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Relationship between Sweetness and Structure of Sweet-Tasting Protein, Brazzein

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To date, only eight sweet-tasting proteins have been known to elicit sweetness. Among them, brazzein (molecular mass of 6.4 kDa) is the smallest sweet-tasting protein isolated from the fruit of the West African plant *Pentadiplandra brazzeana Baillon*. Brazzein has attracted attention as a candidate for sweeteners for the control of obesity, oral health and diabetic management, because of its potential sweetness, sugar-like taste and good stability at high temperature and wide pH ranges. To elucidate the relationship between the structure and sweetness of highly sweet-tasting protein we have constructed several brazzein variants of residues in the flexible loops and the N- and C-termini of brazzein by site-directed mutagenesis. The brazzein variants were expressed in *E. coli* BL21and purified the same method as pET26b(+)-brazzein. The variants of the residues that located in the loop between ***β***-strand III and ***β***-strand II showed similar sweetness to the wild-type brazzein. On the other hand, the variants of the residues that located in the ***β***-strand III, in loop between ***α***-helix, ***β***-strand and the residues in *N*- &*C*- termini increased sweetness. Particularly, His31 and Glu41 residues in the flexible loops and Glu36 residue in the *β*-strand III of the brazzein were the critical residues of the molecule for eliciting sweetness. We have also made multiple mutations of three residues. All double mutations made the molecules sweeter than wild-type brazzein and three single mutants. The increasing order of their sweetness were triple variants> double variants > single variants. These results strongly support the hypotheses that brazzein binds to a non-continuous and multi-sites of the sweet taste receptor. We also found that mutations of Lys5 to Asp or Glu at position 5 of the N-terminal significantly decreased sweetness and mutation of the Glu53 to Arg at position 53 of the C-terminal made the molecules significantly sweeter than brazzein. From these results, we suggest that the positive charge at the Lys5 in the N-terminal was necessary for structural integrity, whereas the charge and length of side chain at position 53 in the C-terminal play an important role in the interaction between brazzein and the sweet taste receptor. Taken together, our findings also support the previous results that mutations increasing the positive charge favor sweet-tasting protein potency.

Biography:

Kwang-Hoon Kong got his PhD from Tokyo University of Japan in 1993. He is the professor of the Department of Chemistry at Chung-Ang University in Korea. He has published more than 90 papers in reputed journals and has been awarded the IBC TOP 100 Scientists in 2012, 2013, 2015, and 2017.