Modifying the Temporal Profile of the High-Potency Sweetener Neotame

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It is possible, using hydrophobic organic acids (such as cinnamate) or hydroxyamino acids (such as serine and tyrosine), to modify the temporal profile of the high-potency sweetener neotame. On the basis of Monte Carlo simulations, it was concluded that it is unlikely that this effect is due to direct interaction between the neotame molecule and the taste modifier. It is shown, using conformational analysis and molecular modeling, that the taste modifiers can adopt low-energy conformers which mimic the proposed active conformation of neotame, which suggests that the modifiers may compete for binding at the receptor site.

Keywords: *Neotame; high-potency sweetener; temporal profile; Monte Carlo simulation; conformational analysis*

INTRODUCTION

Neotame (*1*) is an analogue of the high-potency sweetener aspartame (*2*). Whereas aspartame has a potency of 150−200 times that of sucrose, neotame has a potency of 6000−9000 times that of sucrose on a weight basis. High-potency sweeteners often have temporal profiles different from that of sucrose (different onset time, different time of maximum sweetness, and different duration), which may detract from their usefulness in food systems (*3*). Neotame has a longer time to maximum sweetness and a longer duration than aspartame (*1*). In this paper we describe the discovery that hydrophobic organic acids such as cinnamate and hydroxyamino acids such as serine and tyrosine can modify the temporal profile of neotame, shortening the duration of the sweetness and making it more sucrose-like. We consider two hypotheses to explain this phenomenon.

MATERIALS AND METHODS

Sensory Evaluation. Samples were evaluated at room temperature. Time−intensity profiles were recorded with a computerized data acquisition system. Sweetness intensity was recorded with a mouse on a 0−15 linear scale, and judges were trained to use this scale with standard sucrose solutions, where sweetness rating equals the percent of sucrose concentration (e.g., a 10% sucrose solution corresponds to 10 on the linear scale). Starting at the moment that the sample was taken into the mouth, the sweet sensation was continuously rated by moving the cursor along the line scale, until the point at which sweetness was no longer present. A panel of seven judges rated each sample in three separate sessions. The following time−intensity parameters were extracted from the recordings: maximum intensity reached during the recording; area under the curve, total; rising area under curve (to maximum intensity); falling area under curve (from maximum to disappearance); time to maximum intensity; and duration of sweetness.

Monte Carlo Simulations. Neotame, serine, and tyrosine were modeled as zwitterions. Cinnamate was modeled as an anion. Simulations were carried out using the BOSS program (*4*). A molecule of neotame plus a molecule of a taste modifier (serine, tyrosine, or cinnamate) was modeled in low-energy conformations, starting in configurations with favorable amine−carboxylate orientations. Each pair was modeled in a box of 512 TIP4P (*5*) water molecules. Monte Carlo simulations initially involved 2×10^6 configurations of equilibration, followed by $(100-500) \times 10^6$ configurations of averaging. Every 5×10^5 configurations, the shortest atom−atom distance between protonated amine and unprotonated carboxylate was recorded.

Conformational Analysis. Conformational analysis was carried out using the MacroModel program (*6*). We used the MMFF force field (*7*) with the planar sp^2 nitrogens option. Conformational searching was carried out using the Monte Carlo Multiple Minimum method (*8*), followed by truncated Newton conjugate gradient minimization. Monte Carlo searching continued until extended runs produced no new conformations.

RESULTS AND DISCUSSION

Effect of Taste Modifiers on Neotame Temporal Profile. We have discovered that the temporal profile of neotame may be modified by the addition of hydrophobic acids and certain amino acids (*9*, *10*). Cinnamic acid, various substituted benzoic acids, tyrosine, serine, and threonine were all found to be effective for this purpose. The modifiers used did not have significant tastes of their own at the concentrations used.

Table 1 lists the effect of 5, 10, 25, and 50 ppm of cinnamate on the sweetness temporal profile of 25 ppm of neotame in a cola beverage. The main effect is on the duration of sweetness, as shown by the duration and falling area under curve parameters of Table 1. Cinnamate produces up to 17% reduction in sweetness duration; this reduction is statistically significant ($p < 0.05$) for 10 and 25 ppm of cinnamate. Thus, it appears

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Table 1. Summary of Temporal Profile Data for Colas Sweetened with 25 ppm of Neotame and Various Levels of Cinnamic Acid[a]

		ppm of cinnamic acid			
parameter	control	5	10	25	50
max intensity	8.56	8.71	8.55	8.78	8.68
area under curve	236	254	209	221	213
rising area under curve	56	67	55	55	54
falling area under curve	180	187	154	165	159
time to max intensity	10.8	12.2	10.8	10.6	10.3
duration	59.3	56.9	49.2[b]	50.3[b]	52.9

[a] Parameters are defined under Materials and Methods. [b] Statistically significant difference from control ($p < 0.05$).

that taste modifiers decrease the effect of neotame upon the receptor, either via interaction with neotame or via interaction with the receptor.

Do Modifiers Interact Directly with Neotame? We first considered whether taste-modifying compounds might interact directly with neotame. A priori this seems to be unlikely because neotame is a relatively small molecule with a limited number of functional groups that could interact with the taste modifiers we identified. To investigate possible interactions between neotame and taste modifiers, we carried out Monte Carlo simulations of a molecule of neotame plus a taste modifier molecule in a box of 512 water molecules. Simulations were carried out for neotame plus cinnamate, neotame plus serine, and neotame plus tyrosine. Simulations were started in configurations in which favorable electrostatic and hydrophobic interactions were favored. If there is a strong interaction between neotame and the taste modifier molecule, the two should exhibit a strong tendency to stay closely associated throughout the simulation.

Neotame plus Cinnamate Simulations. Monte Carlo simulations were carried out for 110 million configurations. The starting configuration placed the NH_2^+ group of neotame <4 Å from the carboxylate of cinnamate and the phenyl ring of neotame <4 Å from the phenyl ring of cinnamate. The distance from the NH_2^+ group to the carboxylate oxygens was monitored over the course of the simulation. Figure 1a shows that this distance increased rapidly, and there was little or no tendency for the charged functional groups to reassociate.

Neotame plus Serine Simulations. Monte Carlo simulations were carried out for 360 million configurations. The starting configuration placed the NH_2^+ group of neotame <4 Å from the carboxylate of serine and the carboxylate of neotame <4 Å from the NH_3^+ group of serine. Amine-to-carboxylate distances were monitored over the course of the simulation. Figure 1b shows that, even with two ion-pairing interactions, the molecules do not have a strong tendency to stay together.

Neotame plus Tyrosine Simulations. Monte Carlo simulations were carried out for 500 million configurations. The starting configuration placed the NH_2^+ group of neotame <4 Å from the carboxylate of tyrosine and the carboxylate of neotame <4 Å from the NH_3^+ group of tyrosine. Amine-to-carboxylate distances were monitored over the course of the simulation. Figure 1c shows that the two molecules remained in close contact for a much longer period of time than was seen for the neotame plus serine pair. This may be a result of the fact that fewer Monte Carlo moves are accepted as the molecules become larger; as a result, larger molecules simply take longer to move apart. Nevertheless, the molecules did not show a strong tendency to stay together during the simulation.

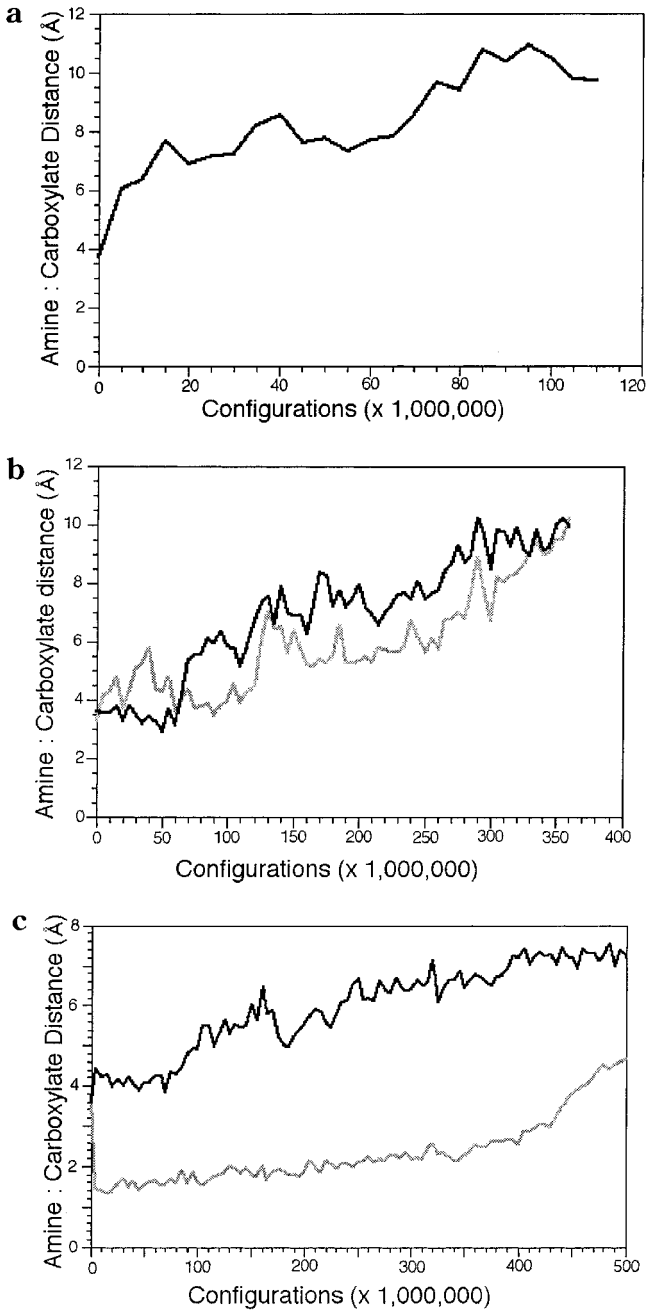

Figure 1. Amine-to-carboxylate distances for neotame plus modifier pairs during the course of Monte Carlo simulations: (a) neotame plus cinnamate; (b) neotame plus serine; (c) neotame plus tyrosine.

We conclude from these simulations that there is not a strong direct interaction between neotame and the taste modifiers examined.

Do Modifiers Compete at the Receptor Site? We previously identified a probable active conformation of neotame (*11*). Here we consider whether the taste modifiers might adopt a similar conformation and compete with neotame for binding at the receptor site.

Cinnamate, serine, and tyrosine were subjected to conformational analysis as described previously (*11*). Low-energy conformers were superimposed onto the proposed active conformation of neotame, as follows. First, the carboxylate group of the taste modifier was matched with the carboxylate group of neotame. Then,

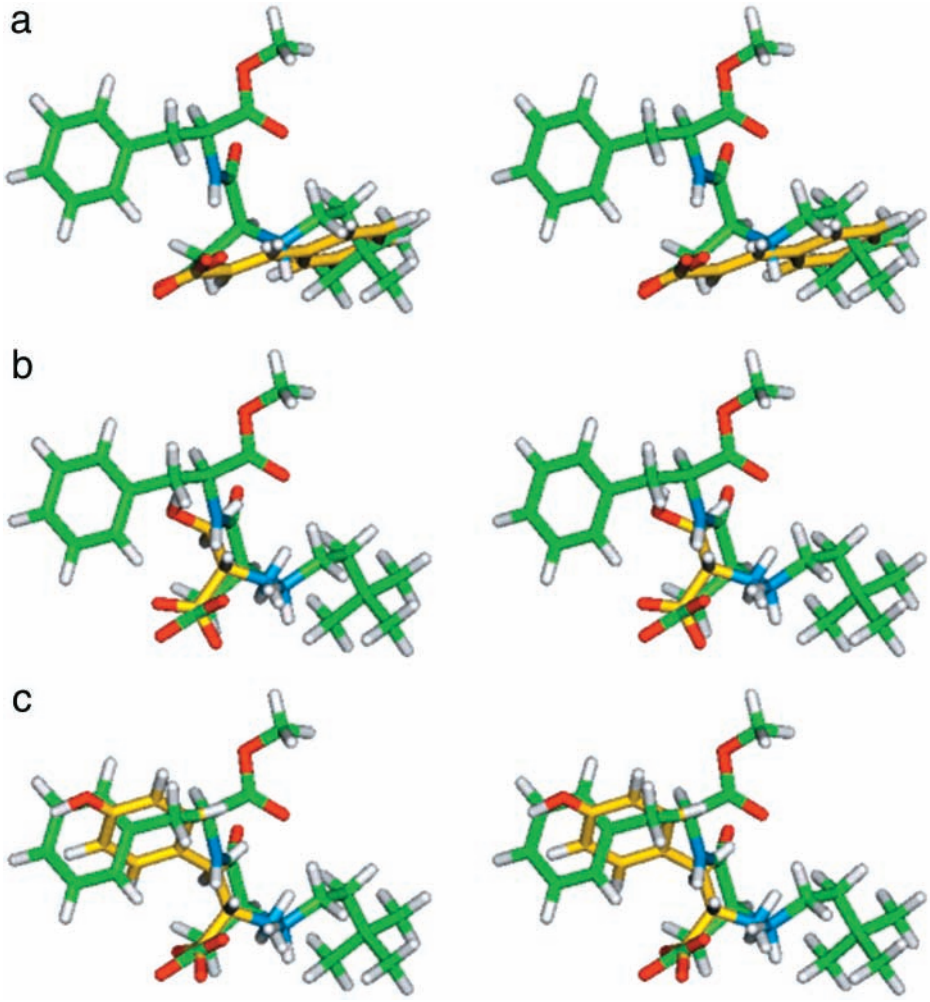

Figure 2. Stereoview of superposition of a low-energy conformer of taste modifier with the proposed active conformer of neotame: (a) neotame plus cinnamate; (b) neotame plus serine; (c) neotame plus tyrosine. For all pairs, red = oxygen, blue = nitrogen, white = hydrogen, green = neotame carbon atoms, and yellow = modifier carbon atoms.

in the cases of tyrosine and serine, the amino group was matched with either the amino group or the amide NH of neotame. Finally, the remainder of the steric bulk of the taste modifier was superimposed to the extent possible onto the neotame structure. In each case, we were able to identify a low-energy conformer that could superimpose well (>75% of modifier van der Waals volume overlapped with neotame). Volume overlaps between taste modifier and neotame are as follows: cinnamate, 97.8 $Å^3$ (80% of total volume); serine, 58.8 $Å^3$ (78%); tyrosine, 97.8 $Å^3$ (77%). The conformer matches are shown in Figure 2. We conclude that it is more likely that taste modifiers exert their effect by competing for binding at the neotame receptor site. The nature of this proposed competition for the receptor site is not entirely clear. It is not manifested in a decreased sweetness intensity but appears to affect the kinetics of the sweetener−receptor interaction.

Conclusion. We have found that it is possible, using hydrophobic organic acids such as cinnamate or using hydroxy amino acids such as serine and tyrosine, to alter the temporal profile of the high-potency sweetener neotame. Computational studies (Monte Carlo simulations and conformational analysis) indicate that it is more likely these compounds act competitively at the receptor than by direct interaction with the sweetener in solution.

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