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THE PLASMA MEMBRANES OF BOVINE TASTE PAPILLAE

POLYACRYLAMIDE GEL ELECTROPHORESIS OF CIRCUMVALLATE MEMBRANE PROTEINS

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SUMMARY

1. Bovine circumvallate papillae and mount papillae were fractionated into cell saps and particulates, from which three membrane fractions (*i.e.* plasma membranes, microsomal membranes, and mitochondrial membranes) were obtained by sucrose density gradient centrifugations. Each of the three membrane fractions and of the cell saps from both papillae was separated into individual protein components by polyacrylamide gel electrophoresis.

2. Polyacrylamide gel electrophorograms of sodium dodecyl sulfate-solubilized membrane proteins showed that plasma membranes from circumvallate and from mount papillae have different protein distributions. Differences were also observed among the other six fractions.

3. Circumvallate plasma membranes contain a total of 21 sodium dodecyl sulfate solubilizable proteins, 14 of which are glycoproteins. Seven of the proteins are major components, five of which are glycoproteins. None of the other five membrane fractions contain as many glycoproteins.

4. At least two of the glycoproteins are unique to this plasma membrane fraction. They are designated as CV-A and CV-B (CV, circumvallate papillae), with apparent molecular weights of about 51000 and 36000, respectively.

INTRODUCTION

The initial phase of taste perception involves the contact between taste stimulants and the taste receptors on the tongue surface. The exact locations and the nature of the taste receptors are not known, though it is generally assumed that taste receptors are proteinacious in nature, and that they are located on the membranes of taste receptor tissues. Since many of the cellular receptors for specific information have been identified on the plasma membranes of various cells (*e.g.* hemagglutionation

Abbreviations: fractions from sucrose density gradient centrifugation are designated as follows: CV, circumvallate papillae; MT, mount papillae; B1, plasma membranes; B2, microsomal membranes; and B3, mitochondrial membranes; Sap, cell sap, the $100000 \times g$ supernatant of whole tissue homogenate.

sites and serological specific antigens of the erythrocytes[1], transplantation antigenic sites of all cells[2], and the insulin receptors of the liver and fat cells[3,4]), it is reasonable to expect, by analogy, the peripheral taste receptors to be present also in the plasma membranes of the taste receptor tissues. One of us (C.H.L.) has proposed that taste specificity may be one of the many functions of the plasma membranes of taste receptor tissues[5]; thus, we have narrowed down the area of search for taste receptors from whole tongue epithelium[6] or whole taste papillae[7] to the plasma membranes of the latter. We began to examine the plasma membranes of the taste-bud containing papillae—the circumvallate papillae of bovine tongue, and to compare with the other intracellular membranes, and the membranes from papillae without taste buds—the mount papillae, at the central-posterior region of the same tongue. We found that ^{14}C-labeled fructose would bind preferentially with plasma membranes of taste papillae in a mixed membrane population[5]. These results suggest the presence of fructose binding sites on the plasma membranes of bovine circumvallate papillae. In order to examine the plasma membranes further, we undertook the study being reported in this communication.

MATERIALS AND METHODS

Materials

Fresh bovine tongues were obtained from a local slaughterhouse, and were kept on ice during transportation. The circumvallate papillae, being rich in taste buds, were used for our studies. The papillae at the adjacent central-posterior area contain no taste buds, and therefore were used for comparisons. The papillae are referred to as mount papillae in this communication, because the central-posterior area in which they were located was raised from the surroundings, as if it were a mount on the tongue surface.

All chemicals were reagent grade. All electrophoretic reagents were purchased from Eastman Kodak Co. Proteins used for molecular weight calibrations: calf intestinal alkaline phosphatase, rabbit muscle lactate dehydrogenase, bovine serum albumin, and egg white lysozyme, were purchased from Sigma Chemical Co.; ovalbumin, beef pancreatic chymotrypsinogen A, trypsin, bovine pancreatic chymotrypsin, and sperm whale myoglobin, from Schwartz-Mann.

Methods

Membrane preparations. Detailed plasma membrane fractionation and isolation procedures were described earlier[5]. From these procedures, the circumvallate papillar homogenates yielded four fractions: cell saps (CV-Sap), plasma membranes (CV-B1), microsomal membranes (CV-B2), and mitochondrial membranes (CV-B3); and the mount papillar (MT) homogenates yielded four similar fractions: MT-Sap, MT-B1, MT-B2, and MT-B3.

The pellated membrane samples were suspended in a small amount of 0.1 M sodium phosphate buffer (pH 7.2). Aliquots were taken for protein determinations by the method of Lowry *et al.*[8]; the rest were used for polyacrylamide gel electrophoresis.

Cell sap proteins. The soluble cell saps obtained from the $100000 \times g$ supernatans were dialyzed against 2 l of 0.15 M NaCl at 4 °C for 24 h, with three changes

of the NaCl solutions. About 20 μg of the dialyzed cell sap proteins were used for electrophoresis.

Membrane solubilization. Membrane proteins were solubilized in 2% sodium dodecyl sulfate in 0.1 M sodium phosphate buffer (pH 7.2) by incubating at 37 °C for 2 h, followed by immersing the sample tubes in a boiling water bath for 1 min. The subsequently cooled samples were treated with 1% 2-mercaptoethanol for 2 h at 37 °C.

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The gels contained 7.5% acrylamide, 0.2% *N,N'*-methylenebisacrylamide, 0.08% *N,N,N',N'*-tetramethylethylenediamine, and 0.05% $(NH_4)_2S_2O_8$ in 0.1 M sodium phosphate buffer (pH 7.2) containing 0.1% dodecyl sulfate. The mixture was pipetted into 5 mm (internal diameter) $\times$ 70 mm gel tubes, overlayed with water, and let polymerize at room temperature for at least 30 min. Protein samples were prepared by a modified Weber and Osborn procedure[9]. For the dodecyl sulfate-solubilized membrane proteins, about 65 to 70 μg of proteins in 100 μl of 0.1 M sodium phosphate (pH 7.2), containing 0.1% dodecyl sulfate and 0.1% 2-mercaptoethanol was mixed with 5 μl of 0.1% bromophenol blue (tracking dye) and 1 drop of glycerol; for the cell sap proteins, about 20 μg of proteins in 50 μl of saline solution was mixed with 50 μl of 0.1% dodecyl sulfate–0.1 M sodium phosphate buffer (pH 7.2), 5 μl of 2-mercaptoethanol, 5 μl of 0.1% bromophenol blue, and one drop of glycerol; for the proteins used for molecular weight calibrations, 10 μg of protein in 100 μl of dodecyl sulfate–sodium phosphate buffer was mixed with 2-mercaptoethanol, bromophenol blue and glycerol. Sample mixtures in duplicates were applied to a pair of gel tubes and were electrophoresed at 8 mA per sample for 2.5 h at room temperature with 0.1% dodecyl sulfate–0.1 M sodium phosphate buffer (pH 7.2) as the electrolyte. One of the pair was stained for all proteins with 0.025% coomassie brilliant blue according to Fairbanks *et al.*[10] except that in Step 3, the sample was washed for 4 h with 10% 2-propanol in 10% acetic acid. The other sample of the pair was first soaked overnight in 25% 2-propanol in 10% acetic acid, followed by washing with 10% 2-propanol in 10% acetic acid[10] to remove dodecyl sulfate, then was stained for glycoproteins with periodic acid–Schiff's reagent according to Zaccharius *et al.*[11]. We found 2-propanol to be more effective than methanol[9,12] in fixing and in removing dodecyl sulfate from the gels. Gels were scanned through a 0.2 mm slit at 560 nm for coomassie blue stained proteins, and at 570 nm for periodate–Schiff's stained glycoproteins, on a Beckman DU spectrophotometer equipped with Gilford linear transport system and recorder.

RESULTS

Typical scanning profiles of the electrophorograms of the circumvallate and mount papillar membrane proteins are shown in Figs 1 and 2, respectively. Coomassie blue stained gels showed the total protein components, and the periodate–Schiff's reagent stained gels showed the positions of the glycoproteins. The carbohydrate positive bands at the gel fronts represented glycolipid materials in the samples[13]. The gel scans for cell sap proteins are shown in Fig. 3. Each protein fraction had its individual scanning profiles, though some similarities were observed between circumvallate and mount samples of the same membranes.

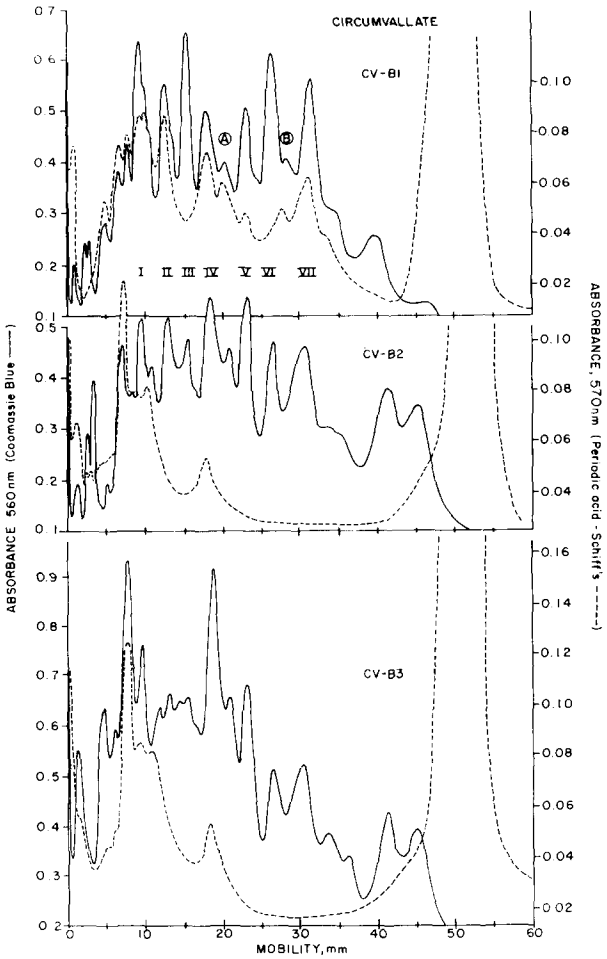

Fig. 1. Typical polyacrylamide gel scans of bovine circumvallate membrane proteins. ——— coomassie blue stained proteins; – – – –, periodate–Schiff's stained glycoproteins. Approx 65 to 70 μg of sodium dodecyl sulfate-solubilized membrane proteins were applied to each gel except that CV-B3 was about 82 μg. The major peaks in the plasma membranes (CV-B1) ar designated by Roman numerals. The two peaks indicated by A and B are the circumvallate-specifi plasma membrane proteins CV-A and CV-B, respectively, as described in the text. B2 represent the microsomal membrane proteins, and B3, the mitochondrial membrane proteins.

Plasma membrane proteins

The gel scans of CV-B1 (Fig. 1, top graph) showed a total of 21 coomassi brilliant blue stained protein peaks, 14 of which were glycoproteins. The apparen molecular weights of the seven CV-B1 major protein components, indicated b Roman numerals in the graph, are summarized in Table I. Five of the seven wer glycoproteins. MT-B1 contained 19 proteins, nine of which were glycoprotein (Fig. 2, top graph). In contrast to the multi-glycoprotein patterns in the plasm membranes (B1), only two or three carbohydrate-containing major peaks were foun in either the microsomal (B2) or the mitochondrial (B3) membranes. This hig

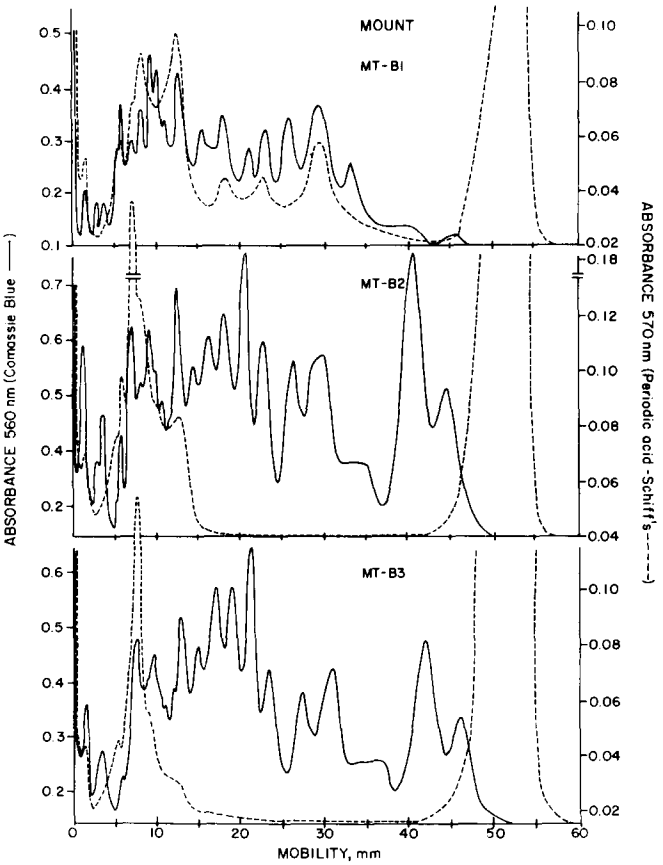

Fig. 2. Typical polyacrylamide gel scans of bovine mount papillar membrane proteins. ———, coomassie blue stained proteins; – – – –, periodate–Schiff's stained glycoproteins. Approx. 65 to 70 μg membrane proteins were applied.

content of glycoproteins was characteristic of the plasma membranes. Glossmann and Neville[12] also found varying numbers of glycoprotein bands in plasma membranes from rat erythrocytes, liver cells and kidney brush borders. Table II shows the comparisons between our results on plasma membrane glycoproteins from bovine tongue papillae and those reported by Glossmann and Neville[12]. The molecular weights of the papillar proteins were determined by comparing their relative electrophoretic mobilities with that of proteins of known molecular weights shown in Fig. 4. The linear relationship between the logarithms of molecular weights and the electrophoretic mobilities of proteins has been established by Shapiro *et al.*[14] and by Weber and Osborn[9].

Circumvallate-specific plasma membrane proteins

There were two minor protein peaks found only in the gel scans of CV-B1. They were designated as the circumvallate-specific plasma membrane proteins CV-A and CV-B, respectively (marked by circled letters A and B in top graph of Fig. 1).

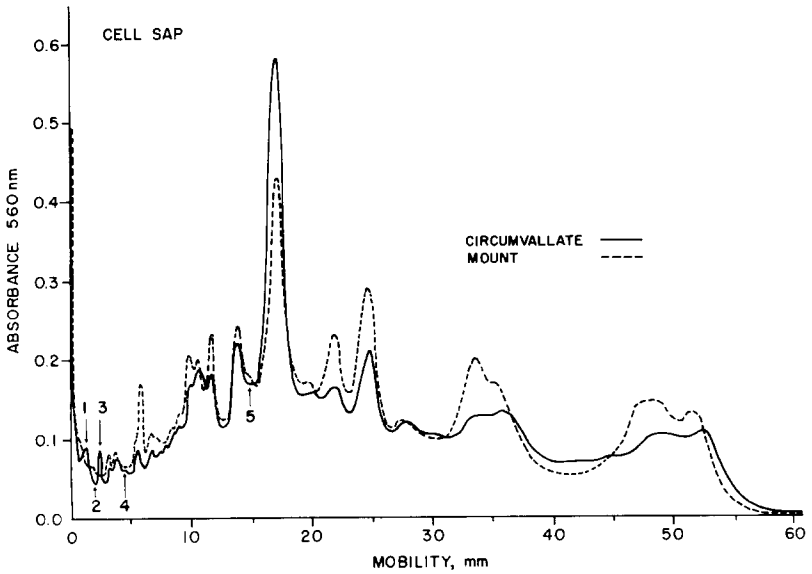

Fig. 3. Typical polyacrylamide gel scans of cell sap proteins of bovine circumvallate (———) and mount (– – – –) papillae. Approx. 20 μg of the soluble proteins were applied. Gels were stained with coomassie blue.

TABLE I

MAJOR PROTEIN COMPONENTS OF BOVINE CIRCUMVALLATE PLASMA MEMBRANES

*Protein**	*Apparent molecular weight***	*Glycoprotein*	*Remarks*
I	84 000	+	possibly contains 2 species
II	72 500	+	possibly contains 2 species
III	64 000	−	
IV	56 000	+	
V	45 000	+	
VI	39 000	−	
VII	31 000	+	specific to circumvallate plasma membrane?

* See Fig. 1.
** See Fig. 4.

Both proteins stained positive with periodate–Schiff's reagent, indicating that they were glycoproteins. The apparent molecular weights of CV-A and CV-B were about 51000 and 36000, respectively. All the other membranes, including the MT-B (Fig. 2) contained neither A nor B, though they all had a protein band that moved slightly ahead of CV-A. This protein had an apparent molecular weight of about 49000, and was not stained by periodate–Schiff's reagent. Therefore, it cannot be considered the same as CV-A.

TABLE II

COMPARISONS ON THE APPARENT MOLECULAR WEIGHTS OF PLASMA MEMBRANE GLYCOPROTEINS

Peak number	*Apparent molecular weight*				
	*Bovine circumvallate papillae**	*Bovine mount papillae***	*Rat erythrocyte****	*Rat liver****	*Rat kidney brush border****
1	> 121 000	> 117 000	> 250 000	> 250 000	> 300 000
2	103 000	101 000	> 250 000	> 250 000	> 250 000
3	94 000	98 000	250 000	250 000	250 000
4	90 000	91 500	170 000	195 000	195 000
5	87 000	88 000	96 000	130 000	130 000
6	83 000	75 000	78 000	96 000	96 000
7	79 000	56 000	70 000		76 000
8	72 500	46 000	68 000		70 000
9	56 000	34 000	41 500		65 000
10	51 000		39 000		60 000
11	45 000				58 000
12	36 000				
13	31 000				
14	28 000				

* See Figs 1 and 4.
** See Figs 2 and 4.
*** From ref. 12.

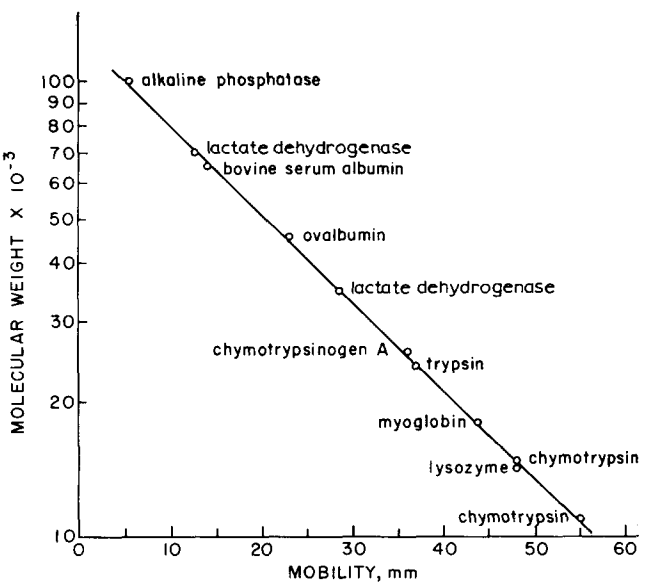

Fig. 4. Semi-logarithmic plot of molecular weights against electrophoretic mobilities of proteins in 0.1% sodium dodecyl sulfate–7.5% polyacrylamide gels.

Besides the two distinct glycoproteins, there is another glycoprotein in the circumvallate plasma membranes that is noteworthy. Protein Peak VII of CV-B1 was not present in MT-B1 (Fig. 2), though it has a protein component moving slightly behind Peak VII. CV-B2 and B3 each had a peak with similar mobility, but was not stained by the Schiff's reagent as the B1 protein. Whether Peak VII represents another CV-specific plasma membrane protein requires further study.

Microsomal membrane proteins

The general pattern of B2 proteins from both papillae were similar, however one notable difference was in the positions of the glycoproteins. A major glycoprotein peak with apparent molecular weight of 54000 in CV-B2 was absent from MT-B2 (Figs 1 and 2, middle graphs). On the other hand, a glycoprotein in MT-B2 (mol. wt about 71500) was not seen in the CV-B2. Both membrane samples showed about 10 major peaks ranging from molecular weight of 121000 to 16600, but the relative sizes of the peaks (*i.e.* the amount of each protein) were different for the two membranes. Particularly, the peak at 7.0 mm (mol. wt about 91500) in MT-B2 contained almost twice as much carbohydrate as the corresponding peak in CV-B2.

Mitochondrial membrane proteins

The major differences between the proteins in CV-B3 and MT-B3 (bottom graphs of Figs 1 and 2) lie in their relative amounts and the amounts of carbohydrate in the glycoproteins in the two tissues. For instance, the height of the coomassie blue stained peak at 7.5 mm (apparent mol. wt 90000) in CV-B3 was more than twice that in MT-B3, although the amount of total protein sample put on the gels were about 82 μg in CV-B3, and about 70 μg in MT-B3; however, when the same peak were stained with periodate–Schiff's reagent, the MT-B3 protein was found to contain much more carbohydrate than the CV-B3 protein. In fact, for the peak in question the ratios of the absorbances of the coomassie blue stained proteins to the Schiff stained proteins were about 8 to 1 in CV-B3, and 3 to 1 in MT-B3. Similarly, there was another major component, a glycoprotein, at 19 mm (mol. wt about 53500) in CV-B3, whereas the corresponding peak in MT-B3 was relatively minor and was carbohydrate-negative.

Cell sap proteins

The electrophoretic scanning profiles of soluble sap proteins from circumvallate and mount papillae are shown in Fig. 3. Their general patterns were similar. Both had identical single major protein peak at 17 mm with apparent molecular weight of about 58600. However, there were three minor protein bands, indicated by numbers 1, 3, and 4, found only in CV-sap; whereas two other proteins (Nos 2 and 5) were present only in MT-sap. Several smaller circumvallate proteins were seen slightly ahead of their corresponding mount proteins.

DISCUSSION

Differences in the electrophoretic patterns were observed between circumvallate and mount proteins from all sources studied, though some resemblance in the overall picture was seen. By studying all the six membrane protein samples together

(Figs 1 and 2), we found that: (1) the plasma membranes (B1) contained more glycoproteins than either of the intracellular membranes; (2) circumvallate membranes contained more glycoproteins than the mount membranes; (3) each of the membranes had its own specific proteins; (4) same membranes from the two papillae contained different proteins; (5) the number and positions of major proteins vary with different membranes; (6) proteins with same mobility existed between any two, or among all three of the cellular membranes; (7) the common proteins might vary quantitatively among the membranes, *i.e.* a minor protein in one membrane might become a major protein in another; and the amount of carbohydrate in a glycoprotein also varied (*cf.* Figs 1 and 2).

Zahler *et al.*[15] also found characteristic electrophoretic patterns for each of the bovine liver organelles they studied. They also observed 20 to 22 protein bands in all membranes (*e.g.* plasma membranes, smooth and rough microsomes, mitochondria and nuclei); some of these were common proteins to several of the organelles studied. Scher and Barland[16] reported several major glycoproteins in the surface membranes of two mouse fibroblast cell lines. On the other hand, Kobylka *et al.*[17] could find only one major glycoprotein band in the erythrocyte membranes of several species.

There seem to be discrepancies between our findings and those by Koyama and Kurihara[7], who did not find any difference in the electrophoretic patterns among proteins from bovine circumvallate, fungiform, and the surrounding epithelium including filiform papillae. We suggest that the reason for their failure to find any differences is that they were using the $10000 \times g$ supernatants from homogenates of the various tissues as their protein sources. This supernatant fraction contains all the cellular materials except whole cells, nuclei, and mitochondria. The combination of using a crude sample and disc gel electrophoresis makes it impossible to detect any difference in a few minor protein species. The differences would be completely masked by the more abundant cellular proteins in this kind of complex protein mixture. These authors have pointed out this might be the case[7].

Our attention was naturally drawn by the two glycoproteins, CV-A and CV-B, found only in the plasma membranes of the circumvallate. This is the first demonstration of the presence of bovine circumvallate-specific plasma membrane proteins. The presence of distinct protein species on the plasma membranes of a taste papilla undoubtedly raises speculations on whether these are "taste-receptor" proteins. However, we consider it too early to draw any meaningful conclusions on their possible functions in taste reception. While we are not assigning any specific roles to them, our findings are significant in their own right, because the mere presence of these proteins is an indication of the specific characteristics of taste papillae and of their plasma membranes, whether they be the taste receptor proteins, or structural proteins, or functional enzymes. The finding that both CV-A and B are also glycoproteins is noteworthy, because many cell surface receptor proteins for specific cellular functions are glycoproteins—*e.g.* the blood group antigens[18] and the receptors for virus-induced tumor transformations[19]. Nevertheless, more studies are necessary before assessment of their significances with respect to taste can be attempted. As an initial step toward this goal, we are planning studies to measure the specific binding activities of these circumvallate-specific plasma membrane proteins to radioisotope-labeled taste stimulants.

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