Large-Scale All-Electron Quantum Chemical Calculation Toward a Sweet-Tasting Protein, Brazzein, and Its Mutants

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1 Introduction

It had been recognized for many years that only small molecules were capable of causing a sweet taste. The search for sweeteners, however, found out naturally occurring sweet-tasting macromolecules, namely sweet proteins, in a variety of West African and South Asian fruits. Thaumatin was first identified as one of the sweet proteins, and then monellin, mabinlin, pentadin, curculin, brazzein, and neoculin were isolated sequentially. Sweet-tasting proteins are expected to be a potential replacement for natural sugars and artificial sweeteners.

The human sweet taste receptor is a heterodimer of two G-protein coupled receptor subunits, T1R2 and T1R3, and broadly responsive to natural sugars, artificial sweeteners, D-amino acids, and sweet-tasting proteins. The three-dimensional structure of the sweet receptor protein T1R2/T1R3 is still unknown, and the exact mode for interaction of sweet-tasting proteins with the T1R2/T1R3 receptor has not yet been elucidated. Very recently the recognition patterns of T1R2/T1R3 for small molecular sweeteners were suggested [1].

We have recently been carrying out computational simulations for saccharides and sweet-tasting proteins [2, 3]. The purpose of this study is to clarify characteristics of sweet-tasting materials and their functions and to search highly functional materials for foods and pharmaceutical agents on the basis of electronic state calculations. This work also aims to elucidate the mechanism for the expression of sweetness. A small protein, *des*-pGlu brazzein, is one of the sweetest protein sweeteners so far discovered and 4,000 times sweeter on a weight basis than a 2% sucrose solution. The protein is composed of a single polypeptide chain bearing 53 amino acid residues and four disulfide bonds [4]. To examine a relationship between the sweetness of protein sweeteners and their electronic properties, we are currently working all-electron quantum chemical calculations on *des*-pGlu brazzein and two different mutants, Glu41Lys and Arg43Ala, using a density functional method program, ProteinDF. The former mutant is sweeter than the brazzein and the latter mutant has a taste like water.

2 Computational Methods

The NMR structure of brazzein was downloaded from Protein Data Bank (PDB code: 2brz) and the structure of *des*-pGlu brazzein (*Fig. 1*(a)) obtained by removal of N-terminal pyro-grutamate from brazzein. Since the structures of two different mutants Glu41Lys and Arg43Ala are not available in the Protein Data Bank, we mutated the amino acid residues Glu41 and Arg43 in *des*-pGlu brazzein to Lys and Ala, respectively, by using ProteinEditor implemented in ProteinDF software package. *Fig. 1*(b) and (c) show the structures of both mutants Glu41Lys and Arg43Ala.

2.1 Molecular Dynamics Calculation and Energy Minimization

We used the commercial software package AMBER9 to carry out molecular dynamics (MD) calculations and energy minimizations on the structures of *des*-pGlu brazzein and two mutants Glu41Lys and Arg43Ala. All protein molecules were solvated with TIP3P model water molecules within 8.0 Å of respective proteins, and MD calculations were conducted for 30ps at 300K to relax local structural distortions. The structures thus obtained were optimized by an energy minimization. The MD calculations and energy minimizations were performed on a 64bit Itanium2 system PC Linux Workstation.

2.2 All-Electron Quantum Chemical Calculation

After energy minimization, the surrounding water molecules were removed and counterions were placed near each charged amino acid residue to neutralize the system using ProteinEditor. The positions of counterions were adjusted by the molecular

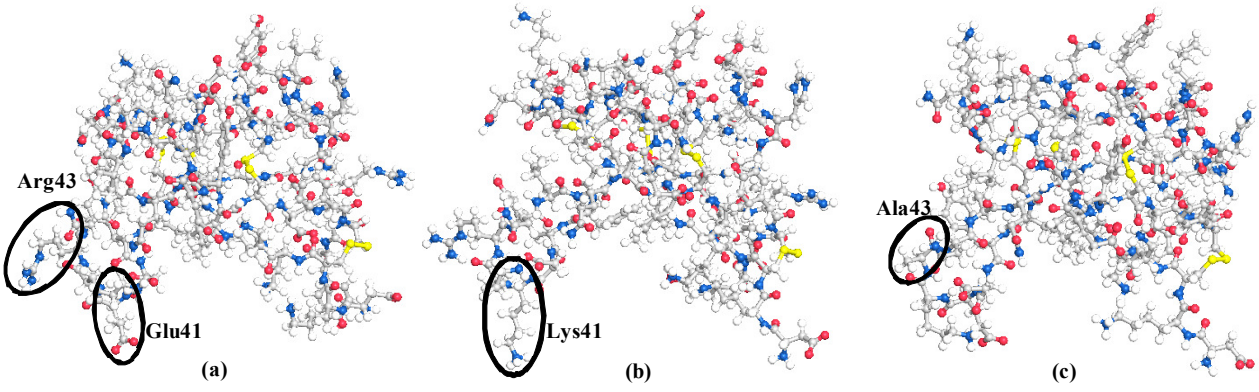

Fig. 1. **Structures of *des*-pGlu brazzein (a) and Two Mutants Glu41Lys (b) and Arg43Ala (c).**

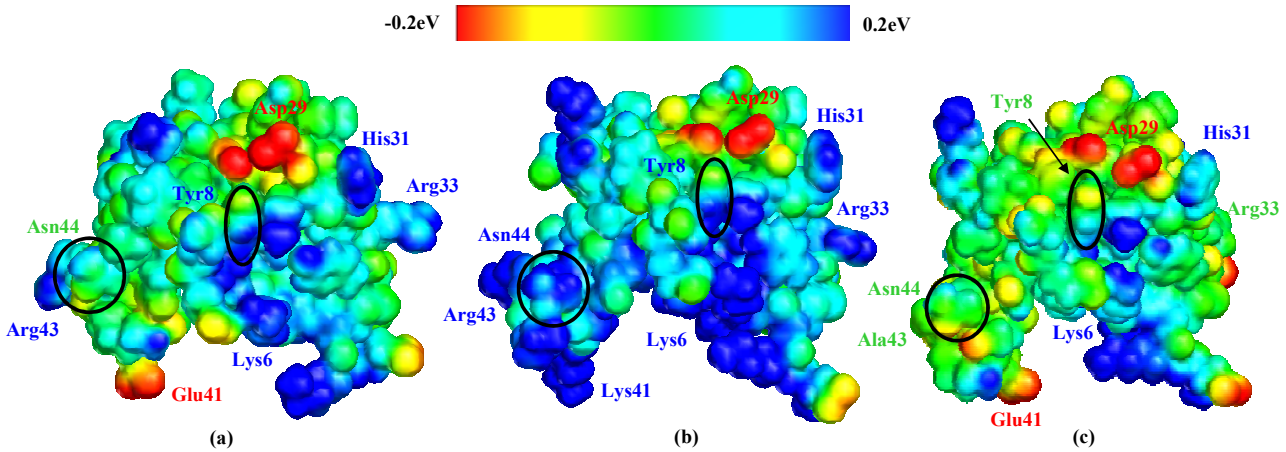

Fig. 2. **Electrostatic Potential Maps of *des*-pGlu brazzein (a) and Two Mutants Glu41Lys (b) and Arg43Ala (c). Color Code: Red Negative Charge, Green Neutral, and Blue Positive Charge.**

mechanics calculation using AMBER9. The resulting structures were subsequently subjected to an all-electron quantum chemical calculation with the Slater and Vosko-Wilk-Nusair (SVWN) functional using ProteinDF. These ProteinDF calculations were carried out on a Core2Duo system PC Linux Cluster (16 Cores) and gave the electrostatic potential (ESP) map and the highest occupied and lowest unoccupied molecular orbitals (HOMO and LUMO).

3 Results and Discussion

3.1 *des*-pGlu brazzein

As shown in *Fig. 2*(a), the calculated ESP map for *des*-pGlu brazzein showed that the basic amino acid residues such as Lys6, His31, Arg33, and Arg43 have positive charge, while the acidic amino acid residues such as Asp29 and Glu41 and the C-terminal Tyr54 residue have negative charge. In addition, we found that positive charge is evident on the neutral amino acid residue Tyr8. A comparison of our computational results with experimental results on the sweetness of mutants [4] suggests that these charged amino acid residues are involved in changing and/or eliciting the sweetness of *des*-pGlu brazzein.

3.2 Two Mutants Glu41Lys and Arg43Ala

The analogous ProteinDF calculations on two mutants Glu41Lys and Arg43Ala were carried out to examine a relationship between the sweetness of protein sweeteners and their electronic properties. The calculated ESP maps for two mutants Glu41Lys and Arg43Ala are shown in *Fig. 2*(b) and (c), respectively. *Table 1* illustrates the electric charge of the selected amino acid residues in *des*-pGlu brazzein and Glu41Lys and Arg43Ala mutants. For Glu41Lys mutant, *Fig. 2*(b) and *Table 1* show that the electric charge of the basic amino acid residues Arg33 and Arg43 and the neutral amino acid residue Tyr8 became more strongly positive; the neutral amino acid residue Asn44 became positively charged. Consequently, the number of amino acid residues bearing positive charge increased and the positive charge widely spread over the mutant. On the other hand, for Arg43Ala mutant, *Fig. 2*(c) and *Table 1* clearly reveal that the positive charge of the basic amino acid residue Arg33 and the neutral amino acid residue Tyr8 was neutralized. Thus, the number of positively charged amino acid residues evidently decreased in this mutant.

Table 1. **Electric Charge of the Selected Amino Acid Residues in *des*-pGlu brazzein and Two Mutants.**

Amino Acid Residue	*des*-pGlu brazzein	Glu41Lys	Arg43Ala
Tyr8	+	++	**0**
His31	++	++	+
Arg33	+	++	**0**
Glu41	–		–
Arg43	+	++	
Asn44	**0**	++	**0**

++: Strongly Positive +: Positive 0: Neutral –: Negative

The neutral amino acid residues such as Tyr8 and Asn44 and the basic amino acid residues such as His31, Arg33, and Arg43 are expected to be closely associated to the interaction with the human sweet receptor protein T1R2/T1R3.

4 Conclusion

The all-electron quantum chemical calculations toward the sweet-tasting protein *des*-pGlu brazzein and two different mutants Glu41Lys and Arg43Ala were performed to explore the relationship between the sweetness of protein sweeteners and their electronic properties. The ProteinDF computations indicate that the charged amino acid residues and charge distribution of sweet-tasting proteins can play important roles in the interaction between the proteins and the human sweet receptor protein T1R2/T1R3.

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