Extraordinary Helicity in Short Peptides via End Capping Design

Behrouz Forood, Hemender K. Reddy, and Krishnan P. Nambiar*

Department of Chemistry, University of California Davis, California 95616

Received January 27, 1994

Designing peptides and proteins that fold into predetermined secondary structures is a challenging problem in modern bioorganic chemistry.[1-3] α-Helix is a landmark secondary structure found in proteins.[4,5] Several laboratories have been investigating the parameters that influence helix stability.[6-16] We and others have recently shown that amino acids with side chains capable of hydrogen bonding with the free NH groups at the amino terminus and the free carbonyls at the carboxy terminus have a stabilizing effect on the helical structure of short peptides.[17-22] These results parallel the prediction of Presta and Rose,[23] and the statistical correlations of Richardson and Richardson,[24] and Harper and Rose[25] that α-helical structures in proteins are stabilized by hydrogen bonding between the unpaired main-chain NH and CO groups and the side chains of amino acids flanking the helical termini. Based on earlier findings and structural considerations, we hypothesized that by increasing the hydrogen bonding capacity of the end capping residues we should be able to increase the helicity of the peptides. To test our hypothesis, we designed novel functional groups that would be efficient amino end capping moieties. We reasoned that sulfur atom at various oxidation states would provide an excellent system for studying the effect of end capping of amino termini. We designed a series of sulfur-containing molecules, each differing in its oxidation state of sulfur, and incorporated them at the amino terminus of a 15-residue alanine-rich peptide. The end capped peptides show remarkable helicity in aqueous solution. The peptide with sulfonate as the end capping moiety showed the highest helicity, confirming the validity of our structural hypothesis.

Our model peptide (X-HN-Ala_4-Glu-Ala_3-Lys-Ala_4-Tyr-Arg-$CONH_2$) has the following features. It contains a glutamic acid at position 5 and a lysine residue at position 9 to introduce an intrahelical salt bridge and to make the peptide water soluble. A tyrosine was introduced at position 14 to enable peptide quantitation by UV absorbance.[14,26] We introduced an arginine at the carboxyl end which we previously found to be the most efficient amino acid in capping the carboxyl end.[18] The negatively charged carboxyl group of Arg-15 was amidated to avoid the repulsive interaction between itself and the negative pole of the helical macro dipole. From our previous studies[18] we had observed aspartic acid to be most effective in amino end capping. Hence, in the present study, we chose sulfur-containing moieties that are isosteric with aspartic acid side chain.

The peptides were synthesized as their carboxamides using 5-[4-(9-fluorenylmethoxycarbonyl)(aminomethyl)-3,5-dimethoxyphenoxy]valeric acid (PAL) resin from Milligen/Biosearch on a Milligen/Biosearch 9050 peptide synthesizer using 9-fluorenylmethoxycarbonyl (Fmoc) chemistry and benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP)/1-hydroxybenzotriazole (HOBT) activation. The peptides were cleaved from the resin using 90% trifluoroacetic acid (TFA)/5% thioanisole/3% ethane dithiol/2% anisole at room temperature for 6 h and precipitated from ice-cold diethyl ether. The peptides were purified on a reversed-phase C_{18} HPLC column, using water and acetonitrile containing 0.1% trifluoroacetic acid as the eluent. The peptide primary structure was confirmed by quantitative amino acid analysis and mass spectrometry.[27] CD spectra were recorded on a Jasco 600 spectropolarimeter, and a total of six scans were averaged to obtain each spectrum. All CD studies were carried out using 10 μM peptide solutions in 10 mM phosphate buffer at pH 7, using 10-mm path length cell. The peptide concentrations were determined by measuring the absorbance of tyrosine residue at 275 nm at 25 °C in 6 M guanidine·HCl.[14,26]

The helix content was measured using 10 μM solution of each peptide by CD at low temperature (0 °C) in 10 mM phosphate buffer at pH 7. The end capped peptides show characteristic CD spectra[28] indicating a relatively high α-helix content with a negative band centered at 222 nm (Figure 1). Helix content is taken as directly proportional to the mean residue ellipticity at 222 nm $[\theta]_{222}$. 100% helicity was estimated using the formula $[\theta]_{222}^{helix} = -40\,000[1 - (2.5/n)]$, where n = number of amino acid residues.[29] According to this method, the mean residue ellipticity at 222 nm for a 15-residue peptide in completely helical conformation is −33 300. α-Helicity was independent of peptide concentration in the range of 10–200 μM, showing that these peptides are monomeric in solution (data not shown). Table 1 shows the α-helix content of the peptides. Mean residue ellipticity $[\theta]_{222}$ is calculated by dividing molar ellipticity by 15 since all peptides contain 15 amino acids. % Helicity = $100[\theta]_{222}/(-33\,300)$.

The potential α-helix inducing effect of various residues, such as thioether, sulfoxide, sulfone, sulfonate, *N*-acetyl, and carboxylate, capable of amino end capping was determined by synthesizing peptides **1**–**7**. Methylthiopropionic acid was activated using HOBT and BOP in 0.6 M *N*-methylmorpholine in

(1) Creighton, T. E. *Proteins: Structures and Molecular Properties*, 2nd ed.; W. H. Freeman and Co.: New York, 1993.
(2) Degrado, W. G.; Regan, L. *Science* **1988**, *241*, 976–978.
(3) Richardson, D. C.; Richardson, J. S. *Trends Biochem. Sci.* **1989**, *14*, 304–309.
(4) Pauling, L.; Corey, R. B.; Branson, H. R. *Proc. Natl. Acad. Sci. U.S.A.* **1951**, *37*, 205–211.
(5) Perutz, M. F. *Nature* **1951**, *167*, 1053–1054.
(6) Chou, P. Y.; Fasman, G. D. *Adv. Enzymol.* **1978**, *47*, 45–148.
(7) Levitt, M. *Biochemistry* **1978**, *17*, 4277–4284.
(8) Sueki, M.; Lee, S.; Powers, S. P.; Denten, J. B.; Konishi, Y.; Scheraga, H. A. *Macromolecules* **1984**, *17*, 148–155.
(9) O'Neil, K. T.; Degrado, W. F. *Science* **1990**, *250*, 646–651.
(10) Merutka, G.; Stellwagen, E. *Biochemistry* **1990**, *29*, 894–898.
(11) Lyu, P. C.; Liff, M. I.; Marky, L. A.; Kallenbach, N. R. *Science* **1990**, *250*, 669–673.
(12) Padmanabhan, S.; Marqusee, S.; Ridgeway, T.; Laue, T. M.; Baldwin, R. L. *Nature* **1990**, *344*, 268–270.
(13) Shoemaker, K. R.; Kim, P. S.; York, E. J.; Stewart, J. M.; Baldwin, R. L. *Nature* **1987**, *326*, 563–567.
(14) Marqusee, S.; Baldwin, R. L. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 8898–8902.
(15) Jackson, D. Y.; King, D. S.; Chmielewski, J.; Singh, S.; Schultz, P. G. *J. Am. Chem. Soc.* **1991**, *113*, 9391–9392.
(16) Degrado, W. F.; Lear, J. D. *J. Am. Chem. Soc.* **1985**, *107*, 7684–7689.
(17) Forood, B.; Feliciano, E. J.; Nambiar, K. P. *Biochemistry* **1992**, *31*, 2199.
(18) Forood, B.; Feliciano, E. J.; Nambiar, K. P. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 838–842.
(19) Bruch, M. D.; Dhingra, M. M.; Gierasch, L. M. *Proteins: Struct. Funct. Genet.* **1991**, *10*, 130–139.
(20) Lyu, P. C.; Wemmer, D. E.; Zhou, H. X.; Pinker, R. J.; Kallenbach, N. R. *Biochemistry* **1993**, *32*, 421–425.
(21) Chakrabartty, A.; Doig, A. J.; Baldwin, R. L. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 11332–11336.
(22) Yumoto, N.; Murase, S.; Hattori, T.; Yamamoto, H.; Tatsu, Y.; Yoshikawa, S. *Biochem. Biophys. Res. Commun.* **1993**, *196*, 1490–1495.
(23) Presta, L. G.; Rose, G. D. *Science* **1988**, *240*, 1632–1641.
(24) Richardson, J. S.; Richardson, D. C. *Science* **1988**, *240*, 1648–1652.
(25) Harper, E. T.; Rose, G. D. *Biochemistry* **1993**, *32*, 7605–7609.
(26) Brands, J. F.; Kaplan, L. J. *Biochemistry* **1973**, *12*, 2011–2024.
(27) The peptide primary structure was confirmed by quantitative amino acid analysis done on a Beckman 6300 high-performance amino acid analyzer at the Protein Structure Laboratory at University of California, Davis, and the molecular weight was determined using electrospray mass spectrometry done at the Advanced Instrumentation Lab at Univeristy of California, Davis.
(28) Davidson, B.; Fasman, G. D. *Biochemistry* **1969**, *8*, 4108–4116.
(29) Scholtz, J. M.; Qian, H.; York, E. J.; Stewart, J. M.; Baldwin, R. L. *Biopolymers* **1991**, *31*, 1463–1470.

0002-7863/94/1516-6935$04.50/0 © 1994 American Chemical Society

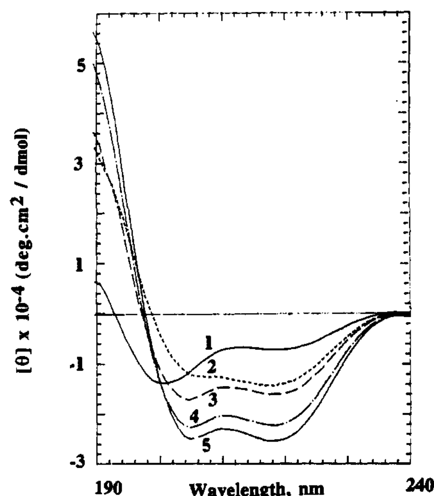

Figure 1. CD spectra of 10 μM solutions of peptides **1–5** at 0 °C in 10 mM phosphate buffer at pH 7.

Table 1. α-Helix Content of Peptides **1–7**

peptide	X	$-[\theta]_{222}$[a] (deg·cm²·dmol⁻¹)	helicity (%)[b]	$-[\theta]_{222}$[c] (deg·cm²·dmol⁻¹)
1	H	5800	17.4	7800
2	$CH_3SCH_2CH_2CO$-	10 700	32.1	11 500
3	$CH_3SOCH_2CH_2CO$-	13 300	39.9	14 500
4	$CH_3SO_2CH_2CH_2CO$-	20 900	62.8	20 400
5	$HOSO_2CH_2CH_2CO$-	24 400	73.3	22 100
6	CH_3CO-	10 700	32.1	12 100
7	$HOCOCH_2CH_2CO$-	21 100	63.4	19 200

[a] Mean residue ellipticity $[\theta]_{222}$ was determined from the CD spectra of a 10 μM solution of peptides at 0 °C in 10 mM phosphate buffer at pH 7. [b] Percentage helicity was calculated as $100([\theta]_{222}/^{max}[\theta]_{222})$. $^{max}[\theta]_{222} = -40\,000\,[1-(2.5/n)]$, where $n = 15$. [c] Mean residue ellipticity $[\theta]_{222}$ was determined from the CD spectra of a 10 μM solution of peptides at 0 °C in 10 mM phosphate buffer at pH 7 containing 1 M NaCl.

DMF for 20 min and coupled to resin-bound peptide **1**. Deprotection and isolation of peptide **2** was carried out in the same manner as described above. Selective oxidation of the thioether group to sulfoxide (peptide **3**) was accomplished using *N*-chlorosuccinimide.[30,31] Two diastereomeric sulfoxides were separated using HPLC and further characterized by mass spectrometry. Oxidation of the thioether group to sulfone was done using performic acid.[32,33] The reaction mixture was purified by preparative HPLC to yield peptide **4**. Sulfonic acid peptide (**5**) was prepared as follows. *S*-Trityl-3-mercaptopropionic acid was activated by HOBT and BOP in 0.6 M *N*-methylmorpholine in DMF for 20 min and coupled to resin-bound peptide **1**. After deprotection and HPLC purification, the peptide containing the thiol group was oxidized using performic acid to yield the sulfonic acid peptide **5**.

The helix content of peptides **1–7** in aqueous solution is shown in Table 1. Peptide **1** shows weak helicity (17.4%). When methylthiopropionic acid is incorporated at the amino terminus (peptide **2**), helicity increases to 32.1%. In peptide **2**, the unfavorable interaction between the positively charged amino terminal NH_3^+ group and the positive pole of the helical macrodipole is absent. This is similar to the *N*-acetylation effect (peptide **6**). Since sulfur in a thioether is a weak hydrogen bonding atom, we cannot expect a noticeable increase in helicity due to the presence of a sulfur atom. When the thioether is oxidized to the corresponding sulfoxide (peptide **3**), the helicity increases to 39.9%, suggesting that the oxygen atom of the sulfoxide is hydrogen bonding to one of the free NH groups at the amino terminus.[34] Complete oxidation of the thioether to the sulfone (peptide **4**) increases the helicity to 62.8%, a major increase over the thioether peptide **2**. This drastic increase in helicity can be attributed to the presence of two oxygen atoms in sulfone that can possibly hydrogen bond with two free NH groups at the amino terminus. The introduction of a sulfonic acid rsidue at the amino terminus (peptide **5**) generates a helicity of 73.3%. Here we have reached the highest oxidation state attainable for a sulfur-containing moiety, providing three oxygen atoms that can possibly hydrogen bond with all free NH groups (three) at the amino terminus. In addition, the sulfonate group, being negatively charged, can also interact favorably with the positive dipole of the helical macrodipole. CD measurements carried out in high salt (1 M NaCl) conditions where charge-dipole interactions would be largely screened show only a minor decrease in helicity (Table 1), suggesting that hydrogen bonding is the major contributor to helix stability. NMR studies on other peptides have shown the presence of a hydrogen bond between the N-cap residue and an unsatisfied main-chain NH.[20] In order to compare the effect of a sulfonate with that of a carboxylate, we synthesized peptide **7**, incorporating a succinyl residue at the amino terminus. Peptide **7** showed 63.4% helicity, a value similar to that of the sulfone peptide **4**. Since the carboxyl group has only two oxygen atoms, it can hydrogen bond with only two NH groups. We find that sulfonate with three oxygen atoms is the best moiety for amino end capping. The CD spectra of the peptides (Figure 1) do not show an isodichroic point, indicating that the capping group contributes to the CD spectrum.

A comparison of CD spectra of peptides clearly shows that an increase in the oxidation state of sulfur atom (which results in a corresponding incrase in the number of hydrogen bonding oxygen atoms) shows a corresponding increase in the α-helicity of peptides. This observation is in complete agreement with our structural hypothesis correlating the number of hydrogen bonding atoms participating in end capping with helix stabilizing/inducing capacity. The trend in α-helix inducing ability was found to be sulfonate > sulfone > sulfoxide > sulfide as predicted. The overall ranking of the residues tested is sulfonate > succinate > sulfone > sulfoxide > sulfide = acetyl Our data clearly demonstrate the feasibility of designing novel molecules which when incorporated into peptides at helical termini can induce helical folding and stabilize folded structures via end capping.

Acknowledgment. This research was supported by a grant from National Institutes of Health (R29 GM 39822).

(30) Shechter, Y.; Burstein, Y.; Patchornik, A. *Biochemistry* **1975**, *14*, 4497–4503.
(31) Walsh, M.; Stevens, F. C. *Biochemistry* **1978**, *19*, 3924–3930.
(32) Means, G. E.; Feeney, R. E. *Bioconjugate Chem.* **1990**, *1*, 2–12.
(33) Hirs, C. H. W. *Methods Enzymol.* **1967**, *11*, 197–199.

(34) Only one of the two diastereomeric sulfoxides shows helix stabilization. The other diastereomer showed very low helicity (17.4%). It is likely that it adopts a conformation that disfavors N-terminal capping.