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Mechanistic insights into the substrate recognition of PPO: toward the rational design of effective inhibitors

Ge-Fei Hao^a, Chang-Guo Zhan^{b,*}, and Guang-Fu Yang^a

^aKey Laboratory of Pesticide & Chemical Biology of Ministry of Education, College of Chemistry, Central China Normal University, Wuhan, P. R. China

^bDepartment of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, Lexington, Kentucky, United States of America

Abstract

“...the obtained structural and energetic insights of the substrate recognition process represent a paradigm shift and a new starting point for structure-based design of novel, more potent PPO inhibitors.”

Keywords

substrate recognition; inhibitor design; agricultural herbicide; variegated porphyria; photodynamic therapy; resistance

Protoporphyrinogen oxidase (PPO) inhibitor design is undergoing a radical transition from laborious to more efficient ways based on the detailed understanding the protein structures and mechanisms in various species [1]. Development of structure-based design methods and understanding of relevant mechanistic studies play a key role in this transition [2–4].

Why are PPO inhibitors important?

PPO is the last common enzyme for the biosynthesis of heme and chlorophyll, catalyzing the oxidation of protoporphyrinogen-IX to protoporphyrin-IX [5]. The inhibition or functional loss of PPO is more than merely blocking the production of heme and chlorophyll. When the enzyme is inhibited, the substrate protoporphyrinogen-IX will accumulate in the cytoplasm and will be slowly oxidized by O₂ in the mitochondrion and chloroplast to produce protoporphyrin-IX. This spontaneous production can have dire consequences: In the presence of light, the photosensitive protoporphyrin-IX generates singlet oxygen that causes lipid peroxidation and cell death [6].

Author for correspondence: Department of Pharmaceutical Science, College of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, 789 South Limestone Street, Lexington, KY 40536, USA Tel.: +1 859 323 3943 Fax: +1 859 257 7585 zhan@uky.edu.

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Due to the crucial role in the life cycle, PPO is of great importance in medical research. For example, partial PPO deficiency in humans causes an inherited disease known as variegated porphyria (VP) characterized by cutaneous photosensitivity and the propensity to develop acute neurovisceral crisis [7]. The symptom of VP and its highly variable penetrance of infected individuals make the study of the nature of PPO causing the disease of great interest. Besides, protoporphyrin-IX is an extremely effective photosensitizer, but it is not useful before activation. Halling *et al.* [8] showed that PPO inhibitors could activate the photosensitizer protoporphyrin-IX and cause its accumulation within tumor cells. Hence, an important medical application of PPO inhibitors is associated with photodynamic therapy (PDT), which has been used in the detection and treatment of cancer [8]. Besides, PPO inhibitors have also been used as herbicides to control weeds [1].

VP characterized by an abnormal pattern of protoporphyrin-IX excretion is a type of acute hepatic porphyria [9,10]. Though the study of VP has been performed for more than fifty years [11,12], the entire molecular mechanism of VP is still unclear. To address this important issue, PPO inhibitor mimicking protoporphyrinogen-IX plays important function. It is hypothesized that the sensitivity of VP patients to light should be similar with the condition in plants. Because inhibition of PPO in plants can also lead the accumulation of photosensitizing protoporphyrin-IX. Hence, PPO inhibitors can be used as chemical probes to study the mechanism of VP. A recent study indicated that the VP-causing mutation affect the catalytic activity of PPO by affecting the ability of PPO to sample the privileged conformations [13]. If novel non-competitive inhibitors could be designed to prevent the release of protoporphyrinogen-IX to cytoplasm, the non-enzymatic oxidation may not happen and the sensitivity of VP patients to light may be largely relieved.

In addition, competitive PPO inhibitors have demonstrated advantageous characteristics including activation of the photosensitizer protoporphyrin-IX. An important medical application of competitive PPO inhibitors is associated with PDT. Hence, the characteristics exhibited by PPO-inhibiting have attracted the attention of chemists worldwide. Great effort has focused on the synthesis of structurally different PPO inhibitors and more than 30 PPO inhibitors have been reported during the last decade, including diphenylethers, phenylpyrazoles, oxadiazoles, triazolinones, thiadiazoles, pyrimidindiones, oxazolidinedione, *N*-phenyl-phthalimides, and others [1]. However, most PPO inhibitors only mimic two of the four pyrrole rings in protoporphyrinogen-IX [14]. To improve the activity of PPO inhibitors, mimicking more pyrrole rings of protoporphyrinogen-IX maybe a good choice. Besides, discovering PPO inhibitors that can selectively accumulate within tumor cells may have a great contribution for the development of cancer treatment through PDT. All of these rely on design of more novel PPO inhibitors with various structures and action mechanisms.

What are the main challenges of PPO inhibitor design?

There are many challenges for the discovery of modern pharmaceuticals. Three major challenges facing the PPO inhibitor design are: (1) understand molecular mechanism concerning the PPO substrate recognition, (2) design inhibitors with novel a protein-ligand

interaction mechanism, and (3) design inhibitors targeting a specific PPO species. Below, we briefly discuss how these challenges can influence the discovery of PPO inhibitors.

Competitive inhibitors can compete with the substrate to bind in the same active pocket. Up to now, all of the available PPO inhibitors are competitive inhibitors to mimic half of the structure of protoporphyrinogen-IX. Hence, understanding the mechanism of the substrate (S) recognition and the structure of the enzyme–substrate (ES) complex is crucial for rational design of competitive inhibitors [15].

One of the grave concerns for modern pharmaceuticals is development of resistance. Up to now, more than 30 PPO inhibitors were discovered, but almost all of the inhibitors discovered in recent decades have similar action mechanism, which is unfavorable to avoid resistance. Therefore, the discovery of PPO inhibitors with novel scaffolds and novel action mechanisms are of great interest, but it has been hampered by the lack of structural and mechanistic understanding of the substrate.

Actually, the most potentially important medical application of PPO inhibitors is associated with PDT [8], which has been used in the detection and treatment of cancer and is also potentially valuable in destroying bacteria and other dangerous organisms. Hence, design of PPO inhibitors targeting specific PPO species is very important. In fact, selectivity is an important but still unresolved problem. Whether pharmaceuticals or agrochemicals, improving selectivity is very challenging. For agrochemicals, the success is to hit the target from species of interest while avoiding inhibit target from mammals and beneficial organisms which may result in negative effect for human and environment. For pharmaceuticals, the success is to hit the specific target isoforms while avoiding inhibit other similar proteins which may result in side effects, such as toxicity. The scientific problem of designing particular selectivity is significantly more complex than improving the potency to a target, because of the multi-factorial nature of the task [16].

How mechanistic studies influence the rational design of PPO inhibitors?

To put this in perspective, mechanistic study means to bridge between a biological target and successful inhibitor design. PPO is only one of the numerous biological targets, but its significance in both pharmaceutical and agrochemical areas makes it in special position. As an agrochemical target, PPO is old. But for pharmaceuticals, PPO is new. No matter whether it is “new” or “old”, which is defined only according to the discovery time of the function, PPO is an important biological resource worthy of further studies. Although there are many available PPO inhibitors, there are still many challenges facing PPO inhibitor design.

In a recent study [17], we computationally simulated and discovered the binding model of protoporphyrinogen-IX with PPO, which was also validated by experimental tests including site-directed mutagenesis. Based on this novel binding model, the substrate recognition mechanism has been identified for the first time, indicating that the protoporphyrinogen-IX binding process should be very fast, but the protoporphyrin-IX leaving process should be much slower. Most importantly, a feedback inhibition mechanism of protoporphyrin-IX for PPO activity was discovered, which is also validated by enzyme kinetic studies.

Previously, we believed that the binding conformation of the substrate is important for competitive PPO inhibitor design. However, after the substrate recognition mechanism was uncovered for the first time, the strategy of PPO inhibitor design could change. As mentioned above, protoporphyrin-IX has a feedback inhibition, which means that the product has a stronger binding than the substrate. Therefore, we should pay more attention on the binding conformation of the product rather than the substrate. An ideal inhibitor should be designed to mimic the binding conformation of product. In addition, the substrate-binding channel in PPO has also been identified. So, the substrate-binding channel of PPO should also be a potential target site for inhibitor design. Such inhibitors will have a novel interaction mechanism different from the existing inhibitors, and should overcome the resistance to competitive inhibitors of PPO. In one word, the obtained structural and energetic insights into the entire binding and leaving process represents a paradigm shift and a new starting point for structure-based design of novel, more potent PPO inhibitors.

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Biography

Chang-Guo Zhan