Low-Energy Conformations of Delicious Peptide, a Food Flavor. Study by Quenched Molecular Dynamics and NMR

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NMR and quenched molecular dynamics investigations of Delicious Peptide are reported. Four families of structures are derived from the molecular dynamics simulation, which can be considered as cyclic, due to hydrogen bonding from the first residue to the end of the chain, or S-shaped. The S-shaped family contains the lowest energy structures, which is consistent with the NMR results obtained. The families of structures display interactions between the acidic and basic parts of the molecule, which is consistent with the currently accepted theory for the flavor-inducing properties of this peptide.

Keywords: *Flavor peptides; NMR; quenched molecular dynamics*

INTRODUCTION

The contribution of amino acids and peptides to the tastes of foods has been known for many years (Tada et al., 1984; Kirimura et al., 1969). The taste of traditional Japanese foods such as sake and soy sauce is assumed to be due to the release of amino acids from natural proteins during fermentation. Amino acids and peptides are also known to be involved in the flavor of meat and cheese (Spanier et al., 1988; Lemiuex and Simard, 1992). The tastes of individual amino acids as well as some short linear peptides have been studied in detail and can be categorized as sweet, savory, sour, or bitter (Kirimura et al., 1969; Nosho et al., 1990). A link between flavor and structure is known to exist for sweet- and bitter-tasting peptides (Nakamura and Okai, 1993). For example, sweet molecules are known to contain a hydrogen bond donor (AH) and a hydrogen bond acceptor (B) with a distance between of about 3.1 Å and potent sweet molecules also contain a hydrophobic group (X) (Goodman et al., 1993).

The Delicious Peptide (Tamura et al., 1989) (Lys-Gly-Asp-Glu-Glu-Ser-Leu-Ala) is an eight-residue linear food flavor peptide first isolated by Yamasaki and Maekawa (1978) from the gravy of beef. The flavor of the compound was found to be the same as that for beef gravy, with a slight accompanying bitter taste. The peptide was sequenced, and the synthesized peptide was found to possess the same beef flavour as the extracted compound.

In the Delicious Peptide, lysine, a basic amino acid, is present at the N terminus, while acidic amino acids constitute the middle part of the peptide. The taste has been found to be produced by the combination of the basic N-terminal region with the acidic middle region of the molecule. If the peptide is fragmented into smaller components, the combination of the shorter peptides Lys-Gly, Asp-Glu-Glu, and Ser-Leu-Ala has the same taste characteristics as the entire eight residue peptide.

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The purpose of this paper is to attempt to establish a link between the primary structure and flavor of the compound with any specific secondary structure. Previous conformational studies of the Delicious Peptide have involved comparison of the structure with the protein monellin, a sweet-tasting protein from the berry of a West African plant (Spanier and Miller, 1993).

It is generally accepted that flexible short linear peptides probably exist in numerous low-energy states in solution (van Gunsteren and Berendsen, 1990) and that a particular conformation may only be adopted when the peptide comes into contact with a specific receptor. An example of this is the neuromodulator Met-enkephalin (Tyr-Gly-Gly-Phe-Met) (Smith et al., 1991; Perez et al., 1992), which has been studied extensively by both experimental and theoretical methods for many years.

In general, the specific structure of oligopeptides is not readily determined by NMR studies in aqueous solutions. However, there are some exceptions; examples are ribonuclease A peptide (Osterhout et al., 1989) and other short peptide protein fragments (Dyson et al., 1992). In recent years, attempts have been made to develop methods for studying the conformational flexibility of peptides in solution by NMR using techniques such as multiconformational evaluation (Cicero et al., 1995; Schmidt et al., 1993) and Monte Carlo methods in which the experimental NMR data are included as statistical weights to obtain low-energy conformations (Vesterman et al., 1989; Nikiforovich et al., 1993; Landis and Allured, 1991).

The initial method of studying the Delicious Peptide was two-dimensional NMR spectroscopy to look for evidence of a specific conformation or a limited number of conformations. Proton NMR studies at 500 MHz found no evidence of a specific conformation or limited conformations and suggested a random coil type of conformation. These findings are demonstrated under Results.

With the experimental evidence in mind, molecular modeling techniques were used to search for low-energy conformations. Many techniques (Vasquex et al., 1994) have been developed for the location of low-energy conformations of flexible molecules in the absence of any experimental evidence. Those available include Monte Carlo simulations (Velikson et al., 1992; Abagyan and Totrov, 1994) as well as simulated annealing (Nayeem

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et al., 1991; Salvino et al., 1993; Li and Scheraga, 1992), usually implemented as an extension of the Monte Carlo algorithm during which searches are carried out at a high temperature and then at progressively lower temperatures. The system should eventually become "frozen" into the lowest energy conformation. This method will lead to only one conformation, so here quenched molecular dynamics (O'Connor et al., 1992) were used to analyze the conformational space available to the Delicious Peptide according to the method outlined for Met-enkephalin and other short linear peptides (Smith et al., 1991; Perez et al., 1992). Quenched molecular dynamics involves the use of high-temperature dynamics followed by minimization. Comparison of the structures obtained was made with the NMR results.

EXPERIMENTAL METHODS

Samples of Delicious Peptide were obtained from the Institute of Food Research, Reading, U.K. The NMR spectra were acquired on Bruker 300 and 500 MHz spectrometers. For each experiment, 10−12 mg of solid peptide was dissolved in a solvent of either D_2O or a mixture of 90% H_2O/10% D_2O. Solvent suppression was carried out by presaturation of the water signal.

COSY (Wüthrich, 1986; Kessler et al., 1988b) experiments were run to confirm the resonance assignment of the individual residues in the peptide. The ROESY (Bax and Davis, 1985) technique was used for sequential assignment of the residue connectivites and to give any secondary structure information. Due to the unfavorable correlation times of short linear peptides in water, it was not possible to use the conventional NOESY experiment to record the presence of NOEs (Kessler et al., 1988b; Williamson and Waltho, 1992). The two-dimensional spectra were recorded with 256 by 4K datasets. The ROESY spectra were recorded with a radiofrequency field strength of 2 kHz using a mixing time of 400 ms at a temperature of 298 K.

Amide temperature coefficients were measured by determination of the values of the amide proton chemical shifts over a temperature range of 5−35 °C and then plotting a graph of the variation of chemical shift with temperature for each residue in the sequence. The gradient of the line for each individual residue gives the temperature coefficient for that particular residue in the sequence.

Molecular modeling calculations were performed using the Quanta 4.0/CHARMM (Brooks et al., 1983) program using an explicit hydrogen model. Molecular dynamics simulations were carried out with a dielectric constant of 80 to simulate the electrostatic effects of an aqueous environment. Molecular dynamics simulations were carried out using the Verlet algorithm with a time step of 1 fs.

The calculation method used was similar to that used by Smith et al. (1991). An initial fully extended conformation present in a zwitterionic form was used as the starting structure. This structure was minimized using a combination of steepest descents and adopted-basis Newton−Raphson algorithms to a root mean square deviation of less than 0.001. The peptide was heated to 1000 K over 10 ps of dynamics, increasing the temperature by 5 K every 50 time steps with a molecular dynamics time step of 0.001 ps. The structure was then allowed to equilibrate at this temperature for a further 10 ps of molecular dynamics. Quenched molecular dynamics were then carried out for a further 600 ps at the same temperature. The co-ordinates of the peptide were saved to a trajectory file every 1 ps, giving a total of 600 conformations for further analysis.

Each of the structures obtained was energy minimized to a root mean square derivative of less than 0.001 kcal/mol using an adopted-basis Newton−Raphson algorithm. Constraints were placed on all of the ω dihedral angles in the molecule with a force constant of 50 kcal/mol to prevent the formation of cis conformations of the peptide bond during the high-temperature quenched molecular dynamics run.

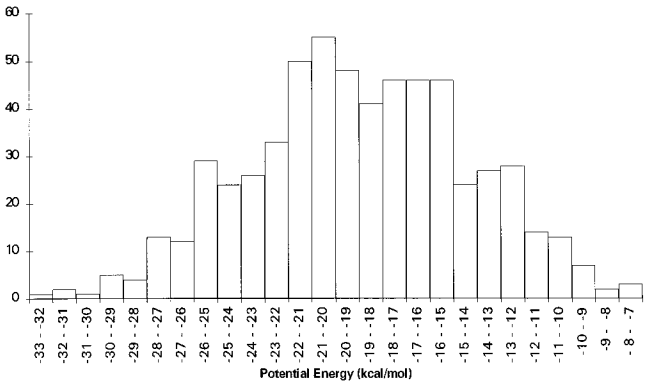

Figure 1. Potential energy histogram obtained from quenched dynamics of Delicious Peptide.

Table 1. NMR Chemical Shift Assignments and Coupling Constants

	chemical shift (ppm)				
residue	NH	CαH	CβH	other	$^3J_{NH-C\alpha}$ (Hz)
Lys-1		4.03	1.90	γCH$_2$, 1.47 δCH$_2$, 1.60 εCH$_2$, 2.98	
Gly-2	8.57	4.00			5.00
Asp-3	8.36	4.71	2.88		7.24
Glu-4	8.24	4.30	1.98	γCH$_2$, 2.45	6.64
Glu-5	8.18	4.30	1.98	γCH$_2$, 2.45	6.60
Ser-6	8.10	4.40	3.82		6.69
Leu-7	8.04	4.34	1.67	γCH, 1.67 δCH$_3$, 0.88 0.84	8.42
Ala-8	8.06	4.34	1.37		7.78

The results showed a Gaussian-like distribution of energies with the lowest energy structures being those that would be the most likely to exist in solution at 300 K (Figure 1). Low-energy conformations were selected that were within 5 kcal/mol of the lowest energy conformation. Each of these low energy conformations was categorized in terms of backbone dihedral (ϕ, ψ) angle ranges and also in terms of backbone hydrogen bonds. The structures were sorted into families using structural similarities based on low root mean square deviations.

Further molecular dynamics calculations were carried out on the lowest energy conformation to check the stability of the structure at room temperature. This involved a 50 ps molecular dynamics run at 300 K using a time step of 0.001 ps with a dielectric constant of 80.

The results of the simulation methods were compared to the constraints obtained from the NMR results to see if the experimental results fitted in with the structures obtained from the simulation methods.

RESULTS

NMR Results. A complete proton spin system assignment was made for the Delicious Peptide mainly using the COSY spectrum, in which clear residue connectivities were seen for each residue in the sequence. Confirmation of one overlapping amide signal was achieved by looking at the ROESY spectrum, whereby the two signals could be distinguished from their connectivities to the next residue in the sequence. The peptide resonance assignments are shown in Table 1. The results of the ROESY spectra taken are shown in Figure 2. Here it can be seen that there are no long-range NOE connectivities recorded. The NOE interactions obtained are from backbone adjacent residues or

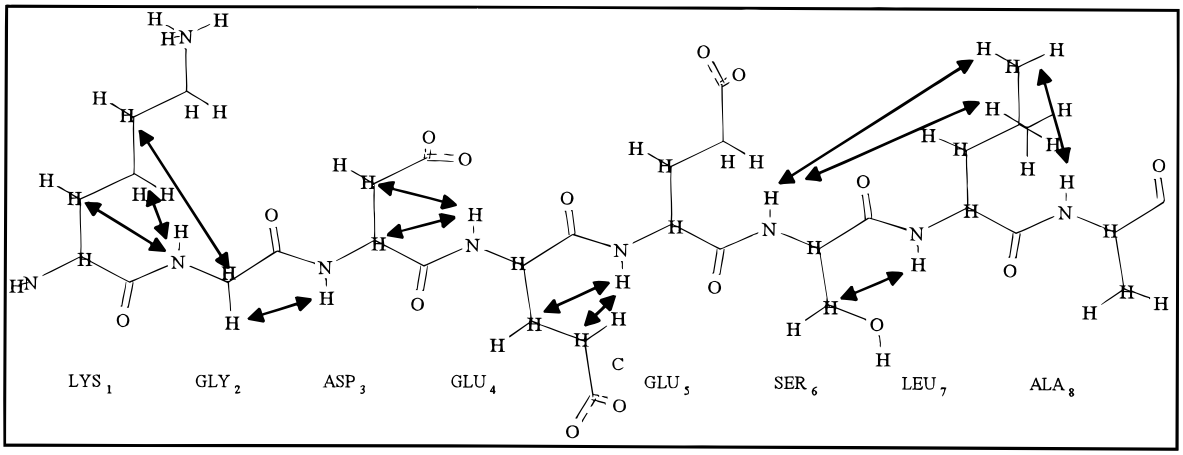

Figure 2. ROE interactions for Delicious Peptide.

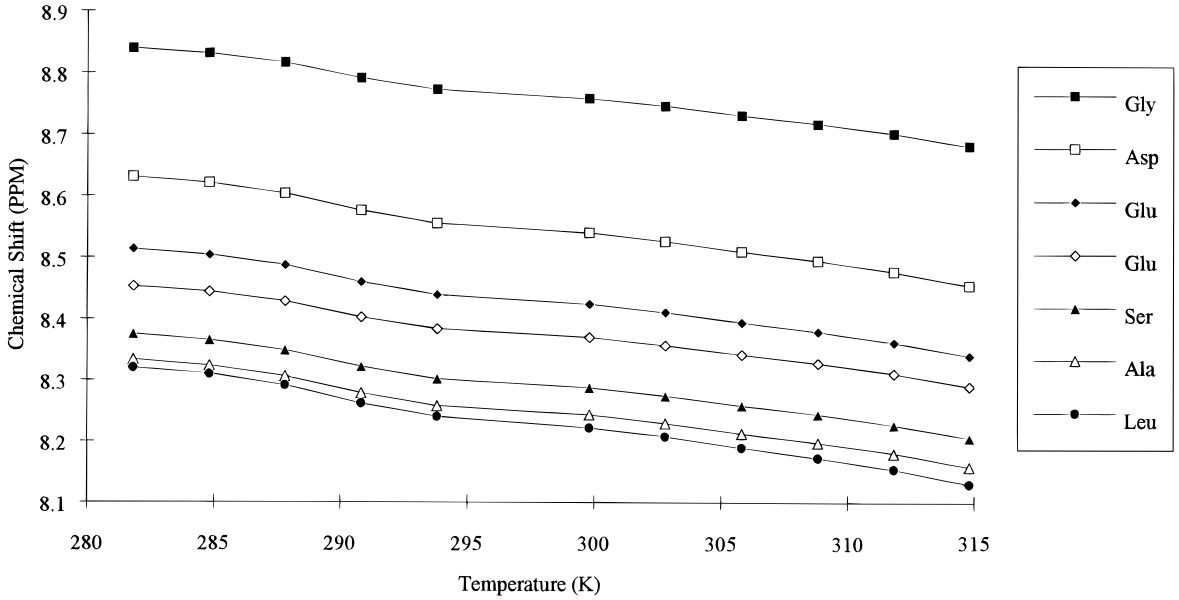

Figure 3. Graph of variation of amide chemical shift with temperature for Delicious Peptide.

from side chains to adjacent backbone residues. This suggests that a random coil type structure is present for the peptide in aqueous solution.

The values obtained for the $^3J_{N\alpha}$ coupling constants are also shown in Table 1. Using a Karplus type equation, it is possible to link the value of coupling constants to the backbone torsion angles in the peptide. Specific values for J couplings have been found to be indicative of the secondary structure of the peptide. The values obtained here (around 7 Hz) are indicative of a random coil mixture of conformations (Williamson et al., 1992).

A graph of the variation in the amide ^{1}H chemical shift resonances of the individual residues with temperature (Figure 3) gives similar straight lines for each of the different residues in the sequence and therefore similar temperature coefficients in each case. The slopes of the graphs are found to be similar in each case. A change in slope for any one residue would suggest a different hydrogen bonding involved in that particular residue. The results suggest that each of the residues in the peptide is involved in hydrogen bonding to a similar extent.

The combination of the above results leads to the suggestion that the Delicious Peptide does not have a specific conformation in water but is present as a random coil series of low-energy conformations with no one conformation present in a sufficiently high concentration to be identified in the NMR spectra.

Simulation. The 600 ps molecular dynamics run results in 600 structures for further analysis by minimization. Although the initial starting structure for the simulation consists of an extended straight-chain peptide, during the quenched dynamics process, the structure is able to coil a round and form a large number of different conformations. This means that a large area of conformational space is explored, the high temperature allowing the peptide to cross over higher energy barriers and explore conformational space. After minimization, the range in energy of the minimized structures is −7 to −33 kcal/mol. Figure 1 shows an energy histogram of the 600 minimized structures considered. The structures can be seen to form a Gaussian type distribution, with the lowest energy ones being those most likely to exist in solution. Any high-temperature conformation present that is unstable at room temperature is expected to be removed by the minimization stage. Figure 4 displays the ϕ, ψ maps generated during the run at 1 ps intervals. Each graph consists of 600 data points. The spread of the points on the graph shows the large area of conformational space sampled during the quenched molecular dynamics run.

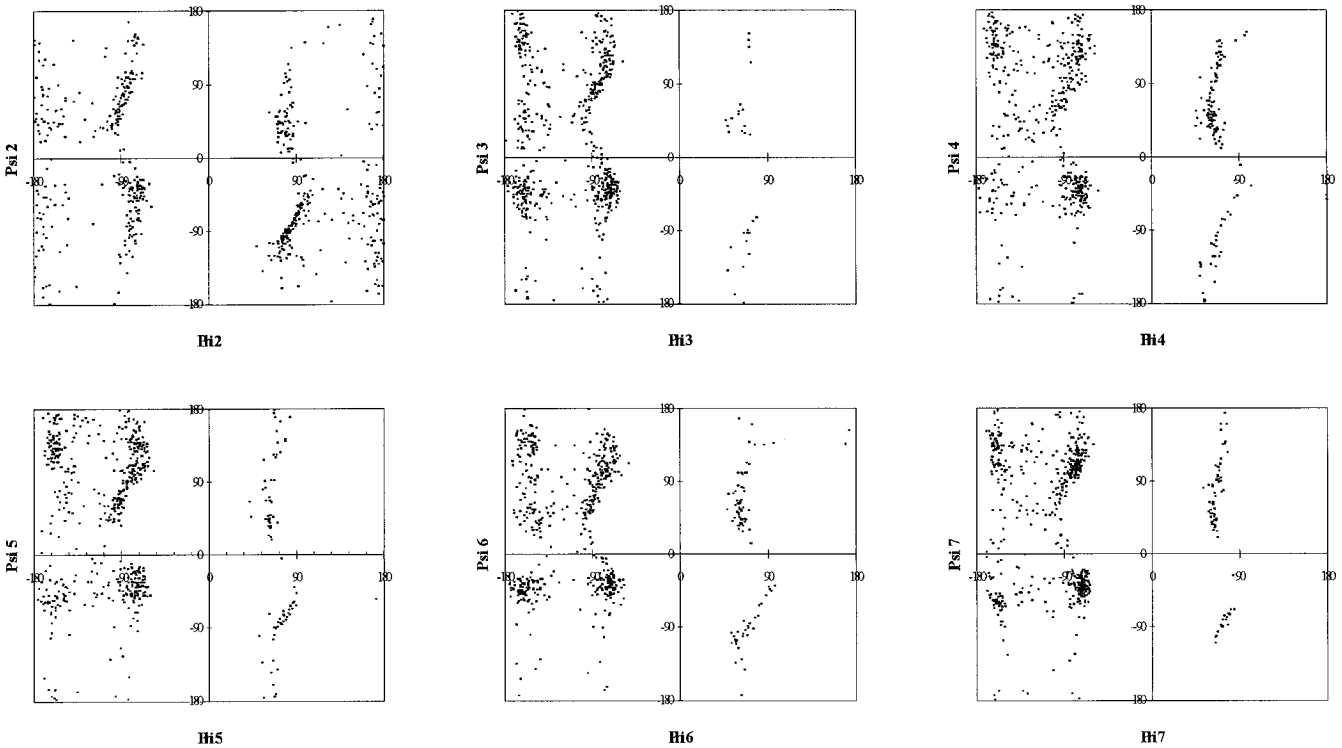

Figure 4. ϕ, ψ maps for the 600 structures of Delicious Peptide (residues 2−7).

Selecting conformations within 5 kcal of the lowest energy structure results in 28 different low-energy conformations for further examination. These structures show a wide variation of structures with the formation of some structural families. Compared to the low-energy conformations obtained for Met-enkephalin (Smith et al., 1991), a greater flexibility is seen. This was likely to be due to the longer length of the chain and the larger size of the peptide involved in this case, with eight residues compared to five residues. This means that more conformational flexibility is expected.

The conformations obtained are categorized in groups according to the number of hydrogen bonds present and other secondary structural elements. The structures obtained at first glance can be divided into two broad categories: those with an extended-chain "round" type conformation, with hydrogen bonds from one end of the structure to the other to give a cyclic type structure, and those which gave a more coiled S-shaped type of structure with interactions between the start of the peptide (Lys-Gly) and the middle acidic portion (Asp-Glu-Glu). This is important because the flavor action is thought to be produced from the combination of the initial Lys-Gly basic residues with the middle acidic (Asp-Glu-Glu) portion of the peptide. Of the round type structures, the cyclic form is produced from either Lysine, the first residue, or glycine, the second residue. The torsion angles of the majority of structures are found to be in, or near, the region of that expected for an α-helical type of structure, although in a few cases the compounds contain the majority of angles in the β-sheet range.

Families of Structures. The 600 minimized structures were sorted into different families of conformations according to root mean square deviations (Figure 5). Four families (F1−F4) of structures are found to exist, which account for two-thirds of those obtained. No particular trends are found among the nonfamily structures. All of the lower energy structures are clustered into groups as shown in Figure 6, while Table 2 gives the variation in torsion angles for the lowest member of each family.

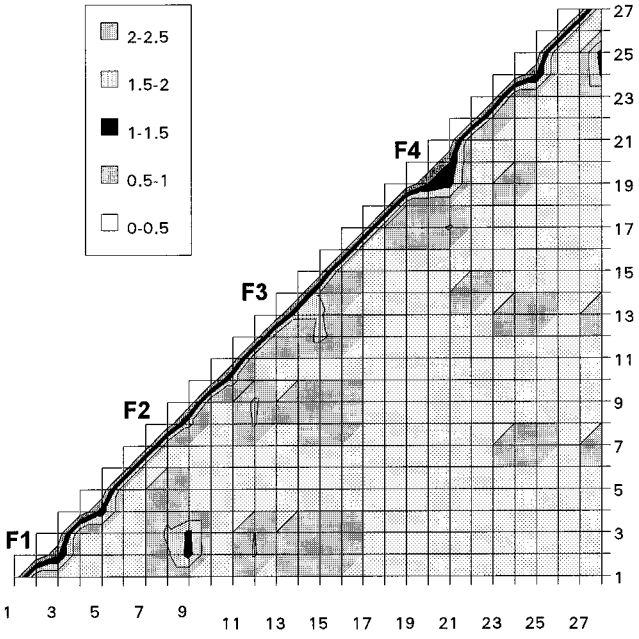

Figure 5. RMS deviations between the 28 low-energy structures and the families of structures formed.

Family F1 contains the largest series of conformations with six low-energy structures. These consist of round cyclic type structures with hydrogen bonds existing in some cases between the first and the last residues. In most cases other hydrogen bonds are found to exist between the middle peptide residues. A subset of structures show a β-turn (Milner-White, 1990; Sibanda et al., 1989) between the first four residues of the peptide (Lys-Gly-Asp-Glu) with a backbone hydrogen bond existing between Lys-1 and Glu-4.

Family F2 contains similar round type conformations with more defined cyclic hydrogen bonds between Lys

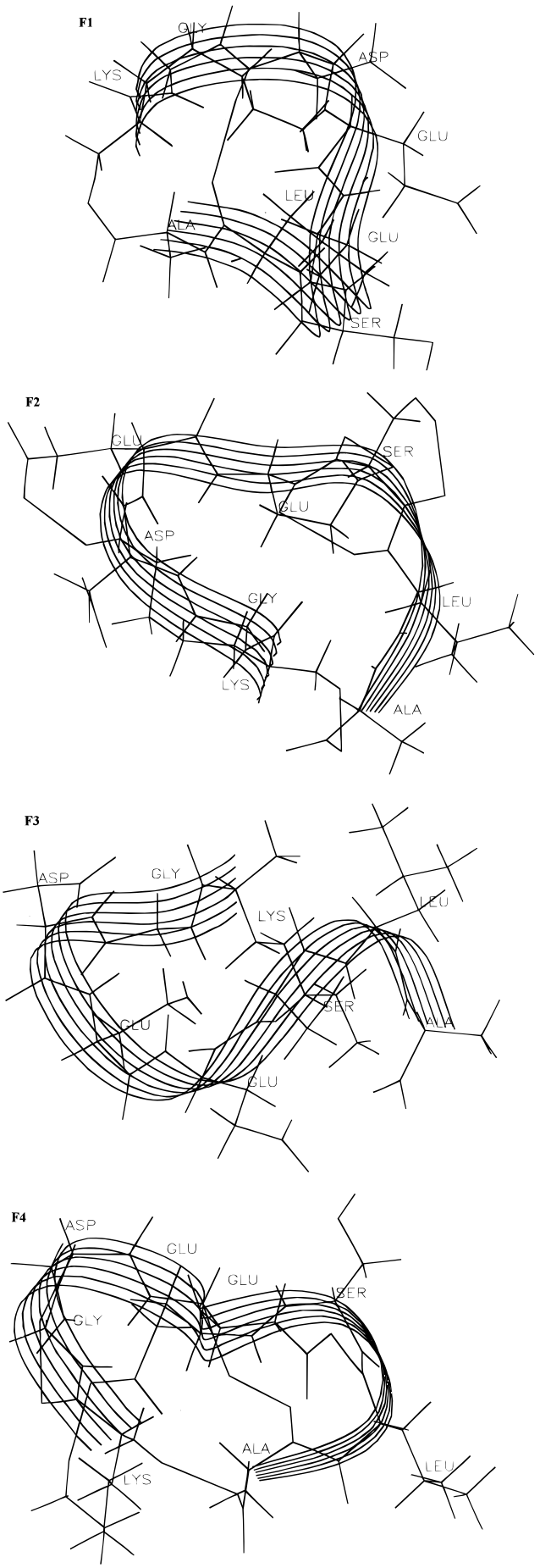

Figure 6. Low-energy structures for the different families of conformations.

Table 2. Dihedral Angles (Degrees) Present in Different Families of Structures

residue	dihedral	F1	F2	F3	F4
Lys-1	ψ	139.998	143.187	−129.293	136.622
Gly-2	ϕ	−146.433	−133.216	96.969	90.822
	ψ	−106.212	80.978	−46.938	−69.517
Asp-3	ϕ	−71.531	−165.765	−64.763	−69.609
	ψ	−43.015	−36.499	−29.776	−27.201
Glu-4	ϕ	−156.034	−126.194	−118.910	−139.878
	ψ	−163.110	−45.381	−35.628	63.980
Glu-4	ϕ	−80.958	−75.129	−144.259	−79.484
	ψ	−29.896	142.472	−98.796	−15.760
Ser-6	ϕ	−71.720	−90.080	−151.531	−59.002
	ψ	−37.686	82.170	−67.494	−55.347
Leu-7	ϕ	−103.251	−153.872	−142.838	−87.696
	ψ	50.972	−107.009	−37.069	−22.615
Ala-8	ϕ	−85.239	−169.068	−136.234	70.274

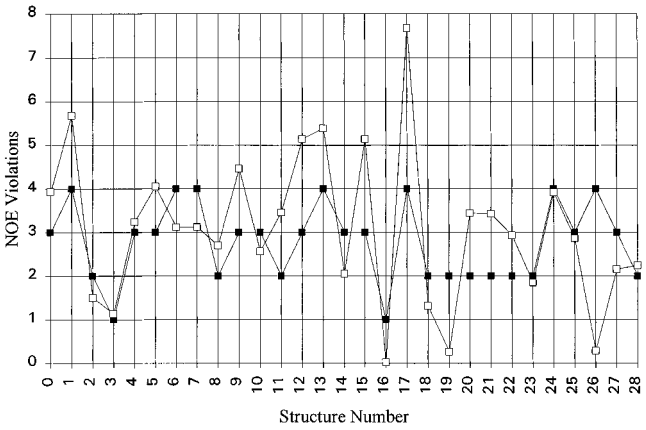

Figure 7. NOE constraint violations: (■) number of distance violations; (□) total amount of violations (Å).

and Ala, although the structures in general are of higher energy than those of F1. No turn type structures exist within this family of structures.

Family F3 contains the lowest energy structures of the quenched dynamics run. They consist of S-shaped coiled structures with few backbone hydrogen bonds, although some side chain hydrogen bonds are found. These structures do not form cyclic type conformations.

Family F4 consists of coiled structures with hydrogen bond interactions in each case between the first four residues in the sequence (Lys-Gly-Asp-Glu), and in most of the residues backbone hydrogen bonds exist to form a cyclic round structure.

Lowest Energy Conformation. The lowest energy conformation consists of no backbone hydrogen bonds, although there are two side chain hydrogen bonds from the Lys residue to the Glu residue and the Ala residue, linking up the different regions in the peptide and suggesting some interaction between these regions. The molecule as a whole consisted of a mainly α-helical range of torsional angles and a coiled type structure.

The lowest energy structure was subjected to a further 50 ps molecular dynamics simulation at 300 K using a time step of 0.001 ps. It is found to be a stable conformation under this time of trajectory. During the course of the further molecular dynamics run, no lower energy structures were obtained. The structure is found to be fairly stable during the course of the run without large changes in the backbone region.

Comparison of Simulation Results with NMR Results. Figure 7 shows a graph of the number of NOE constraint violations and their total value for each of the 28 low-energy structures obtained for the simulation methods as well as the linear starting structure for the

quenched molecular dynamics run. A total of 10 inter-residue NOE constraints were used. The results show that up to four NOE violations are present for any one structure with a maximum violation of eight. Three conformations contain very small (approximately zero) violations of the NOE constraints. Most violations occur between the residues in the latter part of the peptide (Ser-Leu-Ala).

NOE constraints for a peptide usually represent an average structure, and any specific constraint can be the result of an average between more than one conformation. For this reason constraints from NMR data are now often applied as "time-averaged" (Mierke et al., 1994; Abseher et al., 1994) constraints over the course of a molecular dynamics run. This means that a given low-energy conformation may not fit all of the NMR results, especially in the case of a flexible molecule. The structures that best fit the NMR results are not found to be the lowest energy structures present, and this may be because the NMR constraints are derived from an average of all conformations and not just one specific structure.

The results show the low-energy structures obtained from quenched molecular dynamics studies give good agreement with the determined experimental data. The experimental NMR data do not give evidence for any one specific low-energy structure.

Conclusions. The NMR results show the flexibility of the Delicious Peptide in water and that there is no specific conformation present; rather, a mixture of conformations must exist. This is to be expected for the majority of short linear peptides. Our results are used to test the validity of quenched molecular dynamics techniques.

The simulation has successfully sampled a large area of conformational space and produced a number of low-energy conformations that are unlikely to be found by other conventional molecular dynamics techniques. The lowest energy conformation is found to be stable by further molecular dynamics calculations, but this could not be proved to be the global minimum. However, the structure was found to be stable over the course of a 50 ps molecular dynamics run. The quenched molecular dynamics results produce four families of different conformations.

The low-energy conformations obtained suggest an interaction between the basic and the acidic regions of the peptide in the form of backbone and side chain hydrogen bonds. This agrees with the model of how the peptide interacts with the flavor receptor (Spanier and Miller, 1993). In the model of a flavor receptor for savory compounds, "cationic" residues are thought to interact with acidic residues and thus with the taste receptor. A new study by Nakata et al. (1995) has suggested that some changes in position of the acidic and basic fragments in the peptide do not affect the Delicious Peptide taste. This suggests that the interaction of the basic and acidic regions of the peptide, not their sequence, is important for the production of flavor, although these regions must be present at a distance across which they can interact.

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