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Riboflavin-Binding Protein Exhibits Selective Sweet Suppression toward Protein Sweeteners

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Abstract

Riboflavin-binding protein (RBP) is well known as a riboflavin carrier protein in chicken egg and serum. A novel function of RBP was found as a sweet-suppressing protein. RBP, purified from hen egg white, suppressed the sweetness of protein sweeteners such as thaumatin, monellin, and lysozyme, whereas it did not suppress the sweetness of low molecular weight sweeteners such as sucrose, glycine, D-phenylalanine, saccharin, cyclamate, aspartame, and stevioside. Therefore, the sweet-suppressing activity of RBP was apparently selective to protein sweeteners. The sweet suppression by RBP was independent of binding of riboflavin with its molecule. Yolk RBP, with minor structural differences compared with egg white RBP, also elicited a weaker sweet suppression. However, other commercially available proteins including ovalbumin, ovomucoid, β-lactogloblin, myoglobin, and albumin did not substantially alter the sweetness of protein sweeteners. Because a prerinse with RBP reduced the subsequent sweetness of protein sweeteners, whereas the enzymatic activity of lysozyme and the elution profile of lysozyme on gel permeation chromatography were not affected by RBP, it is suggested that the sweet suppression is caused by an interaction of RBP with a sweet taste receptor rather than with the protein sweeteners themselves. The selectivity in the sweet suppression by RBP is consistent with the existence of multiple interaction sites within a single sweet taste receptor.

Key words: egg white, lysozyme, riboflavin-binding protein, sweet suppression, sweetness

Introduction

There have been few studies on the taste effects of proteins because most proteins do not elicit any taste. The major exceptions to this generalization are the sweet proteins such as monellin (Morris and Cagan 1972), thaumatin (Van der Wel and Loeve 1972), brazzein (Ming and Hellekant 1994), and mabinlin (Liu et al. 1993). These sweet proteins were discovered in fruits of tropical plants. Aside from these odd sweet taste–eliciting proteins, protein has important functions by altering many physicochemical properties of foods such as their viscosity, gelation, cohesion, adhesion, elasticity, emulsification, foaming, and so on (Kinsella 1982). Because almost all proteins are apparently tasteless, it is believed that protein contributes to the palatability of food only through these textural effects. However, we found that a common protein, lysozyme in egg white, elicited sweetness (Maehashi and Udaka 1998) with a threshold value of around 7 μM (Masuda et al. 2005). This suggests that proteins having taste properties may be more common that previously thought because this sweet protein was found in an ordinary food, egg. However, this is puzzling because we have never found that egg white itself is sweet. Perhaps, there is also a sweet-inhibiting substance in egg white. There are several taste-modifying proteins found in tropical fruits such as miraculin (Theerasilp and Kurihara 1988), which transforms sour taste into sweet taste, and curculin (Yamashita et al. 1990), which induces a sweet taste with water. However, it would be important to discover a taste-modifying protein in common, widely consumed foods because that information could help us understand how an interaction or a competition between food components affects the taste of food generally. Therefore, in the work reported here, we undertook studies to explain why the sweet protein lysozyme is not perceived as sweet in protein-rich egg white. We tested the hypothesis that egg white contains a natural suppressor of sweet proteins. To this end, we documented and purified a sweet-suppressing protein from egg white and identified it as riboflavin-binding protein (RBP). Subsequently, the sweet-suppressing properties of RBP were characterized, and its mechanism of action is discussed.

Materials and methods

Materials

Fresh white leghorn eggs were obtained from a local food market. Sucrose, glycine, D(±)-phenylalanine, sodium saccharin, myoglobin (from horse skeletal muscle), sodium chloride, citric acid anhydrate, monosodium glutamate monohydrate, and quinine hydrochloride were purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). Sodium *N*-cyclohexylsulfamate (sodium cyclamate) was purchased from Tokyo Chemical Industry Co., Ltd (Tokyo, Japan). Ovalbumin (from chicken egg white), ovomucoid (from chicken egg white), β-lactogloblin (from bovine milk), albumin (from bovine serum), thaumatin, monellin, and ±2-(4-methoxyphenoxy) propanoic acid (PMP) were purchased from Sigma Co. (St Louis, MO). Lysozyme (from chicken egg white, 6-times crystallized) was purchased from Seikagaku Corporation (Tokyo, Japan). Stevioside was obtained from Morita Kagaku Kogyo Co. Ltd (Osaka, Japan). DEAE-Sepharose CL-6B, CM-Sepharose CL-6B, Phenyl Sepharose CL-4B, and Sephadex G-75 were purchased from Amersham Biosciences Corp. (Piscataway, NJ).

Purification of RBP from egg white and yolk

Purification of RBP from chicken egg white and yolk was performed essentially according to the method of Miller and White (1986). Chicken egg white was collected from 40 eggs, diluted with an equal volume of 0.2 M acetate buffer (pH 4.3) and filtered. To the filtrate, 300 ml of buffered DEAE-Sepharose CL-6B (0.1 M acetate buffer, pH 4.3) gel was added. After the DEAE-Sepharose gel was packed into a column and washed with the buffer, RBP was eluted with a linear NaCl concentration gradient of 0–1 M in the buffer. Then the yellow fraction was subjected to ammonium sulfate fractionation. The precipitate that appeared at 55–85% $(NH_4)_2SO_4$ saturation was collected and dissolved in water. This yellow fraction was adjusted to pH 3.14 and was subjected to CM-Sepharose CL-6B (buffered with 25 mM acetate buffer at pH 3.14). After the yellow fraction that included free riboflavin was eluted, the column was washed with 25 mM acetate buffer (pH 5.8) to obtain the apo-form RBP and then it was subjected to gel filtration on a Sephadex G-25 column to remove the acetate salt.

Chicken yolk collected from 40 eggs was diluted with an equal volume of 0.1 M acetate buffer (pH 4.3) and centrifuged at 9000× *g* for 1 h. The supernatant was dialyzed against deionized water for 24 h and then against 0.1 M acetate buffer (pH 4.3) for 24 h. The same procedure as used for egg white was then conducted to obtain yolk RBP. Both RBPs isolated through chromatography were dialyzed against deionized water to remove buffer salt and then lyophilized.

SDS-polyacrylamide gel electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed in 15% gel according to the method of Laemmli (1970). Molecular weight (MW) markers (GE Healthcare Bio-Sciences Corp., Piscataway, NJ) included phosphorylase b for 97 kDa, albumin for 66 kDa, ovalbumin for 45 kDa, carbonic anhydrase for 30 kDa, trypsin inhibitor for 20 kDa, and α-lactoalbumin for 14 kDa. After electrophoresis, the gels were stained with coomassie brilliant blue R-250.

Amino acid sequence analysis

To determine a partial amino acid sequence of sample proteins, in-gel digestion was conducted following the method by Cleveland et al. (1977) using *Staphylococcus aureus* V8 protease (Wako Pure Chemical Industries, Ltd). After electroblotting to a polyvinylidene fluoride membrane (Bio-Rad Laboratories, Inc., Hercules, CA), each separated band of a proteolytic fragment was excised from the membrane and subjected to a protein sequencer PPSQ-20 (Shimazdu Corp., Kyoto, Japan) for amino-terminal analysis. Digestion by L-(tosylamido-2-phenyl) ethyl chloromethyl ketone–treated (TPCK) trypsin (Wako Pure Chemical Industries, Ltd) was also conducted to obtain proteolytic fragments of the sample protein following the method of Hamazume et al. (1987). These fragments were then subjected to the protein sequencer.

Measurement of bacteriolytic activity of lysozyme

The bacteriolytic activity of lysozyme toward *Micrococcus luteus* cells (Sigma Co.) was measured turbidimetrically following the method of Shugar (1952). To a 2.95 ml of 0.2-mg/ml suspension of *M. luteus* in M/15 phosphate buffer (pH 6.24), 50 µl of lysozyme solution was added. The decrease in absorbance was then monitored at 450 nm by a Beckman DU640 spectrophotometer (Beckman Coulter, Inc., Fullerton, CA).

Determination of equisweet concentrations to 0.15 M sucrose for various sweeteners

Various sweeteners were dissolved in deionized water, and their equisweet concentrations to 0.15 M sucrose were determined by paired comparisons test using 6 well-trained subjects (4 females and 2 males, average age = 22 years) from our laboratories. Sweetener solutions were prepared at several concentrations, and the sweetness intensity of each solution was compared with that of 0.15 M sucrose solution that is of moderate sweetness at room temperature. Because of long-lasting aftertaste of some of the sweeteners, tasting was conducted with a 3-min interval between samples.

Measurement of sweet-suppressing activity

The sweet-suppressing activity was assayed by a sensory test using 3 subjects who were chosen from the subjects in the section described above. They were trained according to the procedure described in this section. To measure sweet-suppressing activity of sample, at first 0.5 ml of a sweetener solution at room temperature was held in mouth for 30 s to evaluate its sweetness and then expectorated. Following the evaluation, the mouth was rinsed repeatedly with deionized

water. After the sweetness disappeared totally, a solution of same sweetener mixed with a sample of a potential inhibitor dissolved in deionized water was tasted and its sweetness was compared with the first sweetener solution that did not contain the potential inhibitor. Sweetness intensities were recorded using a 10-cm unstructured line scale with anchor points "not at all" and "equisweet to that without potential inhibitor." Sweet suppression was represented as the percentage of the intensity of sweetness of the sweetener with the potential inhibitor compared with the sweetener without the potential inhibitor.

Results

Survey and identification of sweet-suppressing protein from egg white

Egg white from 5 eggs was diluted with deionized water at twice volume and adjusted to pH 4.2. After boiling for 3 min, it was cooled and centrifuged to remove high MW substances such as ovomucin and ovalbumin. Then it was subjected to CM-Sepharose CL-6B column equilibrated at pH 7.0 to separate it into 3 fractions: flow through, CM-1, and CM-2. The flow through was further separated by a DEAE-Sepharose CL-6B column as flow through, DEAE-1, and DEAE-2 fractions. Thus, 5 fractions were obtained from egg white and examined for their sweet taste–masking effect on the sweetness of lysozyme. Fraction CM-2 was strongly sweet itself because it contained lysozyme from egg white. Whereas the DEAE-1 fraction and the CM-1 fraction had no effect on the taste of lysozyme, the DEAE-2 fraction suppressed the sweetness of 0.2% lysozyme completely. Therefore, the DEAE-2 fraction probably contained a protein having sweet-suppressing activity. We next further purified it on a Phenyl Sepharose CL-4B column to identify it. The peak fraction contained the sweet-suppressing activity was collected and isolated further by gel filtration using Sephadex G-75 column. After the active fraction was dialyzed against deionized water, a protein with a yellow color was obtained. This purified protein was found to have a sweet-suppressing activity at 2% on 0.2% lysozyme sweetness.

This purified protein showed a single band on SDS-PAGE with an estimated MW of 35 kDa. The solution of this purified protein had a yellow color. From the fact of its absorption to DEAE-Sepharose column, its apparent MW of 35 kDa and its yellow color, it was hypothesized to be RBP. To confirm this hypothesis, a partial amino acid sequence of purified protein was determined by sequencing of peptide fragments from digests of V8 protease and TPCK trypsin. As shown in Figure 1, the sequences of 3 fragments P1, P2, and P3 were identical to that of RBP (Hamazume et al. 1984). Thus, the sweet-suppressing protein purified from hen egg white was identified as RBP.

Riboflavin-free (apo-form) RBP was purified from egg white following the established method (Miller and White 1986) described in Materials and Methods. A single protein was obtained with an estimated MW of 35 kDa on SDS-PAGE as shown in Figure 2. This apo-form RBP was examined for its sweet-suppressing activity toward 0.2% lysozyme. Because no difference was detected in the activity of the apo- and holo-forms of RBP, it was considered that riboflavin itself does not have sweet-suppressing activity and also the structure of riboflavin-binding site within RBP molecule does not participate in this activity. Therefore, the apo-form of RBP was used for all subsequent experiments in this study.

Selectivity of sweet-suppressing effect of RBP

The sweet-suppressing effect of egg white RBP on various sweet compounds was examined. Samples tested were 3 sweet proteins, lysozyme, thaumatin, and monellin; 1 sugar, sucrose;

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*       10          20          30          40          50          60
QQYGCLEGDT HKAKPSPEPN MHECTLYSES SCCYANFTEQ LAHSPIIKVS NSYWNRCGQL
                              P1
        70          80          90          100         110         120
SKSCEDFTKK IECFYRCSPH AARWIDPRYT AAIQSVPLCQ SFCDDWYEAC KDDSICAHNW
                                                        P2
        130         140         150         160         170         180
LTDWERDESG ENHCKSKCVP YSEMYANGTD MCQSMWGESF KVSESSCLCL QMNKKDMVAI
                              P3
        190         200         210         219
KHLLSESSEE SSSMSSSEEH ACQKKLLKFE ALQQEEGEE
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Figure 1 The complete amino acid sequence of chicken egg white RBP (Hamazume et al. 1984). **N*-terminal amino acid is pyroglutamic acid. Peptide fragments obtained from proteolytic digestion of purified protein in this study is indicated as P1, P2, and P3.

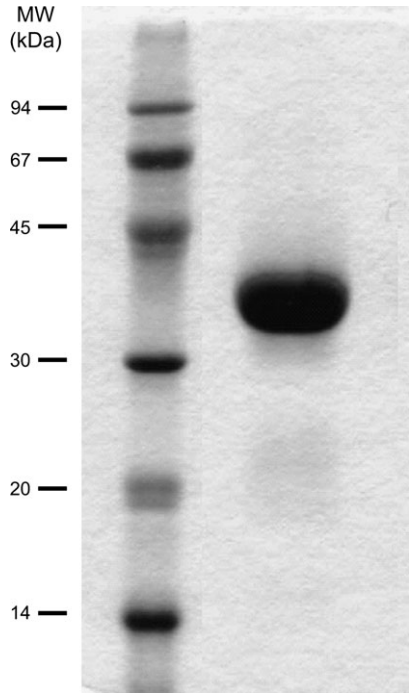

Figure 2 SDS-PAGE of RBP purified from chicken egg white.

1 dipeptide derivative, aspartame; 2 amino acids, glycine and D-phenylalanine; 1 terpenoid glycoside, stevioside; 1 *N*-sulfonylamide, sodium saccharin; and 1 sulfamate, sodium cyclamate. To evaluate the sweet-suppressing effect of RBP toward those sweeteners at the same levels of sweetness intensity, we first determined, for each sweetener, the concentration that produced the same intensity of sweetness as 0.15 M sucrose (see Materials and Methods and Table 1). Next, the effects of RBP on the sweetness of these sweeteners were examined. As shown in Figure 3, RBP elicited a sweet-suppressing effect on lysozyme as well as the other 2 sweet proteins, monellin and thaumatin. In contrast, RBP did not reduce the sweetness of sucrose, aspartame, glycine, D-phenylalanine, stevioside, saccharin, and cyclamate. This result demonstrates that RBP selectively suppresses the sweetness of sweet proteins but not that of low MW sweeteners.

The sweet-suppressing effect of egg white RBP toward multiple concentrations of thaumatin was then examined with the results shown in Figure 4. When RBP was added to each thaumatin solution, the sweetness intensity of each thaumatin solution (0.3, 0.45, and 0.6 μM) was suppressed by addition of RBP. Higher concentrations of thaumatin required higher concentration of RBP for effective sweetness inhibition. On the other hand, sucrose was not affected by RBP even at a lower sucrose concentration of 0.075 M at all.

Perhaps RBP has proteolytic activity against sweet proteins, degrading them thereby causing the loss of sweetness. One of the sweet proteins, lysozyme, is an enzyme with bacteriolytic activity. We reasoned that if lysozyme is degraded by RBP, then lysozyme should lose its enzymatic activity. Therefore, the bacteriolytic activity of the mixture solution of lysozyme and RBP was investigated. That is, 5 μM lysozyme was mixed with 50 or 100 μM RBP, and 0.1 mM lysozyme was mixed with 1 mM RBP under the same conditions as the taste evaluation. After those mixtures were left for 30 min at room temperature, the bacteriolytic activity was determined. No change was found in the enzymatic activity of lysozyme before compared with after the addition of RBP (Table 2). The sample of the mixture of 0.1 mM lysozyme and 1 mM RBP was analyzed by SDS-PAGE, and it was confirmed that the molecular size of lysozyme was also not to be changed during incubation with RBP (Figure 5).

Table 1 Equisweet concentration to 0.15 M sucrose

Sweetener	Concentration[a]
Aspartame	0.72 ± 0.06 (mM)
Glycine	0.54 ± 0.02 (M)
D-phenylalanine	28 ± 2.5 (mM)
Stevioside	0.28 ± 0.03 (mM)
Sodium saccharin	0.51 ± 0.02 (mM)
Sodium cyclamate	9.35 ± 0.95 (mM)
Lysozyme	0.11 ± 0.02 (mM)
Monellin	1.1 ± 0.11 (μM)
Thaumatin	0.30 ± 0.01 (μM)

[a]Mean ± standard error.

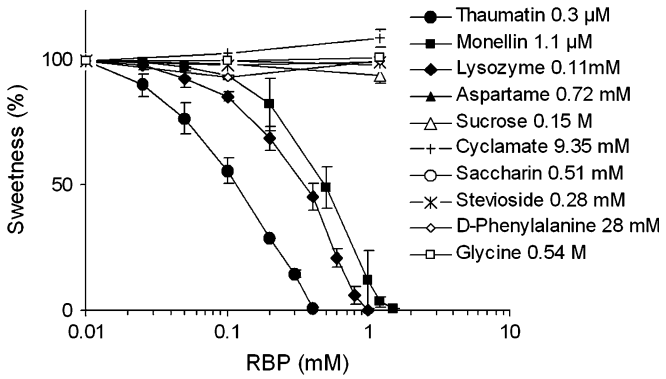

Figure 3 Sweet suppression by white RBP toward various sweeteners. Sweetness intensity of each sweeteners without RBP is expressed as 100%, which elicits a sweetness equivalent to 0.15 M sucrose. Vertical bars indicate standard error.

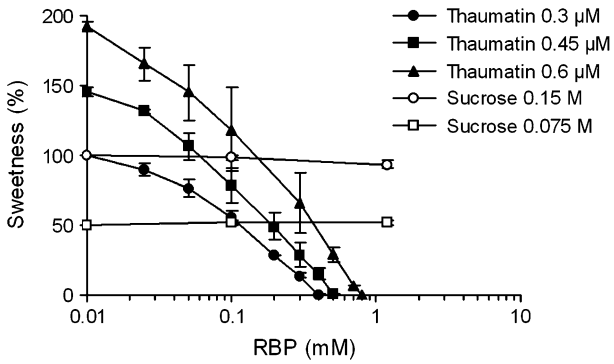

Figure 4 Effect of RBP on the sweetness of multiple concentration of thaumatin and sucrose. Sweetness intensity of 0.3, 0.45, and 0.6 μM of thaumatin without RBP were expressed as 100%, 150%, and 200%, respectively. That of 0.15 M sucrose and 0.075 M sucrose were expressed as 100% and 50%, respectively. Vertical bars indicate standard error.

Table 2 Effect of RBP on the enzymatic activity of lysozyme

Sample solution	Bacteriolytic activity (%)[a]
Lysozyme 5 μM	100
Lysozyme 5 μM + RBP 50 μM	103
Lysozyme 5 μM + RBP 100 μM	103
Lysozyme 0.1 mM[b]	100
Lysozyme 0.1 mM + RBP 1 mM[b]	101
RBP 1 mM[b]	ND

ND: not detected.[a]Bacteriolytic activities of the sample solutions with RBP were expressed as a percentage against those counterparts without RBP. [b]These sample solutions were diluted 100 times for this assay.

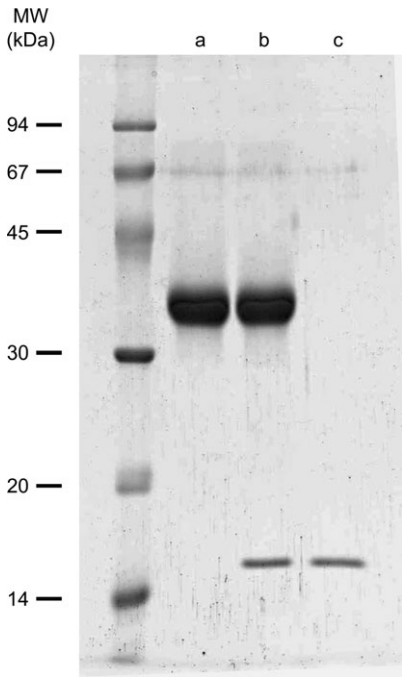

Figure 5 SDS-PAGE of the mixture of lysozyme and RBP. 1 mM RBP **(a)**, the mixture of 0.1 mM lysozyme and 1 mM RBP **(b)**, and 0.1 mM lysozyme **(c)** were analyzed on SDS-PAGE.

From these results, it was concluded that RBP suppressed the sweetness of protein sweeteners without degrading them. Furthermore, the fact that the addition of RBP to lysozyme did not affect the enzymatic activity of lysozyme indicates that RBP does not interact with lysozyme molecule to interfere it with binding to substrate for enzymatic activity. To examine a molecular interaction, mixture of RBP and lysozyme was run on the Sephadex G-75 gel permeation chromatography column and compared its elution profile with each of those eluted singly. As shown in Figure 6, neither shift nor complex of peaks was observed in the elution profile of the mixture. From these results, we concluded that there is no chemical interaction between lysozyme and RBP.

Structural specificity of RBP for sweet suppression

To confirm specificity of RBP as the sweet-suppressing protein, 3 commercially available proteins, ovalbumin, β-lactogloblin, and myoglobin, and 2 RBPs prepared from chicken egg white and yolk were compared for their effect on the sweetness of 0.3 μM thaumatin. All proteins tested in this experiment did not have any taste themselves. The sweetness of 0.3 μM thaumatin mixed with each protein was compared with sweetness of thaumatin alone. As shown in Figure 7, the 3 proteins tested other than egg white and yolk RBP did not affect sweetness of thaumatin, whereas both RBPs suppressed the sweetness of thaumatin. However, the sweet-suppressing activity of yolk RBP was weaker than that of egg white RBP. These results confirmed that the sweet-suppressing effect was specific in

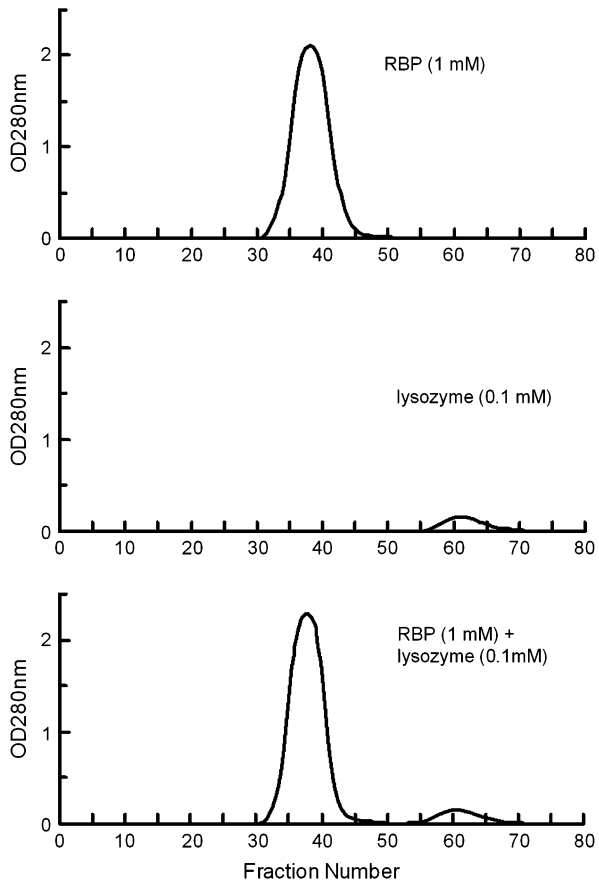

Figure 6 Elution profiles of RBP and lysozyme on Sephadex G-75 column. Samples were run on a Sephadex G-75 column (1.5 × 95 cm) in 10 mM phosphate buffer (pH 7.0) containing 0.1 M NaCl.

RBPs and was not a common feature of proteins. Furthermore, structural differences between white RBP and yolk RBP affected the sweet-suppressing activity.

Effect of prerinse with RBP on the sweetness of protein sweeteners

To further characterize sweet suppression by RBP, we examined the effect of an oral prerinse with RBP solution on the sweetness of protein sweeteners. After 0.5 ml of 1.2 mM RBP solution was held in the mouth for 10 s and expectorated, each protein sweetener solution was immediately evaluated for sweetness intensity compared with the taste of the sweetener without a prerinse. For a control, the effect of PMP prerinse on the sweetness of 0.15 M sucrose was also examined in the same way. The sodium salt of PMP is the well known as an antisweet substance (Schiffman et al. 1999). Therefore, PMP solution was used after neutralization by NaOH. As shown in Figure 8, after a rinse with 1.2 mM RBP, the sweetness intensity of 0.3 μM thaumatin, 1.1 μM monellin, and 0.11 mM lysozyme was reduced. In the same manner, the sweetness intensity of 0.15 M sucrose was reduced after rinsing with 1 mM PMP, suggested as a sweet-receptor

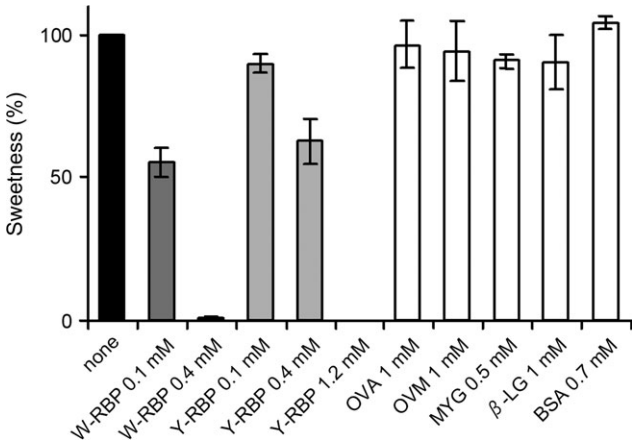

Figure 7 Effects of white and yolk RBP and other proteins on the sweetness of 0.3 μM thaumatin. Sweetness intensity of 0.3 μM thaumatin alone was expressed as 100%. W-RBP, white RBP; Y-RBP, Yolk RBP; OVA, ovalbumin; OVM, ovomucoid; MYG, myoglobin; β-LG, β-lactoglobin; BSA, bovine serum albumin.

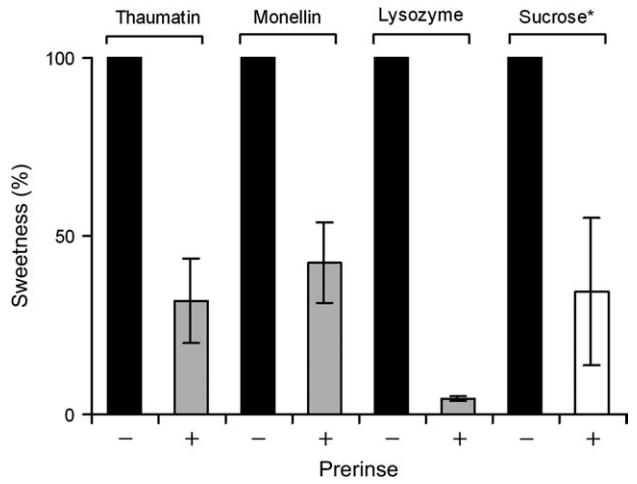

Figure 8 Effect of prerinse with RBP on the sweetness of sweet proteins. −, without prerinse; +, prerinsed. The sweetness of 0.3 μM thaumatin, 1.1 μM monellin, and 0.11 mM lysozyme were evaluated after the mouth was rinsed with 1.2 mM RBP. *The sweetness of 0.15 M sucrose was evaluated after rinse with 1 mM PMP. Vertical bars indicate standard error.

antagonist. We found that 1 mM PMP inhibited the sweetness of 0.15 M sucrose completely when they were mixed (preliminary test data not shown). The sweet-suppressing modality of RBP was similar to that of PMP in which prerinse also reduced sweetness but the effect was weaker than as the mixture, indicating the effect does not last long time.

Effect of RBP on various taste stimuli

Effect of RBP on other, nonsweet, taste stimuli was examined using quinine for bitterness, sodium chloride for saltiness, monosodium glutamate for umami, and citric acid for sourness. As shown in Figure 9, RBP at 1 mM had no effect

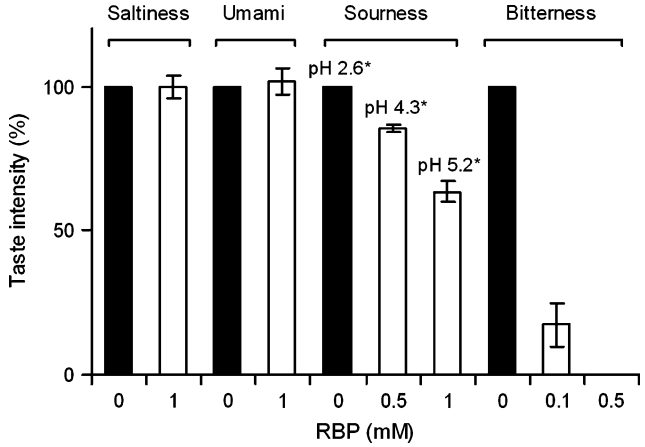

Figure 9 Effect of RBP on various taste stimuli. Saltiness of 0.15 M NaCl, umami of 0.25 mM monosodium glutamate, sourness of 10 mM citric acid, and bitterness of 0.125 mM quinine were used for the standard taste solutions, and each taste intensity of those added RBP was compared and expressed as a percentage against them. *The pHs were measured for citric acid tasted. Vertical bars indicate standard error.

on the saltiness of 0.15 M sodium chloride and on the umami taste of 25 mM monosodium glutamate. The sourness of 10 mM citric acid was decreased by the addition of 0.5 and 1 mM RBP; however, this effect was most likely caused by neutralization of acid by the protein because the pHs of the taste solutions with added 0.5 and 1 mM RBP were 4.3 and 5.2, respectively, whereas that of citric acid alone was 2.6. The bitterness of 0.125 mM quinine was dramatically decreased by 0.1 mM RBP addition and was totally eliminated by 0.5 mM RBP. Thus, it was found that RBP suppressed bitterness in addition of sweetness. Although sodium is well known to be a potent bitter blocker (Keast et al. 2004a), the RBP solution was unlikely to contain sufficient amount of sodium for suppression of bitterness of 0.125 mM quinine because it is presented that approximately half suppression of the bitterness of 0.1 mM quinine was achieved by 100 mM NaCl by Breslin and Beauchamp (1995). Therefore, it was considered that RBP itself suppressed bitterness. This bitter-suppressing property of RBP merits further investigation because there might be a possibility of common mechanism on sweet and bitter suppression.

Discussion

RBP was first isolated from hen egg white by Rhodes et al. (1958). It is a monomeric phosphorylated glycoprotein (Hamazume et al. 1984), consisting of 219 amino acid residues with 9 disulfide bonds (Hamazume et al. 1987). It binds one molecule of riboflavin (Becvar and Palmer 1982) and is involved in the transport of riboflavin from the serum compartment into the chicken oocyte and from the oviduct into the egg white. Although RBP has been well studied and characterized because of its riboflavin-binding activity and its

physiological importance, its function in taste has not previously been investigated. The novel finding reported here is that this protein suppressed the sweet taste of several protein sweeteners completely.

It is known that the family of hen RBP includes 3 proteins from different organs: egg white, egg yolk, and plasma. These proteins are coded for by the same genes, but they undergo different posttranslational modifications. Egg white RBP is synthesized in oviduct, plasma RBP is synthesized in liver, and egg yolk RBP results from proteolytic cleavage of the C-terminal 11 or 13 amino acids from plasma RBP. The amino acid sequence of egg yolk RBP is identical with that of egg white RBP except that the C-termini of these proteins are different (Norioka et al. 1985). It is also known that egg yolk RBP has the same characteristics as white RBP, such as carbohydrate chains attached to both Asn36 and Asn147 residues and phosphate groups bound to some serine residues in the sequence of Ser185 to Ser197 as a cluster (Hamazume et al. 1984); however, their carbohydrate compositions in yolk RBP differ from those in white RBP (Miller et al. 1982). In this study, both RBPs from egg white and egg yolk exhibited sweet-suppressing activity but the activity was higher in egg white RBP than egg yolk RBP. The differences between the 2 RBPs are the C-terminal amino acid sequence and the structure of oligosaccharide chains. These structures might have a relationship to the sweet-suppressing activity but is not likely to be essential because the structural difference did not lead loss of the activity but decrease in it. An interesting fact is that the taste-modifying proteins such as miraculin and neoculin are also glycoproteins, but the role of the oligosaccharide chain on their activity has not been elucidated.

Several nonprotein sweet inhibitors, lactisole (Matholouthi et al. 1994), gymnemic acid (Liu et al. 1992), ziziphin (Yoshikawa et al. 1991), hodulcin (Kennedy et al. 1988), and zinc sulfate (Keast et al. 2004b), have been identified. Lactisole (PMP) suppresses the sweet taste perception by acting as a sweet antagonist. Schiffman et al. (1999) reported that lactisole suppressed the sweetness of sucrose, aspartame, and stevioside, whereas it did not suppress that of thaumatin. Keast et al. (2004b) found that zinc sulfate inhibited the sweetness of most compounds used in their experiments, whereas it did not inhibit that of Na-cyclamate. Interestingly, RBP was effective on the sweetness of proteins but not on the sweetness of low-molecular sweeteners. Therefore, RBP appears to be a sweet inhibitor for protein sweeteners only.

There are many cases of protein–protein interactions due to general properties of hydrophobicity and/or electric charge of proteins (Forsythe and Foster 1950). For example, if RBP reduced the sweetness of protein sweeteners by degrading them, this would be of little interest for those investigating sweet taste mechanisms. Therefore, we needed to know whether RBP has a specific chemical interaction with protein sweeteners. However, for lysozyme, one of the sweet proteins, both its enzymatic activity and its molecule size were unaffected by the addition of RBP, suggesting that RBP did not work by degrading the sweet protein. In additional experiment, we demonstrated that RBP did not form complex with lysozyme on gel permeation chromatography. Moreover, 5 proteins, ovalbumin, ovomucoid, myoglobin, β-lactogloblin, and bovine serum albumin, did not suppress the sweetness of thaumatin. Considering these results, it is unlikely that RBP interacts with protein sweeteners themselves to suppress their sweetness. Furthermore, we found that a prerinse with RBP decreased the sweetness of protein sweeteners that were subsequently tasted, in much the same manner that a PMP prerinse decreases the sweetness of sucrose. This result supports the hypothesis that the mechanism for RBP sweet suppression involves an interaction of RBP with sweet taste receptor. It is suggested that PMP (lactisole) binds to a sweet taste receptor to inhibit sweet taste perception (Jiang, Cui, Zhao, Liu, et al. 2005)

It has been known that old-world monkeys including humans perceive monellin and thaumatin as tasting sweet, whereas these proteins are not perceived as sweet by other species including new-world monkeys, mice, and rats using both electrophysiological and behavioral techniques (Glaser et al. 1978). The detection of sweetness of most or all sweet taste compounds is mediated by heteromeric receptor comprised T1R2 and T1R3, a family of G protein–coupled receptors selectively expressed in subsets of taste receptor cells (Nelson et al. 2001). Recent experiments with heterologous expression of the human/mouse chimeras of T1R3 (Jiang et al. 2004) suggested that the molecular basis for species-specific sensitivity to brazzein depends on a site within the cystein-rich region of human T1R3. Lactisole suppresses the sweet taste perception by humans but not by rats (Sclafani and Perez 1997). This fact was also further studied using heterologous expression of human and mouse T1R2/T1R3, and as a result, 7 residues within the transmembrane domain of human T1R3 were predicted to form a potential-binding pocket for lactisole and are responsible for the sweet receptor's sensitivity to lactisole inhibition (Jiang, Cui, Zhao, Liu, et al. 2005). Jiang, Cui, Zhao, Snyder, et al. (2005) also showed that cyclamate-binding pocket is close to the lactisole-binding pocket within the T1R3 transmembrane domain. Apparently the sweet taste receptor has at least 2 different interaction sites within the T1R3 subunit for 2 types of sweeteners, a lactisole-sensitive and a lactisole-insensitive site. From the results of our study combined with those in the previous reports mentioned above, a mechanism for the sweet suppression of RBP can be proposed. It is hypothesized that RBP interacts competitively with the lactisole-insensitive region within sweet taste receptor molecule, thereby reducing or eliminating the sweetness of protein sweeteners.

This novel sweet-suppressing protein, RBP, will be a useful material for studying taste–structure relationships and to help elucidate the mechanisms underlying sweet taste perception. This discovery will also provide us valuable information for understanding how taste stimuli work in the complex matrix that is food.

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