Conservation of a Pseudomonad-like Hydrocarbon Degradative Ferredoxin Oxygenase Complex Involved in Rhizopine Catabolism in Sinorhizobium meliloti and Rhizobium leguminosarum bv. viciae

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Abstract

In Sinorhizobium meliloti the mocCABR genes have previously been shown to be required for rhizopine (3-O-methyl-scyllino-samine, 3-O-MSI) catabolism. We show that the mocDEF gene cluster is also needed. MocDE(F), which is involved in the catabolism of 3-O-MSI to its demethylated form scyllino-samine (SI) has homology to components that would comprise a ferredoxin-oxygenase system. The mocCABRDEF suite of genes is required for 3-O-MSI catabolism in both S. meliloti and R. leguminosarum bv. viciae. However, SI catabolism in S. meliloti requires mocCABR, whereas only mocCA are required for its catabolism in R. leguminosarum suggesting the two species require different chromosomal genes which act in concert with moc genes for the catabolism of rhizopine.

The rhizobia (Rhizobium, Azorhizobium, Bradyrhizobium, Sinorhizobium and Mesorhizobium) form symbiotic associations with legume plants reducing gaseous dinitrogen to ammonium ions that are readily assimilated by plants. This process occurs in nodules on plant roots. The rhizopine 3-O-methyl-scyllino-samine (3-O-MSI) is important in intra-species rhizobial competition for nodulation (Gordon et al., 1996; Heinrich et al., 1999). Rhizopines are produced in bacteroids within nodules by certain members of Sinorhizobium meliloti and Rhizobium leguminosarum bv. viciae. They are novel compounds in that only the strain which can induce rhizopine synthesis in the nodule can catabolize it in the free-living bacteria (Murphy and Saint 1992; Murphy et al., 1995; Dessaux et al., 1998). The genes for rhizopine synthesis and catabolism have been isolated and are termed mos and moc genes respectively (Murphy et al., 1987; Murphy et al., 1993).

Previously, the mocCABR gene cluster has been shown to be required for 3-O-MSI catabolism in S. meliloti strain L5-30 (Rossbach et al., 1994). Homologies between the deduced proteins from this region and proteins in data bases suggest that MocA and MocC most likely have catabolic roles and MocB and MocR are probably involved in transport and regulation respectively (Rossbach et al., 1994).

More recently, we have shown in R. leguminosarum bv. viciae strain 1a that besides mocCABR two additional genes (mocD and mocE) and a likely third (mocF) are also required for 3-O-MSI catabolism. These genes are involved in the conversion of 3-O-MSI to its demethylated form, scyllino-samine (SI), in the rhizopine degradative pathway (Bahar et al., 1998).

We have used a combination of deletion/complementation studies, Tn5-B20 mutagenesis and DNA sequencing to determine whether these additional genes are also required for 3-O-MSI catabolism in S. meliloti. These studies indicate that situated immediately downstream of mocCABR are the mocDE(F) genes that are also required for 3-O-MSI catabolism (Figure 1). When mocEF and part of mocD are removed from the moc cluster SI (the demethylated form of 3-O-MSI) but not 3-O-MSI is catabolised (Figure 1). The mocDE(F) genes are therefore involved in demethylation of 3-O-MSI to SI.

In R. leguminosarum bv. viciae strain 1a the MocDEF proteins were proposed to form a ferredoxin oxygenase complex. This oxygenase system would use the ferredoxin reductase (MocF) to transfer electrons from NADH to the ferredoxin (MocE). Electrons would then be transferred to the oxygenase (MocD) to convert the methyl group of 3-O-MSI to a hydroxyl group thus forming SI (Bahar et al., 1998). This model is similar to that proposed for the initial steps in the breakdown of xylene by Pseudomonas putida (Suzuki et al., 1991).

There is extensive homology (81%, 82%, 71% identity at the amino acid level, respectively) between MocD, E and F in R. leguminosarum bv. viciae 1a and S. meliloti. Importantly, consensus sequences present in proteins forming a ferredoxin oxygenase complex are present. S. meliloti MocD, the proposed oxidative component, possesses three histidine boxes (His-X-X-His31-His-X- X-His-His-His-145-His-X-X-His-His) located in hydrophobic regions of the MocD protein which is characteristic of oxidative enzymes like xylene-monoxygenase (Suzuki et al., 1991) and alkane hydroxylase (Kok et al., 1989). MocE, a small protein of 106 amino acids has the conserved sequence C-X-H-X15-17-C-X2-H found in Rieske-like proteins. Both proteins lack homology to known ferredoxin oxygenases.
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ferredoxins involved in hydrocarbon breakdown (Morrice et al., 1988; Tan et al., 1993). Although MocF is not essential for 3-O-MSI catabolism it contains two copies of the consensus sequence G-X-G-X2-G-X3-A-X6-G characteristic of NAD-dependent ferredoxin reductase components which interact with ferredoxins in ring hydroxylating oxygenase reactions in hydrocarbon degradation (Zylstra and Gibson, 1989). It is possible the non-essential nature of MocF can be explained by it being substituted by another ferredoxin reductase elsewhere in the genome.

The results presented here differ from those described by Rossbach et al. (1994) who showed that mocCABR were sufficient for 3-O-MSI catabolism. As our study indicates that these genes only catabolised SI it is possible that SI and not 3-O-MSI was utilized in the Rossbach study. These compounds would not be distinguishable using the paper electrophoretic conditions reported.

In S. meliloti mocCABR are required for SI catabolism whereas in R. leguminosarum mocCA are sufficient. Hence, as mocB is a putative transport protein (Rossbach et al., 1994), R. leguminosarum, but not S. meliloti, likely has a gene outside of the moc cluster which can substitute for mocB to transport SI into the cells. Furthermore, this implies that the S. meliloti mocR gene is involved in regulation of both the demethylation of 3-O-MSI to SI and then the further catabolism of SI whereas in R. leguminosarum it only functions in regulation of the demethylation step. This suggests that in R. leguminosarum, the further catabolism of SI may be regulated by another gene, or alternatively, genes other than those in the moc cluster are involved in the catabolism of 3-O-MSI and these may be different in R. leguminosarum and S. meliloti.

In conclusion this study has shown that the moc cluster (mocCABRDE(F)) required for 3-O-MSI catabolism is conserved in S. meliloti strain L5-30 and R. leguminosarum bv. viciae strain 1a. In what are quite distantly related species (Young, 1996) the moc genes are highly homologous. The breakdown of 3-O-MSI proceeds via SI and appears to involve a mocDEF encoded Reiske-like ferredoxin oxygenase system similar to those found in hydrocarbon catabolism in pseudomonads. However, since R. leguminosarum does not require all of the moc genes that S. meliloti requires to catabolize SI it suggests genes
outside of the moc cluster, which act in concert with moc genes, are different in the two species. The rhizopine catabolic genes appear to have an adaptive role utilizing available functions in their host species to enable rhizopine catabolism to proceed.

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