucine were freshly prepared as substrates from 2,3-epoxy-4-methyl-1-pentanol. Incorporation of deuterium from (3*S*)-[3-²H]leucine and loss of deuterium from (3*R*)-[3-²H]leucine in the lipid-core of *H. salinarum* was observed. Taken together with the results of our previous report, involving the incubation of chiral-labeled [5-²H]leucine, these results strongly suggested an involvement of isovaleryl-CoA dehydrogenase in leucine conversion to isoprenoid lipid in halophilic archaea. The stereochemical course of the reaction (*anti*-elimination) might have been the same as that previously reported for mammalian enzyme reactions. Thus, these results suggested that branched amino acids were metabolized to mevalonate in archaea in a manner similar to other organisms.

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