Engineering the biosynthesis of artemisinin

Wim J Quax, Ingy I Abdallah, Hegar Pramastya and Dan Xue, Email: w.j.quax@rug.nl

University of Groningen, The Netherlands

Abstract

Terpenoids represent the largest class of natural products with a diverse array of structures and functions. Many terpenoids have reported therapeutic properties such as antimicrobial, anti-inflammatory, immunomodulatory and chemotherapeutic properties making them of great interest in the medical field. Terpenoids suffer from low natural yields and complicated chemical synthesis; hence there is a need for a more sustainable production method. Metabolic engineering using biosynthetic mevalonate and non-mevalonate pathways provides an excellent opportunity to construct microbial cell factories producing terpenoids. The complexity and diversity of terpenoid structures depends mainly on the action of the terpene synthases responsible for their synthesis. Amorpha- 4, 11-diene synthase (ADS) cyclizes the substrate farnesyl pyrophosphate to produce amorpha- 4, 11-diene as major product. This is considered the first committed and rate-limiting step in the biosynthesis of the antimalarial artemisinin. Here, we utilize a reported 3D model of ADS to perform mutability landscape guided enzyme engineering. A mutant library of 258 variants along sixteen active site residues was created and then screened for catalytic activity and product profile. This allowed for identification of the role of some of these residues in the mechanism. The mutability landscape also helped to identify variants with improved catalytic activity. H448A showed ~4 fold increase in catalytic efficiency and the double mutation T399S/H448A showed that kcat has improved by ~5 times. This variant can be used to enhance amorphadiene production and in turn artemisinin biosynthesis. Our findings provide the basis for the first step in improving industrial production of artemisinin and they open up possibilities for further engineering and understanding of ADS.

Malaria is still an eminent threat to major parts of the world population mainly in sub-Saharan Africa. Researchers around the world continuously seek novel solutions to either eliminate or treat the disease. Artemisinin, isolated from the Chinese medicinal herb Artemisia annua, is the active ingredient in artemisinin-based combination therapies used to treat the disease. However, naturally artemisinin is produced in small quantities, which leads to a shortage of global supply. Due to its complex structure, it is difficult chemically synthesize. Thus to date, A. annua remains as the main commercial source of artemisinin. Current advances in genetic and metabolic engineering drives to more diverse approaches and developments on improving in planta production of artemisinin, both in A. annua and in other plants. In this review, we describe efforts in bioengineering to obtain a higher production of artemisinin in A. annua and stable heterologous in planta systems. The current progress and advancements provides hope for significantly improved production in plants.

Malaria is still a global concern with around 214 million annual cases and 430,000 annual deaths, mainly among of children younger than 5 This fatal disease is caused by Plasmodium sp. particularly Plasmodium falciparum that proliferate in female Anopheles mosquitoes Since the 1940s there has been continuous attempts to halt the spread of the disease and this has succeeded in Europe, North America, and parts of Asia and Latin America. However, not in Sub-Saharan Africa where 80% of the annual malaria patients are found. Besides measures such as vector control and insecticide-treated nets, research and development has led to new drugs and a vaccine. The current preferred therapy is artemisinin combination therapy that is based on artemisinin produced in the natural source Artemisia annua. Artemisinin can also be produced heterologously in the plants Nicotiana in malaria mortality and morbidity. The focus for many years has been to screen traditional medicine to find new antimalarial drugs The malaria drug artemisinin is an example of this and originates from A. annua, a Chinese medicinal plant (Qinghao), commonly known as sweet wormwood. It was discovered by the Chinese researcher You-You Tu and her team in 1972, and was named Qinghaosu Chemically, artemisinin is a sesquiterpene lactone with a unique endoperoxide structure, without the nitrogen containing heterocyclic ring like other antimalarial compounds The in planta accumulation of artemisinin is 0.01–1.4% dry weight depending on the plant variety and artemisinin is stored in the glandular trichomes of A. annua . The current production using plants with a “low” content of artemisinin can only just cover the global need, which have led to an increase in price In 2006, World Health Organization (WHO) recommended artemisinin as

the first-choice treatment for malaria. Rapid emergence of antimalarial drug resistance drew attention to formulation of artemisinin-based combination therapy (ACT) with artemisinin as the primary substance and is now the preferred treatment

Efforts in plant breeding have been challenging due to the heterozygous nature of A. annua, which results in transgenic plants with varying degrees of artemisinin content even though they were generated in the same laboratory. This variation is due to the segregation of the heterozygous wild type progeny leading to a different genetic background than the parent plant. Although several high content lines have been created, the unstable yield in the progeny of these cultivars were insufficient to increase the global supply of artemisinin

Biography:

Wim J Quax was appointed as Professor of Pharmaceutical Biology at the University of Groningen in 1998. He has developed an extensive research group focussing on directed evolution and protein design technology for researching pharmaceutically relevant proteins. One of his focus areas are enzymes catalysing the synthesis of natural products. He has published >300 peer reviewed papers and book chapters and he is Inventor of >30 patents. He is the former Scientific Director of the Groningen Research Institute for Pharmacy (GRIP).

This work is partly presented at International Conference on Biotechnology, Biomarkers & Systems Biology

March 04-05, 2019 | Amsterdam, Netherlands