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Development of a Pedagogical Case Study on Tasting Under the Influence of Miracle Berry Fruit Tablets

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Abstract

Over the past few decades there has been an increased interest in the "the science of cooking." Topics such as caramelization, Maillard reactions and molecular gastronomy have become familiar to even novice home cooks through publications such as, On Food and Cooking, by Harold McGee and Cooks Illustrated, The Science of Good Cooking. The science of cooking can be entertaining, but also is a useful way to communicate chemistry concepts to learners of all ages. The science of cooking investigates the underlying chemical, biological, and physical processes involved in the transformations that food undergoes during the cooking process. Teaching with case studies is an effective teaching tool which can increase student understanding and utilize critical thinking skills. Case studies can illustrate scientific knowledge through inquiry-based activities. This case study investigates sensory perceptions of two major classes of bioorganic compounds: carbohydrates and proteins. Miraculin is a protein isolated from red berries of Richadella dulcifica, which has exhibited taste-altering properties in acidic conditions. Taste receptors bind miraculin and block normal carbohydrate sweeteners at a neutral pH. Upon acidification, miraculin triggers the activation of these taste receptors and produces a "sweet situation" with foods such as orange juice, lemons and other sour solutions. This case study is suitable for high school through upper-division college curricula, and students will evaluate and analyze miraculin activity and apply their understanding to answer inquiry-based questions. Additionally, instructors can choose to include hypothesis development and testing utilizing commercially available miraculin berry tablets.

Keywords: Case Study, Miraculin, Taste Receptors

1. Introduction

When it comes to increasingly complex topics, especially those found within the STEM field, some students encounter difficulty grasping new material and conceptual information. However, implementation of case studies can provide students with a more interactive approach to learning – such exposure to lecture material can potentially better the students' understanding and retention of the material presented. Therefore, this case study was designed for high school chemistry and undergraduate science majors to present and explore the sensory perception of proteins and carbohydrates. The protein under investigation is a glycoprotein that can be isolated from the red berries of Richadella dulcificia and is better known as miraculin. The miraculin protein is notorious for its taste-altering properties under acidic conditions. When introduced into the system, the protein blocks typical carbohydrate sweeteners from binding to their corresponding taste receptors at neutral pH. However, once the pH of the chemical environment drops (this can be accomplished through the introduction of acidic foods such as orange juice, lemons, and other sour solutions), miraculin changes its conformation and triggers a "sweet sensation." In other words, sour foods ingested will be perceived as sweet in the presence of miraculin. Presenting students with such a scenario can pique their interest in the subject of biochemical signaling and protein structures, which, in turn is meant to increase their retention of the subject matter. Students will analyze the activity of miraculin under different conditions through inquiry-based questions when introduced to the case study. In general, this case study was designed to be suitable for high school through upper-division college curriculum. Furthermore, instructors are encouraged to include hypothesis development and testing with commercially available miracle berry tablets in order to increase student engagement.

1.1 Objectives

Upon completion of this case study, students should be able to:

1. Recognize that proteins can act as inhibitors to cell signaling.
2. Recognize that proteins are sensitive to changes in pH.
3. Recognize that proteins must have a specific shape in order to function properly.

1.2 Classroom Management

For high school students this case study takes approximately one 55 minute class period. Students should work in pairs or in a small group at the instructor's discretion. The supplemental storyline should be presented to the students at the beginning as the supplied PowerPoint presentation. Following this storyline, the students should be supplied with the high school supplemental activity to complete. Students should be given approximately 15-20 minutes to complete the activity in small groups, followed by a class discussion. If instructors plan on pairing the activities with the M-berry Miracle Berry Fruit Tablets, then the foods to be tested should include a *minimum* of one food that is sour and one food item that is sweet. However, a larger variety of foods should be tested to gain the best experience. Recommended options are lemon or lime slices, vinegar, orange slices, orange juice, grapes, hot sauce, water, and cheese. Please be aware of any allergies students may have and plan accordingly.

For undergraduate students, this case study will take approximately two hours of homework time along with one 55-minute class period. Before presenting the undergraduate case study activity, students should have a basic understanding of proteins and how they function from previous courses. However, these case study questions can be adapted to include the appropriate level of material for individual courses. Students will work independently outside the classroom to look over the case study storyline, explore online articles and their textbook for more detailed information on the various proteins involved in the signaling pathway. Students should bring their answers to the following class where they will be given 15 minutes to compare with one another in pairs or small groups. Each group will then be assigned a question from the worksheet to present and explain to the class. If sufficient time remains, the instructor may also inquire which questions were most challenging, which could provide information on what topics should be elaborated on in future classes.

2. Transduction of the Miraculin Protein Signal

2.1. Taste Buds

Once the miraculin protein is dissolved in the saliva, it will interact with the sweet taste buds and their chemoreceptors in order to trigger sweet sensations. In general, taste buds are structures in the tongue responsible for detecting the five basic tastes – sour, sweet, salty, bitter, and umami.[1] These structures are only located in circumvallate and fungiform papillae, which are small projections found across the tongue. While the taste buds found in circumvallate papillae detect for bitter sensations along the back of the tongue, those found in fungiform papillae detect for sweet and sour tastes on both the sides and the apex of the tongue.[2]

In terms of structure, taste buds contain three major cell types, including basal cells, sensory cells, and supporting cells. The sensory (gustatory) cells are epithelial cells responsible for the perception of taste through the activation of the sensory (afferent) fibers of three cranial nerves – the facial nerve, glossopharyngeal nerve, or the vagus nerve. The tip of each gustatory cell, otherwise known as gustatory hairs, extend through the taste pore on the surface of the tongue and are covered with numerous taste receptors.[3,4] Once these taste receptors are stimulated with the binding of the appropriate molecule, the sensory signal is carried by one of the cranial nerves to the thalamus and other regions of the forebrain involved in taste recognition through depolarization.[1] It is suggested that different tastes have segregated pathways to the brain – e.g. stimulation of sweet and sour taste receptors leads to the activation of the facial nerve.

2.2. Biochemical Signaling Pathway

2.2.1. g protein-coupled receptors

Signal transduction systems are classified according to the type of receptor involved. For the signal transduction of sweet sensations, G protein-coupled receptors are involved. These receptors indirectly activate enzymes in order to produce secondary messengers, thus resulting in the intended change in metabolic activity.[5] The human sweet taste receptor is a heterodimer composed of two transmembrane proteins, T1R2 and T1R3 (taste type 1 receptors 2 and 3).[6] This receptor is coupled to a heterotrimeric G protein, which includes alpha, beta, and gamma subunits. Once the intended ligand (e.g. the miraculin protein) binds to the receptor, the intrinsic GTPase activity of the G protein is activated. G proteins can take on both an active and inactive conformation, depending on whether GTP or GDP is bound. When inactive, the alpha subunit of the G protein has a GDP molecule bound to it. However, when a ligand binds to the coupled receptor, the intrinsic GTPase activity of the G protein is catalyzed by the GTP-GDP exchange factors that help to replace the bound GDP with GTP. As a result, the G protein is activated.

When activated, two previously buried regions of the G protein (switch I and switch II) become exposed and can interact with appropriate proteins involved in the signaling pathway. The gamma phosphate group of GTP is responsible for this active conformation by hydrogen bonding with amino acid residues in switch I and II. Once that terminal phosphate group is hydrolyzed, those hydrogen bonds are lost, and the G protein reverts to its inactive form by burying the switch sites.[5]

Once activated, the alpha subunit dissociates from the beta and gamma subunits of the G protein. The beta-gamma subunit then interacts with phospholipase C-β2 (PLC-β2), which is part of a class of membrane associated enzymes that function in the cleavage of phosphoester bonds in lipids. PLC-β2 specifically cleaves phosphatidylinositol 4,5-bisphosphate (PIP2) into inositol 1,4,5-triphosphate (IP3) and diacylglycerol (DAG).[6]

2.2.2. activation of ip$_3$-recpetors

The IP_3 molecule binds to a gated receptor calcium channel (IP_3R), which stimulates the release of sequestered calcium ions from the endoplasmic reticulum (ER) of the gustatory cell. The channel is a large tetrameric protein, with each subunit consisting of about 2700 amino acid residues. There are three different subtypes of the receptor – IP_3R1, IP_3R2, and IP_3R3. The form of the receptor specifically involved in this signaling pathway is subtype three, which is the least sensitive in its affinity for IP_3. While the activation of IP_3R requires the binding of IP_3, calcium is also a necessary component in the receptor's activation. Calcium acts to regulate IP_3 binding to the receptor and its effects on the binding differ between the three subtypes. While the effects of calcium on IP_3 binding to IP_3R3 remain unknown, it was determined that the influence of calcium on the receptor is generally biphasic – modest increases in calcium ion concentration enhances the receptor's response to IP_3, whereas higher concentrations can be inhibitory to the binding of the molecule.

It has been suggested that there are two distinct calcium binding sites that regulate the stimulatory and inhibitory effects of calcium and that IP_3 controls which calcium binding site is available – when IP_3 is bound, the stimulatory site is accessible for calcium, while the inhibitory site is blocked. IP_3 initially binds to the IP_3-binding core (IBC) through interactions between its phosphate groups and the receptor's α- and β-domains. These interactions help to bring about a conformational change where the two sides of the receptor are closed together, which initiates the binding of calcium to the stimulatory site. Although the location of the calcium binding site continues to remain unknown, it has been established that the suppressor domain (SD). Which is connected to the IBC via a flexible linkage, plays a significant role in promoting calcium binding.[7]

2.2.3. excitation of nerves

The influx of calcium from the ER activates the transient receptor potential melastatin 5 (TRPM5) channel. This channel specifically carries sodium ions into the cytosol of the cell in order to induce membrane depolarization.[8] The membrane depolarization allows for the secretion of ATP through a gap junction hemichannel known as PANX-1 (PX1). Since gustatory cells do not have the typical vesicular machinery that is required for the exocytosis of neurotransmitters, ATP is instead used as the main form of communication in order to excite neighboring presynaptic cells of the afferent nerve fibers.[9]

3. Case Study Supplemental Materials

Supplemental class activities are available for high school and undergraduate curricula along with suggested answers for the questions posed within the respective activities. The case study storyline is also available as a supplemental document which involves a fictional story about "Holly Berry," a student wrongly accused of drug use in her high school when her teacher overhears a conversation about flavor-tripping. The principal insists that Holly must research and present her findings on the science behind the miracle berry which alters the flavors of some food groups.

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