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Structuring sweetness: What makes Stevia 200 times sweeter than sugar

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Stevia rebaudiana. Credit: public domain

New research from Washington University in St. Louis reveals the molecular machinery behind the high-intensity sweetness of the stevia plant. The results could be used to engineer new non-caloric products without the aftertaste that many associate with sweetener marketed as Stevia.

Although the genes and proteins in the biochemical pathway responsible for stevia synthesis are almost completely known, this is the first time that the three-dimensional structure of the proteins that make rebaudioside A—or 'RebA,' the major ingredient in the product Stevia—has been published, according to the authors of a new paper in the *Proceedings of the National Academy of Sciences*.

"If someone is diabetic or obese and needs to remove sugar from their diet, they can turn to artificial sweeteners made from chemical synthesis (aspartame, saccharin, etc), but all of these have 'off-tastes' not associated with sugar, and some have their own health issues," said Joseph Jez, professor of biology in Arts & Sciences and lead author of the new study.

"Stevias and their related molecules occur naturally in plants and are more than 200 times sweeter than sugar," he said. "They've been consumed for centuries in Central and South America, and are safe for consumers. Many major food and beverage companies are looking ahead and aiming to reduce sugar/calories in various projects over the next few years in response to consumer demands worldwide."

Researchers determined the structure of the RebA protein by X-ray crystallography. Their analysis shows how RebA is synthesized by a key plant enzyme and how the chemical structure needed for that high-intensity sweetness is built biochemically.

To make something 200 times sweeter than a single glucose molecule, the plant enzyme decorates a core terpene scaffold with three special sugars.

That extra-sweet taste from the stevia plant comes with an unwanted flavor downside, however.

"For me, the sweetness of Stevia comes with an aftertaste of licked aluminum foil," Jez said. Many consumers experience this slightly metallic aftertaste.

"The taste is particular to the predominant molecules in the plant leaf: the stevioside and RebA," he said. "It is their chemical structure that hits the taste receptors on the tongue that trigger 'sweet,' but they also hit other taste receptors that trigger the other tastes."

"RebA is abundant in the stevia plant and was the first product made from the plant because it was easy to purify in bulk. Call this 'Stevia 1.0'," Jez said. "But in the leaf are other related compounds with different structures that hit the 'sweet' without the aftertaste. Those are 'Stevia 2.0,' and they will be big."

There are many ways that the newly published protein structure information could be used to help improve sweeteners.

"One could use the snapshot of the protein that makes RebA to guide protein engineering efforts to tailor the types and/or pattern of sugars in the stevias," Jez said. "This could be used to explore the chemical space between 'sweet' and 'yuck'."

"There are also molecules in other plants that are not 'stevias' but can deliver intense sweetness," he said. "We could use the information of how the stevia plant does it as a way of finding those details."

More information: Soon Goo Lee el al., "Molecular basis for branched steviol glucoside biosynthesis," *PNAS* (2019). www.pnas.org/cgi/doi/10.1073/pnas.1902104116

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