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The Effects of Regularly Spaced Glutamine Substitutions on Alpha-Helical Peptide Structures. A DFT/ONIOM Study

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Abstract

The side-chains of the residues of glutamine (Q) and asparagine (N) contain amide groups. These can H-bond to each other in patterns similar to those of the backbone amides in α -helices. We show that mutating multiple Q's for alanines (A's) in a polyalanine helix stabilizes the helical structure, while similar mutations with multiple N's do not. We suggest that modification of peptides by incorporating Q's in such positions can make more robust helices that can be used to test the effects of secondary structures in biochemical experiments linked to proteins with variable structures such as tau and α -synuclein.

Among natural amino acid residues, that of glutamine (Q) has unusual properties due to its amide containing side chain. Another residue, that of asparagine (N), contains a similar amide, but its shorter side chain causes it to be somewhat less flexible, hence less adept at forming stable structures. Amidic H-bonds provide peptides much of the stabilizations of the normal secondary structures. If the additional amides on the side-chains of the Q's form chains of H-bonds, they might affect the relative energies of secondary structures and possibly be used to modify the secondary structures of similar peptides without these substitutions. Several examples come to mind. Among the short peptides (6–8 residues) related to amyloids (abnormal secondary structures), reported to crystallize as *parallel* sheets, six of eight contain at least one Q and five contain at least one N.(1) We have recently shown that the cooperative H-bonding between the Q's in adjacent strands of the amyloid causing sequence, VQIVYK, of the protein, tau, plays an important role in stabilizing the β -sheet of the amyloid.(2) The other sequence thought to cause aggregation of tau, VQIINK,(3,4) also contains both a Q and N. Perutz has published several reports on the cooperative interaction of poly-Q peptides,(5–7) which have been linked to Huntington's disease. A theoretical explanation of the cooperativity in Poly-Q has also recently appeared.(8)

In this paper, we examine the consequences of unnatural sequences containing Q's to demonstrate their effects upon controlling secondary structures. We started with an optimized conformation of the α -helical capped polyalanine peptide, acetyl(Ala)₂₈NH₂. In each of these structures, from one to six A's were mutated to Q's in (for multiple mutations): mutations four residues apart (i and i+4). The i+4 series was chosen so that adjacent Q's would be closest (in space) in an α -helix. The tabulated mutation energies and enthalpies (table 1) were calculated using the following equations:

$$\begin{aligned}\Delta E_{\text{mutation(A} \rightarrow \text{X})} &= (\Delta E_{\text{peptide(A)}} + \Delta E_{\text{Q}}) - (\Delta E_{\text{peptide(Q)}} + \Delta E_{\text{A}}) \\ \Delta H_{\text{mutation(A} \rightarrow \text{X})} &= (\Delta H_{\text{peptide(A)}} + \Delta H_{\text{Q}}) - (\Delta H_{\text{peptide(Q)}} + \Delta H_{\text{A}})\end{aligned}$$

METHODS

We used the ONIOM(9,10) method as programmed in the Gaussian09(11) suite of computer programs. ONIOM divides the system into up to three segments which can be treated at different levels of calculational complexity. Thus, one can treat the essential part of the system at the high level, while the less critical parts of the system might be calculated at the medium or low level. For this study we only used two levels (high and medium). We treated the cores of the peptides (equivalent to a corresponding peptide containing only glycines) and the side chains of the glutamine and asparagine residues at the high level, with only the side chains of the alanines at the medium level. The high level used hybrid DFT methods at the B3LYP/D95(d,p) level. This method combines Becke's 3-parameter functional,(12) with the non-local correlation provided by the correlation functional of Lee, Yang and Parr.(13) In the ONIOM method, there are unsatisfied valences in the high level at the interface between it and medium level. These valences were satisfied by using the default method of capping them with a hydrogen atom in the direction of the connecting atom in the medium level with a C-H distance of 0.723886 times the C-C distance. We used the AM1(14) semiempirical molecular orbital method for the ONIOM medium level. While the B3LYP functional has been recently criticized,(15) we found B3LYP/d95(d,p) reasonably accurate when compared to other functionals combined with moderate basis sets for the H-bonding in Water dimer.(16)

All geometries were completely optimized in all internal degrees of freedom and vibrational calculations performed to assure the geometries are true minima on the PESs as there are no imaginary vibrational frequencies, and to obtain the vibrational frequencies used to calculate the enthalpies at 298K. Detailed discussion of the vibrational spectra will be presented elsewhere. Distortion energies of the backbone were calculated by comparing the high level of the ONIOM calculations (DFT) for the fully relaxed polyalanine helix with the backbones of substituted helices.

The current procedure gave relative energies that agreed well with complete DFT optimizations for a series of five small 3_{10} -helical peptides.(17) We have used this procedure for several previous studies of peptide structures.(2,18–22)

RESULTS AND DISCUSSION

The substituted helices can have two different H-bonding conformations. We determined that the ones with the C=O's of the amides on the side chains pointing towards the N-terminus (acetyl capped) are consistently more stable. The mutations were made starting at the 12th residue (from the N-terminus). The next three mutations were made at residues 16, 20 and 24, followed by the last two at 8 and 4. All data refer to these conformations. Tables 1 and 2 summarize the results. Table 1 clearly shows that multiple i+4 mutations of Q for A in a polyalanine α -helix lead to significant stabilizations of the helices. However similar mutations of N for A do not. Stabilization due to H-bonding between the side chains comes at a sacrifice of a distorted backbone, as indicated. Furthermore, both the increasing stabilization for Q substitutions (as is evident from the mutation energies) and the shortening of the H-bond distances between the side chains (see table 2), particularly near the center of the H-bonding chain involving the Q side chains, with increasing i+4 mutations these mutations lead to apparent cooperative H-bonding by the amides of the side chains in a

manner analogous to those previously reported for formamide chains.(23,24) For example, for 6 Q's the increased interaction energy compared to the all alanine α -helix is -19.3 and the distortion energy is 7.1 kcal/mol. Thus, one would expect an interaction enthalpy of -26.4 kcal/mol if distortion were absent. The total interaction energy for a chain of six H-bonding formamides (obtained by summing over the H-bonding energies)(23,24) is -31.86 kcal/mol, which is a somewhat greater stabilization. This difference can be attributed to the difficulty of simultaneously accommodating all the H-bonds in their optimal geometries, which we call attractive strain.(25) The shorter O...H distances (1.806 Angstroms for the central H-bond) in a chain of six H-bonding formamides compared to 1.862 Angstroms for the corresponding H-bond in this study exemplifies the differences in the H-bonding geometries between the current study and that of the formamide chains.

The data for multiple substitutions of N tell a somewhat different story. No stabilization over the all alanine helix occurs until six N's are substituted. The optimized structures clearly represent local (not global) minima. For the structure containing 6N's, the mutation energy is -5.6 and the distortion energy is 18.5 kcal/mol. Following the reasoning given above for mutation of six Q's, the expected interaction energy between the six N's would be -24.1 kcal/mol if distortion were absent, slightly (2.3 kcal/mol) less than the corresponding value for six Q's. When the sixth N is included, the terminal H-bond in the chain involving the side chains no longer follows the trend of becoming shorter as the number of mutations increases. Apparently, the strain in the backbone has become too great to accommodate increasingly cooperative H-bonds between the side chains. This seems another and more illustrative example of attractive strain.

The present results indicate that the presence of multiple Q's at $i+4$ positions in α -helices tend to stabilize this secondary structure. As a result, properly induced mutations in natural proteins of variable structure such as tau(26) or synuclein(27) whose aggregates are linked to Alzheimer's and Parkinson's diseases might make the α -helical domains more robust which might diminish these protein's abilities to form aggregates in which the α -helical structures become partially converted to β -sheet. Similar mutations might be useful in creating helical bundles in de novo proteins in a manner that might augment the techniques reported by Hecht.(28) On the other hand, substitutions of N's for A's in the $i+4$ positions do not lead to stabilizations of α -helices.

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Figure 1. Helix with three Q's mutated for A's. The changes in the O...H distances for the distorted H-bonds of the backbone are noted (in Angstroms). The H-bonds between the side chains are indicated with red dotted lines.

Table 1

Mutation energies, enthalpies, and deformation energy (kcal/mol). Deformation energies are calculated as the difference between the high-level energies for the backbone only between the polyalanine and the substituted helices.

mutations	Glutamine			Asparagine		
	ΔE	ΔH	$\Delta E_{(deformation)}$	ΔE	ΔH	$\Delta E_{(deformation)}$
1	2.9	2.3	-0.1	3.2	3.0	0.6
2	0.4	-0.5	0.0	7.4	6.7	2.2
3	-3.4	-4.2	1.8	6.1	5.5	4.5
4	-8.6	-9.8	3.4	3.5	3.1	7.9
5	-13.4	-14.5	5.4	0.9	0.8	10.7
6	-19.3	-20.4	7.1	-5.6	-5.2	18.5

Table 2

Calculated O...H distances between Q side-chains starting from the N-terminus (Angstroms).

number of Q's	1 st	2 nd	3 rd	4 th	5 th
2	1.986				
3	1.945	1.947			
4	1.925	1.891	1.914		
5	1.921	1.879	1.871	1.901	
6	1.907	1.866	1.862	1.868	1.903