Large-scale Synthesis of Farnesyl Diphosphate as Substrate for Recombinant Sesquiterpene Synthases

Thore Frister¹, Steffen Hartwig¹, Sascha Beutel¹, Thomas Schepeler¹

¹Institute of Technical Chemistry, Leibniz University Hanover, Callinstr. 5, D-30167 Hanover, Germany

Introduction

Farnesyl diphosphate (FPP) is a key intermediate of the terpenoid pathway in all living organisms. In eucaryotes FPP is converted to a broad range of essential metabolites including sterols and dolichols, which play an significant role in diseases of the cardiovascular system. Therefore FPP and its derivatives attracted a considerable amount of attention in pharmaceutical research. In plants FPP is the precursor for the biosynthesis of sesquiterpenes, a large and structurally diverse class of natural products. These compounds exhibit often a strong smell and are thus frequently used as fragrance compounds in costly perfumes and scented household goods. These days, the majority of terpene production is still based on the isolation from plant derived raw materials. The increasing need of terpenes especially in the emerging markets leads to higher prices and demands new, climate-independent, reliable processes for the production of terpenes. Recent advances in the recombinant terpen synthase related research have shown an in-vitro approach to certain sesquiterpenes based on chemical synthetic FPP (see paper Optimizing recombinant expression of patchoulol from P. cablin and enzymatic FPP biosynthesis as a model system for high level sesquiterpene production², Hartwig et al.). For the development of a semisynthetic approach based on the biotransformation of FPP large amounts of this high value substrate are required.

Experiments

FPP-Synthesis and Downstreaming

The procedure according to Keller et al. was found to be the most convenient method for a scale-up. Using this synthetic approach, the renewable, plant derived starting material farnesol can be phosphorylated stepwise in a fast and cost effective reaction at low temperatures. The reaction was carried out in a 250 ml flask equipped with a magnetic stirrer. During the reaction, the solution was cooled in a water bath to 37°C to avoid product losses due to decomposition. For the product isolation, the crude reaction mixture was applied on a silica plug using 0.7 kg silica gel 60 (230-400 mesh) as stationary phase in a 40 cm column with a diameter of 8 cm. The eluent consisted initially of a mixture of i-ProOH / 25% NH₄OH (6:4) which was subsequently changed to a higher ratio of i-ProOH to accelerate the elution of phosphate esters. A pressure reduction valve was installed to maintain a constant flow rate of 10 mL/min. This fast purification step delivers a FPP enriched solution, which contains small amounts of the mono- and triphosphate esters as well.

Discussion and Outlook

We present an up-scaling of the FPP-synthesis based on the method developed by Keller et al. The downstream-process was optimized in order to produce large amounts of substrate without time-consuming purification and extensive buffer adjustment. Even though the product contains small amounts of other phosphate esters, no enzyme inhibition was noticed. Because of the still challenging exact quantification of FPP in a mixture of polyphosphate esters, the downstreaming of FPP needs to be improved. For that purpose fast liquid chromatography in combination with cation-exchange membrane adsorbers is a promising technique for the purification of prenyl diposphates.

Literature


Acknowledgements

This work is funded by the European Regional Development Fund (ERDF): Innovation Network “Refinement of Plant Resources” (ZIM 8-80130940) We would like to thank Prof. Berger and Dr. Krings (Institute of Food Chemistry, University Hannover) for assistance with terpene analytics.

Fig. 1 Examples of terpenes produced in nature, with FPP as a key substrate

Fig. 2 Synthesis of FPP according to Keller et al. using the plant derived starting material farnesol.

Fig. 3 HPLC-chromatogram of a mixture of farnesylphosphate esters.

Fig. 4 GC-chromatogram of the enzymatic conversion of FPP.