Recognition and Stabilization of an α-Helical Peptide by a Synthetic Receptor

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The design of synthetic receptors that recognize functional groups on the surface of a protein is poorly developed in comparison to inhibitors that bind in enzyme active sites.[1] Yet, surface interactions, mediated by complementary shape and charge distribution,[2,3] often are essential for the functional role of a protein. The extensive solvation of charged and polar functional groups on the protein exterior introduces complexities into surface recognition. Approaches that make use of metal binding[4] and multiple charge−charge interactions[5] have targeted secondary structural units such as the α-helix. Our strategy has been to use hydrogen-bonding groups in recognizing and stabilizing α-helices as models for protein surfaces.[6] Herein we report that a tetraguanidinium-based receptor (**1**) binds strongly to a peptide with four aspartate residues (**2**) and stabilizes it in an α-helical conformation.

Molecular-modeling studies showed that **1** wraps around an ideal right-handed α-helix conformation of peptide **2**, in a coiled left-handed helix, with an almost perfect matching of each guanidinium moiety with the corresponding carboxylate group of the aspartate residues. Molecular dynamics at 300 K showed that the doubly coiled structure was stable after 500 ps[7] (Figure 1) while the α-helix of the peptide alone was destroyed after only a few ps.

The synthesis of guanidinium receptors **1** and **3**−**6** has been reported (Figure 2).[8] The mean spacing between the guanidinium central carbons of **1** in its outer helical conformation is 8.9 Å (Figure 1) and complements the carbon−carbon distances of the carboxylates of **2** in an inner α-helix (7.0 Å, Figure 1). These molecules have been shown to adopt a helical conformation around sulfate anions.[8] Peptides **2**, **7**, and **8** were
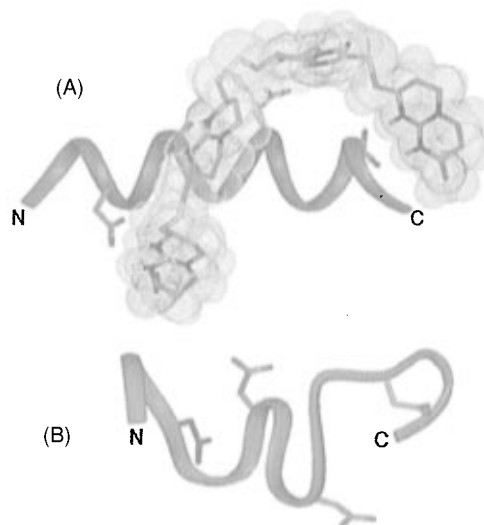

Figure 1. Last structures obtained after 500 ps trajectories of unrestrained molecular dynamics for (**A**) complex between tetraguanidinium (**1**) and DDDD (**2**) peptide and for (**B**) **2** alone.
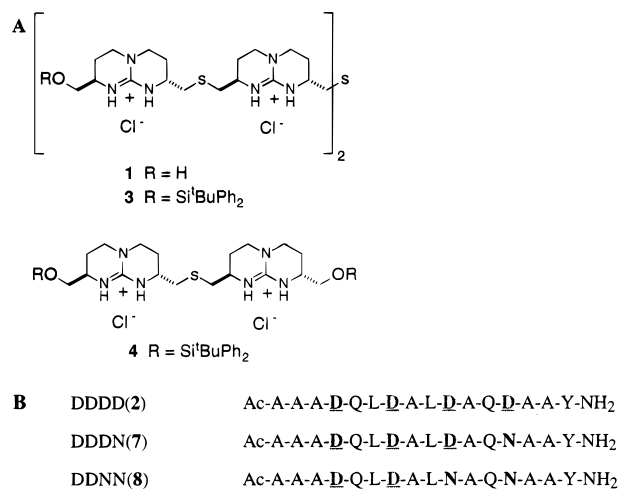

Figure 2. Receptors and peptide sequences used in binding studies. (**A**) The *all-S* enantiomers of structures **1** and **3** are designated **5** and **6**, respectively. (**B**) Amino acid sequences.

synthesized[9] to include a C-terminal Tyr to assist concentration determination[10] and C- and N-terminal capping to reduce helix macrodipole effects. The *all-R* and *all-S* enantiomers of the silyl-protected tetraguanidinium and the di(hydroxy)tetraguanidinium were assayed for their binding behavior to the tetracarboxylate form[11] of peptide **2** by CD spectroscopy. Increasing amounts of tetraguanidinium (0−250 μM) were added to a standard solution of the target peptide (∼50 μM) in 10% H$_2$O/90% CH$_3$OH. CD spectra showed a marked increase in α-helicity (minima at 222 and 208 nm) upon addition of the tetraguanidinium solution. The resulting binding curves were fitted by a 1:1 binding model, and the calculated association constants are collected in Table 1. To probe the stoichiometry of the association, we conducted a Job CD titration[12] between peptide **2** and receptor **3** in 10% H$_2$O/90% CH$_3$OH. Maximum

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(1) Silverman, R. B. *Mechanism-Based Enzyme Inactivation: Chemistry and Enzymology*; CRC Press: Boca Raton, FL, 1988.
(2) Nassar, N.; Horn, G.; Herrmann, C.; Scherer, A.; McCormick, F.; Wittinghofer, A. *Nature* **1995**, *375*, 554−560.
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(4) Mallik, S.; Plunkett, S. D.; Dhal, P. K.; Johnson, R. D.; Pack, D.; Shnek, D.; Arnold, F. H. *New J. Chem.* **1994**, *18*, 299−304.
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(7) Conditions: heating and equilibrating for 50 ps, followed by 450 ps at 300 K. Aspartate side chains are shown along the peptide ribbon backbone. Solvent effects were simulated by performing the molecular dynamics in a medium of ϵ = 4r_{ij}. AMBER force field parameters and Insight/Discover programs (Biosym/MSI) were used.
(8) Sanchez-Quesada, J.; Seel, C.; Prados, P.; de Mendoza, J.; Dalcol, I.; Giralt, E. *J. Am. Chem. Soc.* **1996**, *118*, 277−278.

(9) Peptides were synthesized using standard solid phase BOP/HOBt chemistry with Fmoc amino acids in DMF. Electrospray mass spectrometry yielded molecular weights of 1661.2 for DDDD (calculated 1661.7), 1660.8 for DDDN (calculated 1661.0), and 1659.9 for DDNN (calculated 1660.0).
(10) Brandts, J. F.; Kaplan, L. J. *Biochemistry* **1973**, *12*, 2011−2024.
(11) Carboxylate forms of the peptides were made by dissolving the peptide in water and addition of ammonium hydroxide to pH > 11. Lyophilization and aqueous cationic exchange with Amberlite-IRA 120+ tetramethylammonium ion exchange resin and relyophilization afforded the corresponding tetramethylammonium salts.

Table 1. Binding Affinities of Peptide Substrates with Guanidinium Receptors and Helix Stabilization on Binding

sequence	receptor	K_a (M^{-1})	$H_i{}^a$	$H_f{}^b$	Δ^c	$\Delta\Delta^d$
DDDD (**2**)	**1**	$(3.40 \pm 1.2) \times 10^5$	21.1	45.3	24.2	142
	3	$(1.56 \pm 0.6) \times 10^5$		61.2	40.1	143
	5	$(2.90 \pm 1.3) \times 10^5$		39.3	18.2	106
	6	$(2.41 \pm 0.9) \times 10^5$		50.6	29.5	132
	4^e	$(8.52 \pm 3.2) \times 10^3$		29.1	8	43
DDDN (**7**)	**3**	5.65×10^4	29.9	44.7	14.8	45
DDNN (**8**)	**3**	3.80×10^4	55.7	50.4	−5.3	−10

^{a} Initial percentage helicity.[13] ^{b} Final percentage helicity, at 2 equiv of receptor. ^{c} Change in percentage helicity ($\Delta = H_f - H_i$). ^{d} Percentage stabilization ($\Delta\Delta = \Delta/H_i$). ^{e} Uncorrected for **4** CD absorbance.

signal change in the Job titration was observed at 0.5 mol fraction of the peptide, indicative of 1:1 complexation.

Detailed information on the binding process was derived from NMR studies of the tetraaspartate peptide **2** alone and in the presence of the nonprotected, *all-R* tetraguanidinium receptor **1**.[15] In 10% H$_2$O /90% CH$_3$OH, both bound and unbound peptides show strong N$_i$N$_{i+1}$, and N$_i$N$_{i+2,3}$ and α_iN$_{i+3}$ NOEs, which are typical for helical conformations. Nevertheless, the first three N-terminal (Ala 1−3) and last two C-terminal (Ala 15, Tyr 16) residues including the capping acetyl and carboxamide motifs appeared to be structured only in the presence of receptor **1**, as suggested by the occurrence of a continuous series of N$_i$N$_{i+1}$ NOEs of the now nondegenerate amide protons. Furthermore, for all non-Asp residues, the conformation sensitive H$^\alpha$ protons shifted drastically upfield by as much as 76% upon addition of **2** to a total helicity of 44%, in comparison to 25% for the peptide alone. This helical stabilization is in good agreement with the CD results and was shown to be independent of changes in peptide concentration. The long series of sequential and medium-range NN NOEs combined with the strong upfield shift of the H$^\alpha$ protons upon addition of receptor **1** indicates that the helical conformation spans the whole sequence, a rather unusual feature in a peptide of this size. A direct indication of 1:1 complex formation through interaction between the four negatively charged Asp residues of the peptide

(12) Blanda, M. T.; Horner, J. H.; Newcomb, M. *J. Org. Chem.* **1989**, *54*, 4626−4636.

(13) Percentage helicity was determined from $f = [(\Theta_{obsd} - \Theta_0)/(\Theta_{100} - \Theta_0)] \times 100$, where Θ_{obsd}, Θ_0, and Θ_{100} represent the measured ellipticity, the zero ellipticity (random coil), and 100% helicity (as calculated), respectively, and f is the percent (or fraction, f) helix. The 100% helical state is given by Helix$_{100\%}$ = $-40\,000(1 - 2.5/n)$, where n is the number of amide bonds.[14] Zero percent helicity was determined experimentally by thermal denaturation of the DDDD peptide in pure water. The measurement gave -2060 (deg cm^2 dmol^{-1}) and was used as Θ_0 in the percent helix calculations for all peptide sequences.

(14) Chakrabartty, A.; Schellman, J. A.; Baldwin, R. L. *Nature* **1991**, *351*, 586−588.

(15) Spectra were recorded with 1 or 2 mM samples in 90% MeOH-d_3/ 10% H$_2$O on a Varian VXR500 spectrometer at 5, 15, and 25 °C. Spectral width was 5000 Hz with 1×2K data points in the F2 and F1 dimensions, zero-filled to 2×4K, and processed with shifted sine bell window functions and base line corrections in both F1 and F2 dimensions. Assignment was done applying the standard two-step procedure described by Wüthrich[16] using (scalar) through-bond TOCSY MLEV-17 spin lock sequence of 80 ms or COSY and through-space ROESY(mixing time 150 ms) or NOESY- (mixing times 100−400 ms) experiments. Residual water signal was eliminated by the WATERGATE pulse sequence, and spectra were referenced to dioxane as internal standard. Total helicity was calculated based upon the H$^\alpha$ chemical shift deviation ($\Delta\delta$H$^\alpha$) with respect to random coil values[17] by percent helix = $\Sigma(\Delta\delta$H$^\alpha)/(n(-0.38))$, where -0.38 ppm was taken to be the value for 100% helix and n, the number of amide bonds, is 17.

(16) Wüthrich, K. *NMR of Proteins and Nucleic Acids*; John Wiley Sons: New York, 1986.

(17) Rizo, J.; Blanco, F. J.; Kobe, B.; Bruch, M. D.; Gierasch, L. M. *Biochemistry* **1993**, *32*, 4881−4894.
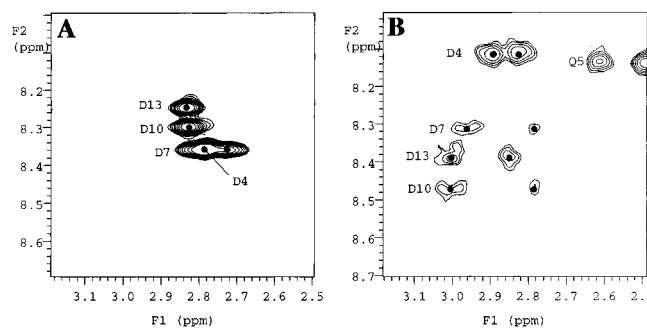
Figure 3. (A) Expansion of TOCSY spectrum (Asp-β region) of **2** in 90% MeOH at 288 K. (B) Expansion of TOCSY spectrum (Asp-β region) of **2** in the presence of **1** in 90% MeOH at 288 K.
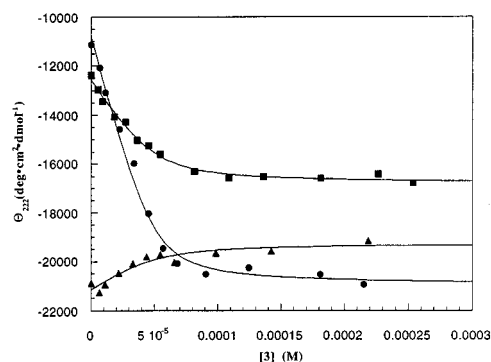

Figure 4. Binding Curves for CD titrations of (a) receptor **3** with DDDD (●) sequence, (b) **3** with DDDN (■), and (c) **3** with DDNN (▲) in 10% H$_2$O/CH$_3$OH at 25 °C.

and the four positively charged residues of guanidinium receptor **1** comes from analysis of TOCSY spectra of both the free peptide and the complex (Figure 3). As shown in Figure 3A, in the absence of receptor **1**, peptide **2** shows in the amide β-Asp region only a moderate amide chemical shift dispersion (0.12 ppm) and degenerate β-protons for three Asp residues (residues 4, 10, and 13). This situation changes dramatically in the presence of the receptor. In the same region, the spectrum of the complex (Figure 3B) shows a much larger amide chemical shift dispersion (0.40 ppm) and, even more relevant, a clear differentiation between diastereotopic β-protons for all β-Asp residues with $\beta\beta'$ chemical shift differences as large as 0.22 ppm. This splitting of Asp β-protons is a strong indication of a well-defined structure, similar to that observed in proteins with a particular tertiary structure.

Two additional peptide sequences were synthesized based directly on DDDD peptide **2**. The primary amido group of Asn was used to approximate a neutral isostere for aspartate. CD binding studies between receptor **3** and peptides DDDN (**7**) and DDNN (**8**) showed a correspondence between the number of aspartates and the degree of α-helical stabilization. The strongest binding occurs between the tetraaspartate peptide and the tetraguanidinium molecules, clearly pointing to the key role played by guanidinium−carboxylate interactions. An association constant of $1-2 \times 10^5$ M^{-1} between **3** and DDDD (**2**) in 10% H$_2$O/90% CH$_3$OH leads to a stabilization (Figure 4) of the α-helical conformation by almost 150%.