Chelerythrine Chloride and Urease inhibitor

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Inhibition of ruminal microbial urease is of particular interest due to its crucial role in regulating urea-N utilization efficiency and nitrogen pollution in the livestock industry. Chelerythrine chloride was selected as a potential urease inhibitor by inhibiting the GTPase activity of UreG and interaction between UreG and nickel, which were the key steps in disrupting urease maturation. The inhibition potency of chelerythrine chloride against UreG provided new evidence for strategies to develop novel urease inhibitors targeting UreG to reduce nitrogen excretion from ruminants.

Keywords: urease inhibitor ; UreG ; chelerythrine chloride ; ammonia production ; rumen

1. Introduction

Urea is commonly used as a cost-efficient replacement of feed proteins to provide the sole nitrogen source for urease [1][2][3]. In rumens, urease catalyzes the hydrolysis of urea into ammonia, which can be synthesized into microbial protein to support the requirements for animal growth, meat or milk production [4]. However, the high urease activity leads to excessive ammonia production, which not only results in the explosion of toxic ammonia in the blood, but is also converted to volatile ammonia and escapes into the environment [5][6]. Emissions of nitrogen have been recognized as one of the major drivers in the production of greenhouse gases and wastewater and soil pollution. The livestock industry contributes approximately 65 $Tg{\cdot}N{\cdot}yr^{-1}$, which is roughly one-third of the global human-induced nitrogen emissions [7]. Ruminant nitrogen is the major source of livestock pollution, with approximately 46 $Tg{\cdot}N{\cdot}yr^{-1}$, equivalent to 71% of the total nitrogen emissions from livestock [7]. For ruminants, the dietary nitrogen utilization efficiency is only about 25% [8][9], with as much as 60–90% of the feed nitrogen being excreted [10]. Taken together, regulation of urease activity is crucial to reduce ammonia emissions and improve the efficiency of urea-N utilization in ruminants, and urease inhibitors have been recognized as one of the most effective strategies.

There are many kinds of urease inhibitors, including those that attack the active center of urease, such as urea analogues [11], hydroxamic acids [12], phosphoramide and their derivatives [13], as well as other inhibitors that interact with the flap regions near active centers, such as 1, 4-benzoquinone [14], catechol [15], and some heavy metal ions [16]. However, only acetohydroxamic acid (AHA) is a commercially available urease inhibitor approved by the Food and Drug Administration for the treatment of humans and animals [17]. Unfortunately, significant adverse effects, including deep-vein phlebothrombosis, lower-extremity phlebitis, and malformation of embryos, have been reported [18]. In rumens, AHA was found to degrade rapidly, and inhibit the growth of rumen microorganisms other than urease-producing bacteria [19]. In recent years, plant-derived natural compounds (including that shown in Cover picture [20][21][22][23]) have been preferred for their low toxicity, chemically stability, eco-friendliness and high efficiency at low concentrations [24]. Gnetegha Ayemele et al. [25] screened giant milkweed as an alternative plant-derived feed additive, which could improve nitrogen utilization efficiency by inhibiting protozoa in ruminants without impairing fermentation. Limited studies have found that natural compounds such as terpenoids, phenolic compounds, alkaloids, and other substances were inhibitory towards the activities of plant and microbial ureases [26]. These findings suggest that natural compounds have great potential as urease inhibitors.

The active center of urease is deeply buried in apo-urease, with an unobserved wide-open state [27]. Additionally, urease is a highly specific substrate of urea [28], which makes it challenging to design new urease inhibitors based on the active center. Accessory protein UreG, which is a GTPase involved in transferring nickel to the active center and activating urease upon GTP hydrolysis, plays an important role in urease maturation [29]. It has been reported [30][31] that UreG receives nickel from the accessory protein UreE through the formation of an UreE-UreG complex. Subsequently, nickel-charged UreG delivered nickel to the active center of apo-urease by forming an UreG-UreFH complex and apo-urease/UreGFH super-complex, in which GTP hydrolysis induced the conformational changes of UreG, and promoted the

insertion of nickel into the active center. Moreover, UreG and urease maturation are relatively conserved among different urease-producing bacteria [32]. Disruption of the urease maturation process by abolishing UreG function has been proposed as a new strategy to develop urease inhibitors.

2. Screening of urease inhibitor

Given the importance of UreG GTPase activity and the simplicity of GTPase activity detection, we set the GTPase activity of UreG as an indicator to preliminary screen for potential inhibitors from 1130 natural compounds. Most of the top 20 compounds with better inhibition potency were alkaloids, among which nitidine chloride, chelerythrine chloride, chelerythrine, sanguinarine chloride, sanguinarine and dihydrochelerythrine were the major bioactive ingredients of *Zanthoxylum nitidum*. Some studies [43,44] found that *Z. nitidum* and its bioactive ingredients were used to treat gastritis diseases, and inhibited the growth of *H. pylori*. The mechanism of anti-gastritis and inhibiting *H. pylori* activity remains unclear, which may be related to the decrease in UreG GTP activity.

To better identify potent inhibitors of urease, the IC_{50} of compounds with stronger inhibition potency towards UreG GTP activity, as well as the binding energy and inhibitor constant obtained using molecular docking, were used for further screening. Chelerythrine chloride is considered to be the most potential inhibitor of urease, with the lowest IC_{50} and K_i values and the highest binding energy. Li et al. [33] demonstrated that when the final concentration of *H. pylori* UreG was 5 μM, the IC_{50} value of coptisine was 89.86 μM. In this study, the IC_{50} value of coptisine chloride was 86.62 μM, with a final concentration of 3 μM UreG, and the IC_{50} value of 18.13 μM for chelerythrine chloride was significantly lower than that of coptisine chloride. Additionally, chelerythrine chloride and coptisine chloride, two alkaloids, had similar binding energy and K_i, which were different from that of the caffeoylquinic acid isochlorogenic acid C. Notably, when the final concentration of natural compounds was 100 μM, the inhibition rate of isochlorogenic acid C against UreG activity was stronger than that of chelerythrine chloride. However, when their final concentration was lower than 100 μM, the fitting curve of chelerythrine chloride was steeper with weaker GTPase activity, suggesting that it is better to evaluate the inhibition potency of compounds using multiple additive concentrations.

3. The mechanism of chelerythrine chloride as a urease inhibitor of UreG

UreG is thought to act as a bridge that transfers nickel from UreE to apo-urease upon the formation of the UreE-UreG and UreDFG complexes. The key to UreG serving as a target to design urease inhibitors is to disrupt the urease activation process by blocking nickel delivery to UreG. Here, we further explored whether nickel binding to UreG was suppressed by chelerythrine chloride as well as its underlying mechanism. The ITC results show that chelerythrine chloride reduced the molar ratio, binding affinity and $-\Delta H$ value in between nickel and UreG, inhibiting nickel binding to UreG. Furthermore, chelerythrine chloride induced a secondary structure change of UreG, and formed a pi-anion interaction with Asp41. The crystal structure of the UreGFH complex [31] revealed that the Asp37 residue (Asp41 residue in the study) generated charge-charge repulsion with γ-phosphate upon GTP binding, which induced the formation of a salt bridge between Glu42 and Arg130, and the motion of “zip-up” between β2 strand and β3 strand, subsequently propagating the conformational changes of UreG in the CPH motif, which was the key site for nickel binding. Chelerythrine chloride may disrupt the biological function of CPH motif through the induced structure change of UreG and interaction with Asp41 residue. Our previous study [34] also found that the Asp41 residue was the key residue affecting the binding of nickel and UreG. A recent study [35] demonstrated that the cmpd4 compound formed a hydrogen bond with Thy15 residue of *H. pylori* UreG, and inhibited the activities of UreG and *H. pylori.* The results of Thy15 are consistent with those of our Thy19.

Yang et al. [35] first proposed and confirmed that targeting the metallochaperone UreG to design urease inhibitors could disrupt the urease maturation process, and provided two effective urease inhibitors of colloidal bismuth against *H. pylori* activity only through UreG, not apo-urease. Li et al. [33] demonstrated that coptisine inactivation of *H. pylori* urease involved binding to the urease active site sulfhydryl group and accessory protein UreG. These results show the potential of UreG serving as a target for developing urease inhibitors. Chelerythrine chloride not only inhibited the GTPase activity of UreG from a predominant ruminal microbial urease, but also suppressed nickel binding to UreG, which would interfere with urease maturation. Moreover, in clinical practice, the antimicrobial, antibacterial, anti-inflammatory and antiplatelet activities of chelerythrine have been extensively studied [36][37]. In the livestock industry, pharmacokinetic analysis concluded that the addition of chelerythrine to feed was safe due to the first pass effect after intestinal and liver metabolism [38]. These characteristics of chelerythrine chloride provide a solid foundation for the development of chelerythrine chloride as a natural feed additive in ruminants.

References

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