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Recognition of Flexible Peptides in Water by Transition Metal Complexes

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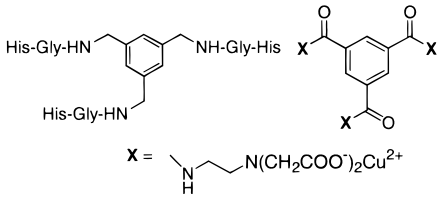
ABSTRACT



This paper describes the design, synthesis, and evaluation of transition metal complexes capable of recognizing flexible histidine-containing peptides in aqueous medium (25 mM HEPES buffer, pH = 7.0, 25 °C). When the pattern of metal ions on a complex matches with the pattern of histidine moieties on the peptide, strong interaction ($K = 1.2 \times 10^6$ M^{-1}) can be achieved. The complex was highly selective (>200:1) in discriminating similar flexible peptides differing only by one glycine unit.

Recognition of peptides is very important for hormone action, immune response, and other biochemical events.[1] Peptide therapeutics rely on strong binding of the peptide drugs to their biological targets. Detection of disease marker peptides has the potential of early diagnosis of the disease. Thus, recognition of peptides has attracted considerable attention in recent years. Several groups have reported strong and selective binding to peptides[2] and proteins[3] by designed artificial receptors in aqueous media. Combinatorial approaches to peptide recognition have been demonstrated.[4] Synthetic receptors are reported to recognize and bind to specific sites of proteins and disturb biologically important protein−protein interactions.[5] To our knowledge, these artificial receptors selectively bind to proteins or peptide molecules through hydrogen bonding, ion-pair, or hydrophobic interactions and often the binding constants are low (<50 000).

We are interested in the construction of metal complexes capable of binding strongly to histidine patterns of flexible peptides in water. Herein, we report that if the distribution of the metal ions on a complex is complementary to the histidine pattern of a peptide, a strong and selective binding can be achieved. Recognition based on metal−ligand interactions has several advantages compared to the approaches reported in the literature for peptide recognition. Hydrogen bonding and ion-pair interactions are particularly weak in a competitive solvent, e.g, water.[6] On the other hand, the strength and kinetics of metal−ligand interactions in water can be tuned by the choice of metal ions, the ligands positioning the metal ions, and the pH of the medium.[7] The

(1) Hecht, S. M., Ed. *Bioorganic Chemistry: Peptides and Proteins*; Oxford University Press: New York, 1998.

(2) (a) Ngola, S. M.; Kearney, P. C.; Mecozzi, S.; Russell, K.; Dougherty, D. A. *J. Am. Chem. Soc.* **1999**, *121*, 1192−1201. (b) Hossain, A. Md.; Schneider, H.-J. *J. Am. Chem. Soc.* **1998**, *120*, 11208−11209. (c) Breslo, R.; Yang, Z.; Ching, R.; Trojandt, G.; Obodel, F. *J. Am. Chem. Soc.* **1998**, *120*, 3536−3537. (d) Hioki, H.; Still, W. C. *J. Org. Chem.* **1998**, *63*, 904−905. (e) Peczuh, M. W.; Hamilton, A. D.; Sanohez-Quesada, J.; Mendoza, J.; Haack, T.; Giralt, E. *J. Am. Chem. Soc.* **1997**, *119*, 9327−9328.

(3) (a) Souers, A. J.; Virgill, A. A.; Rosenquist, A.; Fenuik, W.; Ellman, J. A. *J. Am Chem. Soc.* **1999**, *121*, 1817−1825. (b) Bernardi, A.; Cocchia, A.; Brocca, P.; Sonnino, S.; Zuccotto, F. *J. Am. Chem. Soc.* **1999**, *121*, 2032−2036. (c) Hamuro, Y.; Calama, M. C.; Park, H. S.; Hamilton, A. D.; *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 2680−2683.

(4) (a) Hioki, H.; Clark Still, W. *J. Org. Chem.* **1998**, *63*, 904−905. (b) Breslow, R.; Yang, Z.; Ching, R.; Trojandt, G.; Odobel, F. *J. Am. Chem. Soc.* **1998**, *120*, 3536−3537. (c) Torneiro, M.; Clark Still, W. *Tetrahedron* **1997**, *53*, 8739−8750. (d) Sasaki, S.; Takagi, M.; Tanaka, Y.; Maeda, M. *Tetrahedron Lett.* **1996**, *37*, 85−88.

(5) (a) Bishop, A.; Kung, C.; Shah, K.; Witucki, L.; Shokat, K. M.; Liu, Y. *J. Am. Chem. Soc.* **1999**, *121*, 627−631. (b) Park, H. S.; Liu, Q.; Hamilton, A. D. *J. Am. Chem. Soc.* **1999**, *121*, 8−13. (c) Hirota, S.; Endo, M.; Hayamizu, K.; Tsukazaki, T.; Takabe, T.; Kohzuma, T.; Yamaguchi, O. *J. Am. Chem. Soc.* **1999**, *121*, 849−855. (d) Zutshi, R.; Brickner, M.; Chmielewski, J. *Curr. Opin. Chem. Biol.* **1998**, *2*, 62−66.

(6) Frey, P. A.; Clelard, W. W. *Bioorg. Chem.* **1998**, *26*, 175−192.

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metal ions, depending on the oxidation state, will have defined spectroscopic properties. These properties can be used to obtain structural information about the recognition process.[8]

To demonstrate the pattern-matching concept for peptide recognition, we have designed and synthesized water-soluble peptides with three histidine moieties at particular distances apart (12−18 Å, **t_H**, **t_GH**, **t_AH**, **t_QH**, and **t_GGH**, Figure 1). This corresponds to the inter-histidine distances

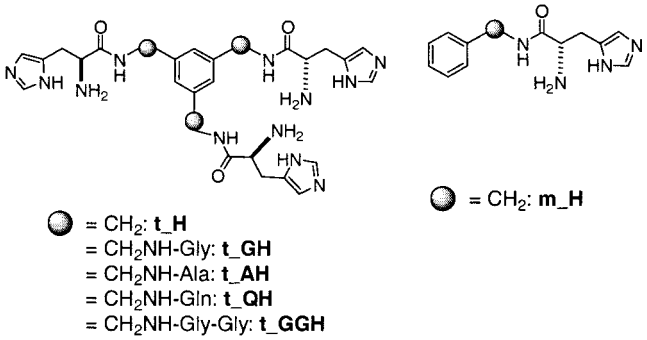


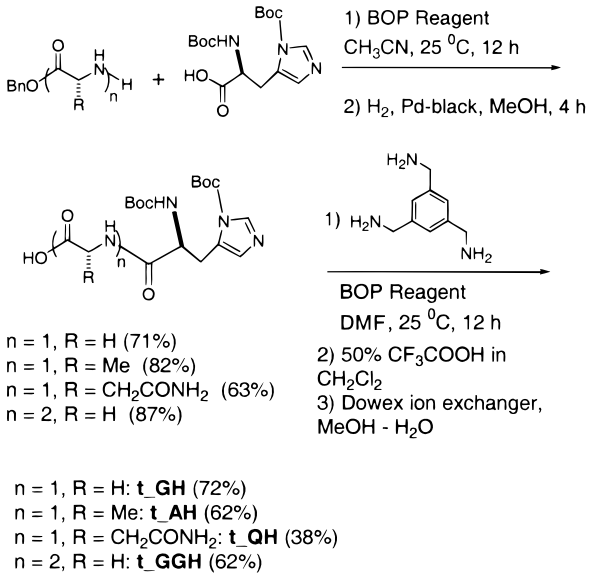
Figure 1. Structures of the histidine-containing peptides used for recognition in water (pH = 7.0).

(His 3, His 10, and His 15) on the surface of the protein carbonic anhydrase (bovine erythrocyte). The peptides were designed using the Spartan software (Version 5.0.3, Wavefunction Inc., Irvine, CA). The molecules were first energy minimized employing the Merck molecular mechanics force field. Then conformational searches (systematic) were performed to identify the lowest energy conformation(s) of the molecules. The mono histidine peptide **m_H** (Figure 1) was used as the control for the recognition studies.

From modeling, the distance among the imidazole groups of the histidines was found to increase by ∼4 Å when the peptide length increased by a glycine unit. The inter-histidine distances changed from 12 Å for **t_H** to 16−18 Å for **t_GH**. Because of flexibility, the estimated distance for **t_GGH** was measured to vary from 18 to 23 Å. The peptide **t_GGH** was designed to probe the role of flexibility and length in the recognition process. Insertion of another glycine unit (**t_GGGH**) decreased the water solubility, rendering it unsuitable for our studies. The peptide **t_AH** has the hydrophobic methyl group; **t_QH** has the hydrophilic amide moiety in the side chain. Replacing the alanine of **t_AH** with a phenylalanine (**t_FH**) made the peptide insoluble in water (pH = 7.0).

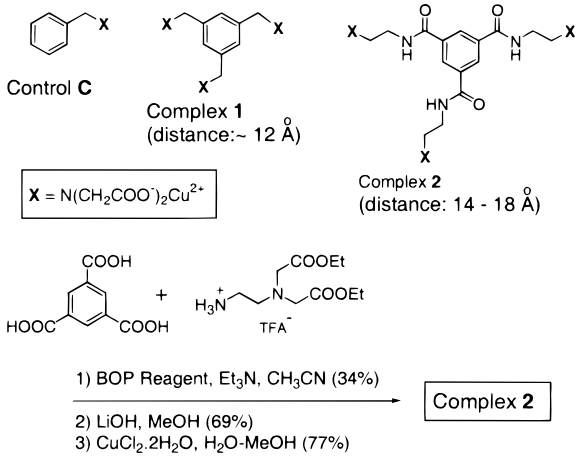
The syntheses of ligands[9] are depicted in Scheme 1. Benzyl ester derivatives of the appropriate amino acid were coupled with *N*,*N*-di-Boc histidine using the BOP reagent.[10] After deprotection of the benzyl group (H_2, Pd black), the resultant free acid was combined with 1,3,5-tris(aminomethyl)benzene.[11] Finally the Boc groups of histidines are cleaved by trifluoroacetic acid followed by ion exchange (Dowex macroporous resin, MeOH−H_2O as the eluant) to yield the free peptides.

Scheme 1. Synthesis of the Peptides



The transition metal complexes **1** and **2** (structures and synthesis are shown in Scheme 2) were designed and synthesized for selective and strong binding to these peptides. The distances among the three Cu^{2+} ions were estimated by molecular modeling (using the software Spartan, energy minimization employing the Merck molecular mechanics force field followed by systematic conformational search) and are also indicated in Scheme 2. Complex **1** can position three metal ions ∼12 Å apart. Complex **2** is flexible, and

Scheme 2. Structures of the Transition Metal Complexes and the Synthesis of Complex **2**



(7) Lippard, S. J.; Berg, J. M. *Principles of Bioinorganic Chemistry*; University Science Books: Mill Valley, CA, 1994.

(8) Kendrick, M. J.; May, M. T.; Plishka, M. J.; Robinson, K. D. *Metal in Biological Systems*; Ellis Horwood: Chichester, England, 1992; pp 17−48.

the distances among the cupric ions were estimated to be 14−18 Å. Complex **C** with one cupric ion was used as the control in these studies. The imminodiacetate−Cu^{2+} moiety was selected for these recognition studies in aqueous medium because of its wide use in affinity chromatography purification of proteins.[12] Elemental analyses indicated that **C** complexed one cupric ion while **1** and **2** incorporated three cupric ions. Determination of Cu^{2+} by UV/vis spectrometry (employing EDTA) for these complexes also indicated the proper number of cupric ions (Supporting Information).

Interactions of the peptides and the metal complexes were followed by isothermal titration microcalorimetry (ITC).[13] ITC is a rapid method of determining the binding constant (K), enthalpy (ΔH), and stoichiometry (n) of the interactions simultaneously. It has been used to study small-molecule interactions (including metal−ligand interactions),[14] protein−ligand interactions,[15] and protein−protein and other interactions.[16]

ITC experiments (Model ITC-4200 from Calorimetry Sciences, Provo, UT) were conducted in aqueous solutions (25 mM HEPES buffer, pH = 7.0, [peptide] = 0.80−5.0 mM, [Cu^{2+} complex] = 0.1−0.5 mM, 25.0 °C). The raw data were corrected for the heats of dilution of the appropriate peptides and processed using the software provided by the manufacturer (Bindworks 3.0). Typical raw and processed data are shown in Figure 2 (titration of **t_GH** with complex **2**).

The peptides have two potential sites for metal coordination: the α-amino and the imidazole moieties of the histidine residues. At pH = 7.0, the α-amino groups remain mostly protonated ($pK_a = 9.2$) while the imidazole nitrogen atoms are unprotonated ($pK_a = 6.8$). Experimental conditions were optimized with variation of pH, concentration of the buffer, and the identity of the buffer. It was found that the affinity was highest at pH = 7.0 (compared to pH = 6.0 and 8.0). Buffer identity (HEPES, MES, MOPS) and concentration (25 mM, 50 mM, 100 mM) did not affect the binding process.

Stability constants and the thermodynamic parameters of the interactions are shown in Table 1. The mono-histidine peptide **m_H** was also found to have low affinity for all the metal complexes (**C**, **1**, and **2**). Strong and selective binding

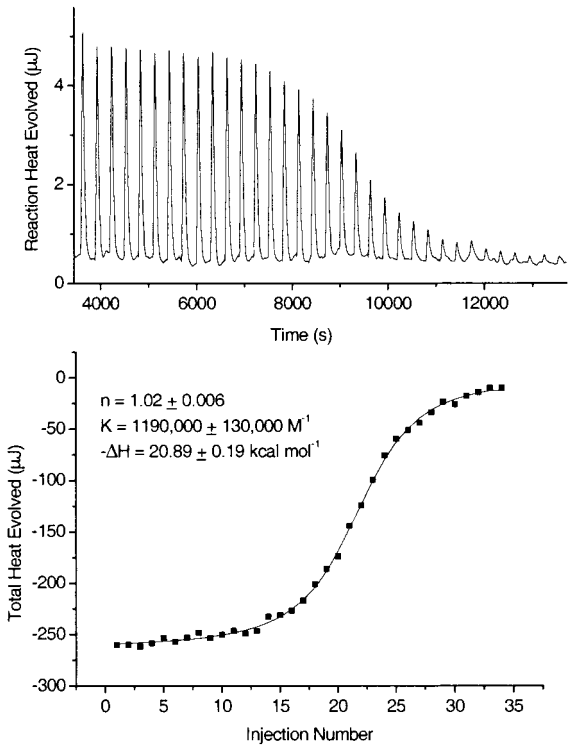


Figure 2. Raw and processed data for the titration of **t_GH** with complex **2** ([**2**] = 50 μM; [**t_GH**] = 600 μM; 25 mM HEPES buffer, pH = 7.0, 25.0 °C; 40 × 5 μL injections).

was achieved by employing the flexible metal complex **2**. With the distance-matched peptide (**t_GH**), a strong affinity (1.2×10^6 M^{-1}) was obtained. This appears to be due to a relatively low loss of entropy upon binding. A shorter peptide (**t_H**) or a longer peptide (**t_GGH**) was found to decrease

Table 1. Binding Constants, Enthalpy, Entropy, and the Stoichiometry of Binding for Complexes **C**, **1**, and **2** with the Histidine Peptides (25 mM HEPES buffer, pH = 7.0, 25.0 °C; 40 × 5 μL Injections)

system	n	$10^{-3}K$, M^{-1}	$-\Delta H$, kcal mol^{-1}	$-\Delta G$, kcal mol^{-1}	$-T\Delta S$, kcal mol^{-1}
C−m_H	1.12 ± 0.1	(4.5 ± 0.8)	11.6 ± 0.8	5.0	6.6
C−t_H	0.31 ± 0.01	(56 ± 12)	22.1 ± 1.5	6.5	15.7
C−t_GH	0.44 ± 0.06	(23.5 ± 2)	23.8 ± 0.5	6.0	17.8
1−m_H	3.89 ± 0.06	(9.5 ± 1)	6.9 ± 0.2	5.4	1.5
1−t_H	0.82 ± 0.01	(104.6 ± 32)	25.8 ± 0.9	6.8	18.9
1−t_GH	0.92 ± 0.01	(32.6 ± 3.7)	30.2 ± 0.7	6.1	24.1
1−t_GGH	1.25 ± 0.03	(50.3 ± 7.6)	21.9 ± 0.9	6.4	15.4
1−t_AH	1.06 ± 0.01	(15.4 ± 1.3)	29.6 ± 0.8	5.7	23.8
1−t_QH	0.61 ± 0.02	(77 ± 15)	32.7 ± 2.1	6.7	26.1
2−m_H	2.63 ± 0.21	(1.7 ± 0.2)	18.4 ± 2.5	4.4	14.0
2−t_H	0.80 ± 0.02[a]	(104.4 ± 22.8)	30.0 ± 1.5	6.8	23.2
2−t_GH	1.02 ± 0.006	(1190 ± 130)	20.8 ± 0.2	8.3	12.6
2−t_GGH	0.68 ± 0.003[a]	(243.6 ± 24.5)	28.0 ± 0.4	7.3	20.7
2−t_AH	0.61 ± 0.04[a]	(5.1 ± 1)	51.5 ± 6.8	5.0	46.3
2−t_QH	0.47 ± 0.03[a]	(41.1 ± 7.5)	71.0 ± 6.7	6.3	64.7

[a] Possibly reflects the formation of oligomeric species as the distances are not matched. No isobestic points were observed for these titrations using UV spectrometry.

(9) Characterization data for the peptides and the metal complexes are available as Supporting Information.

(10) Castro, B.; Dormoy, J.-R.; Dourtoglou, B.; Evin, G.; Selve, C.; Zieglar, J.-C. *Synthesis* **1976**, 751−757.

(11) Michcell, M. S.; Waller, D.-L.; Whelan, J.; Bosnich, B. *Inorg. Chem.* **1987**, *26*, 396−400.

(12) (a) Vunnum, S.; Natarajan, V.; Cramer, S. *J. Chromatogr. A* **1998**, *818*, 31−41. (b) Muller, K. M.; Arndt, K. M.; Bauer, K.; Pluckthun, A. *Anal. Biochem.* **1998**, *259*, 54−61. (c) Mahiou, J.; Abastado, J. P.; Cabanie, L.; Godeau, F. *Biochem. J.* **1998**, *330*, 1051−1058.

(13) (a) Doyle, M. L. *Curr. Opin. Biotech.* **1997**, *8*. 31−35. (b) Wadso, I. *Chem Soc. Rev.* **1997**, 79−86.

(14) (a) Berger, M.; Schmidtcher, F. P. *Angew. Chem., Int. Ed.* **1998**, *37*, 2694−2696. (b) Wu, C.; Chen, W.-Y.; Lee, J.-F. *J. Colloid Interface Sci.* **1996**, *183*, 236−242.

(15) (a) Taquet, A.; Labarbe, R.; Houssier, C. *Biochemistry* **1998**, *37*, 9119−9126. (b) Hileman, R. E.; Jennings, R. N.; Linhardt, R. J. *Biochemistry* **1998**, *37*, 15231−15237.

(16) (a) Ladbury, J. E.; Chowdhury, B. Z. *Biocalorimetry: Applications of Calorimetry in the Biological Sciences*; John-Wiley & Sons: New York, 1998. (b) Jelesarov, I.; Bosshard, H. R. *J. Mol. Recogn.* **1999**, *12*, 3−18. (c) Haq, I.; Trent, J. O.; Chowdhury, B. Z.; Jenkins, T. C. J. *Am. Chem. Soc.* **1999**, *121*, 1768−1779.

the binding constant. Higher loss of entropy offsets the enthalpy gain for these interactions.

A hydrophobic side chain on the peptide (**t_AH**) had a devastating effect on the affinity for the metal complexes (K ~15 000 for **1**; 5000 for **2**), again due to unfavorable entropy changes. Introduction of a hydrophilic side chain on the peptide (**t_QH**) also led to large entropy losses and consequently decreased the affinity for the metal complexes. With the flexible metal complex **2**, 1:1 binding was observed only when the distances were matched (**t_GH**). The stoichiometry was found to be less than 1 when the distances were not matched. This possibly indicates the formation of oligomeric species among the peptide and the metal complex **2** (high enthalpy but high entropy loss). On the other hand, the more rigid metal complex **1** was prone to forming 1:1 complexes with the peptides.

The binding constants are graphically depicted in Figure 3. The binding parameters are given in Table 1. A proposed

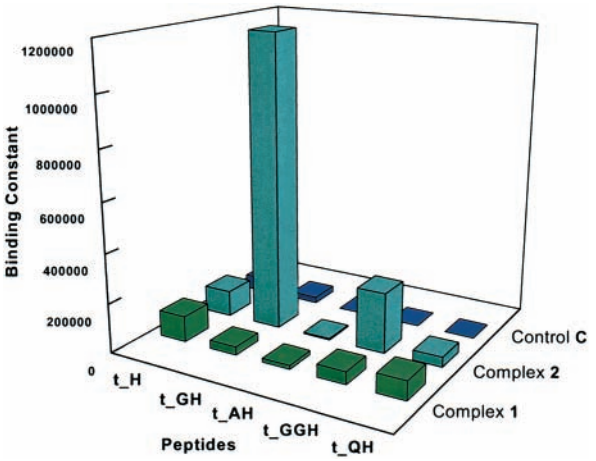


Figure 3. Binding constants for complexes **C**, **1**, and **2** with the histidine peptides (25 mM HEPES buffer, pH = 7.0, 25.0 °C).

structure of the complex **2**.**t_GH** is shown in Figure 4. The structure was generated by docking the individual structures (**2** and **t_GH**) using the MM3 force field of the modeling software CAChe (Oxford Molecular Inc.)

To demonstrate that the cupric ions are involved in binding, the systems were studied by EPR spectroscopy (9.445 GHz, field 2000−4000 G, 25 °C) in the solution phase (25 mM HEPES buffer, pH = 7.0). As an illustrative example, the value of $g_{||}$ was found to be 2.31 for complex **2** (0.8 mM solution). After addition of the peptide **t_GH** (1.6 mM), $g_{||}$ decreased to 2.25. These values matched well with the reported values for iminodiacetate−Cu^{2+} binding

(17) Dhal, P. K.; Arnold, F. H. *Macromolecules* **1992**, *25*, 7051−7059.
(18) Connors, K. A. *Binding Constants*; Wiley: New York, 1987; pp 141−215.

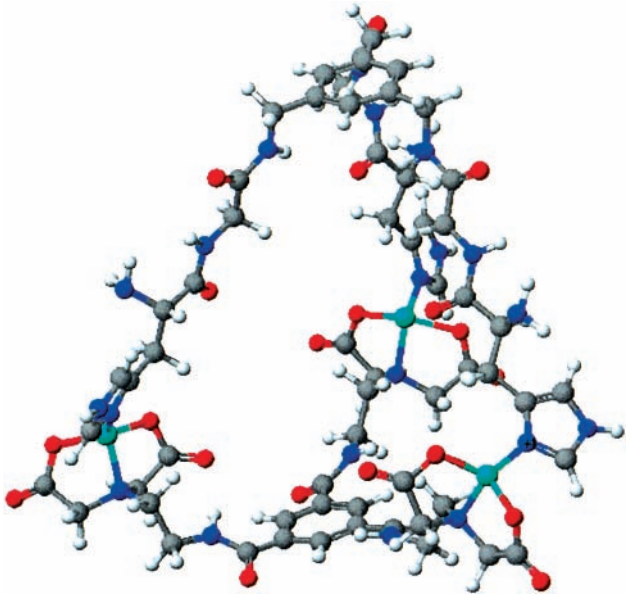


Figure 4. Proposed structure of **2**.**t_GH**.

to imidazole moieties of organic molecules.[17] In addition, ITC titrations between the peptides and ligands without Cu^{2+} ions failed to detect any binding.

The interactions between the metal complexes and the histidine-containing peptides were also followed using UV−vis spectrometry (25 mM HEPES buffer, 25 °C). The absorption maxima for the metal complexes (0.5 mM) shifted from 720 to 665 nm upon sequential addition of the peptides (2 mM, 10 × 100 μL additions). Analysis of the resultant titration curves[18] corroborated the stoichiometry and the binding constants obtained by ITC. The position of absorption maxima for the resultant complexes (665 nm) indicated that each cupric ion was coordinating to one histidine unit.[17]

In conclusion, metal complex **2** binds to its matched trihistidine (**t_GH**) strongly and selectively. Complex **2** favors **t_GH** compared to other peptides differing by only one amino acid unit (selectivity 230:1 with **t_AH**, 30:1 with **t_QH**), a shorter peptide (selectivity 10:1 with **t_GH**), or a longer peptide (selectivity 5:1 for **t_GGH**).

Acknowledgment. This work was supported by an NSF-CAREER award (CHE-9896083) and an NIGMS-AREA grant (1R15 59594-01, NIH). The microcalorimeter was purchased through an NSF-EPSCoR award to North Dakota.

Supporting Information Available: Characterization data for the peptides (^{1}H and ^{13}C spectra) and metal complexes (elemental analysis) and ITC titration data (raw and processed) for the results reported in Table 1. This material is available free of charge via the Internet at http://pubs.acs.org.

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