BIOCHEMISTRY OF SHORT-CHAIN ALKANES:
EVIDENCE FOR AN ELONGATION/REDUCTION/C1-ELIMINATION PATHWAY

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1. Introduction

C7-C11 alkanes accumulate in the oleo resins of several Pinus species native to western North America, most notably in the xylem oleoresin of Jeffrey pine, Pinus jeffreyi Grev. & Balf. (Savage et al., 1990a). Biological production of light hydrocarbons is of interest because they possess the same excellent combustion properties as petrochemical hydrocarbons in gasoline. Whereas production levels of short-chain alkanes in plants are insufficient to provide an economically viable fuel source, the genes encoding the alkane biosynthetic pathway may provide a biotechnological resource for engineering fermentation organisms with the capability to convert biomass to an alkane-based fuel. However, the feasibility of transgenic alkane biosynthesis depends upon the complexity of the alkane biosynthetic pathway.

Aliphatic hydrocarbons in other organisms appear to arise via acyl lipid intermediates. Long-chain waxy hydrocarbons in plants, animals and microalgae apparently are synthesized by elongation of a C16 or C18 acyl CoA to generate an acyl thioester one carbon longer than the hydrocarbon product (Kolattukudy, 1987; Vaz et al., 1988). The long-chain acyl CoA is then reduced to an aldehyde and free CoA, and the former undergoes either decarbonylation to form a hydrocarbon and carbon monoxide (Cheesbrough and Kolattukudy, 1984, 1988; Dennis and Kolattukudy, 1991, 1992), or decarboxylation to form a hydrocarbon and carbon dioxide, as has been recently demonstrated for hydrocarbon biosynthesis in insects (Reed et al., 1994; Mpuru et al., 1996). n-Pentane found in developing peanuts also appears to arise from an acyl lipid, albeit directly from the lipoxygenase-catalyzed degradation of linoleic acid without the involvement of an aldehyde intermediate (Pattee et al., 1970, 1974). In an early study of short-chain alkane biosynthesis in P. jeffreyi (Sandermann et al., 1960), the labeling pattern of n-[14C]heptane generated by incubating tissue slices with [2-14C]acetate was consistent with chain elongation via a fatty acid synthase-type polymerization of acetate units. However, this study did not determine whether the aliphatic carbon chain arises by decarbonylation or decarboxylation of octanal (generated from the reduction of a C8 thioester), or by degradation of a longer-chain fatty acyl group without involvement of an aldehyde intermediate.

We recently developed an in vivo experimental system using P. jeffreyi xylem sections that rapidly incorporate radiolabel from [14C]acetate into n-heptane (Savage et al., 1996a). Here, we examine the effect of an aldehyde trapping reagent (hydroxylamine) and a thiol reagent (β-mercaptoethanol) on the incorporation of radiolabel into n-heptane and putative biosynthetic intermediates. These results, along with observation of the direct in vivo conversion of 14C]octanal to n-[14C]heptane, suggest that the pathway to the short-chain alkane involves the decarbonylation or decarboxylation of octanal to generate n-heptane.
2. Results

Incubation of xylem sections with \[^{14}\mathrm{C}\]acetate not only resulted in radiolabel incorporation into \(n\)-heptane, but also into octanal and 1-octanol. Incorporation of radiolabel into the \(C_3\) alcohol was unexpected because there are no reports of alcohol intermediates in long-chain hydrocarbon biosynthesis, and 1-octanol has not been reported to accumulate in \(P.\ jeffreyi\) xylem or xylem oleoresin (Savage et al., 1996a). Thus, potential roles of both octanal and 1-octanol in the biosynthetic pathway to \(n\)-heptane were further investigated.

Early evidence for long-chain aldehydes as precursors of long-chain alkanes was provided by \[^{14}\mathrm{C}\]acetate feeding studies of wax biosynthesis in garden peas (\textit{Pisum sativum}), where \(\beta\)-mercaptoethanol inhibited radiolabel incorporation into alkanes and stimulated radiolabel incorporation into long-chain aldehydes (Buckner and Kolattukudy, 1973). Co-incubation of \(P.\ jeffreyi\) xylem sections with 5 \(\mu\mathrm{Ci}\) \[^{14}\mathrm{C}\]acetate and 1 to 50 \(\mathrm{mM}\) \(\beta\)-mercaptoethanol similarly resulted in inhibition of \(n\)-heptane biosynthesis relative to incubations with \[^{14}\mathrm{C}\]acetate alone, although radiolabel accumulated into both octanal and 1-octanol in the presence of \(\beta\)-mercaptoethanol.

Hydroxylamine serves as a useful probe for biosynthetic pathways involving aldehyde intermediates by reacting with free aldehydes to generate metabolically inactive oximes. To determine whether octanal is a precursor to both \(n\)-heptane and 1-octanol, xylem sections were co-incubated with 5 \(\mu\mathrm{Ci}\) \[^{14}\mathrm{C}\]acetate and 0.1 to 1 M hydroxylamine. Radiolabel was incorporated into octyl oximes in co-incubations with hydroxylamine, and increasing concentrations of the reagent inhibited radiolabel incorporation into \(n\)-heptane and 1-octanol, with a corresponding accumulation of radiolabel into octyl oximes.

To more directly assess the role of octanal and 1-octanol in \(n\)-heptane biosynthesis, xylem sections were incubated with \[^{14}\mathrm{C}\]1-octanol and \[^{14}\mathrm{C}\]octanal. Incubation of xylem sections with 10\(^5\) dpm \[^{14}\mathrm{C}\]octanal resulted in the incorporation of \(^{14}\mathrm{C}\) into \(n\)-heptane, although the majority of radiolabel was recovered as 1-octanol. Incubation of xylem sections with 10\(^5\) dpm \[^{14}\mathrm{C}\]1-octanol also resulted in incorporation of radiolabel into \(n\)-heptane, and a small amount of radiolabel was incorporated into octanal. To determine whether 1-octanol is first oxidized to the aldehyde before conversion to \(n\)-heptane, xylem sections were co-incubated with 10\(^5\) dpm \[^{14}\mathrm{C}\]1-octanol and 500 \(\mathrm{mM}\) hydroxylamine. No radiolabel was incorporated into \(n\)-heptane, but \(^{14}\mathrm{C}\) was incorporated into octyl oximes.

3. Conclusions

Taken together, these results support a pathway for the biosynthesis of \(n\)-heptane whereby acetate is polymerized via a typical fatty acid synthase reaction sequence to yield a \(C_5\) thioester, which subsequently undergoes a two-electron reduction to generate a free thiol and octanal, the latter of which alternately undergoes an additional, reversible, reduction to form 1-octanol or direct loss of \(C_1\) to generate \(n\)-heptane. Evidence supporting the role of acyl lipids in this pathway include the labeling pattern of \(n\)-\(^{14}\mathrm{C}\)heptane generated from \([2\-^{14}\mathrm{C}]\)acetate (alternately labeled carbons beginning with \(C_1\)) previously reported (Sandermann et al., 1960), and the rapid incorporation of radiolabel into \(n\)-heptane from \[^{14}\mathrm{C}\]acetate reported here. Evidence for the central role of octanal in \(n\)-heptane and 1-octanol biosynthesis is provided by the inhibition of radiolabel incorporation into \(n\)-heptane by \(\beta\)-mercaptoethanol, with corresponding accumulation of label in both the \(C_3\) aldehyde and alcohol. Hydroxylamine inhibition of radiolabel incorporation into both \(n\)-heptane and 1-octanol, with corresponding accumulation of radiolabel into octyl oximes, provides additional evidence that octanal serves as a precursor of both the alkane and the alcohol. Incorporation of radiolabel into \(n\)-heptane and 1-octanol from \[^{14}\mathrm{C}\]octanal, and the hydroxylamine-sensitive incorporation of radiolabel into \(n\)-heptane from \[^{14}\mathrm{C}\]1-octanol, confirm the direct relationship of the aldehyde to the alkane and the reversibility of the aldehyde to alcohol reduction. Further evidence supporting this pathway, including the effect of the \(\beta\)-keto acyl acyl carrier protein synthase inhibitor cerulenin on \(n\)-heptane biosynthesis, has recently been published (Savage et al., 1996b).