Development of a Taste Sensor with Lipid/Polymer Membranes for Detection of Non-Charged Bitter Substances Based on Allostery

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論 文 名　：Development of a Taste Sensor with Lipid/Polymer Membranes for Detection of Non-Charged Bitter Substances Based on Allostery
(アロステリーに基づく脂質高分子膜を用いた味覚センサによる非荷電苦味物質の検出に関する研究)

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論 文 内 容 の 要 旨

Taste sensors have been developed over several decades and successfully applied in the food and pharmaceutical industries. However, detecting non-charged bitter substances remains a significant challenge due to their reliance on potentiometric determination, which primarily depends on changes in the surface charge density of lipid/polymer membranes caused by interactions with charged substances. To address this limitation, a novel sensing approach utilizing lipid/polymer membranes for non-charged bitter substances is required.

In 2019, Yoshimatsu, Toko, and colleagues proposed a surface modification approach to detect non-charged bitter substances like caffeine. This innovative method employs hydroxybenzoic acids (HBAs) as membrane-modifying agents, with 2,6-dihydroxybenzoic acid (2,6-DHBA) playing a key role. The 2,6-DHBA-modified membrane demonstrated enhanced responses to caffeine, theophylline, and theobromine, with sensor responses increasing proportionally with sample concentration. The response principle for caffeine detection was discussed as an allosteric mechanism. Allostery, a phenomenon commonly observed in enzymes and receptors, involves ligand binding at one site of a receptor molecule affecting interactions with the same or a different ligand at a distant site. However, the specific conditions necessary for this mechanism—such as the optimal concentration of the modifier and the interactions between the modifier and caffeine—remain unclear.

In addition to methodological challenges, ethical concerns and toxicity risks associated with human sensory panels—particularly when the drug's toxicity profile is incomplete—underscore the necessity of using taste sensors to evaluate the bitterness of non-charged pharmaceuticals, such as xanthine-based compounds.

This dissertation aims to develop an allostery-based taste sensor for detecting non-charged bitter substances, including pharmaceutical compounds, as a reliable alternative to human sensory panels. The thesis comprises five chapters. In addition to the introductory and concluding chapters, the core chapters address the following topics:

Chapter 2 investigates the mechanism underlying caffeine detection in taste sensors from two perspectives. First, experimental investigations clarify the effect of membrane modifiers on caffeine detection. Results demonstrate that modifiers with higher hydrophobicity are more likely to adsorb onto the membrane, while membranes modified with HBAs with greater degree of dissociation respond more effectively to caffeine. Additionally, optimizing the membrane surface charge density enhances sensitivity to caffeine.

Subsequent ^{1}H-NMR measurements confirmed interactions between caffeine and HBAs. The absence of chemical shifts in aniline—a control substrate—indicates that intermolecular H-bonds and π–π interactions between HBAs and caffeine form stable complexes in solution. NOESY measurements further reveal that caffeine and 2,6-DHBA stack together, suggesting H-bonding between the hydroxy group of HBA and the carbonyl group or nitrogen within caffeine's imidazole ring.

A comparison of taste sensor results with ^{1}H-NMR data validates the allosteric mechanism underlying caffeine detection. This mechanism involves a transition from intramolecular H-bonds within HBAs to intermolecular H-bonds between HBAs and caffeine. Disruption of HBAs' intramolecular H-bonds by caffeine enables ionized H^{+} to return to the membrane, thereby generating a positive sensor response.

Chapter 3 focuses on enhancing sensor sensitivity to caffeine and its analogs through a three-step process. First, the key structural features required for the allosteric mechanism were identified, revealing that 2,6-DHBA possesses structural elements conducive to this mechanism. Second, the potential of novel modifiers was validated by testing three candidates: 2,6-dihydroxyterephthalic acid (2,6-DHTA), 1,3-dihydroxy-2-naphthoic acid, and 3-bromo-2,6-dihydroxybenzoic acid (3-Br-2,6-DHBA). The results demonstrated that both 2,6-DHTA and 3-Br-2,6-DHBA significantly improved the sensor's sensitivity to caffeine. Finally, the modified sensors were evaluated with caffeine analogs. Sensors incorporating 2,6-DHTA or 3-Br-2,6-DHBA exhibited enhanced sensitivity to these analogs, with the 3-Br-2,6-DHBA-modified sensor showing excellent repeatability and selectivity for caffeine.

Chapter 4 evaluates the bitterness of non-charged pharmaceuticals, including xanthine-based substances, using the 3-Br-2,6-DHBA-modified sensor. Concentration-dependent experiments and selectivity tests were conducted with compounds such as etofylline, proxyphylline, diprophylline, acefylline, doxofylline, and pentoxifylline. The results indicate that the response principle for xanthine-based substances aligns with the allosteric mechanism observed for caffeine detection. Sensory tests quantified the bitterness intensity of these substances, revealing a proportional relationship between the taste sensor responses and the perceived bitterness intensity. Additionally, the sensor is utilized to assess the bitterness of pentoxifylline, a xanthine derivative unsuitable for sensory testing. These results highlight the 3-Br-2,6-DHBA-modified taste sensor as a valuable complementary tool for assessing the bitterness of substances with a xanthine scaffold. Finally, the evaluation scope was expanded to include non-xanthine derivatives, such as allopurinol, broadening the application of the sensor.

Chapter 5 summarizes the findings of Chapters 2–4, affirming allostery as an effective approach for detecting non-charged bitter substances, including caffeine and xanthine-based pharmaceuticals. This research highlights the potential of taste sensors as reliable alternatives to human sensory panels, providing valuable insights for assessing the bitterness of non-charged compounds.