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Unusual features of a recombinant apple α-farnesene synthase

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Abstract

A recombinant α-farnesene synthase from apple (*Malus* × *domestica*), expressed in *Escherichia coli*, showed features not previously reported. Activity was enhanced 5-fold by K^+ and all four isomers of α-farnesene, as well as β-farnesene, were produced from an isomeric mixture of farnesyl diphosphate (FDP). Monoterpenes, linalool, (*Z*)- and (*E*)-β-ocimene and β-myrcene, were synthesised from geranyl diphosphate (GDP), but at 18% of the optimised rate for α-farnesene synthesis from FDP. Addition of K^+ reduced monoterpene synthase activity. The enzyme also produced α-farnesene by a reaction involving coupling of GDP and isoprenyl diphosphate but at <1% of the rate with FDP. Mutagenesis of active site aspartate residues removed sesquiterpene, monoterpene and prenyltransferase activities suggesting catalysis through the same active site. Phylogenetic analysis clusters this enzyme with isoprene synthases rather than with other sesquiterpene synthases, suggesting that it has evolved differently from other plant sesquiterpene synthases. This is the first demonstration of a sesquiterpene synthase possessing prenyltransferase activity.

Keywords: α-Farnesene; Prenyltransferase; Sesquiterpene; Terpene synthase; Farnesyl diphosphate; *Malus domestica*; Apple

1. Introduction

(*E*,*E*)-α-Farnesene (3,7,11-trimethyldodeca-1,3*E*,6*E*,10-tetraene) is a sesquiterpene hydrocarbon produced by many plant species in a range of tissues, in response to pathogens (Huang et al., 2003), or on wounding by herbivores (Boevé et al., 1996; Mercke et al., 2004; Pare and Tumlinson, 1999; van den Boom et al., 2004; Vuorinen et al., 2004). Production of α-farnesene is thought to play a role in plant defence by attracting predators and parasitoids (Pare and Tumlinson, 1998); however, α-farnesene is also an attractant to codling moth (Yana et al., 2003) and a sex pheromone in mice (Morgan et al., 2004). In plants (*E*,*E*)-α-farnesene is often produced along with other sesquiterpenes (Köllner et al., 2004) or as a minor product resulting from genetic engineering (Deligeorgopoulou and Allemann, 2003).

Rupasinghe et al. (2000) partially purified α-farnesene synthase from apple skin as a 108 kDa oligomer with an optimum pH for activity of 5.6 and an absolute requirement for either Mg^{2+} or Mn^{2+}. Kinetic studies on this impure native protein showed optimal activity at 20 °C while half-maximal activity was retained at 0 °C. A cDNA encoding a α-farnesene synthase, essentially identical to the enyme we are now reporting (sharing 574/576 amino acids) was isolated from ripe apple skin by Pechous and Whitaker (2004). The predicted molecular mass was 66 kDa. Protein expressed in vitro from lysed cells, but without further purification, showed α-farnesene synthase activity using (*E*,*E*)-farnesyl diphosphate (FDP) as a precursor, with minor quantities of β-ocimene produced when incubated with

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the monoterpene precursor geranyl diphosphate (GDP). The α-farnesene produced was 99.5% the (*E*,*E*)-isomer. From attempts to solubilise in vitro-produced protein, the authors suggested that improperly folded protein was also capable of synthesising (*E*)-nerolidol. Trace amounts of (*E*)-nerolidol and β-farnesene were also produced in assays with impure soluble enzyme. In contrast to the native partially purified enzyme, maximal activity was observed at pH 7.0–8.0.

Additional α-farnesene synthase genes have been isolated from angiosperms: pear (GeneBank Accession No. AY566286) and cucumber (Mercke et al., 2004), and the gymnosperms: loblolly pine (Phillips et al., 2003), and spruce (Martin et al., 2004). However, with the exception of the pear gene, the sequences have little similarity to those from apple. In each case, (*E*,*E*)-α-farnesene is the main product from FDP. The cucumber enzyme also uses GDP to make the monoterpene, β-ocimene. A mammalian or insect equivalent does not yet appear to have been isolated. With the importance of α-farnesene production to apple (Pare and Tumlinson, 1999; Pechous et al., 2005; Rowan et al., 2001; Rupasinghe et al., 2000; Yana et al., 2003), we carried out extensive kinetic studies on a purified recombinant enzyme from 'Royal Gala' apple. These studies revealed major differences in enzymic properties from those reported for the native apple α-farnesene and other sesquiterpene synthases and revealed a hitherto unreported prenyltransferase activity associated with this sesquiterpene synthase.

2. Results and discussion

2.1. Characterisation of recombinant α-farnesene synthase

The recombinant enzyme, which functioned as a monomer (Fig. 1), demonstrated a broad pH optimum (pH 7–8.5) and K_m values for FDP (~3 μM), Mg^{2+} (~700 μM) and Mn^{2+} (~15 μM), similar to other sesquiterpene synthases (Crock et al., 1997; Steele et al., 1998). Activity with FDP was enhanced 5-fold on addition of K ions (Table 1). Optimal K^+ concentrations were determined to be 30–50 mM ($K_m \sim 3$ mM). The addition of Na^+ did

Table 1
Effect of metal ions on the relative activity of semi-purified recombinant α-farnesene synthase in the presence of saturating (25 μM FDP)

Metal ion	V_{rel} (%)
Mg/K	100
Mn/K	41
Mg/Mn/K	69
Mg	16
Mn	13
Mg/Mn	18
Mg/Na	23
Mn/Na	15
Mg/Mn/Na	24

Concentrations used were 7 mM $MgCl_2$, 150 μM $MnCl_2$, 50 mM KCl, 50 mM NaCl. Data were the mean of three independent measurements and the experiment was carried out twice. SEM was <5% of the mean.

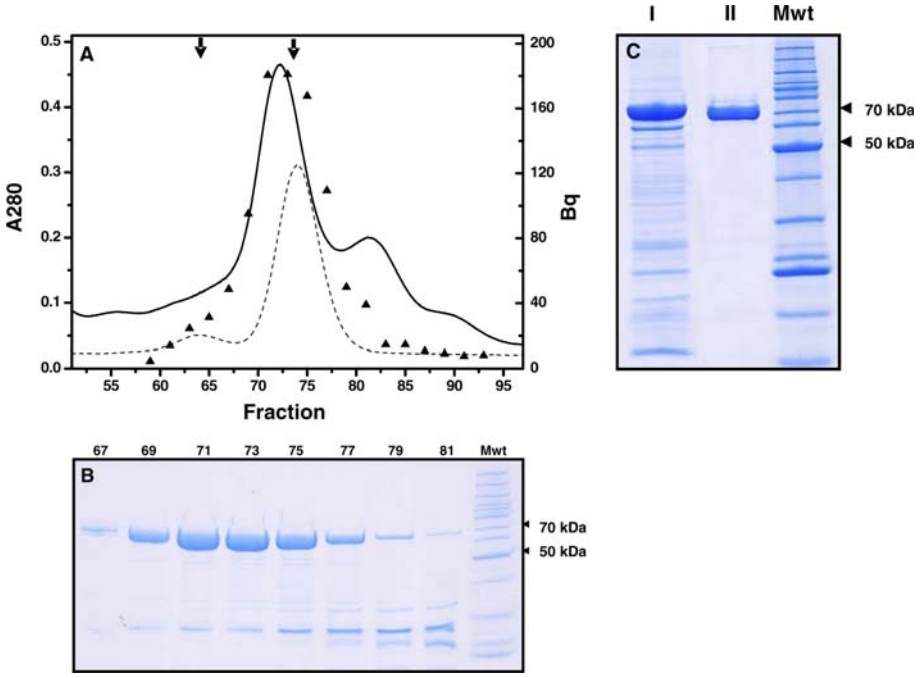

Fig. 1. Purification of recombinant α-farnesene synthase. (A) Gel filtration profile of purified recombinant apple $(His)_6$-α-farnesene synthase (——), BSA standard (------) and corresponding activity profile (▲) on G200 Superdex. BSA monomer and dimer peaks are indicated by down arrows. (B) SDS–PAGE gels of a subset of corresponding fractions from the gel filtration purification of recombinant apple $(His)_6$-α-farnesene synthase with molecular mass markers indicated. (C) Semi-purified (I) and purified (II) recombinant α-farnesene synthase used in kinetic analysis. Specific activity determinations and experiments with deuterated precursors were carried out using (II). Molecular mass markers are indicated.