Molecular cloning and functional expression of a new aphid isoprenyl diphosphate synthase

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Aphids are important insect pests in temperate regions, damaging crop plants by sucking nutrients from the phloem and by transmitting plant viruses [1]. Chemical control of certain aphid species is becoming extremely difficult due to resistance to insecticides [2]. In this context, the development of novel pest control products that specifically target aphids is highly desirable. To this end, we decided to focus on aphid isoprenyl diphosphate synthases (IPPS) as potential biorational target sites. Short-chain *E*-IPPSs are a class of prenyltransferases that are central to isoprenoid metabolism. This group includes geranyl diphosphate synthase (GPPS), farnesyl diphosphate synthase (FPPS) and geranylgeranyl diphosphate synthase (GGPPS) which synthesize geranyl diphosphate (C10), farnesyl diphosphate (C15), and geranylgeranyl diphosphate (C20), respectively.

First, we undertook the characterization of aphid FPPS. In most organisms, FPPS is a key enzyme in isoprenoid biosynthesis, supplying sesquiterpene precursors for several classes of essential metabolites (i.e. sterols, dolichols, ubiquinones, carotenoids, and substrates for farnesylation of proteins). In insect, FPPS is involved in the biosynthetic pathway of juvenile hormone. This hormone plays an important role in maintaining juvenile characteristics during the development of insects, but also in the maturation of the reproductive system [3, 4]. In many aphid species, FPPS is also predicted to play a key role in the biosynthetic pathway of the alarm pheromone E-β-farnesene.

A standard PCR cloning strategy was used to isolate the cDNAs of FPPSs from from four aphid species (*Aphis fabae, Acyrthosiphon pisum, Megoura viciae and Myzus persicae*). Identification of the deduced amino acid sequences as FPPSs was confirmed by BLASTP analysis. The predicted translated products have conserved sequence domains required by all *E*-IPPSs for substrate binding and catalytic activity [5]. However, the aphid sequences encode proteins displaying an apparently rare susbtitution (Phe/Tyr→Gln; Q281) at position -4 relative to the first aspartate-rich motif, a feature only shared with type-I lepidopteran FPPSs [6]. Our results also indicate that the *M. persicae* gene encodes two different isoforms, which vary by the presence or absence, in the N-terminus, of a mitochondrial targeting motif. We expressed both *M. persicae* isoforms in *E. coli* for functional characterization. The recombinant enzymes expressed were shown to be active using a standard acid lability assay. However HPLC and LSC analysis of reaction products revealed that the recombinant enzymes yielded geranyl diphosphate as its major product, which suggests a predominant role in geranyl diphosphate formation, as opposed to farnesyl diphosphate production as originally hypothesized.

In aphids, GPPS is supposed to play a key role in sexual phermone and alarm pheromone biosynthesis and represents a promising target for the development of novel biorational

insecticides. Moreover, the aphid GPPS reported here is only the second animal GPPS cloned, which provides new insight into the regulatory mechanism of product chain-length specificity used by an important class of prenyltransferases.

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