Phytochemistry 125 (2016) 106–111

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Synthesis of rebaudioside A from stevioside and their interaction model with hTAS2R4 bitter taste receptor

Ramit Singla, Vikas Jaitak *

Centre for Pharmaceutical Sciences and Natural Products, School of Basic and Applied Sciences, Central University of Punjab, Bathinda(Pb) 151 001, India

ARTICLE INFO

Article history:
Received 16 October 2015
Received in revised form 15 January 2016
Accepted 7 March 2016
Available online 11 March 2016

Keywords:
Stevia rebaudiana (Asteraceae)
Steviol glycosides
Homology model
Ramachandran plot
hTAS2R4
β-1,3-Glucanase
Transglycosylation

ABSTRACT

Steviol glycosides (SG's) from *Stevia rebaudiana* (Bertoni) have been used as a natural low-calorie sweeteners. Its aftertaste bitterness restricts its use for human consumption and limits its application in food and pharmaceutical products. In present study, we have performed computational analysis in order to investigate the interaction of two major constituents of SG's against homology model of the hTAS2R4 receptor. Molecular simulation study was performed using stevioside and rebaudioside A revealed that, sugar moiety at the C-3′′ position in rebaudioside A causes restriction of its entry into the receptor site thereby unable to trigger the bitter reception signaling cascade. Encouraged by the current finding, we have also developed a greener route using β-1,3-glucanase from *Irpex lacteus* for the synthesis of de-bittered rebaudioside A from stevioside. The rebaudioside A obtained was of high quality with percent conversion of 62.5%. The results here reported could be used for the synthesis of rebaudioside A which have large application in food and pharmaceutical industry.

1. Introduction

Gustatory reception in human has five widely accepted Descriptors: sweet, umami, bitter, salty, and sour. Taste receptor cells (TRCs) and signaling elements have been recognized in the oral cavity as well as taste buds present on the tongue (Chandrashekar et al., 2006). Sweet taste receptor (STR) belong to class C of G-protein coupled receptor (C-GPCR) family (Adler et al., 2000). Sweet taste reception is commenced by the activation of heterogenic receptor, made up by a combination of hTAS1R2 and hTAS1R3 protein (Mayank and Jaitak, 2015). In contrast to the single receptor-based detection of sweet taste, bitter taste receptors belong to frizzled receptor family of G-protein coupled receptor (Fredriksson et al., 2003). They exhibit unique but partially overlapping molecular receptive ranges because the transduction of bitter taste in humans is mediated by 25 receptors of hTAS2R gene family that are cluster of chromosomes 5p15, 7q31 and 12p13 (Singh et al., 2011). hTAS2Rs are between 290 and 333 amino acids long, have seven transmembrane helices (TM1-TM7), a short extracellular amino terminal and an intracellular carboxyl-terminal (Upadhyaya et al., 2015).

Steviol glycosides (SGs) are natural sweeteners and belongs to the category of ent-kaurene diterpenes (Jaitak et al., 2008) and are extracted from a native shrub of Brazil and Paraguay, *Stevia rebaudiana* Bertoni (Stevia). The sweetener mixture mainly consists of stevioside (6–10%), rebaudioside A (2–4%) and other minor constituents up to 0.1–1%. (Jaitak et al., 2008; Singh et al., 2011). Stevioside is reported to be 250–300 times and rebaudioside-A is 300–400 times sweetener than sucrose (Kochikyan et al., 2006). Although, concentration of stevioside in *S. rebaudiana* leaves is higher than rebaudioside-A but aftertaste bitterness of stevioside and its low solubility in water restricts its use for human consumption and limits its application in food and pharmaceutical products. Rebaudioside-A, one of the other major constituents next to stevioside in *S. rebaudiana* leaves is preferred than stevioside, being more sweetener, high solubility in water and devoid of aftertaste bitterness (McChesney, 2006; Nabors, 2001; O'Donnell and Kearsley, 2012). In Dec 2008, US Food and Drug Administration (FDA) granted approval to stevia containing a minimum of 95% rebaudioside-A (Generally Recognized As Safe, GRAS) as general purpose sweetener for use in foods and beverages (Carakostas et al., 2008). The French Food Safety Agency (AFSSA), Food Standards Australia New Zealand (FSANZ) EFSA, European Food Safety Authority (EFSA) evaluated rebaudioside-A and certified a positive safety assessment (Boileau et al., 2012; GRAS notice 000354). In September 2009, France authorised the use of rebaudioside-A as a sweetener at the national level. In comparison to stevioside, the percentage of rebaudioside-A in *S. rebaudiana* leaves is low, and also non-availability of rebaudioside-A genotypes limits

* Corresponding author.
E-mail address: vikasjaitak@gmail.com (V. Jaitak).

http://dx.doi.org/10.1016/j.phytochem.2016.03.004
0031-9422/

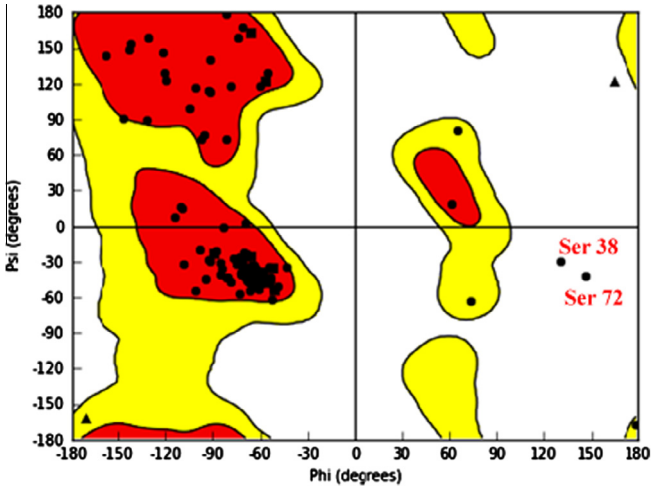

Fig. 1. Ramachandran plot for hTAS2R4.

its commercialization. The choice therefore, falls on the synthesis of rebaudioside-A from stevioside.

Out of 25 receptors, the hTAS2R4 receptor is involved in bitter taste reception activation by stevioside and rebaudioside A (Hellfritsch et al., 2012). But, the molecular/structural mechanism behind the bitterness is still unknown. In the present study, we have developed a homology model for bitter receptor and determined the structural requirements of a ligand for bitter taste receptor activation. We have also proposed a strategy for the synthesis of highly de-bittered rebaudioside A from stevioside by enzymatic biotransformation.

2. Results and discussion

2.1. Homology model construction and validation

Due to the unavailability of the crystal structure of hTAS2R4 receptor, a homology model of hTAS2R4 receptor was prepared.

Table 1
Dock scores, protein–ligand interactions of hTAS2R4 for SG.

S. No.	Ligand	Dockscore (kcal/mol)	Ligand interaction
1	Stevioside	−13.385	Val 70 , Tyr 147, Glu 172, Asn 169 Ile 170. Lys 262, Gln 249, Met 257, Asp 258
2	Rebaudioside A	−8.761	

Models of hTAS2R4 were generated by I-TASSER, that utilizes threading, *ab initio* replica-exchange Monte Carlo modeling simulations and structural refinement (Yang et al., 2015b). Eight templates having PDB id 4GRV, 4BWB, 4N6H, 4IAR, 2Z73, 2DJH, 4BUO and 4EA3 were found to be optimum to build the homology model. Models were evaluated from C-score.

Subsequently final model was selected by the assessment of Ramachandran plot (Fig. 1) created by RAMPAGE server to determine if the model is folded correctly about the solved crystal structure of proteins available in protein data bank (Lovell et al., 2003). The overall G-score as evaluated by Procheck was -0.11 for hTAS2R4. The quality factor calculated by ERRAT was 91.409.

These outcomes suggested that the model constructed is of high quality. All GPCR family proteins shares common structural features of seven hydrophobic transmembrane helices with an extracellular amino (N) terminus and an intracellular carboxyl (C) terminus. The constructed model consisted of an extracellular topological domain, a transmembrane domain and cytoplasmic topological domain (Fig. 2a).

2.2. Binding site analysis

The binding site of homolog was determined using sitemap application of maestro 9.0. The primary amino acid residues that are participating in the binding site were Phe 84, Val 85, Phe 88, Met 89, Phe 168, Leu 177, Ser 180, Ser 184, Ser 243, Thr 246, Leu 247, Tyr 250, Leu 266 and Thr 270 (Fig 2b). Upon 3D analysis of the binding site, it was revealed that the binding cavity was smaller in size and volume.

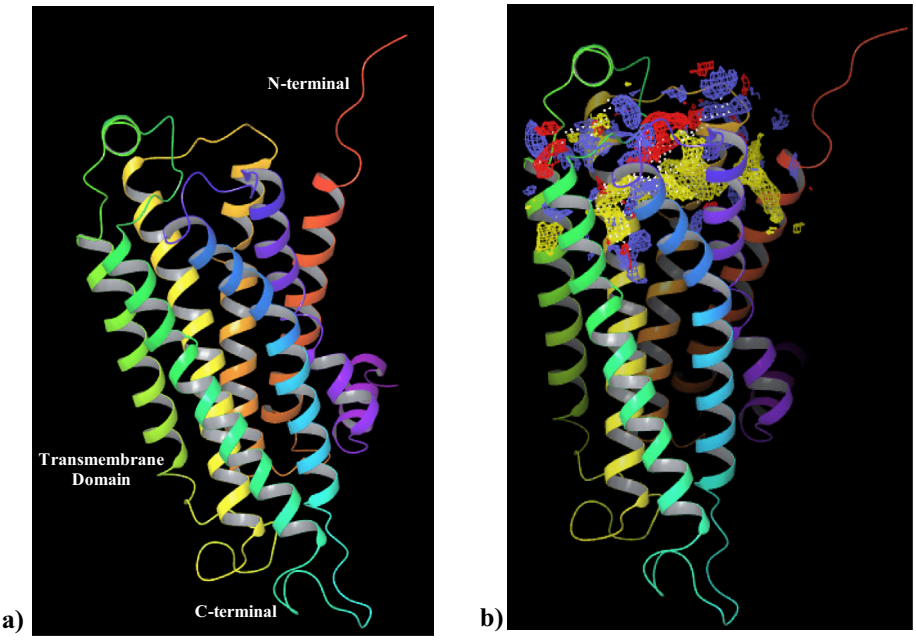

Fig. 2. 3D structure of constructed homology model of hTAS2R4 receptor, helix 1(Red), helix 2 (orange), helix 3 (yellow), helix 4 (light green), helix 5 (dark green), helix 6 (blue) and helix 7 (purple) (a) Binding site in constructed model of h hTAS2R4, Hydrophobic map (yellow mesh), hydrogen-bond donor map (blue mesh), hydrogen-bond acceptor map (red mesh) (b). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

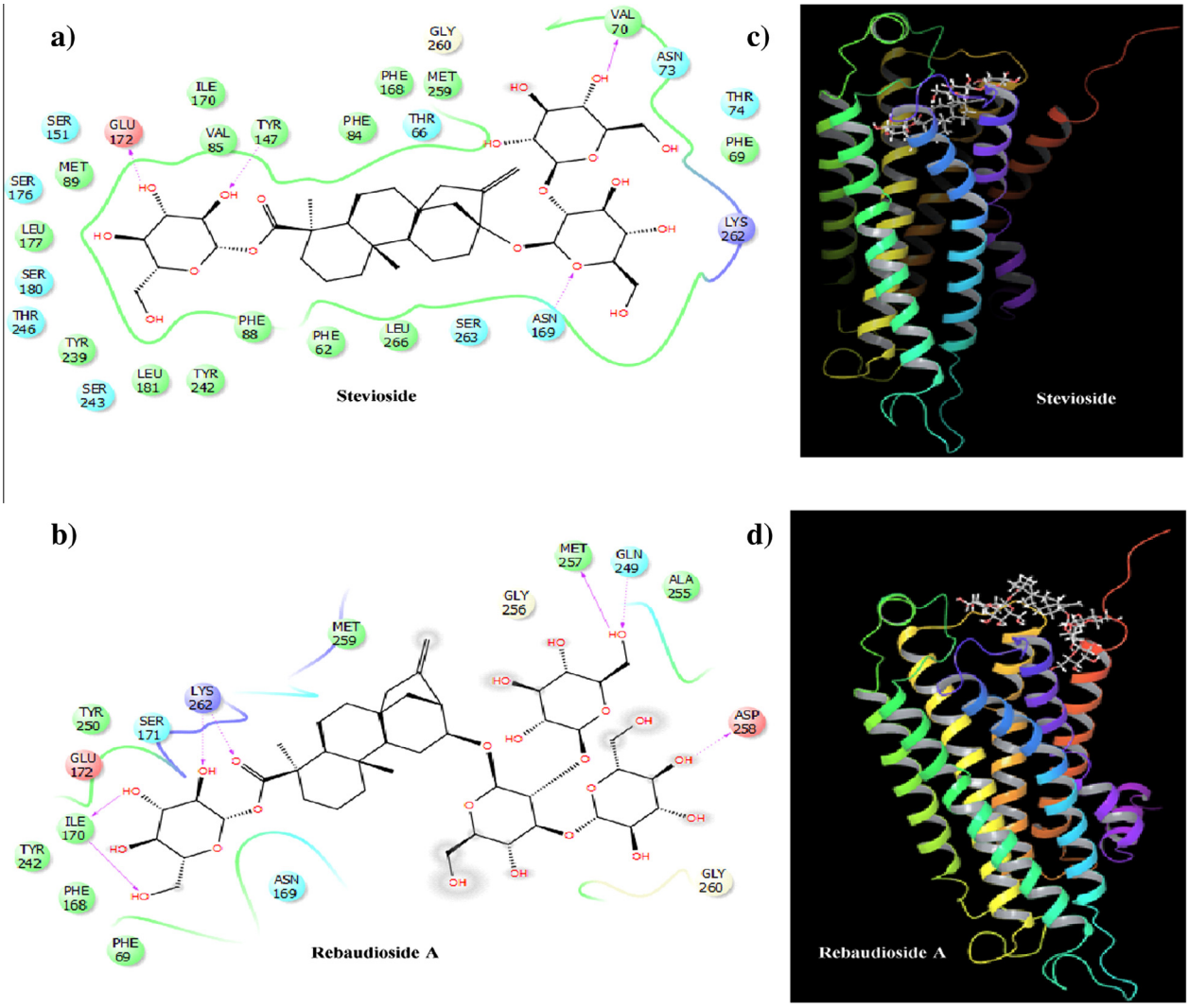

Fig. 3. Ligand interaction diagram of stevioside (a) and rebaudioside A (b); stevioside (c) and rebaudioside A (d) in the binding site of hTAS2R4 receptor.

Molecular interactions of stevioside and rebaudioside A was analysed with bitter taste receptor hTAS2R4. Stevioside was having docking score of −13.587 kcal/mol (Table 1). The van der waals forces contribute 30.47%, and H-bond contributes 44.2% of the dock score. Stevioside display an H-bond interaction with backbone via Val 70 (OH′′′′O═C, bond length 1.85 Å) and side chain via Tyr 147 (OH′′′′OH, bond length 2.34 Å), Glu 172 (OH′′′′OH, bond length 2.00 Å) and Asn 169 (O′′′′NH, bond length 2.43 Å) of the receptor cavity (Fig. 3a). Rebaudioside A showed very feeble binding affinity with the receptor cavity as reflected by docking score of −8.761 kcal/mol. Rebaudioside A display an H-bond interaction with the backbone, via Ile 170 (OH′′′′OH, bond length 2.00 Å) and Met 257 (OH′′′′OH, bond length 1.74 Å). It showed H-bond interaction with side chain and participating amino acid residue were Gln 249 (HO′′′′HN, bond length 2.06 Å), Asp 258 (OH′′′′OH, bond length 2.10 Å) and Lys 262 (C═O′′′′HN, bond length 2.27 Å and HO′′′′HN, bond length 2.12 Å) (Fig 3b). Due to the spatial arrangement of the sugar moieties, the rebaudioside-A was not able to interact optimally with the receptor cavity.

Numerous reports have been published on the limited bitterness profile of rebaudioside A, (Boileau et al., 2012; Carakostas et al., 2008; Hellfritsch et al., 2012; Kochikyan et al., 2006) which is the outcome of its restricted interaction with the bitter receptor. Current molecular simulation study also suggests its limited interaction with the hTAS2R4 receptor. The docking experiment revealed that the SGs interact with the binding site of the bitter receptor (Fig 3c and d). Upon analysing the interaction profile of stevioside and rebaudioside A, it came to light that due to structural similarity, rebaudioside A and stevioside lies in the same binding cavity sharing a couple of amino acids including Phe 69, Phe 168. Phe 169, Ile 170, Glu 172, Met 259, Gly 260 and Lys 262. But due to the presence of sugar residue at the C-3′′ position, rebaudioside A was unable to enter deep inside the binding cavity which is needed for the receptor activation (Pydi et al., 2012, 2014) (Fig 3c and d). Whereas, in case of stevioside the absence of additional sugar residue at the C-3′′ position, it was able to make a strong contact with the receptor site and through extensive hydrophobic interactions initiate a cascade of signaling involved in the taste reception. As a significant proportion of stevioside in steviol glycoside, thereby it can be speculated that the characteristic after bitterness is associated with the higher content of stevioside. Rebaudioside A was unable to trigger the bitter taste signaling due to the presence of additional glucose moiety.

In a recent *in vitro* study, it has been observed that stevioside stimulate the hTAS2R4 bitter receptor and the result obtained were found to corroborate with our molecular simulation study (Hellfritsch et al., 2012). Therefore, preparation of rebaudioside A lies in an important niche, where its semisynthetic intervention not only involve importance in the food industry but also in pharmaceutical industry.

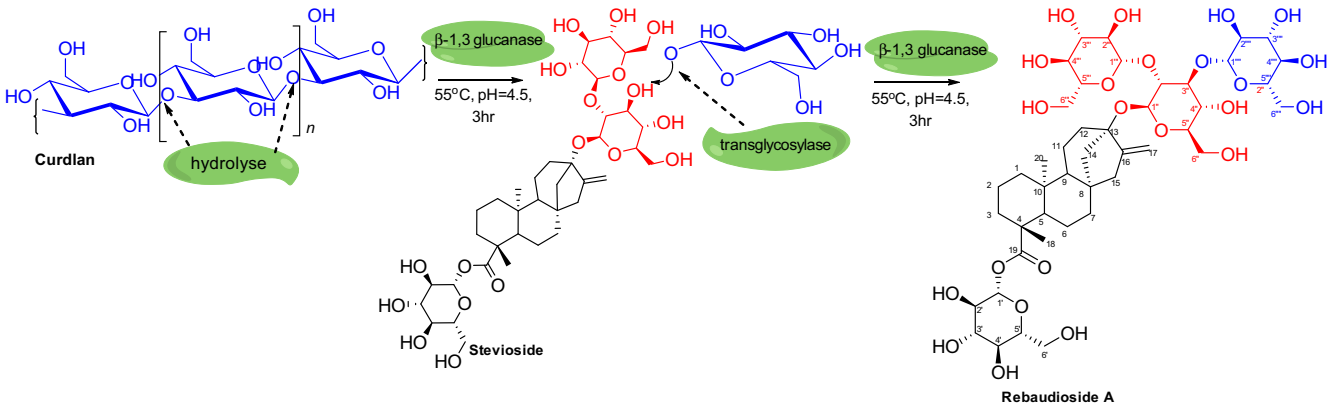

Fig. 4. Transglycosylation of stevioside to rebaudioside A.

2.3. Synthesis of rebaudioside-A

In recent times, the importance of developing greener route for the synthesis of pharmaceutically cardinal moieties has been a center of interest in the medicinal chemists due to its industrial and environmental importance (Roschangar et al., 2015).

Enzymatic synthesis attracted a considerable attention and contributed a significant role in the development of greener routes. By the process of enzymatic transglycosylation, there is a formation of the glycosidic bond during the transfer of a sugar residue from one glycoside to another (Jaitak et al., 2009a,b; Nishihashi et al., 1983; Wang et al., 2015; Yang et al., 2015a)

$$\text{Percent Conversion} = \frac{\text{Amount of rebaudioside A formed}}{\text{Amount of stevioside decreased} \times 1.2} \times 100$$

$$\text{where "1.2" is calculated as} = \frac{\text{Molecular weight of rebaudioside A}}{\text{Molecular weight of stevioside}}$$

In the present study, we have synthesized rebaudioside A from stevioside by enzymatic biotransformation using β-1,3-glucanase from *Irpex lacteus*. The transglycosylation executed by the used enzyme is involved of two *in-situ* steps. In the first step by the property of β-1,3-glucanase it cleaves the glucose moiety from the donor curdlan followed by selectively transferring at the C-3″ position of stevioside with β-configuration in the second step (Fig. 4). The temperature, pH, time, the concentration of curdlan and enzyme plays a significant role in the product yields. Stevioside and curdlan were taken in a ratio of 1:2. The reaction condition was optimized at 55 °C, in a citric acid buffer of pH 4.5, reaction time was 3hrs. The reaction product was formed in maximum yield with enzyme activity of 3.425 units/g. The reaction product was purified by column chromatography, and the percent conversion rate was calculated as 62.5%. The characterization of the product was performed by NMR, HRMS, melting point and data was found to be consistent with previous literature (Singh et al., 2009). In ^{1}H NMR, anomeric proton at δ_H 5.28 (1H, d, J = 8 Hz) of H-1′′′′ indicates the β configuration of glucose moiety attached to C-3′′. The other three anomeric values 5.64 (1H), 5.02 (1H), 4.95 (1H) represent the three more glucose moieties in rebaudioside-A. In C^{13} NMR, δc at 14.89 (C-20) and δc 28.04 (C-18) indicates the presence of two methyl groups. δc value at 175.6 and δc 104 indicating the presence of carbonyl carbon (C-19) and for the exocyclic C=C (C-17) group respectively. The shifting of the δc from 78.0 to δc 86.27 indicated the attachment of a glucose moiety at C-3′′ (Jaitak et al., 2009a,b). Additional peaks for the sugar moiety attached to the C-3′′ position were observed to be δ_C 78.46, 74.35, 73.59, 70.01 and 61.02. Melting point was found to be 245–247 °C.

3. Conclusion

In the present study, we have investigated the binding mode of two SG's, stevioside and rebaudioside A against hTAS2R4 receptor via the computational method. Stevioside and rebaudioside A have structural similarity thereby bind at same receptor site hTAS2R4 receptor. Rebaudioside-A was not able to enter the receptor site effectively thereby failing to initiate bitter taste response due to the presence of a sugar residue at the C-3′′ position. By this reason, the rebaudioside A has shown superior quality taste profile that makes it a valuable additive in food and pharmaceutical industry. Encouraged by our findings, we have developed a highly efficient and greener route for the semi-synthesis of rebaudioside A from stevioside using enzymatic reaction. The developed protocol could be used for the synthesis of high-quality rebaudioside A from bittered stevioside for meeting the rising demands of low calorific natural sweeteners in food and pharmaceutical industry.

4. Experimental

4.1. Homology model development and validation

Primary sequence of hTAS2R4 taste receptor (taste receptor type 2, member 4) from *Homo sapiens* (Q9NYW5) was retrieved from the Universal Protein Resource (http://www.uniprot.org/) and used as targets for homology modeling. To find suitable templates for hTAS2R4, BlastP search was performed against Protein Data Bank (PDB). From searching results, it was inspected that no appropriate template was found that share more than 35% of sequence identity, and they were not able to satisfy query coverage of 100%. Erstwhile studies on GPCR have been reported that homology models of improved quality and reliability can be achieved using multiple templates (Larsson et al., 2008; Yarnitzky et al., 2010). Homology modeling was performed using the I-TASSER (Iterative Threading ASSEmbly Refinement) web server of University of Michigan, USA maintained by Zhang Lab (Roy et al., 2010). Upon submission of the query sequence, I-TASSER retrieves templates of proteins having similar folds (or super-secondary structures) from the PDB library by LOMETS (Local Meta-threading Server) (Wu and Zhang, 2007). LOMETS identified eight templates with PDB id 4GRV, 4BWB, 4N6H, 4IAR, 2Z73, 2DJH, 4BUO and 4EA3. I-TASSER server excises the continuous fragments from the selected/ identified PDB templates and then reassembles them into

a complete models by utilizing replica-exchange Monte Carlo simulations with the threading unaligned regions (mainly loops) built by *ab initio* modeling (Zhang, 2008).

Low free-energy states of the models are identified by SPICKER through clustering the decoys (Zhang and Skolnick, 2004b). Next step proceeds with fragment assembly simulation that is guided by TM-align for removing any steric clashes and to refine the global topology of the cluster decoys (Zhang and Skolnick, 2004a). The final five complete model of hTAS2R4 is obtained by REMO that optimises the hydrogen-bonding network (Li and Zhang, 2009). I-TASSER server also evaluates the quality of the models from C-score, which is based on the impact of threading template alignments and the convergence parameters of the structure assembly simulations. TM-score is also calculated which is a recently proposed scale of measuring the structural similarity between two structures. In TM-score, the small distance is weighted stronger than the big distance that makes the score insensitive to the local modeling error as with the case of RMSD calculations. A TM-score >0.5 indicates a model of correct topology and a TM-score <0.17 means a random similarity. The refined models were checked using procheck, ERRAT available at http://services.mbi.ucla.edu/SAVES/ and RAMPAGE. The ones with best structural features and geometry were selected for the molecular simulation study.

4.2. Ligand modeling

The 2D structure of ligands were constructed using the maestro 9.0 software and saved in sdf format (standard data format). The 2D structures were converted to the 3D structure using the Ligprep module of maestro 9.0. This module adds hydrogens, eliminates any discrepancies between bond length and angle.

4.3. Ligand-binding pocket determination

Ligand binding site was calculated using sitemap application of maestro 9.0. This application performs calculations on the total protein to locate binding sites whose size, functionality, and extent of solvent exposure using the OPLS-2005 force field to determine the binding pocket.

4.4. Molecular docking

The minimized homolog protein prepared by protein preparation wizard was utilized in the docking simulation. Molecular docking calculations were carried out using glide package of maestro 9.0. Affinity grid of 46 × 46 × 46 grid points and spacing 0.25 was generated using the Grid Generation module of maestro 9.0 was generated for the homolog protein around the binding site detected using sitemap module of maestro 9.0. Glide docking was performed using the extra percision mode analysis of Maestro 9.0. The van der waals radii of ligand was taken as 0.5 Å and charge cut off as 0.15. This program calculates the van der waals forces, hydrogen bond interactions and electrostatic forces. Docking simulation was performed using the OPLS-2005 force field. Force field refined ten ligand-receptor possess which were further minimized to yield a single pose, selected on the basis of scoring function. The scoring function combines weighted Coulomb/van der Waals protein–ligand interaction energies, the terms favoring binding affinity (hydrophobic enclosure, neutral–neutral hydrogen-bond motifs, charged-charged hydrogen-bond motifs, pi-cation interactions), and the various penalty terms (desolvation penalties and ligand strain). Ligand interaction module was utilized for to calculate the schematic representation of the residue–ligand interaction between ligands and the active site of hTAS2R4 taste receptor model.

4.5. Enzymes and substrates

Stevioside for the synthesis was isolated from *S. rebaudiana* and characterized according to our earlier published protocol (Jaitak et al., 2009a,b). β-1,3-Glucanase from *I. lacteus* and Curdlan from *Alcaligenes faecalis* were obtained from Sigma Chemical Co. Sodium citrate and citric acid of AR grade were obtained from SDFCL.

4.6. Synthesis of rebaudioside A

Stevioside (200 mg) and curdlan (400 mg) was dissolved in citrate buffer (0.1 M with pH 4.5) and β-1,3-glucanase 3.425 U/g was added. The reaction mixture was shaking in an incubator for 3hr at 55 °C. The reaction mixture was cooled to room temperature and the enzyme was deactivated by boiling at 100 °C. Progress of the reaction was monitored by TLC using solvent system ethyl acetate/ethanol/H_2O (8:2:1.2, v/v/v) (Jaitak et al., 2009a,b). The developed plate was dried and spots were visualized by spraying with 5% sulfuric acid. Reaction mixture was passed through the Dianion HP-20, followed by washing with methanol. Methanol fraction was dried over rota vapor and recrystallized using cold ethanol to yield 150 mg of pure Rebaudioside A. Product was confirmed by, NMR, HRMS and melting point determination.

Rebaudioside A: MP 245–247 °C, R_f value 0.45, ethyl acetate: ethanol:water (8:2:1.2, v/v/v), ^{1}H NMR (400 MHz, DMSO): 5.64 (1H, d, J = 8 Hz), 5.28 (1H, d, J = 8 Hz), 5.02 (1H, t, J = 8 Hz) and 4.95 (1H, d, J = 8 Hz). ^{13}C-NMR (100 MHz, DMSO): δc 14.89, 18.56, 19.79, 21.14, 28.04, 37.48, 38.70, 39.20, 40.03, 41.04, 41.64, 43.10, 43.20, 47.0, 53.23, 56.52, 60.56, 61.02, 61.34, 68.90, 69.48, 70.01, 70.32, 72.44, 73.59, 74.35, 75.98, 76.49, 76.88, 76.98, 77.42, 78.46, 78.79, 78.88, 79.12, 85.40, 86.27, 94.45, 96.45, 102.47, 102.98, 104.0, 152.78, 175.63. HRMS (ESI): m/z calc. for $C_{44}H_{70}O_{23}$ $[M-H]^-$: 965.4230; found 965.5040.

Acknowledgements

Authors are grateful to Department of Science and Technology, New Delhi, India for providing financial assistance during the work. Authors are also thankful to the Honourable Vice - Chancellor for providing the necessary facilities for the Central University of Punjab, Bathinda, India.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phytochem.2016.03.004.

References

Adler, E., Hoon, M.A., Mueller, K.L., Chandrashekar, J., Ryba, N.J., Zuker, C.S., 2000. A novel family of mammalian taste receptors. Cell 100, 693–702.
Boileau, A., Fry, J., Murray, R., 2012. A new calorie-free sugar substitute from the leaf of the stevia plant arrives in the UK. Nutr. Bull. 37, 47–50.
Carakostas, M., Curry, L., Boileau, A., Brusick, D., 2008. Overview: the history, technical function and safety of rebaudioside A, a naturally occurring steviol glycoside, for use in food and beverages. Food Chem. Toxicol. 46, S1–S10.
Chandrashekar, J., Hoon, M.A., Ryba, N.J., Zuker, C.S., 2006. The receptors and cells for mammalian taste. Nature 444, 288–294.
Fredriksson, R., Lagerström, M.C., Lundin, L.-G., Schiöth, H.B., 2003. The G-protein-coupled receptors in the human genome form five main families. Phylogenetic analysis, paralogon groups, and fingerprints. Mol. Pharmacol. 63, 1256–1272.
GRAS notice 000354, 2015. GRAS Notice 000354: High Purity Rebaudioside A – 97%, <http://www.fda.gov/ucm/groups/fdagov-public/@fdagov-foods-gen/documents/document/ucm269561.pdf>as downloaded on 17-11-2015.
Hellfritsch, C., Brockhoff, A., Stähler, F., Meyerhof, W., Hofmann, T., 2012. Human psychometric and taste receptor responses to steviol glycosides. J Agri. Food Chem. 60, 6782–6793.

Jaitak, V., Gupta, A., Kaul, V., Ahuja, P.S., 2008. Validated high-performance thin-layer chromatography method for steviol glycosides in *Stevia rebaudiana*. J. Pharm. Biomed. Anal. 47, 790–794.

Jaitak, V., Kaul, V.K., Kumar, N., Singh, B., Savergave, L., Jogdand, V., Nene, S., 2009a. Simple and efficient enzymatic transglycosylation of stevioside by β-cyclodextrin glucanotransferase from Bacillus firmus. Biotechnol. Lett. 31, 1415–1420.

Jaitak, V., Singh, B., Kaul, V., 2009b. An efficient microwave-assisted extraction process of stevioside and rebaudioside-A from *Stevia rebaudiana* (Bertoni) from *Stevia rebaudiana* (Bertoni). Phytochem. Anal. 20, 240–245.

Kochikyan, V., Markosyan, A., Abelyan, L., Balayan, A., Abelyan, V., 2006. Combined enzymatic modification of stevioside and rebaudioside A. Appl. Biochem. Microbiol. 42, 31–37.

Larsson, P., Wallner, B., Lindahl, E., Elofsson, A., 2008. Using multiple templates to improve quality of homology models in automated homology modeling. Protein Sci. 17, 990–1002.

Li, Y., Zhang, Y., 2009. REMO: A new protocol to refine full atomic protein models from C-alpha traces by optimizing hydrogen-bonding networks. Proteins Struct. Funct. Bioinf. 76, 665–676.

Lovell, S.C., Davis, I.W., Arendall, W.B., de Baker, P.I.W., Word, J.M., Prisant, M.G., Richardson, J.S., Richardson, D.C., 2003. Structure validation by Calpha geometry: phi,psi and Cbeta deviation. Proteins 50, 437–450.

Mayank, Jaitak, V., 2015. Interaction model of steviol glycosides from *Stevia rebaudiana* (Bertoni) with sweet taste receptors: a computational approach. Phytochemistry 116, 12–20.

McChesney, J.D., 2006. Stevia: The Genus Stevia, edited by A. Douglas Kinghorn (University of Illinois at Chicago). Taylor & Francis, London. 2002. xii + 211 pp. 7 × 10 in. $90.00. ISBN 0-415-26830-3. J. Nat. Prod. 69, 860.

Nabors, L.O.B., 2001. Food Science and Technology: Alternative Sweeteners, third ed. Marcel Dekker Inc., NY, U. S., Revised and Expanded.

Nishihashi, H., Matsubayashi, T., Katabami, Tadashi., Matsuda, Ken.-ichi., 1983. Production of β-1,3-glucosyl steviosides. Dainippon Ink and Chemicals, Inc., Japan; DIC Fine Chemical Co., Ltd., p. 10.

O'Donnell, K., Kearsley, M.W., 2012. Sweeteners and Sugar Alternatives in Food Technology, second ed. Wiley-Blackwell.

Pydi, S.P., Bhullar, R.P., Chelikani, P., 2012. Constitutively active mutant gives novel insights into the mechanism of bitter taste receptor activation. J. Neurochem. 122, 537–544.

Pydi, S.P., Singh, N., Upadhyaya, J., Bhullar, R.P., Chelikani, P., 2014. The third intracellular loop plays a critical role in bitter taste receptor activation. Biochim. Biophys. Acta Biomembr. 1838, 231–236.

Roschangar, F., Sheldon, R., Senanayake, C., 2015. Overcoming barriers to green chemistry in the pharmaceutical industry–the Green Aspiration Level™ concept. Green Chem. 17, 752–768.

Roy, A., Kucukural, A., Zhang, Y., 2010. I-TASSER: a unified platform for automated protein structure and function prediction. Nat. Protoc. 5, 725–738.

Singh, N., Pydi, S.P., Upadhyaya, J., Chelikani, P., 2011. Structural basis of activation of bitter taste receptor T2R1 and comparison with Class A G-protein-coupled receptors (GPCRs). J. Biol. Chem. 286, 36032–36041.

Upadhyaya, J., Singh, N., Bhullar, R.P., Chelikani, P., 2015. The structure–function role of C-terminus in human bitter taste receptor T2R4 signaling. Biochim. Biophys. Acta Biomembr. 1848, 1502–1508.

Wang, Y., Chen, L., Li, Y., Li, Y., Yan, M., Chen, K., Hao, N., Xu, L., 2015. Efficient enzymatic production of rebaudioside A from stevioside. Biosci. Biotechnol. Biochem., 1–7

Wu, S., Zhang, Y., 2007. LOMETS: a local meta-threading-server for protein structure prediction. Nucleic Acids Res. 35, 3375–3382.

Yang, J., Shen, J., Wang, Y., Fan, G., 2015a. Process for Preparing High Purity Rebaudioside A, p. 16, Peop. Rep. China.

Yang, J., Yan, R., Roy, A., Xu, D., Poisson, J., Zhang, Y., 2015b. The I-TASSER Suite: protein structure and function prediction. Nat. Methods 12, 7–8.

Yarnitzky, T., Levit, A., Niv, M.Y., 2010. Homology modeling of G-protein-coupled receptors with X-ray structures on the rise. Curr. Opin. Drug Discov. Dev. 13, 317–325.

Zhang, Y., 2008. I-TASSER server for protein 3D structure prediction. BMC Bioinf. 9, 40.

Zhang, Y., Skolnick, J., 2004a. Scoring function for automated assessment of protein structure template quality. Proteins: Struct. Funct. Bioinf. 57, 702–710.

Zhang, Y., Skolnick, J., 2004b. SPICKER: a clustering approach to identify near-native protein folds. J. Comput. Chem. 25, 865–871.