Chemoreceptor Proteins from Taste Buds

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Purification of two protein fractions from tongue epithelium has been reported. These were postulated to be receptors initiating the sensations of sweetness and of bitterness. The "sweet-sensitive protein" forms complexes with sugars. The strengths of the complexes parallel the sweetness of the sugars, and fall within the range anticipated. It has a molecular weight of about 150,000, is isoionic at pH 9.1, is nearly homogeneous, and catalyzes the dehydrogenation of sugars. The K_m for glucose and for fructose are in good agreement with the equilibrium constants for complex formation with these sugars. It is unlikely that the "bitter-sensitive protein" is the true bitter receptor, although it occurs mainly in the area of the tongue which is sensitive to bitter-tasting compounds. The binding constants for bitter compounds do not parallel the bitterness of the compounds, and differ from the binding constants expected by factors of 10^2 to 10^3.

The mechanisms by which compounds of various tastes stimulate the gustatory receptors of the tongue have been of interest for many years. One theory, popular for some time, was that the stimulus compounds affected the rate of some chemical reaction in the cells of the taste buds by acting as substrates, activators, or inhibitors, and that the altered reaction rate in some manner resulted in the production of nerve impulses by the receptor cells. Beidler (1954, 1962) has summarized evidence against the likelihood that this theory is correct. Briefly stated, this evidence consists of the observations that the taste response is nearly independent of the temperature of the receptor, and that the response to high concentrations of stimulus compounds is nearly independent of pH over a rather wide range. Beidler noted that such properties would not be expected if the receptor mechanism was based on the rate of a reaction, but are entirely consistent with the hypothesis that the crucial event is the formation of a weak complex between the stimulus compound and some receptor molecule. The formation of the stimulus-receptor complex is presumed to lead somehow to the initiation of nerve impulses. Beidler also suggested that the receptor molecules are probably located on or near the cell surfaces, since responses can be recorded from the afferent nerve fibers within 30 milliseconds of the time the tongue is exposed to stimulus compounds, and many compounds which have highly toxic intracellular effects (cyanide, for example) elicit taste responses in a reversible manner.

Beidler's hypothesis, that the initial event in taste stimulation is the formation of a weak complex, is further supported by his observation that taste responses can be related to the concentrations of the stimulus compounds through simple adsorption isotherms. By assuming the existence of receptor sites which can adsorb stimulus compounds, and that the taste response is proportional to the number of sites occupied by the stimulus compound, he derived the following equation (Beidler, 1954):

$$C/R = C(1/R_m) + 1/KR_m$$

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Table I. Equilibrium Constants for Binding of Sugars by a "Sweet-Sensitive Protein" from Bovine Tongues, and Taste Thresholds for These Sugars

Sugar	$1/K$ [a]	Threshold [b]
Fructose	$1 \times 10^{-3}M$	$6 \times 10^{-3}M$
Sucrose	$1.2 \times 10^{-2}M$	$1 \times 10^{-2}M$
Glucose	$3.7 \times 10^{-2}M$	$1.2 \times 10^{-2}M$
Galactose	$1 \times 10^{-1}M$	$3 \times 10^{-2}M$
Mannose	$3 \times 10^{-1}M$	$3 \times 10^{-2}M$

[a] Data from Dastoli and Price (1966).
[b] Data from Pfaffman (1959).

In this equation, C is the concentration of the stimulus compound, R is the response at that concentration, R_m is the maximal response obtained when the sites are saturated, and K is the association constant for the interaction of the stimulus compound with the receptor site. Electrophysiological measurements of taste responses with varying concentrations of stimulus compounds led to experimental verification of the predicted relation (Beidler, 1954, 1962), and this equation is often referred to as the basic taste equation.

"SWEET-SENSITIVE PROTEIN"— A RECEPTOR MOLECULE?

Purification from bovine tongue epithelium of a protein fraction having some of the properties expected of the sweet taste receptor molecule has been reported recently (Dastoli and Price, 1966). The assay method was based on the fact that the optical properties of proteins often change when proteins form complexes with other compounds or ions, and involved measurement of alterations in the absorption spectrum and refractive index of protein when sugars were placed in solution with it. The isolation procedure was based on ammonium sulfate fractionation; the fraction containing material which is soluble in 20% ammonium sulfate, but insoluble in 40% ammonium sulfate, contained the sugar-complexing material.

Using the magnitude of the alteration in refractive index of the protein as a measure of response, the interaction of sugars with this material could be adequately described by Beidler's equation (Dastoli and Price, 1966). The equilibrium constants obtained for interaction of five sugars with the protein are shown in Table I. The arrangement of these sugars in order of

their equilibrium constants corresponds to the order of their sweetness in vivo. The reciprocal of the equilibrium constant can easily be shown equal to the concentration of stimulus compound at which half of the receptor sites are occupied. Since Beidler's basic taste equation is based upon the premise that the response is directly proportional to the number of receptor sites that are occupied by stimulus molecules, and since the concentration range between threshold and near-saturation of the sites is usually less than 100-fold, one would expect that the threshold concentration for taste would be lower than the reciprocal of the association constant for interaction of the stimulus compound with the receptor molecule, but the threshold and the reciprocal of the association constant should be of the same order of magnitude. One can then ask, are the equilibrium constants for interaction of the "sweet-sensitive protein" with sugars consistent with the hypothesis that this protein is, in fact, a sweet taste receptor? The values for $1/K$ and for the taste thresholds for the five sugars tested are shown in Table I, and the equilibrium constants are within the range expected from in vivo taste sensitivities for most of the sugars. For fructose, the association constant is five to 10 times higher than what might be expected, but when one considers the fact that the threshold values are for humans and the preparation is from cows, the over-all agreement can be considered satisfactory.

Another prediction that follows from the taste response is that the formation of complexes between the sugar and the receptor should occur over a wide range of pH. While this has not been subjected to extensive study, the complexing of fructose by the "sweet-sensitive protein" has been measured with varying pH, with the results showing the response to high fructose concentrations to be essentially constant from pH 5.5 to 10.5 (Dastoli and Price, 1966).

Hiji *et al.* (1968) found sugar-complexing activity in extracts of rat tongues. The material was soluble in 28% ammonium sulfate, but insoluble in 42% ammonium sulfate, thus resembling the material prepared from bovine tongues. Little, if any, activity was found in extracts of skeletal muscle or intestinal epithelium. Treating the rats with colchicine, which results in loss of most of the taste buds, also resulted in dramatic decreases in the amount of sugar-binding protein in the extracts of tongue epithelium. Cats have little ability to taste sugars, and extracts of cat tongue epithelium showed very little of the sugar-complexing protein. The evidence that the "sweet-sensitive protein" is a sweet taste receptor is far from compelling, but is consistent with the properties one would expect of the receptor for sweet taste. The question of whether or not it is truly a taste receptor molecule is likely to remain unsettled for some time to come. In view of the parallelisms between the in vivo interactions of the receptors with sugars and the in vitro interactions of the "sweet-sensitive protein" with sugars, it seems likely that the protein is, at the very least, a convenient model of the physiological receptor for sweet taste stimuli.

"SWEET-SENSITIVE PROTEIN"—SOME PROPERTIES

Further purification and partial characterization of the "sweet-sensitive protein" from bovine tongues has also been reported (Dastoli *et al.*, 1968b). When the 40% ammonium sulfate precipitate was subjected to gel filtration, it separated into two components. One component, amounting to about 20% of the total material, showed no sugar-complexing properties. The other component formed complexes with sugars, and was subjected to further study. It was hydrolyzed, and quantitatively analyzed for amino acid composition. The amino acids recovered from this procedure corresponded to 101% of the amount expected if the material had been an unconjugated protein, leading to the conclusion that the material was a protein, and that if it contains prosthetic groups they must make up only a small fraction of the total molecular weight. An isoionic point at pH 9.3 was calculated from the amino acid composition. This compared well with the isoionic point, pH 9.1, determined by electrophoresis and by titration. A molecular weight of about 150,000 was obtained from gel filtration and from ultracentrifugation.

The protein appeared to be nearly homogeneous, as evidenced by its migration as a single band when electrophoresed on thin layers of alumina or in polyacrylamide gel, and by its emergence as a single peak from columns of Bio-Gel P-150 or Cellex-CM (Dastoli *et al.*, 1968b). In spite of the apparent homogeneity of the material, there is good reason for believing it to consist of more than one molecular species. Preparations made from single tongues differed from each other in relative affinity for different sugars, and the preparations for which data were reported represented pooled material from several tongues (Dastoli and Price, 1966). Unless one ignores the individual differences, it is difficult to avoid the conclusion that the pooled material contained at least two protein species. The physical homogeneity may be presumed to reflect similarity of the two (or more) proteins with respect to molecular weight and isoionic point. Anderson *et al.* (1963), in an electrophysiological study of sweet taste responses in dogs, found that some afferent nerve fibers fired only when the tongue was exposed to fructose or to sucrose, while others showed a broad sugar specificity. These results imply the existence of at least two kinds of sweet taste receptor sites.

The "sweet-sensitive protein," in the presence of a suitable electron acceptor, catalyzes the dehydrogenation of reducing sugars (Price and Hogan, 1969). Using nicotinamide adenine dinucleotide (NAD^+) as the acceptor, the K_m for glucose at pH 7.1 was found to be 51mM, in good accord with the value of $1/K$, reported by Dastoli and Price (1966) to be 37mM at pH 7.0. Although the kinetics of the reaction are pH-dependent, the kinetic parameters vary by less than a fivefold range as the pH is varied between pH 5.9 and pH 9.4 (the upper and lower limits used). The reaction with fructose is considerably slower than with glucose, and this results in a good deal of scatter in the data and a corresponding uncertainty in assigning numerical values to the kinetic parameters. The value of K_m is between 1 mM and 3 mM, comparing favorably with the value of 1 mM previously reported for $1/K$ (Dastoli and Price, 1966). These data suggest that catalytic activity and the binding of sugars are properties of the same protein in the preparation. Should this prove to be the case, enzymological techniques offer

potentially promising approaches to defining the nature of the sugar-protein interactions. They also raise the somewhat disturbing possibility that the "sweet-sensitive protein" is unrelated to taste, but is simply a glucose dehydrogenase present in the tissue, and the reported sugar-protein complexes were nothing more than binary complexes between an enzyme and substrates or substrate analogues. These would be stable with time because the reaction mixture contained no electron acceptor. An alternative interpretation is that the protein is related to taste, and has evolved from a sugar-binding protein which was already available—namely, a glucose dehydrogenase. A third possibility is that the catalytic activity is an incidental consequence of the interaction between sugar and the protein, and is of neither physiological nor evolutionary significance. Finally, there is the possibility that the glucose dehydrogenase activity represents a contaminant in the preparations. It would be difficult to eliminate this possibility entirely, but in view of the nearly homogeneous nature of the preparations and of the similarities in the binding constants for glucose and for fructose, it seems unlikely.

Little can be said at present regarding the groups involved in the interaction. The magnitude of the free energy changes calculated from the association constants, about -1 to -4 kcal. per mole, suggest that something no stronger than one or two hydrogen bonds is involved in binding the sugars to the protein (Dastoli and Price, 1966). If an ionizable group on a protein side chain was complexing the sugars, one would expect K_m to change by a factor of 10-fold per unit pH in the vicinity of the pK of that group. No such dramatic dependence of K_m on pH was observed over the range from pH 5.9 to 9.4 (Price and Hogan, 1969). While this might appear to eliminate from consideration all ionizable side groups except carboxylate anions (pK 4.5) and guanidinium cations (pK 12.5), "abnormal" values for pK are common enough in ionizable groups on proteins that no firm conclusion can be justified.

Shallenberger and Acree (1967) have suggested that the carbonyl oxygens and amide hydrogens of a protein backbone might be the loci of interaction with the sugars. This hypothesis is consistent with the available data, but it will be very difficult to put it to a critical experimental test. Those workers have discussed the question of which groups on the sugars are likely to be involved in the interaction elsewhere in this symposium.

"BITTER-SENSITIVE PROTEIN"—A RECEPTOR?

Dastoli *et al.* (1968a) reported isolating a material, claimed to be the receptor for bitter-tasting compounds, from the epithelium of pig tongues. This material, which was soluble in 40% ammonium sulfate but insoluble in 60% ammonium sulfate, appeared to be localized in the back of the tongue, which is the area most sensitive to bitter taste stimuli. When assayed by a refractometric method, it was found to form complexes with four bitter-tasting compounds. Although the authors cited no source of information on the bitterness of the compounds, they claimed that the equilibrium constants were in excellent accord with the relative bitternesses. Based on this alleged correlation, they concluded that the material is the bitter receptor. This conclusion has been subjected to severe criticism (Price, 1969), the basis of which is summarized in Table II. The reciprocals of the association constants reported by Dastoli *et al.* (1968a) and the taste thresholds for these compounds are listed in Table II. It is unlikely that the differences among the association constants for the first three compounds listed in the table are significant, but even if one assumes that they are, there is no apparent correlation with the taste thresholds. Furthermore, by the same line of reasoning discussed with respect to sweet taste, one would expect the values of $1/K$ to differ from the taste thresholds by factors of less than 10- to 20-fold. In the interaction of the "bitter-sensitive protein" with the four compounds, $1/K$ ranges from 100 times threshold for caffeine to over 6500 times threshold for brucine. Even when one makes reasonable allowances for the species differences which might exist, the thresholds being for humans, these are remarkable discrepancies, and certainly offer no basis for concluding that the equilibrium constants are in excellent agreement with the bitternesses measured in vivo. Indeed, the evidence suggests that the "bitter-sensitive protein" is neither the physiological receptor for bitter taste stimuli, nor even a convenient model approximating the properties of the receptor.

Table II. Equilibrium Constants for Binding of Bitter Compounds by a "Bitter-Sensitive Protein" from Porcine Tongues, and Taste Thresholds for These Compounds

Compound	$1/K$ [a]	Threshold [b]
Quinine · HCL	$3.9 \times 10^{-3}M$	$3 \times 10^{-5}M$
Brucine · HCL	$4.6 \times 10^{-3}M$	$7 \times 10^{-7}M$
Naringen	$5.1 \times 10^{-3}M$	...
Caffeine	$7.8 \times 10^{-3}M$	$7 \times 10^{-4}M$

[a] Data from Dastoli *et al.* (1968a).
[b] Data from Pfaffman (1959) and Scholl and Munch (1937).

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