Sweeteners and inhibitors of sweetness

Pour Some Sugar on Me

PIOTR KRAJEWSKI
Institute of Physical Chemistry
Polish Academy of Sciences, Warsaw
S. I. Witkiewicz High School No. 64 in Warsaw
bitex@gazeta.pl

Dr. Piotr Krajewski works with the PAS Institute of Organic Chemistry. He educates young people; his pupils are very successful in national and international chemistry competitions.

Research conducted in recent years has greatly improved our understanding of the underlying mechanisms of sweet taste, as well as its inhibitors. A number of sweeteners and inhibitors discovered by Polish researchers have been patented

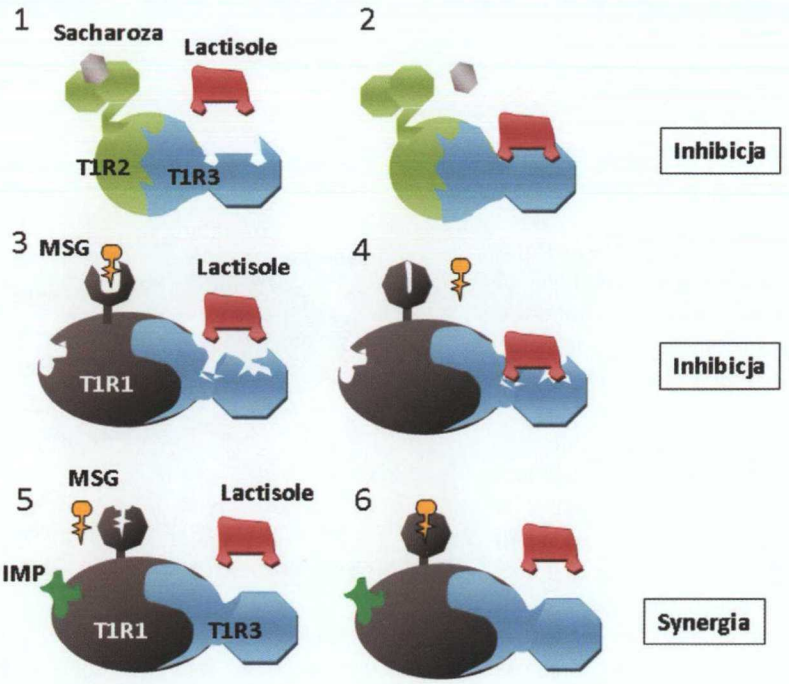
One autumn afternoon in 1996, at one of the laboratories at the PAS Institute of Organic Chemistry, two PhD students were busy drinking coffee instead of working. They were chatting about chemistry; one was describing the NMR spectroscopy methods he used to define the structure of a complex natural compound, isolated from a particular fungus. The other, a certain Piotr, was waxing lyrical about the scientific advances likely to be achieved using his own method of determining the spatial structures of organic compounds. The first, bored with Piotr's drawn out story and driven by a terrible habit, reached for a vial filled with a white substance standing on the bench. He licked his finger, dipped it into the white crystals, and tasted them. He was pleasantly surprised to discover that the substance was extremely sweet - in fact far sweeter than sucrose. Piotr immediately repeated his colleague's appallingly risky experiment, confirming his fascinating and surprising discovery. The "tested" organic compound, assigned the symbol LacBn by Piotr, was one of a series of several compounds he obtained as part of his PhD experiments. LacBn is a well-known compound, first synthesized by two Russian researchers in 1982. Predictably, the students were unable to restrain their natural curiosity and "tested" all the remaining compounds thoroughly. They discovered that some were completely flavorless, a few were bitter, but LacBn was the only one that tasted sweet. And so the story of a new, synthetic sweetener being discovered by chance repeated itself.

Accidental taste discoveries

In 1879, Ira Remsen at the Johns Hopkins University in Baltimore discovered saccharin also quite by chance. In 1937, Michael Sveda at the University of Illinois identified the sweet taste of sodium cyclamate, likewise accidentally. James Schlatter working at G.D. Searle discovered the now famous aspartame in 1965. Two years later, Karl Clauss and Harald Jensen from Hoechst AG stumbled across the discovery of acesulfame potassium. Sucralose, an extremely sweet derivative of saccharin, was discovered under particularly unusual circumstances: in 1976, at Queen Elizabeth College in London, the researcher Shashikant Phadnis - originally from India - was asked by his boss to test a few chemical substances. Unfortunately Phandis misunderstood the instruction and thought he was asked to taste it; the language mishap led to the discovery of a new synthetic sweetener. All these sweeteners, with the exception of cyclamate, are still used on a massive scale.

Sweetness vs. chemical structure

Piotr, a naturally inquisitive man, decided to test the connection between the sweet taste of LacBn derivatives and their chemical structure. Fortunately new derivatives are easy to obtain from relatively cheap reagents. LacBn, the main compound, contains a fragment of NHCH2C6H5. Piotr modified the fragment slightly by substituting one of the hydrogen atoms in the CH2 group with a methyl group. The compound obtained this way is chiral, which means its molecules can exist in two forms that are mirror images of each other and cannot be superimposed, similarly to a pair of shoes. He obtained a racemic mixture containing equal levels of dextro and levo isomers, and was disappointed that the new derivative, rac-LacFea, was not sweet. This briefly dampened



Andrzej Dębowski

The sweetness receptor comprises two proteins – T1R2 and T1R3 – while the umami (savory taste) receptor comprises T1R1 and T1R3. The receptors share the T1R3 protein (blue). Lactisole inhibits the sweetness of substances that bind with the VFT fragment of the T1R2 protein. Lactisole also inhibits umami by blocking monosodium glutamate (MSG). IMP can result in a 15-fold increase of intensity in the perceived taste of MSG through synergy

his enthusiasm for designing new sweeteners. It has long been known that enantiomers frequently have different biological properties. And so Piotr obtained a levo form – (S)-LacFea, which turned out to be tasteless – and a dextro form – (R)-LacFea, which was extremely sweet. Since rac-LacFea is not sweet, and (R)-LacFea is, it follows that its enantiomer, (S)-LacFea, must somehow block the perception of sweet taste by inhibiting its receptors. This process was confirmed by a series of simple experiments in which the inhibitor was added to solutions of various sweet substances or their mixtures. None of the substances – saccharose, glucose, sweet amino acids, honey, several commercial sweeteners containing aspartame, sorbitol, sodium cyclamate, saccharin and all the sweeteners Piotr synthesized himself – resisted the inhibitor. In short, everything that started off as sweet lost its sweetness. It is a fascinating example – perhaps the first in history – where one enantiomer is sweet while the other is an inhibitor of sweet taste.

Further research indicates that substituting the oxygen atom in the 5-member ring with a CH2 group in LacBn results in the formation of CpBn, which is even sweeter (over 250 times sweeter than sucrose) than the original compound. The presence of a phenyl ring (C6H5) is key, since derivative compounds that lack this ring are not sweet. Substituting any of the hydrogen atoms in the phenyl ring with another group (methyl, nitrogen-containing, hydroxyl or a chlorine atom) eliminates the sweet taste. It is important that there is a single carbon atom between the nitrogen atom and the phenyl group; if there are two carbon atoms, the derivative is not sweet. When the carbon atom is removed, the sweet taste disappears with it. Substituting the 5-member ring in LacBn for a 6-member ring (6-LacBn) also eliminates the sweet taste.

Focusing on sweetness

Prior to 1999, no sweet taste receptors had been isolated and characterized. Attempts to elucidate the action of sweet substances could not be conducted in the same manner as enzyme inhibitors with a known spatial structure; instead, it was necessary to use models which remain speculative until the point when the receptor structure is characterized. There are several hypotheses concerning sweetness. The first significant theory, based on the supposition that a specific receptor exists on the surface of cells forming taste buds, was published in *Nature* in 1967 by Robert Shallenberger and Terry Acree. According to the theory, in order to be sweet, an organic substance must contain a hydrogen bond donor (AH, usually OH or NH groups) and a Lewis base acceptor (B, usually the oxygen atom from the C=O group) separated by between 2.5 and 4.0 Å (10-10 m), which react with the complementary AH-B receptor pair to form two hydrogen bonds.

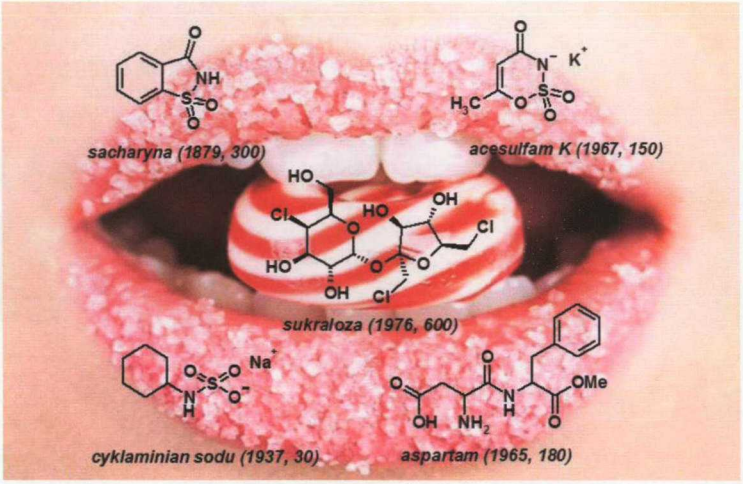
In 1991, Jean-Marie Tinti and Claude Nofre, researchers from the University of Lyon, formulated the multipoint attachment theory

(MPA). It allowed them to propose a structure for lugduname, the most potent sweet substance to date (220,000-300,000 times sweeter than sucrose).

Piotr (the present author, of course) and his colleagues from the PAS Institute of Organic Chemistry wished to determine the sweetness of the substances they isolated. The procedure was simple: they prepared a 10% solution of the sweetener, and the researchers took turns comparing the intensity of the taste against a 10% sucrose solution. They all agreed that the sweetener was significantly sweeter. Next, they prepared several solutions of the sweetener at lower concentrations, and tasted them in random order. The aim was to identify the concentration of the substance at which the sweetness of the solution was the same as the sucrose solution. They calculated the sweetness intensity by dividing the concentration of the sucrose solution by the concentration of the sweetener solution, for example 10%/0.5% = 200. The results must be interpreted with great care to prevent exaggerating the sweetness. The method was used to test several different substances, finding several to be at least 200-250 times sweeter than a 10% sucrose solution. In comparison, the popular sweetener aspartame is approx. 180 times sweeter than sucrose.

Receptors revealed

Over the course of the last decade or so, many scientific breakthroughs have been made contributing to our understanding of sweetness. In 1999, scientists from the University of California at San Diego and the National Institutes of Health identified three genes coding for protein receptors of sweet and umami tastes. The latter is a savory taste, the fifth alongside the four traditionally described tastes, first described by Prof. Kikunae Ikeda in 1908 at the Imperial University in Tokyo. Ikeda also demonstrated that the substance responsible for our perception of umami taste is monosodium glutamate, an amino acid encoded by DNA. It turns out that the receptors belong to the group of G protein-coupled receptors (GPCR). This protein family is described by the symbol hT1R, where h denotes human, T - taste, and R - receptor. Further studies reveal that a fully-functional sweet taste receptor must comprise two proteins, hT1R2 and hT1R3, while the umami receptor must comprise hT1R1 and hT1R3. The two types of receptor share a common element in the hT1R3 protein. Specialized receptors are present in the cellular membrane of taste buds, found in clusters of 50-100, mainly in the mouth, but also in the digestive tract. Sweetness receptors bind their corresponding compound in a lock-and-key fashion; in this instance, the 'key' can be sucrose (ordinary sugar) or aspartame. The binding process causes the receptor protein to change shape, which in turn starts cascades of biochemical reactions leading to processes such as the release of calcium ions from intracellular reservoirs. This results in the depolarization of the cell, or a change in the electrical potential difference between the cellular membrane and the outside of the cell. Groups of specialized taste buds are connected to neurons which conduct electrical signals to the appropriate parts of the brain. The umami taste receptors function in a similar way; however, they are activated by molecules with a different chemical structure than those that stimulate sweetness receptors, such as the previously mentioned monosodium glutamate.



The chemical structures of low-calorie synthetic sweetener molecules show a surprising variety. Sweeteners are listed here by name, with their year of discovery and their sweetness vs. sugar in brackets

Inhibitors of sweetness

In confectionery products, sugar is used as much more than simply a substance providing sweetness; it is also an essential structural ingredient. In jams, jellies and preserves, a high sugar concentration plays an antibacterial role. Certain kidney disorders mean that patients need to drink a highly concentrated glucose syrup. Sorbitol, a sugar alcohol (hydrogenated form of carbohydrate), is added to various products as a humectant. However, excessive sweetness can also cause problems, which in

turn can be solved by adding sweetness inhibitors. Inhibitors can also improve the flavor of synthetic sweeteners that have a tendency to linger with an unpleasant aftertaste. There are also potential applications of sweetness inhibitors in weight loss diets: when people who are "hooked" on candy feel a desperate need to reach for something sweet, an inhibitor can make the flavor of their favorite pastry or chocolate less appealing.

One of the most commonly used sweetness inhibitors is lactisole, a sodium salt derived from propionic acid, discovered by Michael Lindley from Tate & Lyle in the early 1980s. Adding a small amount of lactisole inhibits the sweetness of sugar and other sweet substances by over 80%, while improving the taste of dishes by revealing other hidden flavors. It is worth noting that the (S) entantiomer of lactisole is an inhibitor, while the (R) enantiomer is inactive. Another well-known sweet inhibitor is gymnemic acid, isolated from the leaves of *Gymnema sylvestre*, a herb native to the tropical forests of the Indian subcontinent. Chewing the leaves makes sweetened drinks taste like water.

Studying sweetness inhibitors allows us to better understand the interactions between various molecules and sweet taste receptors. It has been demonstrated that lactisole and its structural analogues bind to the hT1R3 protein in the human receptor.

Venus flytrap

Lactisole blocks the sweet taste of substances that bind with a fragment of the hT1R2 protein known as the Venus flytrap (VFT) protein, named after the plant that attracts and kills flies and other small insects using specially adapted traps. The VFT of the sweet receptor functions in a similar way: if an appropriately shaped molecule finds itself in the "trap", the protein closes in on it to activate the receptor. However, attaching a lactisole molecule prevents new molecules from being captured. Lactisole also inhibits umami taste by blocking monosodium glutamate through an analogous mechanism. Inhibitors discovered at the PAS Institute of Organic Chemistry are likely to bind to both receptors in a similar manner.

Sweetness enhancers

If substance A has a sweetness of X and substance B a sweetness of Y, we might expect a mixture of A and B to have a sweetness of X+Y. However, the sweetness of such a mixture is frequently significantly higher – a phenomenon known as the synergistic effect. It is frequently used in the food industry, since it allows manufacturers to reduce costs per unit of sweetness. Additionally, mixtures of sweeteners may taste much better than the individual components. This effect is used in products such as Coca-Cola Zero, in which aspartame is combined with another sweetener. The case is similar for umami taste. A molecular-level explanation of the synergistic effect has recently been proposed. It states that molecules that activate the umami receptor bind to a fragment of VFT, while IPM and GMP bind to a molecule of the same protein, very close to VFT, stabilizing the closed conformation. Recent achievements in molecular biology have made it possible to discover molecules able to enhance the sweetness of sucrose similarly to the way in which IMP and GMP enhance the taste of glutamate. This substance, assigned the symbol S6419, makes ordinary sugar taste around four times sweeter; as a result, the same effect is achieved using just a quarter of the sucrose that would be required usually, which means reduced production costs, and – even more importantly – a positive impact on consumers' health.

A patent application was filed at the Patent Office in early 2009; it has since been expanded throughout Europe, the US, and China. ■

Further reading

Li X., Staszewski L., Xu H., Durick K., Zoller M., Adler E. (2002). Human receptors for sweet and umami taste. *PNAS, 99*, 4692-4696.

Zhang F., Klebansky B., Fine R. M., Xu H., Pronin A., Liu H., Tachdjian C., Li H. (2008). Molecular mechanism for the umami taste synergism. *PNAS, 105*, 20930-20934.

Zhang F., Klebansky B., Fine R. M., Liu H., Xu H., Servant G., Zoller M., Tachdjian C., Li H. (2010). Molecular mechanism of the sweet taste enhancers. *PNAS, 107*, 4752-4757.

David Webb, The Botanical Society of America

The Venus flytrap plant attracts and kills flies and other small insects by using specially adapted traps. The VFT region of the sweet receptor functions in a similar way: if an appropriately shaped molecule finds itself in the "trap", the protein closes in on it to activate the receptor