Metal Ion-Induced Hetero-Block α-Helical Coiled Coil

Ayumi Kashiwada,* Yohei Nakamura, and Kiyomi Matsuda*

Department of Applied Molecular Chemistry, College of Industrial Technology, Nihon University, Narashino, Chiba 275-8575

Received September 2, 2004; E-mail: a5kasiwa@cit.nihon-u.ac.jp

To construct polypeptides that undergo a hetero-block α-helical coiled coil structure change due to metal ion-induced self-assembly formation, we designed two kinds of 18-residue polypeptides, Pep3 and Pep4, each with a metal binding site. Pep3 and Pep4 were designed based on the sequence of the isoleucine zipper polypeptide forming the triple-stranded α-helical coiled coils. While Pep3 and Pep4 are too short to form a parallel triple stranded α-helical coiled coil individually, they form the hetero-block α-helical coiled coil structure, $(Pep3)_3$–Ni^{2+}–$(Pep4)_3$ in the presence of the Ni^{2+} ions. Circular dichroism spectroscopy and size-exclusion gel-filtration chromatography analyses revealed the formation of the $(Pep3)_3$–M^{n+}–$(Pep4)_3$ hetero-block α-helical coiled coil complex.

De novo design has suggested that polypeptides have been increasingly employed to gain new insights into the formation of particular protein structures from certain amino acid sequences. A considerable number of papers have been reported on amino acid sequences to form important structures, such as α-helical bundles,[1–3] β-sheets,[4,5] and α/β proteins.[6–8] In recent years, α-helical coiled coils have been focused on as the subjects of extensive protein designs.[9–18] The α-helical coiled coils are formed as an oligomerization motif, commonly occurring at the interface between separate protein chains. The α-helical coiled coils, which comprise two or more α-helices that wrap around one another, have been found in many cytoskeletal and contractile systems,[19] transcription regulators,[20] viral envelope proteins,[21–23] and so on.

In general, the hallmark of the coiled coil is a seven-residue heptad repeat, $(abcdefg)_n$, with a predominance of hydrophobic residues at the buried *a* and *d* positions, and charged residues frequently at the *e* and *g* positions.[11–13,18] The residues at the *e* and *g* positions contain oppositely charged residues to form stable inter-helice salt bridges.[14,18]

The designed α-helical coiled coils, which undergo a drastic change in their conformation in response to external stimuli, such as ligand binding and covalent modification, can be used as a tool to control the associations and functions of domains to attach to polypeptides. Metal binding among the various external stimuli can be considered an attractive approach. In recent years, many efforts have been extended to studies of metalloprotein structures. Thus, the factors required for metal binding have been well understood.[24–27]

There are several examples of *de novo* designed α-helical coiled coils induced by metal ions. For example, the driving force for the complex formation between a metal ion and a ligand, such as the bipyridyl group, contributes to the stabilization of the α-helical coiled coils.[28–30] The binding of lanthanoides to γ-caboxyglutamic acids at the solvent-exposed site has been intimately connected to the folding of double-stranded α-helical coiled coils.[31]

Isoleucine zipper polypeptides that form the triple-stranded α-helical coiled coil in an aqueous solution have been designed and prepared.[18] Based on the isoleucine zipper polypeptide (IZ), metal binding sites were constructed in the hydrophobic core of IZ. His or Cys residues were introduced into the hydrophobic position of IZ.[32–34] The polypeptides have a random structure in an aqueous solution in the absence of metal ions. On the other hand, in the presence of a metal ion, the polypeptides fold into the triple-stranded α-helical coiled coil structure.

In this paper, we report on the design of two kinds of 18-residue polypeptides that undergo metal ion-induced self-assembly to form a hetero-block α-helical coiled coil structure. This hetero-block system, drastically induced by a certain metal ion, will have a great possibility of breaking new ground in peptide engineering, such as a vectorial electron-transfer system and a metal ion-sensitive polypeptide hydrogel.

Results and Discussion

Design of Metal Induced Polypeptides. Pep1 consists of 37 amino acid residues containing the heptad, $I_aA_bA_cI_dE_eK$-$(E)_fK_g$ (Fig. 1). The Ile residues at the *a* and *d* positions form a hydrophobic core in the triple stranded coiled coil. Pep2 is a metal-binding polypeptide containing a –H–X–X–X–H– site inside the α-helical coiled coil structure. We substituted two His residues for the Ile residues at the 17*d* and 21*a* positions of Pep1, designated as Pep2 (Fig. 1). Similar isoleucine zipper polypeptides to Pep1 and Pep2 have already been designed and synthesized by Tanaka et al.[18,32]

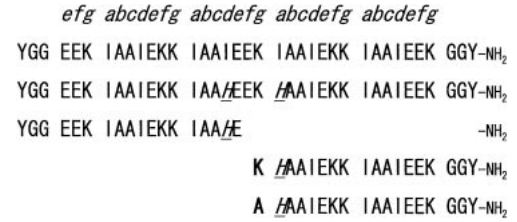

Fig. 1. Amino acid sequences of the metal ion-induced polypeptides used in this study.

Published on the web July 1, 2005; DOI 10.1246/bcsj.78.1291

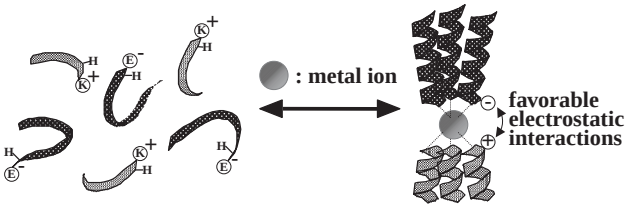

Fig. 2. Schematic view of the hetero-block α-helical coiled coil formation from Pep3 and Pep4 in the presence of a metal ion.

In this study, we designed a new metal ion-induced coiled coil self-assembly system using two kinds of short polypeptides, such as Pep3 and Pep4, containing a His residue, respectively. Pep3 and Pep4 have the same sequences of the 1st to 18th and 20th to 37th residues as Pep2 (Fig. 1). In this system, three Pep3s and three Pep4s form a hetero-block α-helical coiled coil structure, $(Pep3)_3$–M^{n+}–$(Pep4)_3$, in the presence of the metal ion (Fig. 2). The Pep2-metal complex forms a Λ facial isomer from the results of the CPK modeling around the metal binding site. In the $(Pep3)_3$–M^{n+}–$(Pep4)_3$ complex, we also made a CPK model. As a result, the $(Pep3)_3$–M^{n+}–$(Pep4)_3$ complex is considered to form the same Λ facial isomer as the Pep2-metal complex. In the metallopeptide, one face is formed by His residues of the three Pep3s, and the other face by His residues of the three Pep4s. On the other hand, due to the steric instability and the electric repulsions of hydrophilic amino acids around the metal binding site, the homo-block α-helical coiled coil structure, $(Pep3)_3$–M^{n+}–$(Pep3)_3$ or $(Pep4)_3$–M^{n+}–$(Pep4)_3$, should be destabilized.

A successfully designed analogue, which undergoes a metal ion-induced folding transition, should be so unstable that it is unfolded in the absence of the metal ion. Pep3 and Pep4 are too short to form a parallel triple stranded coiled coil individually. Therefore, Pep3 and Pep4 are expected to form a random coil structure in the absence of the metal ion.

Structural Characterizations of Designed Polypeptides. The CD spectra of Pep1 and Pep2 in a pH of 7.0 Tris-HCl buffer solution are shown in Fig. 3. Pep2 in the absence of metal ions was a random coil structure, with a CD minimum of around 200 nm. On the other hand, Pep2 in the presence of Ni^{2+} ions showed the α-helical structure, with CD minima at 208 and 222 nm. These results were the same as previous results of concerning similar isoleucine zipper polypeptides.[32]

The CD spectra of Pep3, Pep4, and an equimolar binary Pep3/Pep4 mixture are shown in Fig. 4. Neither the polypeptides nor the polypeptide mixture showed the α-helical structure in the absence of Ni^{2+} ions. These results suggest that α-helical coiled coil structures are difficult to be formed because of the short chains of the polypeptides (the Pep3 and Pep4). In contrast, in the presence of Ni^{2+} ions, all polypeptides samples showed the α-helical structure, with CD minima at 208 and 222 nm. The higher $[\theta]_{222}$ value for the equimolar binary mixture of the Pep3 and Pep4 was to be noted. These CD data indicate that the metal ion-induced α-helical coiled coil structure is constructed, and a more stable coiled coil consists of the Pep3 and Pep4 units. In Ni^{2+}-complexed Pep3/Pep4, the intensity of the CD spectrum was weaker than that of Ni^{2+}-complexed Pep2, indicating that the complex contained a partially unfolded coiled coil around the metal binding site. In contrast, the α-helical coiled coil structure formed from only Pep3 or Pep4 has less stability because of the steric hindrance and the electric repulsions of hydrophilic amino acids around the metal binding site. Furthermore, the CD spectrum of an equimolar binary Pep3/Pep5 mixture was measured in order to estimate the contribution of the electric interaction around the metal binding site. In the presence of Ni^{2+} ions the sample showed a less-stable α-helical structure (Fig. 5). This result indicated that the electric interaction between the *e* position at the C-terminal of the Pep3 and the *g* position at the N-terminal of the Pep4 played an important role in complex forming, as shown in Fig. 2.

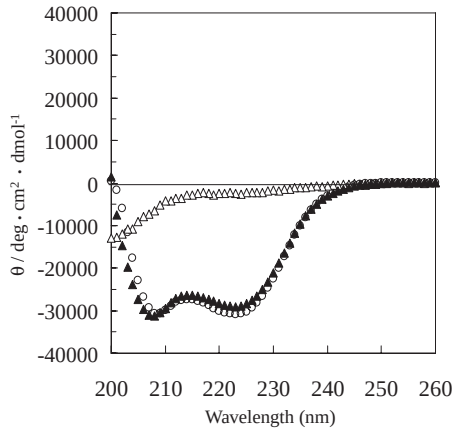

Fig. 3. Circular dichroism spectra of Pep1 (○) and Pep2 in the absence (△) and presence (▲) of $NiCl_2$. The measurements were performed in 10 mM sodium phosphate buffer containing 0.1 M NaCl (pH 7.0) at 20 °C. The polypeptide concentrations were 20 μmM.

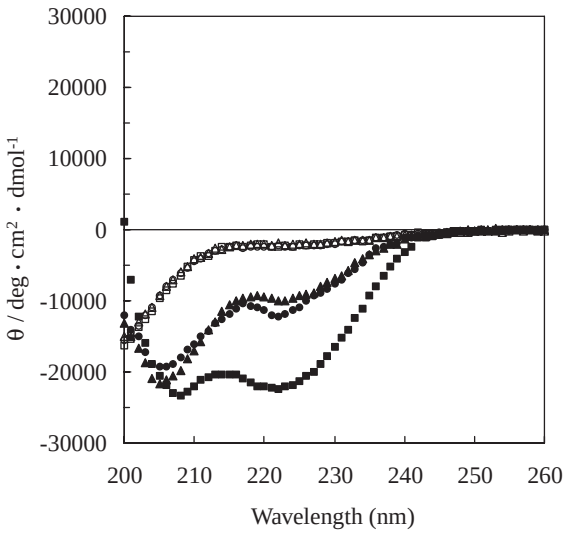

Fig. 4. Circular dichroism spectra of Pep3 (circles), Pep4 (triangles), and an equimolar binary Pep3/Pep4 mixture (squares) in the absence (open symbols) and presence (closed symbols) of $NiCl_2$. The measurements were performed in 10 mM sodium phosphate buffer containing 0.1 M NaCl (pH 7.0) at 20 °C. The polypeptide concentrations were 40 μM.

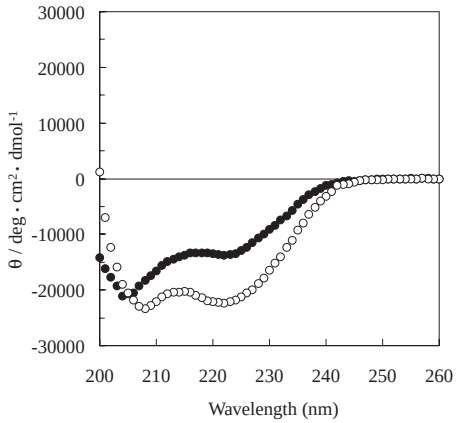

Fig. 5. Circular dichroism spectra of an equimolar binary Pep3/Pep5 mixture (●) and Pep3/Pep4 mixture (○) in the presence of $NiCl_2$. The measurements were performed in 10 mM sodium phosphate buffer containing 0.1 M NaCl (pH 7.0) at 20 °C. The polypeptide concentrations were 40 μM.

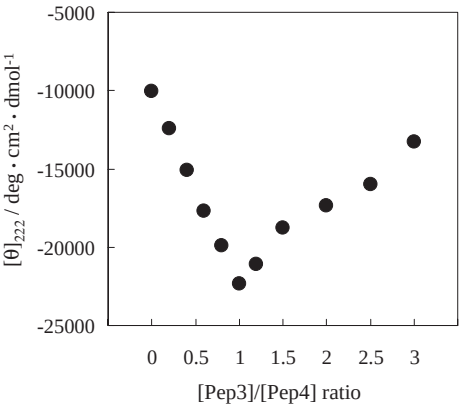

Fig. 6. The relationship between [Pep3]/[Pep4] ratio and the mean residue ellipticity at 222 nm in the presence of $NiCl_2$. The measurements were performed in 10 mM sodium phosphate buffer containing 0.1 M NaCl (pH 7.0) at 20 °C. The total polypeptide concentrations were 40 μM.

Figure 6 shows the $[\theta]_{222}$ values of a Pep3/Pep4 mixture with different compositions in the presence of Ni^{2+} ions. The $[\theta]_{222}$ value increased to a Pep3/Pep4 ratio of 1, and then decreased, suggesting that the Pep3 and Pep4 interacted strongly in a 1:1 (3:3) ratio.

The oligomerization state of polypeptide was determined by Sephadex G-50 gel filtration chromatography. In the presence of Ni^{2+} ions, the Pep2 was eluted at a fraction corresponding to the trimer, judging from the elution of Pep1. Furthermore, the oligomerization state of the equimolar binary mixture of Pep3 and Pep4 was analyzed. The mixture of Pep3/Pep4 showed the fraction corresponding to the hexamer in the presence of Ni^{2+} ions (Fig. 7). To determine the composition of the hexamer from the Pep3/Pep4 mixture, an HPLC analysis was practiced. The result showed that the eluted fraction of hexamer contained Pep3 and Pep4 in a ratio of 1.0:1.1 after normalization for the extinction coefficients, suggesting that Pep3 and

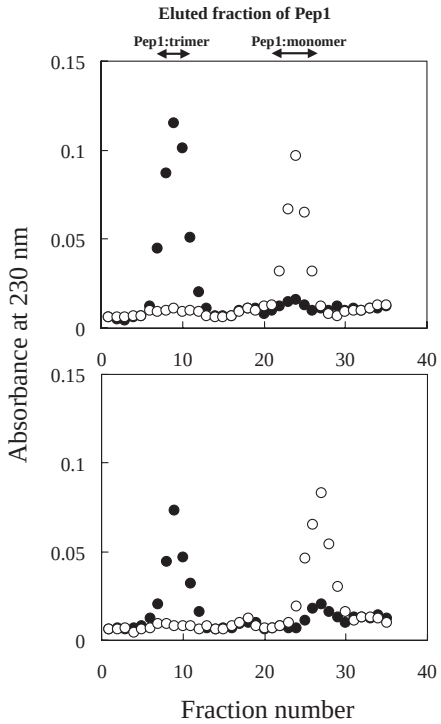

Fig. 7. Analysis of the eluted fraction of Pep2 (A) and an equimolar binary Pep3/Pep4 mixture (B) in the presence (●) or absence (○) of $NiCl_2$ using Sephadex G-50 column. The elution was performed in 10 mM sodium phosphate buffer containing 0.1 M NaCl and 40 μM $NiCl_2$ (pH 7.0) at 20 °C. The arrows indicate the eluted position of the standards, Pep1 in 10 mM sodium phosphate buffer (trimer) and Pep1 in 6 M guanidine hydrochloride solution (monomer).

Pep4 formed a stable hetero-block α-helical coiled coil structure, $(Pep3)_3$–Ni^{2+}–$(Pep4)_3$, in the presence of Ni^{2+} ions.

The thermal stability of the polypeptides in the presence of Ni^{2+} ions was investigated (Fig. 8). An equimolar binary mixture of Pep3 (T_m = 31 °C) and Pep4 (T_m = 28 °C) had a T_m of 43 °C. Thus, the mixture of Pep3 and Pep4 had a higher stability than that of single polypeptides. It is obvious from thermal stability measurement that the $(Pep3)_3$–Ni^{2+}–$(Pep4)_3$ complex was predominantly formed from the Pep3/Pep4 mixture.

$(Pep3)_3$–Ni^{2+}–$(Pep4)_3$ complex formation is highly dependent on the pH. Figure 9 shows a pH titration curve to determine the effect of the pH on complex formation. The pH titration curve was obtained by monitoring the $[\theta]_{222}$ value. As the pH was decreased, the α-helicity estimated from the $[\theta]_{222}$ value decreased. The folding transition took place over approximately from pH 5.5 to pH 7.0, with the transition midpoint occurred at about pH 6.0. The imidazolyl group of a His residue has a pK_a value of approximately 6.0, and so under an acidic condition the imidazolyl group is protonated and does not act as a ligand. The observed pH-dependent folding transition of the complex may correlate with protonation–deprotonation of the imidazolyl group of a His residue. This also indicates that the His residue of the Pep3 or Pep4 acts as a ligand for Ni^{2+}, and plays an important role in complex formation.

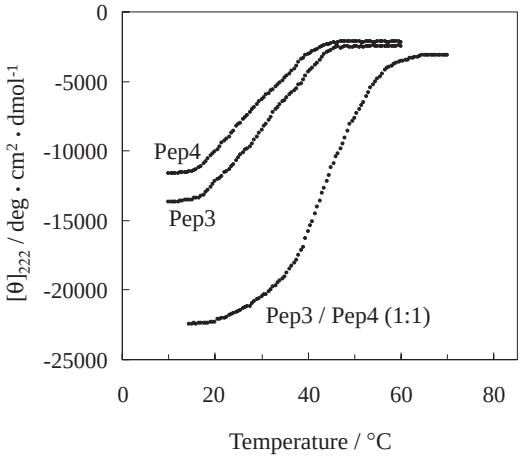

Fig. 8. Thermal melting curves of Pep3, Pep4, and an equimolar binary Pep3/Pep4 mixture in the presence of $NiCl_2$. The mean residue ellipticities at 222 nm are plotted as a function of temperature. The measurements were performed in 10 mM sodium phosphate buffer containing 0.1 M NaCl (pH 7.0). The polypeptide concentrations were 40 μM.

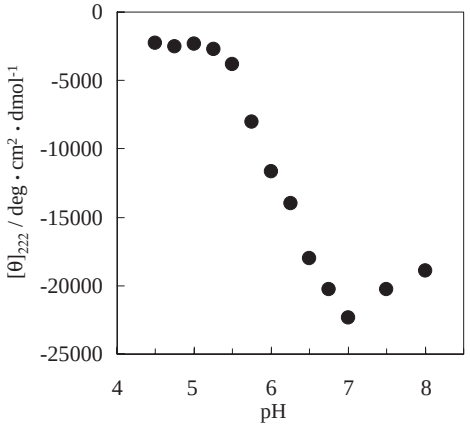

Fig. 9. The relationship between pH and the mean residue ellipticity at 222 nm of an equimolar binary Pep3/Pep4 mixture in the presence of $NiCl_2$. The measurements were performed in 10 mM sodium phosphate buffer containing 0.1 M NaCl (pH 7.0) at 20 °C. The total polypeptide concentrations were 40 μM.

Conclusion

The metal ion-induced hetero-block α-helical coiled coil was constructed by the two kinds of 18 residues short polypeptides based on an isoleucine zipper polypeptide. Double-stranded and triple-stranded helical bundles have been designed in response to a metal ion.[28–34] In contrast, a metal ion-induced coiled coil block structure has not been constructed. The hetero-block coiled coil assembled system can control two different functional domains responding to external stimuli. Therefore, this system has a great possibility of breaking new ground in peptide engineering.

Experimental

Peptide Synthesis. All polypeptides used in this study were synthesized by the solid-phase synthesis method using Rink amide resin, N^{α}Fmoc(9-fluorenylmethoxycarbonyl)-protected amino acids, HBTU (*N*-[(1*H*-benzotriazol-1-yl)(dimethylamino)methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide), and HOBt (1-hydroxybenzotriazole). Deprotection and cleavage of polypeptides from the resin were performed by a treatment with TFA (trifluoroacetic acid)/1,2-ethanedithiol/anisole/ethyl methyl sulfide (93/1/3/3, v/v) for 2 h. Following the cleavage reaction, polypeptides were purified by a reverse-phase HPLC on a YMC-Pack ODS-A column (10 mm i.d. × 250 mm, 5 μm, YMC Inc., Japan) eluted at 4 cm^3/min. with linear acetonitrile/water gradients containing 0.1% (v/v) TFA over the course of 30 min. The final products were characterized by analytical HPLC and MALDI-TOF mass spectrometry, *m*/*z*: 3971 for Pep1 (calculated 3971); 4018 for Pep2 (calculated 4018); 1954 for Pep3 (calculated 1954); 1953 for Pep4 (calculated 1952); 1897 for Pep5 (calculated 1896).

Circular Dichroism (CD) Measurements. All CD measurements were performed on a Jasco J-820 spectropolarimeter by using a 2 mm path-length cuvette. The polypeptide concentration was determined from the tyrosine absorbance at 275 nm in 6 M guanidine hydrochloride solutions.[35] The mean residue ellipticity, $[\theta]$, is given in deg·cm^2·dmol^{-1}.

CD spectra were measured in a 10 mM sodium phosphate buffer containing 0.1 M sodium chloride (pH 7.0) in the presence or absence of Ni^{2+} ions (40 μM of nickel(II) chloride). The polypeptide concentrations were 20 μM for Pep1 and Pep2, and 40 μM for Pep3 and Pep4.

The effect of the Pep3/Pep4 ratio on the α-helical content was determined to monitor $[\theta]_{222}$ as a function of the Pep3/Pep4 ratios in the range between 0 and 3. The total polypeptide concentration was 40 μM.

The thermal transition curves were monitored $[\theta]_{222}$ as a function of the temperature with a 10 mm path length cuvette. The temperature was increased at a rate of 0.5 °C/min. The total polypeptide concentration was 40 μM.

The effect of the pH on the α-helical content was determined to monitor $[\theta]_{222}$ as a function of pH from 4.5 to 8.0.

Size Exclusion Gel Filtration Chromatography. The polypeptide samples were dissolved in 0.15 cm^3 of sodium phosphate buffer (10 mM, pH 7.0, containing 0.1 M sodium chroride) in the presence of Ni^{2+} ions (40 μM of nickel(II) chloride). The samples were applied to a Sephadex G-50 column (0.6 (i.d.) × 11 cm), and were eluted with the same buffer containing 40 μM of nickel(II) chloride at pH 7.0. One eluted fraction of 0.1 cm^3 was collected and monitored at a wavelength of 230 nm. The total polypeptide concentration was 80 μM.

This work has been partly supported by a Grant for High Technology Research Projects, Minister of Education, Culture, Sports, Science and Technology of Japan.

References

1 S. P. Ho and W. F. DeGrado, *J. Am. Chem. Soc.*, **109**, 6751 (1987).

2 T. M. Handel, S. A. Williams, and W. F. DeGrado, *Science*, **261**, 879 (1993).

3 R. B. Hill, D. P. Raleigh, A. Lombardi, and W. F. DeGrado, *Acc. Chem. Res.*, **33**, 745 (2000).

4 A. Pessi, E. Bianchi, A. Crameri, S. Venturini, A. Tramontano, and M. Sollazzo, *Nature*, **362**, 367 (1993).

5 J. S. Nowick, *Acc. Chem. Res.*, **32**, 287 (1999).
6 M. Beuregard, K. Goraj, V. Goffin, K. Heremans, E. Goormaghtigh, J. Ruysschaert, and J. A. Martial, *Protein Eng.*, **4**, 745 (1991).
7 A. N. Fedorov, D. A. Dolgikh, V. V. Chemeris, B. K. Chernov, A. V. Finkelstein, A. A. Schulga, Y. B. Alakhov, M. P. Kirpichnikov, and O. B. Ptitsyn, *J. Mol. Biol.*, **225**, 927 (1992).
8 T. Tanaka, H. Kimura, M. Hayashi, Y. Fujiyoshi, K. Fukuhara, and H. Nakamura, *Protein Sci.*, **3**, 419 (1994).
9 S. Y. M. Lau, A. K. Taneja, and R. S. Hodges, *J. Biol. Chem.*, **259**, 13253 (1984).
10 K. T. O'Neil and W. F. DeGrado, *Science*, **250**, 646 (1990).
11 J. C. Hu, E. K. O'Shea, P. S. Kim, and R. T. Sauer, *Science*, **250**, 1400 (1990).
12 R. S. Hodges, *Curr. Biol.*, **2**, 122 (1992).
13 P. B. Harbury, T. Zhang, P. S. Kim, and T. Alber, *Science*, **262**, 1401 (1993).
14 N. E. Zhou, C. M. Kay, and R. S. Hodges, *Protein Eng.*, **7**, 1365 (1994).
15 R. S. Hodges, *Biochem. Cell Biol.*, **74**, 133 (1996).
16 S. F. Betz, P. A. Liebman, and W. F. DeGrado, *Biochemistry*, **36**, 2450 (1997).
17 J. S. Johansson, B. R. Gibney, J. J. Skalicky, A. J. Wand, and P. L. Dutton, *J. Am. Chem. Soc.*, **120**, 3881 (1998).
18 K. Suzuki, H. Hiroaki, D. Kohda, and T. Tanaka, *Protein Eng.*, **11**, 1051 (1998).
19 C. L. Day and T. Alber, *J. Mol. Biol.*, **301**, 147 (2000).
20 A. Tropsha, J. P. Bowen, F. K. Brown, and J. S. Kizer, *Proc. Natl. Acad. Sci. U.S.A.*, **88**, 9488 (1991).
21 Y. G. Yu, D. S. King, and Y. K. Shin, *Science*, **266**, 274 (1994).
22 M. Rabenstein and Y. K. Shin, *Biochemistry*, **34**, 13390 (1995).
23 W. Weissenhorn, L. J. Calder, A. Dessen, T. Laue, J. J. Skehel, and D. C. Wiley, *Proc. Natl. Acad. Sci. U.S.A.*, **94**, 6065 (1997).
24 L. Regan, *Trends Biochem. Sci.*, **20**, 280 (1995).
25 G. R. Dieckmann, D. K. McRorie, D. L. Tierney, L. M. Utschig, C. P. Singer, T. V. Ohalloran, J. E. PennerHahn, W. F. DeGrado, and V. L. Pecoraro, *J. Am. Chem. Soc.*, **119**, 6195 (1997).
26 C. E. Laplaza and R. H. Holm, *J. Am. Chem. Soc.*, **123**, 10255 (2001).
27 K. B. Musgrave, C. E. Laplaza, R. H. Holm, B. Hedman, and K. O. Hodgson, *J. Am. Chem. Soc.*, **124**, 3083 (2002).
28 A. Y. Kornilova, J. F. Wishart, and M. Y. Ogawa, *Biochemistry*, **40**, 12186 (2001).
29 M. Gochin, V. Khorosheva, and M. A. Case, *J. Am. Chem. Soc.*, **124**, 11018 (2002).
30 A. Fedorova, A. Chaudhari, and M. Y. Ogawa, *J. Am. Chem. Soc.*, **125**, 357 (2003).
31 W. D. Kohn, C. M. Kay, B. D. Sykes, and R. S. Hodges, *J. Am. Chem. Soc.*, **120**, 1124 (1998).
32 K. Suzuki, H. Hiroaki, D. Kohda, H. Nakamura, and T. Tanaka, *J. Am. Chem. Soc.*, **120**, 13008 (1998).
33 X. Li, K. Suzuki, K. Kanaori, K. Tajima, A. Kashiwada, H. Hiroaki, D. Kohda, and T. Tanaka, *Protein Sci.*, **9**, 1327 (2000).
34 T. Kiyokawa, K. Kanaori, K. Tajima, and T. Tanaka, *Biopolymers*, **55**, 407 (2000).
35 J. R. Brandts and K. J. Kaplan, *Biochemistry*, **12**, 2011 (1973).