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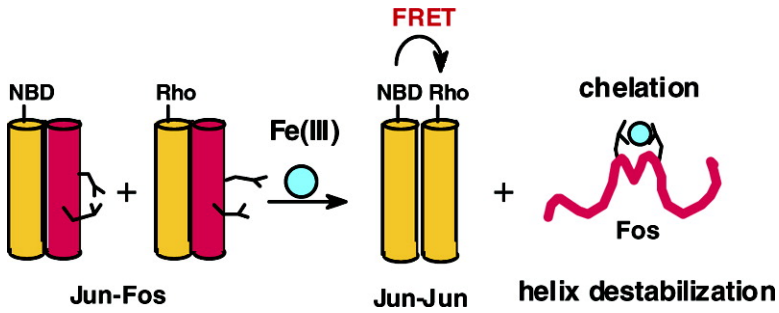
J. Am. Chem. Soc., **2004**, 126 (48), 15762-15769• DOI: 10.1021/ja046870o • Publication Date (Web): 12 November 2004



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Control of Peptide Structure and Recognition by Fe(III)-Induced Helix Destabilization

Shiroh Futaki,* Tatsuto Kiwada, and Yukio Sugiura*

Contribution from the Institute for Chemical Research, Kyoto University, Uji, Kyoto 611-0011, Japan

Received May 27, 2004; E-mail: futaki@scl.kyoto-u.ac.jp; sugiura@scl.kyoto-u.ac.jp

Abstract: Helical peptide segments that change their conformation due to external stimuli have often been employed in peptide-based molecular devices and materials. Using helices containing a pair of the iminodiacetic acid derivatives of lysine (Ida), we show that metal-induced helix destabilization is a promising approach to functional switching, especially for helices that are intrinsically stable. By *i* and $i + 2$ positioning of the Ida residues in a 17-residue model peptide, a significant decrease in the helical content was observed by the addition of Fe(III), whereas Fe(II) had no influence on the stability of the helix. The possibility of redox control of the helical structure was exemplified by the reduction of Fe(III) to Fe(II) using $Na_2S_2O_4$ followed by the subsequent reoxidation. Mutual recognition of the transcription factor Jun-derived leucine-zipper peptide segment with the Fos leucine-zipper segment containing Ida residues was also modulated in the presence of Fe(III).

Introduction

The α-helix is one of the essential components in the structural formation and mutual recognition of proteins, and the eventual exertion of their biological functions. Control of the stability and association of helices is therefore among the major challenges in peptide engineering aimed at developing peptide-based novel devices and materials.[1−20] To stabilize the helical structure, approaches have been reported that employ covalent cross-linking such as the disulfide or the amide formation.[2−8] Approaches using a noncovalent interaction such as ion pairs and metal chelates have also been introduced.[9−16] These bondings have most often been designed to be formed between amino acids located at the *i* and $i + 4$ positions in order to stabilize the helical structures. These approaches are especially useful when the designed helices do not have a tendency of favoring helix formation in the absence of cross-linking. Alternatively, considerable structural changes have been observed for the peptides bearing azobenzene and related cross-linking according to their cis−trans transition by light irradiation,[17−20] where the one structure stabilizes the helical structures and the other destabilizes them. If peptide segments with a strong helix formation tendency can be destabilized by metal chelation, these systems can be applicable to novel modules for switching protein conformation and mutual recognition. This approach is especially useful when the helical structure is stable enough and the metals do not significantly contribute to increasing the helix stability.

As a metal chelate system, we focused on the systems employing a couple of iminodiacetic acid derivatives of lysine (Ida) as a model (Figure 1). Ruan et al. reported that the alignment of two Ida groups having the same orientation to the helices, i.e., at positions *i* and $i + 4$, stabilized the helical structures in the presence of various divalent metals such as Co(II), Ni(II), Cu(II), Zn(II), and Cd(II).[10,11] Hamachi utilized

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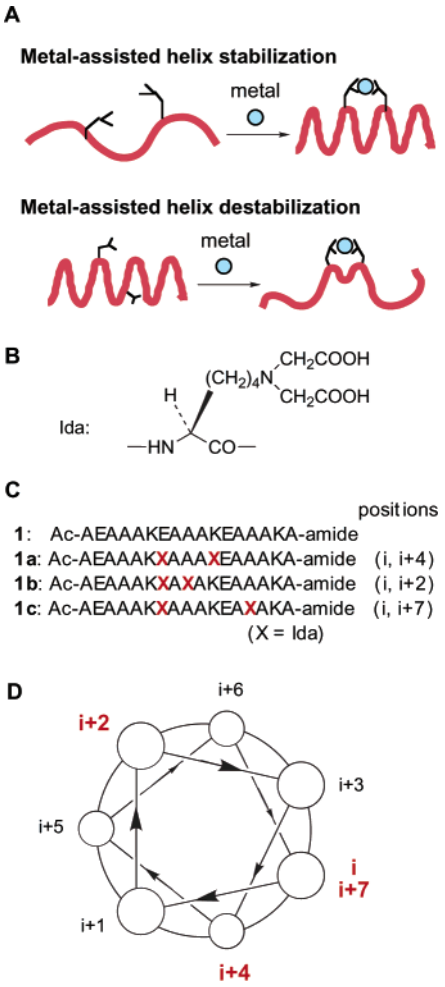

Figure 1. Schematic representation of metal-assisted helix stabilization and destabilization (A), and structures of the iminodiacetic acid derivative of lysine residue (Ida) (B), and Ida-containing helical peptides **1a**−**c** (C) (Ac = acetyl). Topologies of positions $i + 2$, $i + 4$, and $i + 7$ versus position i are shown in helical wheel projection (D), where 3.5 amino acid residues form a turn of the helix instead of the typical 3.6 residues for simplification. Abbreviations for the amino acid residues are: X, Ida; A, Ala; E, Glu; K, Lys.

this system to switch the enzyme activity of RNase S where the S peptide containing two Ida residues made a complex with the S-protein;[12−14] the RNase activity was switched on and off in accordance with the stabilization of the helical structure of the S peptide by the addition of Cu(II) and its depletion by ethylenediaminetetraacetic acid (EDTA).[12] As mentioned above, most of the approaches using a metal switch are intended to switch on by the addition of metals with the eventual stabilization of the helical structures. If *destabilization* of the helices by the addition of metals can be utilized to switch the function on, the range of possible designs of switchable molecules will be widened.

In this study, to ascertain if helix destabilization can be a driving force of functional switching, we studied the relationships between the positions of the iminodiacetic acid pairs in the helical peptides and the their conformation in the presence and absence of Fe(III). Simultaneously, we found that a metal switch can also be accomplished by the redox regulation of the Fe(III)−Fe(II) transition. We then applied this idea to the recognition switch of leucine zipper proteins, Jun and Fos, which were evaluated using fluorescent resonance energy transfer

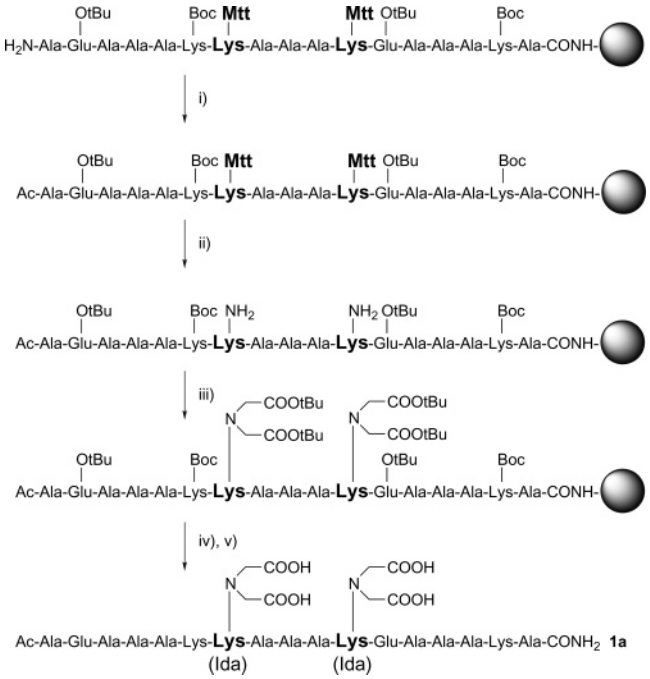

Figure 2. Preparation of Ida-containing peptide **1a** using direct conversion of Lys to Ida on solid-phase resin. (i) Ac_2O, diisopropylethylamine (DIEA)/DMF, 20 °C, 1 h; (ii) 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP)−DCM (1:4), 20 °C, 3 h; (iii) Bu_4NI, $BrCH_2COOtBu$, DIEA/DMF, 20 °C, 3 h; (iv) TFA−ethanedithiol (95:5), 20 °C, 2 h; (v) HPLC. Mtt = 4-methyltrityl.

(FRET). To facilitate the preparation of the helical peptides, we have newly developed an approach to convert lysine to Ida in one pot on the Fmoc-solid-phase resin.

Results and Discussion

Facile Synthesis of Peptides Bearing Iminodiacetic Acid Groups. For the synthesis of peptides which contain the iminodiacetic-acid-derivatized amino acids (Ida), Ruan and Hamachi, respectively, used a strategy where the N^{α}-Boc and Fmoc derivatives were first prepared and the peptide segments were then constructed by solid-phase peptide synthesis.[10−14] However, the preparation of these derivatives involved several steps of time-consuming reactions and purification, which may hamper the accessibility of this approach even though the approaches to control the peptide/protein function using Ida seem very attractive. To facilitate the preparation of the Ida-containing peptides, we developed an alternative approach in which the lysines are directly converted to their iminodiacetic acid derivatives, namely Ida, on the solid phase resin. The Ida-containing peptides can thus be very easily obtained.

Our approach employs the commercially available 4-methyltrityl derivative of lysine [Lys(Mtt)] (Figure 2).[21] After completion of the peptide chain construction on the Fmoc-solid-phase resin, the Mtt group of lysine is selectively removed by the treatment of 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) in dichloromethane (DCM) (1:4), where other protecting groups and peptide anchoring on the resin remain intact.[22] The peptide resin with the free ϵ-amino groups of lysine is then treated with

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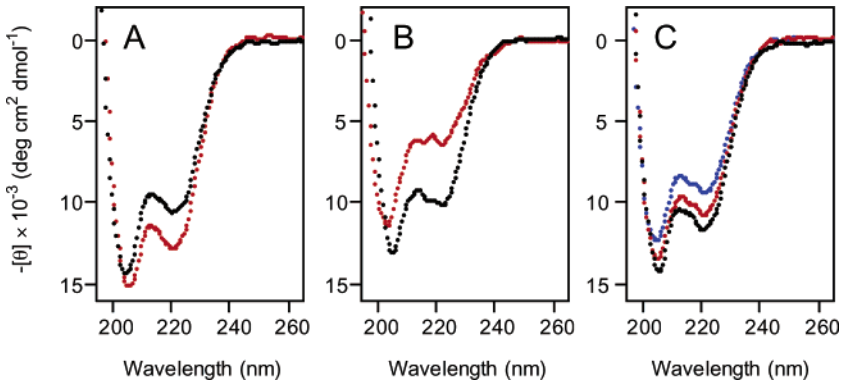

Figure 3. CD spectra of peptides **1a**−**c** in the absence (black) and presence of equimolar Fe(III) (red) at pH 7.0. (A) **1a**; (B) **1b**; (C) **1c**. Peptides, 100 μM; Fe(III) ($FeCl_3$), 100 μM; solvent, H_2O; temperature, 20 °C. Blue in part C, in the presence of 500 μM $FeCl_3$.

tetra-*n*-butylammonium iodide (Bu_4NI) and *tert*-butylbromoacetate in the presence of diisopropylethylamine (DIEA) at 20 °C to achieve complete diiminoacetylation of the ϵ-amino group of lysine. Treatment of the peptide resin containing *tert*-butyl esters of the Ida moieties with trifluoroacetic acid (TFA) in the presence of ethanedithiol (EDT) will give the desired Ida-containing peptides. The ϵ-amino groups of the lysines, which should not be converted to Ida, are protected with the Boc group. To ascertain the feasibility of this approach, we prepared a 17-residue model peptide **1a** in which Ida is placed at positions 7 and 11 (Figure 1C).

The peptide chain of **1a** was constructed on the Rink amide resin[23] using the Fmoc-solid-phase method.[24,25] The peptide resin was treated with HFIP-DCM (1:4) at room temperature for 3 h, followed by Bu_4NI (20 equiv), $BrCH_2COOtBu$ (50 equiv), and DIEA (25 equiv) at room temperature for 3 h. Any unreacted reagents were easily removed by a dimethylformamide (DMF) wash of the peptide resin. Subsequent treatment with TFA−EDT (95:5) at room temperature for 2 h and reverse-phase high performance liquid chromatography (RP-HPLC) purification gave the desired peptide **1a** in high purity. The total yield based on the starting resin was 33%. Based on the HPLC analysis of the final purification, the conversion of Lys to Ida was judged to be substantially quantitative, since no major peaks corresponding to the peptides due to the inadequate conversion from Lys to Ida were observed (see Supporting Information Figure S1). Thus, using our approach, the Ida-containing peptide was conveniently synthesized in one pot in a sufficient yield.

Effect of the Topology of Ida on the Stability of Helical Structures. The design of the Ida-containing peptide **1a** was based on the peptide **1** which was designed by Marqusee and Baldwin to form a monomeric helical structure in water by stabilization through salt bridges between Glu and Lys at the *i* and $i + 4$ positions (Figure 1).[26] To assess the topological effect of Ida in the absence and presence of metal ions on the structure of the peptides, which has a preference for helical conformation, two more analogues of **1**, i.e., peptides **1b** and **1c**, were synthesized. Peptide **1a** has two Ida residues in the middle of the molecule which are placed at positions 7 and 11 (i and i+4). These positions correspond to one turn of the helix, and as have been often reported, are expected to stabilize the helical conformation via chelate or cross-link formation between the two Ida moieties. In contrast, peptide **1b** has Ida residues at positions 7 and 9 (i and i+2), where the position of the first Ida is the same as **1a** but the second Ida locates to position 9 which corresponds to the other side of the helix (see Figure 1D). If the tendency of helix formation of the peptide by itself is too high, the peptide would still form a helical structure even in the presence of metal ions. However, when the effect of the stabilization by the chelation is more predominant, the helical structure of the peptide should be destabilized by the addition of metals. The peptide **1c** has Ida moieties at positions 7 and 14 (*i* and $i + 7$). These two Ida moieties would come to the same side of the helix but could be separated by two turns of the helix. Jackson et al. reported that disulfide formation between 2-amino-6-mercaptohexanoic acid at *i* and $i + 7$ of the α-helix significantly improved the helical stability.[2] It would be interesting whether chelate formation between the same positions also increases the stability of the helix. As for the metal ion, we have employed Fe(III); there is a considerable difference in the stability among the Fe(III)− and Fe(II)−EDTA complexes [log K_{ML} 25.1 for Fe(III) and 14.3 for Fe(II)].[27] The conversion of the oxidation states of the iron ion may lead to a structural change in the above Ida-containing peptides, and therefore, redox control of the helical structures may be expected.

The effect of chelate formation on the peptide conformations of **1a**, **1b**, and **1c** was examined. Figure 3 shows the CD spectra of an aqueous solution of these peptides (pH 7.0) at 20 °C in the absence and presence of Fe(III). In the absence of Fe(III), these peptides produced spectra of predominantly α-helical structures with double minima around 208 and 222 nm. The $[\theta]_{222}$ values, which are a measure of the α-helical content,[28] of these peptides were -1.1×10^4, -1.0×10^4, and -1.2×10^4 deg cm^2 dmol^{-1}, respectively. On the other hand, $[\theta]_{222}$ of peptide **1** under the same conditions was found to be -1.8×10^4 deg cm^2 dmol^{-1} (see Figure S2 in Supporting Information). These results suggested that the replacement of the amino acids of **1** by the Ida moieties resulted in a reduction of the helical content; however, the helical structure itself was retained in the peptides **1a**−**c**. The addition of an equimolar amount of Fe(III) ($FeCl_3$) to the peptide solutions caused a specific effect on the peptide structures. In the case of peptide **1a**, the helical tendency increased to give a $[\theta]_{222}$ value of -1.3×10^4 deg cm^2 dmol^{-1},

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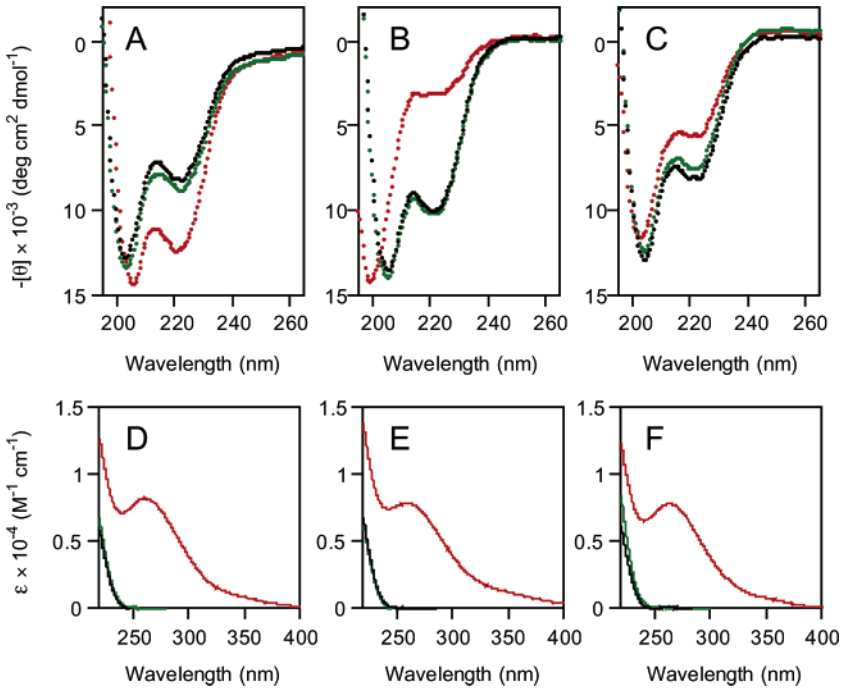

Figure 4. CD (A−C) and UV−vis (D−F) spectra of peptides **1a**−**c** in the presence and absence of Fe(III) and Fe(II) at pH 3.8. (A and D) peptide **1a**; (B and E) **1b**; (C and F) **1c**. Solvent, H_2O; temperature, 20 °C. Black, in the absence of metal; red, in the presence of Fe(III) ($FeCl_3$) (100 μM); green, in the presence of Fe(II) ($FeCl_2$) (100 μM).

an almost 18% increase from that in the absence of Fe(III). On the other hand, a considerable decrease in the helical contents by the addition of Fe(III) was observed for peptide **1b** to yield a decrease in the helical content of 34% ($[\theta]_{222}$: -6.6×10^3 deg cm^2 dmol^{-1}). The effect of Fe(III) on the peptide structure was not significant in the case of **1c** to give a decrease in helicity of 8% ($[\theta]_{222}$: -1.1×10^4 deg cm^2 dmol^{-1}); even the addition of 5 equiv of Fe(III) gave a decrease of 23% ($[\theta]_{222}$: -9.3×10^3 deg cm^2 dmol^{-1}).

Peptide **1** has been designed to possess equivalent numbers of lysine and glutamic acid as charged residues (three residues each) to give a neutral net charge at pH 7. In the case of peptide **1a**, a glutamic acid and a lysine are replaced by Ida residues. In the case of peptides **1b** and **1c**, a glutamic acid and an alanine are replaced. Since the respective Ida residue bears two carboxylates in its side chain, peptides **1a**−**c** can have at most 3−4 negative net charges at pH 7, which may produce some effect on the stability of the helices. Therefore, we examined the effect of helix stabilization and destabilization by Fe(III) at pH 3.8, where a lower degree of repulsion among the negative charges of carboxylates is expected. In the aqueous solution of peptides **1** and **1a**−**c** at pH 3.8, the $[\theta]_{222}$ values or the helical contents of these peptides were slightly lower than those at pH 7.0 in the absence of Fe(III) (Figure 4A−C).[29] However, the effect of the addition of Fe(III) on the peptide structures was significantly greater than that of pH 7.0. In the case of peptide **1a**, the helical content increased by almost 50% with the addition of Fe(III) ($[\theta]_{222}$ in the absence and presence of 100 μM Fe(III): -8.3×10^3 and -1.2×10^4 deg cm^2 dmol^{-1}, respectively). The decrease in the helical contents by the addition of Fe(III) for the peptides **1b** and **1c** was 68% ($[\theta]_{222}$ in the absence and presence of Fe(III): -1.0×10^4 and -3.2×10^3 deg cm^2 dmol^{-1}) and 30% ($[\theta]_{222}$ in the absence and presence of Fe(III): -8.6×10^3 and -6.0×10^3 deg cm^2 dmol^{-1}), respectively.

The chelate formation of these peptides in the above solution (pH 3.8) with Fe(III) was ascertained by observation of the UV−vis spectra (Figure 4D−F). The absorption spectra after the addition of Fe(III) ($\lambda_{max} = 260$ nm, $\epsilon = 8280$ M^{-1} cm^{-1}) was very similar with that reported for the Fe(III)−ethylenediaminetetraacetic acid (EDTA)−H_2O complex.[30] Thus an Fe(III)-induced conformational switch was attained by disposition of the Ida moieties to appropriate positions in the helices. Even when the Ida residues were placed at positions i and $i + 2$, they formed a complex with Fe(III) that produced a significant destabilization on the helical structure and the extent of conformation change was the largest among the three peptides examined. Although there were different effects on the addition of Fe(III) for the peptides **1a**−**c**, the UV−vis spectra of the respective peptides, producing almost the same ϵ values with each other (**1b**: $\lambda_{max} = 258$ nm, $\epsilon = 7935$ M^{-1} cm^{-1}; **1c**: $\lambda_{max} = 264$ nm, $\epsilon = 7910$ M^{-1} cm^{-1}), suggested that the differences were not due to the inefficient chelate formation but the positions of the Ida residues in the peptides.

Possibility of Redox Regulation of the Helical Structure. Helical structures of the peptides **1a**−**c** were significantly affected by the addition of Fe(III). On the other hand, the effect of the addition of an equimolar amount of Fe(II) on the CD spectra of **1a**−**c** was negligible as has been expected (Figure 4A−C). In their UV−vis spectra, absorption around 260 nm was also not observed (Figure 4D−F). To assess the possibility of redox control of the helical structure, $Na_2S_2O_4$ (10 equiv) was added to the aqueous solution of the complex of **1b** and Fe(III) at pH 3.8 (Figure 5, left). Reduction of Fe(III) to Fe(II)

(29) Similarly, a 17% decrease in the helicity at pH 3.8 was observed for peptide **1** when compared at pH 7.0 (see Figure S2 in Supporting Information).

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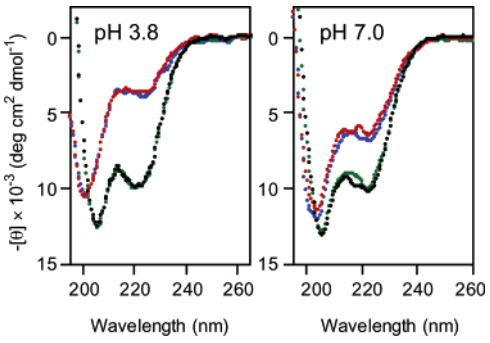

Figure 5. Reversible conformational switch of the peptide **1b** by the Fe(III)−Fe(II) transition at pH 3.8 and 7.0. Peptide, 100 μM in H_2O; temperature 20 °C. Black, in the absence of metal; red, in the presence of Fe(III) ($FeCl_3$, 100 μM); green, reduction by 1mM $Na_2S_2O_4$; blue, reoxidation by vortex.

released the peptide from the structural restriction by the metal chelation and produced a CD spectrum that was essentially identical to that obtained in the absence of metal ions (Figure 5). The air oxidization of the iron ion by vortexing the peptide solution again gave a CD spectrum comparable with that of the **1b**−Fe(III) complex, suggesting that the helical structure was reversibly controlled in accordance with the transition of the oxidized and reduced states of the iron ion. A similar transition was also observed in the UV−vis spectra (data not shown). Reversible control was also possible at pH 7.0 (Figure 5, right).

Modulation of Jun−Fos Mutual Recognition by Fe(III)-Induced Destabilization of Fos Structure. The above results indicated that, by using Ida residues, effective control of the helical structure was possible. Especially, in contrast to the previous reports on the metal-mediated stabilization of helical structures, we have pointed out that a metal switch could be used to destroy helical structures, as was shown for peptide **1b** in which Ida residues were placed at the i and $i + 2$ positions. As our second application of this approach, the switch of the interaction between the leucine zipper segments derived from the transcription regulatory proteins Jun and Fos was studied. It has been reported that, in the absence of Fos, Jun forms a homodimer, whereas in the presence of Fos, Jun preferentially forms a heterodimer with Fos.[31,32] The leucine zipper segments of these proteins are responsible for their mutual recognition. These leucine zipper segments form stable coiled-coil helical structures. Therefore, the destruction of helical structure of the Fos-derived leucine zipper segment may lead to the recognition switch of the Jun−Fos heterodimer to produce the Jun−Jun homodimer (Figure 6A).

Peptide **2a** was designed based on the Fos leucine zipper segment **2**, which was reported to preferentially form a heterodimer with the leucine zipper segment from Jun **3** both through the hydrophobic interaction between leucine and other hydrophobic residues at positions d and a and the electrostatic interaction between amino acids at positions e and g of these peptide segments.[31−33] In **2a**, two Ida residues are placed at positions 19 and 21, and the Ida residues were arranged into positions c (originally A) and e (originally Q) so as not to interfere with the possible salt bridges or hydrophobic interaction among the two peptide segments. Peptide **2a** was similarly prepared as already mentioned without difficulty.

The effect of Fe(III) on the helical conformation of **2a** was determined (Figure 7). The CD spectra of peptide **2a** (30 μM) gave a double minima around 208 and 222 nm, suggestive of helical conformations in the absence of the Jun peptide as has been already reported.[31] A significant effect of the pH was also observed on the destabilization of the helical structures of peptide **2a**. At pH 3.8, the addition of 1 equiv of Fe(III) gave a 50% decrease in helicity, whereas only a 12% decrease by the same amount of Fe(III) at pH 7.0. More than 5 equiv of Fe(III) were necessary to attain a decrease of 50% (Figure 7).[34] Although the higher degree of conformational switching of peptide **2a** by Fe(III) was induced at pH 3.8 rather than at pH 7.0, neutral pH was employed in the latter part because the detailed ways of recognition between Jun and Fos peptides have been studied using neutral solutions.

We have also examined the effect of Fe(III) on helix destabilization of the peptides when Ida residues were placed at different pairs of i and $i + 2$ positions in other regions of the helices (**2b** and **2c**, Figure 6B). Although there was a significant difference in the helical contents of the respective peptide segments in the absence of Fe(III), the degree of helix destabilization by 5 equiv of Fe(III) was similar (~50%) for each peptide (Figure 8).

We next examined whether this metal-mediated helical conformation switch of the Fos peptide is applicable to the recognition switch between the Jun and Fos leucine zipper peptides. To monitor the recognition switch, we have synthesized nitrobenzofurazan (NBD)-labeled and tetramethylrhodamine (Rho)-labeled Jun peptides **3a** and **3b** (Figure 6B). For Ida-containing Fos peptides, **2a** was employed here, since **2b** and **2c** have substitutions in amino acids that may affect hydrophobic and electrostatic interactions and eventual heterodimer formation between the Jun and Fos peptides. The Jun peptide **3** is reported to form a homodimer having a dissociation constant in the submicromolar range in the absence of the Fos peptide.[31,32] When an equimolar amount of **3a** and **3b** is employed, homodimer formation between these peptides was monitored by analysis of the subsequent FRET (Figure 9A). As mentioned above, the Jun peptide preferentially forms a heterodimer with the Fos peptide over the homodimer formation. When **2a** is added to the above system, preferential formation of the Jun−Fos heterodimer can be expected, which would result in observing a decrease in FRET. The addition of Fe(III) to the solution would destabilize the helical structure of Fos peptide **2a**. Subsequent failure in the heterodimer formation of with Jun would regenerate Jun−Jun homodimers, the formation of which will be observable through increase of the FRET efficiency between **3a** and **3b** (Figure 9A).[35]

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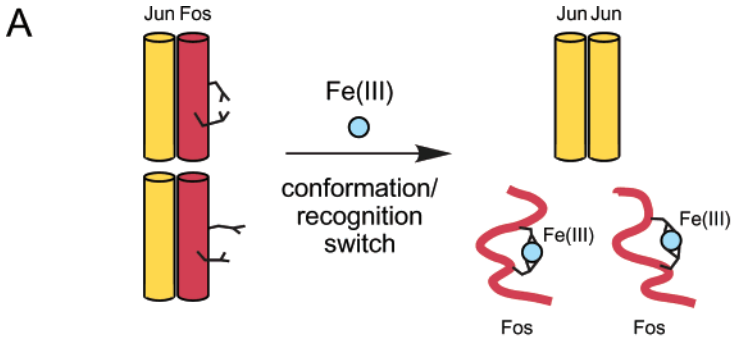
(34) The presence of buffer seems to weaken the effects of Fe(III) on the helix destabilization. When 10 mM (3-morpholino)propanesulfonic acid (MOPS) containing 100 mM NaCl was employed, almost 10 equiv of Fe(III) were needed to destabilize the helical conformation of **2a**−**c** by ~50%, presumably because MOPS can interact with Fe(III) to hinder the complex formation with the peptide (data not shown).
(35) The peptide segment corresponding to the Jun leucine zipper domain was constructed on a Rink amide resin using Fmoc-solid-phase peptide synthesis. After the N-terminus was labeled with tetramethylrhodamine or NBD using 5(6)-carboxytetramethylrhodamine succinimidyl ester and 4-chloro-7-nitrobenzofurazan, the peptide resins were treated with TFA−EDT (95:5) at room temperature for 2 h and then purified by HPLC.



B

2: Ac-GLTDTLQAETDQLEDKKSALQTEIANLLKEKEKLEFILAAY-amide
2a: Ac-GLTDTLQAETDQLEDKKS**X**L**X**TEIANLLKEKEKLEFILAAY-amide
2b: Ac-GLTDTLQAETDQLE**X**K**X**SALQTEIANLLKEKEKLEFILAAY-amide
2c: Ac-GLTDTLQAETDQLEDKKSALQT**X**I**X**NLLKEKEKLEFILAAY-amide

3: Ac-GRIARLEEKVKTLKAQNSELASTANMLREQVAQLKQKVMNY-amide
3a: NBD-GRIARLEEKVKTLKAQNSELASTANMLREQVAQLKQKVMNY-amide
3b: Rho-GRIARLEEKVKTLKAQNSELASTANMLREQVAQLKQKVMNY-amide

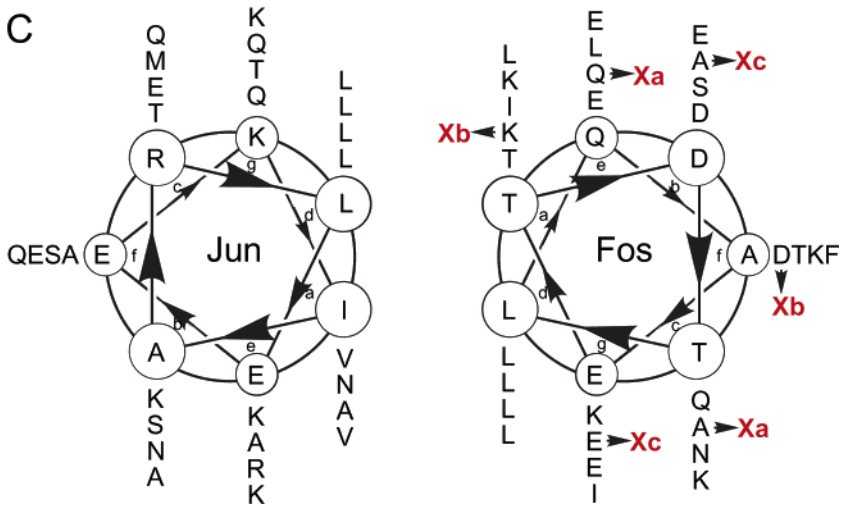


Figure 6. Design of the Fe(III)-induced recognition switch system. Schematic representation of Jun−Fos recognition switch through helix destabilization of an Ida-containing Fos peptide in the presence of Fe(III) (A). Peptide sequences of **2**, **2a**, **2b**, and **2c** (derived from Fos) and **3**, **3a**, and **3b** (from Jun) (X = Ida) (B). Schematic representation of the positions of Ida in Jun−Fos heterodimer forming a parallel coiled coil (C). Abbreviations for the amino acid residues are as follows: X, Ida; A, Ala; D, Asp; E, Glu; F, Phe; G, Gly; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; and Y, Tyr. **Xa**, **Xb**, and **Xc** represent the position where amino acids in the Fos peptide **2** are substituted by Ida in peptide **2a**, **2b**, and **2c**, respectively.

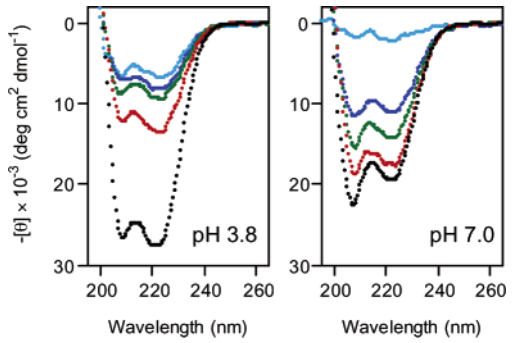


Figure 7. Effect of pH on the structural destabilization of peptide **2a** by Fe(III) at pH 3.8 and 7.0. Peptide, 30 μM in H_2O containing 10 mM NaCl; temperature 20 °C. Black, in the absence of metal; red, green, blue, and light blue in the presence of Fe(III) ($FeCl_3$, 30, 90, 150, and 300 μM, respectively).

Formation of the Jun−Jun homodimer was assessed by the experimental FRET efficiencies (E_{FRET}) (see Experimental Section and ref 36), $E_{FRET} = 1 - I_{DA}/I_D$. An equimolar mixture of NBD-Jun **3a** and Rho-Jun **3b** (6 μM each) in the absence of peptide **2a** gave an E_{FRET} of 0.26, suggesting homodimer formation (Figure 9B and C). The addition of peptide **2a** (12 μM, 1 equiv to total amount of Jun peptides) yielded only a slight decrease in E_{FRET}; however, E_{FRET} in the presence of 5 equiv of the peptide **2a** (60 μM) became 0.12, suggestive of the switch from the Jun−Jun homodimer to the Jun−Fos heterodimer. The subsequent addition of Fe(III) to the above mixture again allowed the recovery of E_{FRET}. Fe(III) (2 or 3 equiv) added to peptide **2a** (final concentration of Fe(III), 120 or 180 μM) yields an E_{FRET} value comparable to that in the absence of the peptide **2a** (Jun−Jun homodimer). Therefore, mutual recognition of the peptides and eventual association states were able to be controlled using the Ida containing peptide and Fe(III).

Conclusion

In this study, we have shown that peptide structures can be effectively stabilized or destabilized in the presence of Fe(III) by the appropriate positioning of Ida residues in helical peptides.

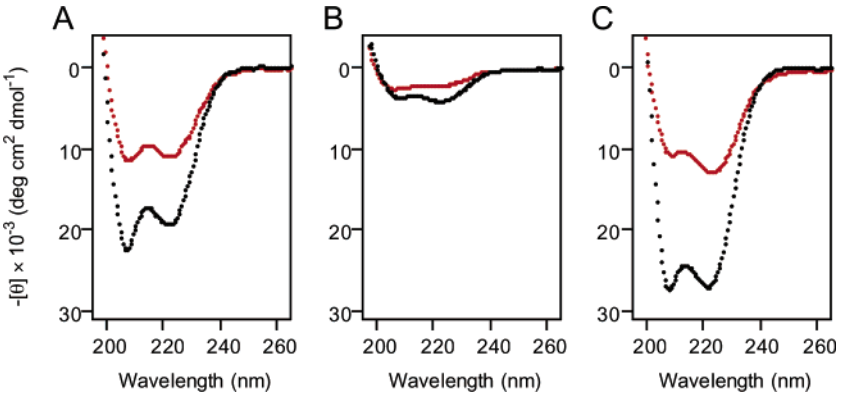


Figure 8. Effect of Ida positions on Fe(III)-induced helix destabilization. CD spectra of peptides **2a** (A), **2b** (B), and **2c** (C) are shown. Peptides, 30 μM in H_2O containing 10 mM NaCl (pH 7.0). Black, in the absence of metal; red, in the presence of Fe(III) ($FeCl_3$, 150 μM).

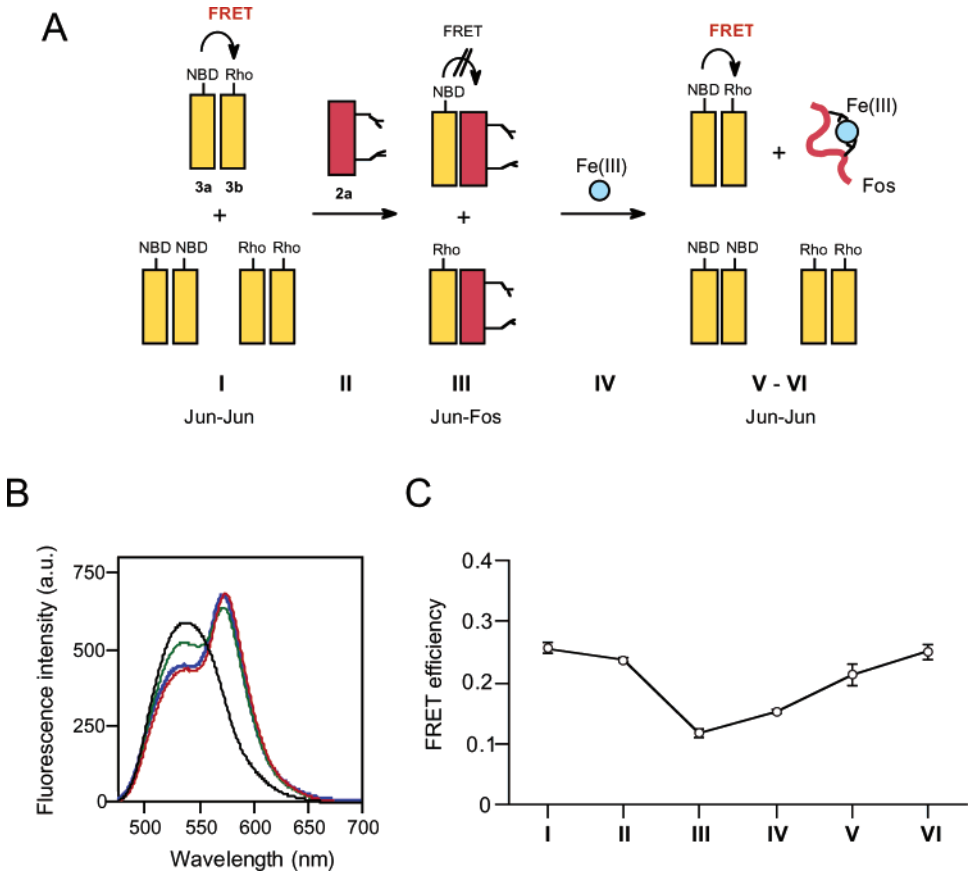


Figure 9. Schematic representation of Fe-mediated recognition switch of Jun and Fos (A) and FRET efficiency (C). **I**, NBD-Jun **3a** and Rho-Jun **3b** (6 μM each in H_2O containing 10 mM NaCl, pH 7.0) in the absence of Fos peptide **2a**; **II** and **III**, in the presence of **2a** (12 μM and 60 μM, respectively); **IV**−**VI**, in the presence of **2a** (60 μM) after addition of Fe(III) ($FeCl_3$) (60, 120, 180 μM, respectively). Fluorescence spectra corresponding to the association states **I** (red), **III** (green), and **VI** (blue) and the spectrum of **3a** (black) are shown in part B. Error bars in part C represent the mean ± standard error of 3 trials.

The helix destabilization approach using metals is a promising approach to switch the peptide conformation and function, especially when the peptides have a strong tendency for helical formation. Since Fe(II) failed to make a chelate with the Ida-containing peptides, redox regulation of the helical structure of the Ida-containing peptides was possible in accordance with the transition between Fe(III) and Fe(II). The recognition switch of the leucine zipper peptide segment from Jun was also achieved using an Ida-containing Fos peptide **2a** in the presence and absence of Fe(III), which was assessed using FRET between the NBD- and Rho-labeled Jun peptides **3a** and **3b**. In addition, we have established a novel approach for preparing Ida-containing peptides using the conversion of Lys to Ida on the Fmoc-solid-phase resin. The concept of structural switching by helix destabilization as illustrated in this study would open new possibilities for the design of functional molecules and devices.

Materials and Methods

General. The Fmoc-amino-acid derivatives and peptide resin were purchased from NovaBiochem. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOFMS) was measured using an Applied Biosystems Voyger-DE STR. High performance liquid chromatography (HPLC) conditions used for the characterization of the synthetic peptides are as follows: column, Cosmosil 5C18-AR-300 (4.6 × 150 mm); eluate, A = H_2O containing 0.1% CF_3COOH,

B = CH_3CN containing 0.1% CF_3COOH; flow, 1 mL/min; detection, 215 nm. UV−vis absorption spectra were recorded on a Beckman DU640 spectrometer at 20 °C. CD spectra were obtained using a Jasco J-720 spectrometer at 20 °C. For the experiments using Fe(II), the solvent was deoxygenated by an N_2 purge for 30 min prior to dissolving the peptides.

Preparation of Ida-Containing Peptides. The peptide chain of **1a** was constructed by Fmoc-solid-phase peptide synthesis on a Rink amide resin using a Shimadzu PSSM8 peptide synthesizer with its standard protocol. A benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP)[37]/1-hydroxybenzotriazole (HOBt)/4-methylmorpholine (NMM) coupling system was employed as the coupling system. As amino acid derivatives, Fmoc-Ala, Fmoc-Glu(O*t*Bu), Fmoc-Lys(Boc) (for Lys) and Fmoc-Lys(Mtt) (for Lys that is converted to Ida) were employed. The N-terminal of the peptide resin was acetylated using acetic anhydride in the presence of NMM. After construction of the protected peptide resin, the Mtt group was removed from the peptide resin by the treatment of HFIP−DCM (1:4) at 20 °C for 3 h. Subsequent treatment of the peptide resin with tetra-*n*-butylammonium iodide (20 equiv), bromoacetic acid *tert*-butyl ester (50 equiv) and DIEA (25 equiv) in DMF (20 °C for 3 h) gave a peptide resin bearing the *tert*-butyl esters of Ida. The final deprotection of the peptide resin using trifluoroacetic acid (TFA)−ethanedithiol (EDT) (95:5) at 20 °C for 2 h followed by reverse-phase HPLC purification gave the desired sample **1a**. Yield from the starting resin, 33%. MALDI-TOFMS: 1845.9 [Calcd for $(M + H)^+$: 1845.0]. Retention time in HPLC, 14.0 min (gradient: 10−40% B in A over 30 min). Other Ida-containing peptides were similarly prepared as stated above. The yields and characterization data are shown in the Supporting Information.

Preparation of NBD-Labeled Jun Peptide 3a. The peptide chain was prepared using Fmoc-solid-phase peptide synthesis on Rink amide resin as mentioned above. The N-terminal of the peptide resin was treated with 4-chloro-7-nitrobenzofurazan (NBD-Cl) (50 equiv) and DIEA (50 equiv) at 20 °C for 6 h. The peptide resin was then treated with TFA−EDT (95:5) at 20 °C for 2 h, and HPLC purification gave the desired sample. Yield from starting resin, 3%. MALDI-TOFMS: 4853.5 [Calcd for $(M + H)^+$: 4852.5]. Retention time in HPLC, 12.5 min (gradient: 30−70% B in A over 40 min).

Preparation of Rho-Labeled Jun Peptide 3b. This peptide was similarly prepared as **3a** except that the N-terminal was treated with 5(6)-carboxytetramethylrhodamine N-hydroxysuccinimidyl ester (1 equiv) and DIEA (1 equiv) at 20 °C for 6 h instead of NBD-Cl and DIEA. Yield from starting resin, 18%. MALDI-TOFMS: 5102.7 [Calcd for $(M + H)^+$: 5101.9]. Retention time in HPLC, 12.4 min (gradient: 30−70% B in A over 40 min).

Fluorescence Resonance Energy Transfer (FRET).[36] The fluorescence analyses of the peptides were carried out using a RF-5300PC fluorescence spectrophotometer (Shimadzu) in a right-angle geometry. The fluorescence spectra were collected over a broad range of emission wavelengths (λ_{ex} = 464 nm and λ_{em} = 220−800 nm for the FRET spectrum with spectral bandwidths of 5 nm). Experimental FRET efficiencies were obtained using

$$E = 1 - I_{DA}/I_D$$

where I_{DA} and I_D are the measured donor fluorescence intensities at 535 nm in the presence and in the absence of acceptor, respectively.

Acknowledgment. This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Supporting Information Available: Synthetic data of peptides of **1b−c** and **2a−c**, HPLC of the peptide **1a**, and CD spectra of the peptide **1** at pH 3.8 and 7.0 are shown. This material is available free of charge via the Internet at http://pubs.acs.org.

JA046870O

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