# Molecular Recognition of Proteins: Sequence-Selective Binding of Aspartate Pairs in Helical Peptides

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The design of synthetic agents that recognize and bind to functional groups on protein surfaces is largely unexplored. This is surprising since such receptors might have important applications in the disruption of physiologically important protein−protein interactions[1] or, when polymer bound, in protein purification.[2] In contrast, the recognition of DNA has been extensively studied and sequence-selective binding to a large number of base pairs has been demonstrated.[3] The surface of a protein represents a difficult challenge for molecular recognition studies as it contains a large and complex arrangement of highly solvated functional groups. The distribution of the groups will depend critically on the conformation of the peptide backbone at the protein surface (α-helix, β-sheet, β-turn, etc.). A major goal will thus be to design synthetic receptors that bind to these functional groups in a way that is selective for both peptide sequence and secondary structure. High selectivity in protein recognition will most likely be achieved by targeting polar residues on the surface with complementary hydrophobic interactions providing additional binding energy. As a first step to developing a general solution to protein surface recognition, we are investigating the design of synthetic receptors that complement two or more residues on different secondary structural features. In this communication we employ short helical peptides as models of a protein surface and demonstrate sequence-selective recognition of a pair of aspartate residues by a rigidified bis-guanidinium receptor.

Other than recent work by Sasaki[4] and Voyer,[5] there have been few reports of synthetic receptors for oligopeptide substrates. In our approach, we have exploited the ability of alkylguanidinium groups to bind strongly to carboxylates in polar solvents.[6] In particular, we have synthesized receptor **1**,[7] in which the rigid scaffold orients two guanidinium units to interact with two carboxylates spaced by 4−5 Å in an approximately parallel arrangement (Figure 1). The corresponding half-receptor **2** (cyclopentylguanidinium hydrochloride) was used as a control. Receptor **1** has high solubility and low absorbance in the far-UV region, making it well suited for study by both NMR and circular dichriosm (CD) spectroscopy. We have prepared, as target substrates, a family of 16-mer peptides in which two aspartate groups are located at different positions ($i + 3$, $i + 4$, $i + 11$) along the chain. These peptides were designed to possess significant α-helical character[9] in 10% water/methanol. The calculated structure in Figure 2 shows that **1** is well suited to bind to the $i + 3$ peptide via four hydrogen bonds to the two carboxylates. Strong binding should, in turn, lead to an increased stabilization of the helical conformation.[10]

i+3: Ac-Ala-Ala-Gln-**Asp**-Ala-Ala-**Asp**-Ala-Ala-Ala-Ala-Ala-Gln-Ala-Ala-Tyr-$CONH_2$
i+4: Ac-Ala-Ala-Gln-**Asp**-Ala-Ala-Ala-**Asp**-Ala-Ala-Ala-Ala-Gln-Ala-Ala-Tyr-$CONH_2$
i+11: Ac-Ala-Ala-Gln-**Asp**-Ala-Ala-Ala-Ala-Ala-Ala-Ala-Ala-Gln-Ala-**Asp**-Tyr-$CONH_2$

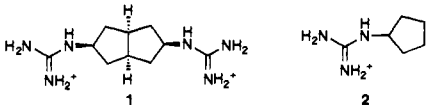

**Figure 1.** Sequences of peptide substrates and structures of guanidinium receptors.

Using NMR and CD spectroscopy, titrations[12] of receptors **1** and **2** were carried out with each synthetic peptide in 10% water/methanol at 25 °C. All peptides were used as their bis-tetramethylammonium salts. Addition of receptor **1** to a solution of peptide $i + 3$ causes sharpening and progressive shifting (Figure 3a) of the amide resonances by as much as 0.13 ppm. These are large changes considering that each amide remains partially solvated and probably samples multiple conformations. The binding affinity of **1** (determined by nonlinear regression

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(1) Franciskovich, J.; Houseman, K.; Mueller, R.; Chmielewski, J. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 765−768.

(2) 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.

(3) For leading references, see: (a) Mrksich, M.; Dervan, P. B. *J. Am. Chem. Soc.* **1993**, *115*, 9892−9899. (b) Strobel, S. A.; Doucette-Stamm, L. A.; Riba, L.; Housman, D. E.; Dervan, P. B. *Science* **1991**, *254*, 1639−1642.

(4) Tabet, M.; Labroo, V.; Sheppard, P.; Sasaki, T. *J. Am. Chem. Soc.* **1993**, *115*, 3866−3868.

(5) Voyer, N.; Deschênes, D.; Bernier, J.; Roby, J. *J. Chem. Soc., Chem. Commun.* **1992**, 134−136.

(6) Fan, E.; Van Arman, S. A.; Kincaid, S.; Hamilton, A. D. *J. Am. Chem. Soc.* **1993**, *115*, 369−370. For other examples of synthetic receptors that function in competitive solvent mixtures, see: (a) Kelly, T. R.; Kim, M. H. *J. Am. Chem. Soc.* **1994**, *116*, 7072−7080. (b) Kato, Y.; Conn, M. M.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1994**, *116*, 3279−3284. (c) Schiessl, P.; Schmidtchen, F. P. *Tetrahedron Lett.* **1993**, *34*, 2449−2452. (d) Kneeland, D. M.; Ariga, K.; Lynch, V. M.; Huang, C.-Y.; Anslyn, E. V. *J. Am. Chem. Soc.* **1993**, *115*, 10042−10055. (e) Williams, D. H.; Searle, M. S.; Mackay, J. P.; Gerhard, U.; Maplestone, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 1172−1178. (f) Faruta, H.; Magda, D.; Sessler, J. L. *J. Am. Chem. Soc.* **1991**, *113*, 978−985. (g) Constant, J. F.; Fahy, J.; Lhomme, J. *Tetrahedron Lett.* **1987**, 1777−1780.

(7) Receptor **1**, *endo,endo*-3,7-diguanidinobicyclo[3.3.0]octane dihydrochloride, was synthesized by reductive amination of bicyclo[3.3.0]-3,7-octanedione with benzylamine and $NaBH_3CN$ (70%), followed by hydrogenation (82%), guanidinylation using di-(Boc)-substituted pyrazolecarboxamidine[8] (73%), deprotection with trifluoroacetic acid (98%), and Dowex chloride ion exchange. Stereochemistry was confirmed by X-ray crystallography performed on the dibenzyl dihydrochloride intermediate. All compounds were satisfactorily analyzed by $^1H$ and $^{13}C$ NMR spectroscopy and by either high-resolution mass spectroscopy or elemental analysis. Synthetic peptides were analyzed by amino acid analysis and FAB mass spectroscopy.

(8) Bernatowicz, M. S.; Wu, Y.; Matsueda, G. R. *Tetrahedron Lett.* **1993**, *34*, 3389−3392.

(9) Huyghues-Despointes, B. M. P.; Scholtz, J. M.; Baldwin, R. L. *Protein Sci.* **1993**, *2*, 1604−1611.

(10) For an earlier example of conformational selectivity in synthetic receptors, see: Vicent, C.; Hirst, S. C.; Garcia-Tellado, F.; Hamilton, A. D. *J. Am. Chem. Soc.* **1991**, *113*, 5466−5467.

(11) Still, W. C.; Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Lipton, M.; Liscamp, R.; Chang, G.; Hendrickson, T.; DeGunst, F.; Hasel, W. MacroModel v3.5a., Department of Chemistry, Columbia University, New York, NY 10027.

(12) Peptide concentrations were determined initially by quantitative amino acid analysis and thereafter by UV based on the tyrosine absorbance (in 6 M guanidinium chloride, 100 mM phosphate, pH 7.00) with $\epsilon_{274}$ = 1450 $M^{-1}$ $cm^{-1}$. All NMR titrations were carried out in a solvent mixture of 10% $H_2O$, 45% $CD_3OD$, and 45% $CD_3OH$ (volume percentages). The $CD_3OD$ reduces the intensity of the exchanging amide resonances, but was added to economize $CD_3OH$. Solvent suppression was achieved using the 1331 pulse sequence.[13] In CD titrations, the degree of helicity was determined by monitoring the ellipticity at 222 nm using the average of 301 data points collected over 60 s. Ellipticity is reported as mean residue ellipticity using $\Theta_{222} = (\Theta_{obs} \times 100)/(LNC)$, where $\Theta_{obs}$ is the measured ellipticity (mdeg), $L$ is the path length (0.1 cm), $N$ is the number of amino acids in the peptide (16), and $C$ is the peptide concentration (in millimolar). Fractional helicity was estimated as described by Baldwin.[14]

(13) Hore, P. J. *J. Magn. Reson.* **1983**, *54*, 539−542.

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

(15) Wilcox, C. S. In *Frontiers in Supramolecular Organic Chemistry and Photochemistry*; Schneider, H. J., Dürr, H., Eds.; VCH: Weinheim, 1990; pp 123−143. We are grateful for Professor Wilcox's provision and support of the HOSTEST computational tools.

(16) Receptor **1** data were analyzed using a binding model with 1:1 receptor/substrate stoichiometry; receptor **2** data were analyzed using a binding model with 1:1 receptor/carboxylate stoichiometry and no cooperativity. Binding analysis for receptor **2** complexation assumes (a) that binding affinity of **2** with each peptide carboxylate is identical ($K_1 = K_2$) and (b) that the chemical shift change ($\Delta\delta_1$) of the half-bound 1:1 peptide/**2** complex is equivalent to half the chemical shift change ($\Delta\delta_2$) of the fully bound 1:2 peptide/**2** complex. See: Friedrichsen, B. P.; Powell, D. R.; Whitlock, H. W. *J. Am Chem. Soc.* **1990**, *112*, 8931−8941.

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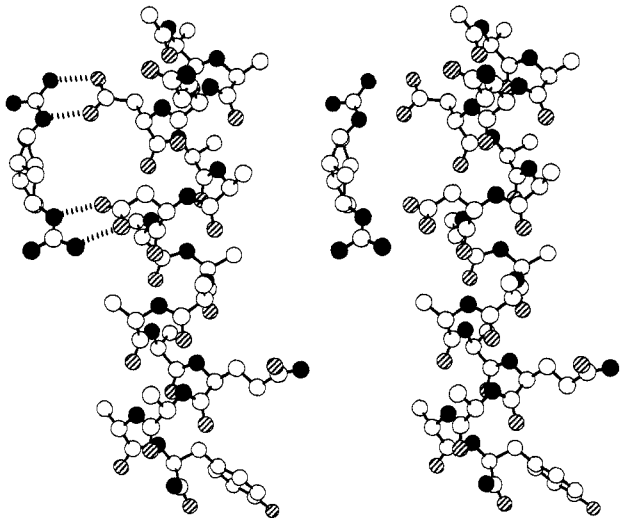

**Figure 2.** Stereoview of the calculated[11] low-energy conformer of the $(i + 3)$/**1** complex. Intermolecular hydrogen-bonding interactions are indicated as hashed bonds, and hydrogen atoms have been omitted for clarity.

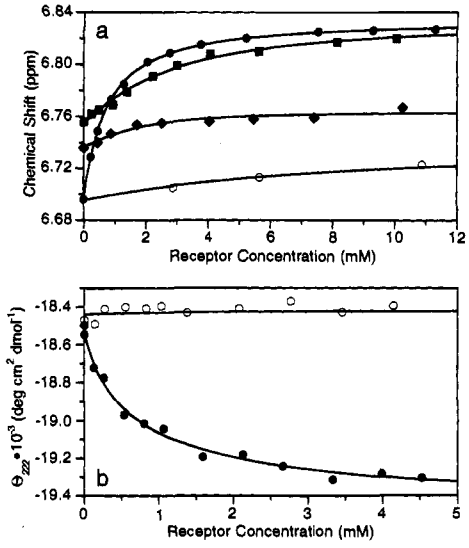


**Figure 3.** (a) $^1$H NMR titrations (glutamine side chain amide resonances) of guanidinium receptors with selected peptide substrates in 10% water/methanol at 25 °C, peptide concentration 1.54−1.66 mM. (b) CD titrations of guanidinium receptors with $i + 3$ in 10% water/methanol at 25 °C, peptide concentration 0.42−0.71 mM.[16] ●: $(i + 3)$/**1**, ■: $(i + 4)$/**1**, ◆: $(i + 11)$/**1**, ○: $(i + 3)$/**2**.

analysis[15] of shifts of the glutamine side chain amide NMR resonances) was greatest for $i + 3$ and was reduced by nearly 3- and 6-fold for peptides $i + 4$ and $i + 11$, respectively (Table 1). Binding affinities determined for each peptide by CD spectroscopy (Figure 3b) were consistent within experimental error.

CD spectra of peptides $i + 3$ and $i + 4$ in 10% water/methanol at 25 °C indicated that they were approximately 50% helical[17] ($\Theta_{222}$ = −18 510 and −18 100 deg·cm$^2$·dmol$^{-1}$, respectively). Addition of 10 equiv of **1** results in a significant (6−9%) increase in peptide helicity. This indicates that the receptor is binding preferentially (though not exclusively) to helically oriented peptides. In contrast, the inherent helical stability of peptide $i + 11$ was lower (26%, $\Theta_{222}$ = −12 400 deg·cm$^2$·dmol$^{-1}$) and addition of **1** induced a slight decrease in helicity. Assuming a 1:1 guanidinium/carboxylate complex,[18] it is not possible for **1** to bind to a helical conformation of $i + 11$ because of the long distance between the aspartates. Instead, the receptor may bind to a random coil or partially helical geometry, accounting for the reduced binding affinity and small shift in the conformational equilibrium.

(17) It was verified by CD spectroscopy that there is no change in the mean residue ellipticity of these peptides over a range of 40−582 μM, indicating that they remain monomeric throughout this concentration range.

**Table 1.** Association Constants ($K_a$) for Complexes of Receptors **1** and **2** with Peptide Substrates[a]

| peptide | $K_a$ ($M^{-1}$) with **1** | $K_a$ ($M^{-1}$) with **2** |
|---|---|---|
| $i + 3$ | 2200 | 23 |
| $i + 4$ | 770 | 23 |
| $i + 11$ | 390 | 26 |

[a] Values were determined from $^1$H NMR titrations in 10% water/methanol and were calculated as described in ref 16. Uncertainties in all NMR titrations were estimated to be ±10%.

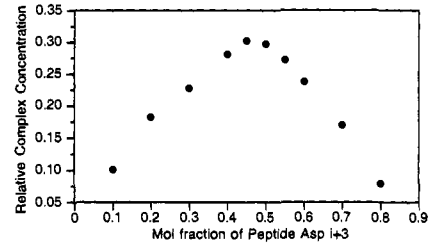


**Figure 4.** Job titration of peptide $i + 3$ with receptor **1** in 10% water/methanol at 25 °C. The total concentration is 2.5 mM, and the glutamine side chain amide resonance is analyzed.

To determine the effect of ionic strength[19] and single-point binding on peptide conformational stability, analogous control NMR and CD titrations were carried out using the half-receptor **2**. The binding affinity of **2** was very weak (<30 $M^{-1}$) for all peptide substrates. Each titration was carried out to a maximum concentration of **2** that was at least 4 times higher than the highest concentration of **1** to ensure that equivalent ionic strength had been surpassed. The very weak binding of **2** indicates that increasing ionic strength makes a negligible contribution to the observed changes. Furthermore, these results strongly imply that, for receptor **1**, binding and peptide conformational changes are due to a 1:1 receptor/substrate complex with both aspartate carboxylates and both receptor guanidiniums simultaneously involved. This was confirmed by a Job NMR titration[20] for the $(i + 3)$/**1** complex (Figure 4), which showed that maximal complex formation occurs at ~0.5 mol fraction of peptide, indicative of a 1:1 peptide/receptor binding stoichiometry. Molecular modeling studies[11] suggest that 1:1 complexation of **1** is favorable with α-helical conformations of the $i + 3$ and $i + 4$ peptides. In the (experimentally) preferred $(i + 3)$/**1** complex, the receptor is oriented with a slight twist (38°) in the left-handed helical direction (Figure 2), while in the $(i + 4)$/**1** complex, the peptide and receptor are nearly collinear. The origin of the $(i + 3)/(i + 4)$ specificity is not yet clear. We are currently preparing a second generation of receptors with additional electrostatic and hydrophobic interactions to bind to a larger area of protein surface.

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(18) For the $i + 4$ and $i + 11$ peptides an alternative 2:1 peptide/**1** binding arrangement may exist; however, the low affinity of simple guanidinium receptors for acetate ($K_a \approx 30$ $M^{-1}$) argues against significant multimeric association.

(19) Scholtz, J. M.; York, E. J.; Stewart, J. M.; Baldwin, R. L. *J. Am. Chem. Soc.* **1991**, *113*, 5102−5104.

(20) Connors, K. A. *Binding Constants*; John Wiley and Sons: New York, 1987.