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**PAPER**

# Dipicolylamine as a unique structural switching element for helical peptides†‡

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Developing novel methods for metal-induced switching of peptide structures expands the design principles of functional biomolecules and biomaterials. Here, a simple method for on-resin synthesis of dipicolylamine (Dpa)-containing peptides was developed. Whereas addition of divalent metal ions such as Fe(II) and Cu(II) to a peptide bearing a pair of Dpa moieties at the *i* and *i* + 4 positions led to the formation of a 1 : 1 complex of Dpa with metals, addition of Ni(II) yielded a cross-linked structure of Dpa–metal (2 : 1). This feature was utilized for the selective detection of Ni(II) using the peptide–Fe(II) complex. Repeated switching of the helical structure was also achieved by multiple additions of divalent metal ions to the peptide.

## Introduction

The design of a structurally switchable peptide is a key area of research in peptide engineering, with the goal of producing sophisticated molecular devices and biomaterials.[1–3] Specific modification with chelating moieties yields peptides with the ability to alter their function in response to the presence of metal ions.[2,3] Previously, we reported the successful control of peptide recognition, channel current gating and DNA binding through a structural switch composed of peptides modified with iminodiacetic acid (Ida) moieties.[3] Increasing the availability of other chelating systems would increase design flexibility in creating novel switching systems. The present study describes the applicability of dipicolylamine (Dpa) (Fig. 1b) a typical chelating moiety used in metal sensor systems and labelling reagent[4] for use as a metal ion-triggered structural switching in helical peptides.

(a)

**1** : Ac-WEAAAREAAAREAAARA-$NH_2$

**2** : Ac-WEAAARXAAAXEAAARA-$NH_2$

**3** : Ac-WEAAARZAAAZEAAARA-$NH_2$

(b)

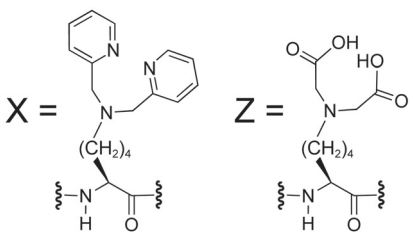


**Fig. 1** (a) Amino acid sequences of the peptides. (b) Structure of a Dpa (X) or Ida (Z)-containing amino acid residue.

## Results and discussion

Cross-linkage of peptide side chains at *i* and *i* + 4 positions is a widely used strategy to stabilise helical peptides.[5] Ida-mediated cross-linking at these positions is also highly effective for stabilising helical structures.[2d,g] To assess the usefulness of Dpa-mediated cross-linking, we examined the feasibility of *i* and *i* + 4 stabilisation in the presence of metal ions. A well-characterised peptide monomer that adopts a helical structure in water was employed as the test peptide (**1**).[6] A pair of Dpa moieties were introduced into this peptide at the *i* and *i* + 4 positions (**2**) (Fig. 1a). For preparation of peptide **2**, we developed a novel and convenient method for the on-resin conversion of lysine to Dpa (Scheme 1). The peptide chain was constructed on the resin using standard Fmoc-based solid-phase peptide synthesis (SPPS) with acetylation of the N-terminus. After removal of the 4-methyltrityl (Mtt) groups by hexafluoroisopropanol (HFIP)–dichloromethane (DCM) (1 : 4),[3a] the peptide resin was treated successively with 2-pyridinecarboxaldehyde and $NaBH(AcO)_3$ to convert the ε-amino moiety of lysine to Dpa. This was then followed by final deprotection with trifluoroacetic acid (TFA)–ethanedithiol (EDT) (95 : 5). Reversed phase HPLC

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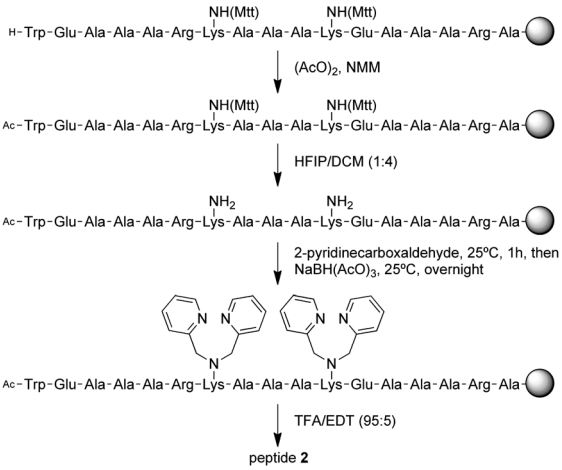
‡ Electronic supplementary information (ESI) available: CD spectra of peptides. See DOI: 10.1039/c2ob07118e




**Scheme 1** Synthesis of a peptide bearing two Dpa moieties. Mtt = 4-methyltrityl group on the ε-amino group of lysine.

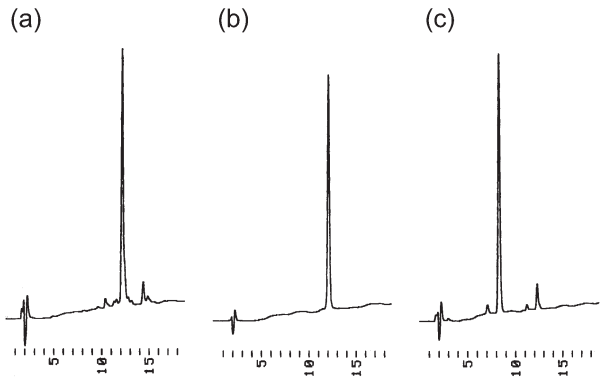



**Fig. 2** HPLC chromatograms of (a) crude peptide **2** after deprotection and detachment from the resin using TFA–EDT (95 : 5) and (b) purified **2**. The purity of the peptide before on-resin conversion of the ε-amino moiety of Lys to Dpa (c) was comparable to that of the sample shown in (a). Column: Cosmosil 5C18-AR-II (4.6 × 150 mm), eluate: A = $H_2O$ containing 0.1% TFA, B = $CH_3CN$ containing 0.1% TFA, gradient: 20%–35% B in A over 15 min, flow: 1.0 mL $min^{-1}$, detection: absorbance at 215 nm. For (c), the peptide was cleaved from part of the resin by TFA–EDT (95 : 5).

(RP-HPLC) and matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOFMS) analyses indicated the complete conversion of lysine to Dpa with no prominent side reactions producing peptide **2** in satisfactory yield (total yield of peptide from resin: 30%). Fig. 2 shows HPLC chromatograms of a purified sample and the crude peptide after TFA treatment. As all of the processes were carried out on a solid-phase support, no purification of intermediates was necessary.

Structural alteration of the peptides in response to divalent metal ions was then assessed with circular dichroism (CD) spectroscopy by monitoring the molar ellipticity at 222 nm, $[\theta]_{222}$, a widely used metric of relative helicity. Peptide **3**, which contained Ida moieties at the same positions as Dpa in peptide **2**, was also prepared as a control (Fig. 1). In the absence of metal ions, the helical content of peptide **3** was significantly lower than that of peptide **1** ($[\theta]_{222}$: $-1.4 \times 10^4$ *vs.* $-2.2 \times 10^4$ deg $cm^2$ $dmol^{-1}$

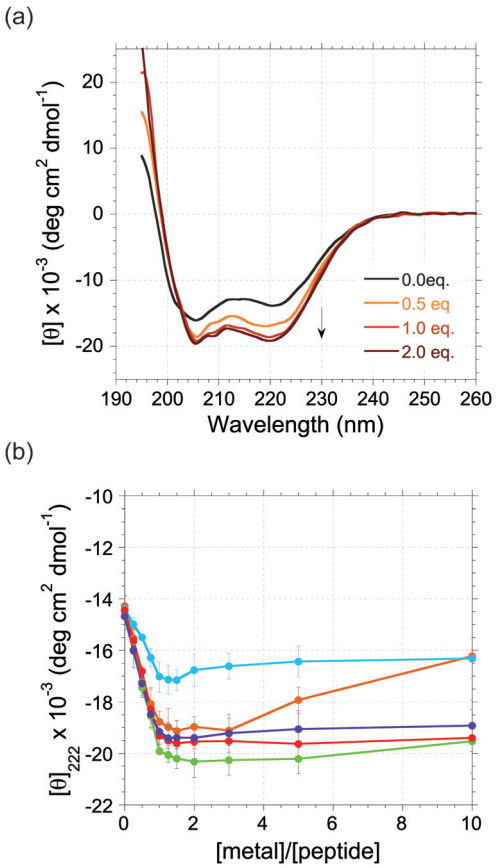



**Fig. 3** (a) Changes in the CD spectra of peptide **3** induced by addition of Fe(II). (b) The molar ellipticity of peptide **3** at 222 nm ($[\theta]_{222}$) as a function of the ratio of metal ions to each peptide [Fe(II), orange; Cu(II), blue; Ni(II), green; Co(II), red; Zn(II), purple]. The data points represent the average of three experiments (±SD). The sample contained 30 μM peptide in 10 mM MOPS containing 100 mM NaCl (pH 7.0).

for peptide **3** and **1**, respectively) (Fig. 3 and Fig. S1‡), presumably due to the electrostatic repulsion between negatively charged Ida moieties in the peptide **3**, hampering the helix formation. However, the addition of one equivalent of divalent metal, such as Fe(II), Ni(II) or Zn(II), resulted in the increase of helicity, suggesting the efficient stabilisation of the helical structure of peptide **3** by intramolecular cross-linking formation (Fig. 3b).§

Alternatively, the helicity of peptide **2** in the absence of metal ions was almost similar with that of peptide **1** ($[\theta]_{222}$: $-2.0 \times 10^4$ *vs.* $-2.2 \times 10^4$ deg $cm^2$ $dmol^{-1}$, respectively) (Fig. 4a). Peptide **1** was designed to stabilise its helical structure by electrostatic interaction between the glutamic acid and the arginine residues with $i$ and $i + 4$ positions,[6] while the glutamic acid at position 7 and arginine at position 11 of peptide **1** were replaced by Dpa moieties in peptide **2**. Considering that this substitution to eliminate an ion pair should reduce the factor of the helix stabilisation in peptide **2** while the peptides **1** and **2** had a similar extent of helicity, there must be some interaction among Dpa moieties in

§ The addition of Fe(II) ion more than two equivalents to the peptide resulted in decrease in the helicity; excess Fe(II) could lead to an alteration in the coordination structure of peptide **2** with Fe(II).[2d]

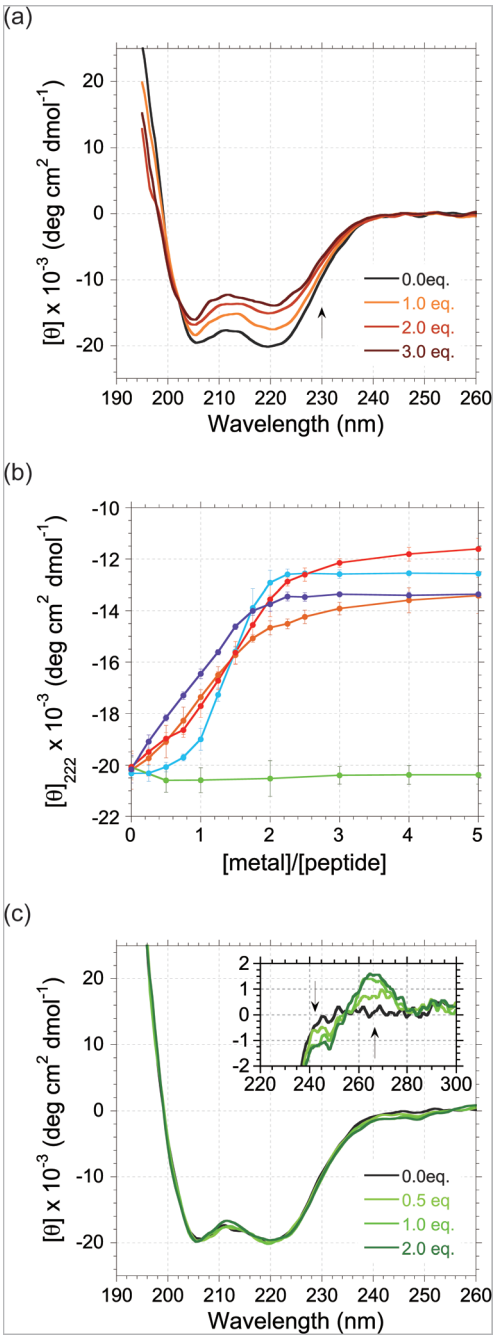



**Fig. 4** Changes in the CD spectra of peptide **2** induced by addition of (a) Fe(II) and (c) Ni(II). The inset shows a magnified view around 260 nm. (b) The molar ellipticity of peptide **2** at 222 nm ($[\theta]_{222}$) as a function of the ratio of metal ions to each peptide [Fe(II), orange; Cu(II), blue; Ni(II), green; Co(II), red; Zn(II), purple]. The data points represent the average of three experiments (±SD). The sample contained 30 μM peptide in 10 mM MOPS containing 100 mM NaCl (pH 7.0).

peptide **2** that compensates the loss of the stabilisation effect. It has been reported that interaction among aromatic amino acids such as phenylalanine placed at $i$ and $i + 4$ position may contribute to stabilise the helical structure.[7] Therefore, hydrophobic or aromatic interaction among the Dpa moieties placed on the same face of the helix in peptide **2** could also be beneficial to stabilise the helical structure.

In contrast to the result obtained with peptide **3**, a considerable decrease in helicity was observed in peptide **2** after the addition of Fe(II) (Fig. 4a). The decrease reached a plateau at approximately two equivalents of Fe(II), yielding an approximately 18% decrease in helicity ($[\theta]_{222}$: $-2.0 \times 10^4$ *vs.* $-1.4 \times 10^4$ deg cm$^2$ dmol$^{-1}$ in the presence of two equivalents of Fe(II)); although the structural alteration was not highly magnificent, successful control of peptide functions have often been achieved with this range of helical structural alteration.[2d,3] A similar tendency in helix destabilisation was observed with the addition of Cu(II), Co(II) and Zn(II) (Fig. 4b). The above results suggested that the Dpa moiety in peptide **2** forms a 1 : 1 complex with these metals instead of forming the 2 : 1 complex observed in peptide **3**. Electrostatic repulsion between the two Dpa–metal complexes in peptide **2** should prevent the formation of a helical structure, which would explain the contrasting responses observed between peptides **2** and **3** after the addition of metal. In contrast, no structural changes were observed in peptide **1** following the addition of these metal ions (Fig. S1‡). Interestingly, no significant decrease in helicity was observed for peptide **2** following the addition of Ni(II) (Fig. 4c). However, the spectral change observed around 260 nm strongly suggested complex formation between Dpa with Ni(II) (Fig. 4c inset).¶ This change was saturated by the addition of one equivalent of Ni(II). These results suggested that, in contrast to the complexes formed with other metals, peptide **2** forms a mononuclear complex with Ni(II) with a restricted conformation of Dpa moieties.[4d] In addition, as with the other metal ions, the structural alteration induced by Ni(II) was completely reversed following the addition of an excess of EDTA (1 mM) (Fig. S2‡).

In order to analyse the detailed mode of the peptide **2** binding with metal ions, we carried out isothermal titration calorimetry (ITC) measurements. Titration of Ni(II) to peptide **2** yielded exothermic enthalpy changes (Fig. 5a). The obtained heat of reaction was well fit by the equation for one to one binding model, yielding 220 nM as the dissociation constant ($K_d$) (Table 1). In contrast, a clear two-step alteration in the heat of reaction was observed in the binding of peptide **2** to Zn(II) (Fig. 5b). This result strongly supported our assumption that peptide **2** forms a 1 : 2 complex with Zn(II). The dissociation constant of the peptide **2** for Zn(II) in the each binding step ($K_{d1}$ and $K_{d2}$) was established by fitting the obtained heat to the equation for sequential binding model with two binding site. The $K_d$ values of peptide **2** for Fe(II) or Co(II) were similarly determined (Fig. S3‡, Table 1).‖ The order of the affinity established by these studies is Zn(II) > Co(II) > Ni(II) > Fe(II) when the $K_{d1}$ values for Zn(II), Co(II) and Fe(II) are compared with the $K_d$ for Ni(II).

To further investigate the relationship between linker lengths and the modes of complex formation of peptide **2** with Ni(II), peptides with different alkyl chain-lengths connecting the

¶There is a possibility that the conformation change of Dpa moieties may also influence the CD signal at 222 nm as in the case of other aromatic amino acid residues.[8] However, this effect may not be critical, if any, in the Dpa moieties of peptide **2**; as shown in Fig. 4c, no significant change in the CD signal at 222 nm was observed by the addition of Ni(II) to the peptide **2** in contrast to a marked change in that at 260 nm.
‖The affinity of peptide **2** for Cu(II) was not determined by ITC since only a marginal amount of heat generated by the binding was observed.

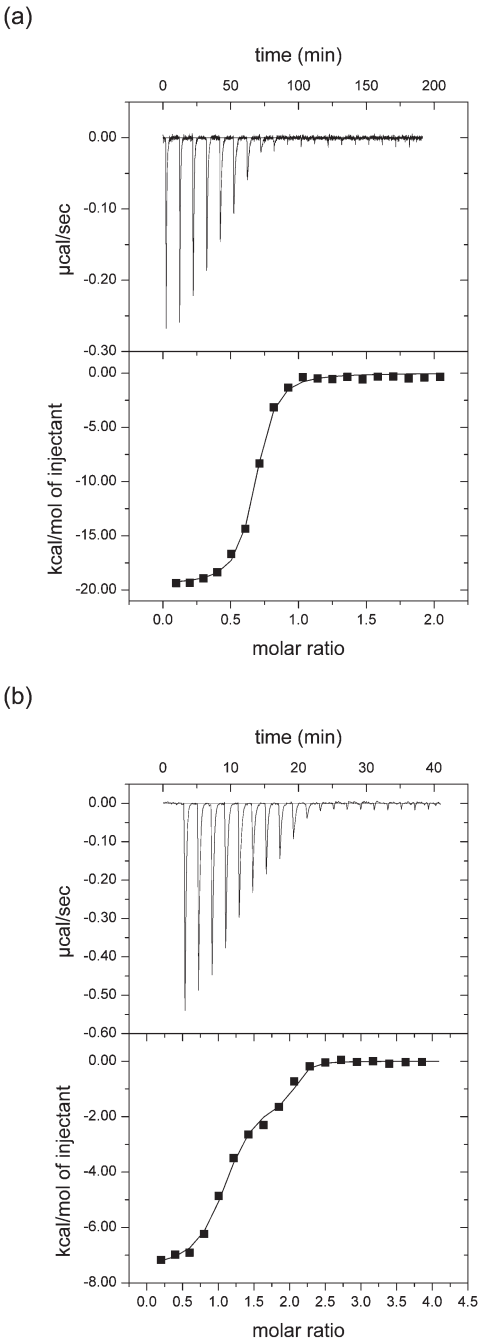



**Fig. 5** ITC measurement in the complex of peptide **2** with (a) Ni(II) and (b) Zn(II). The sample contained 30 µM peptide in 10 mM MOPS containing 100 mM NaCl (pH 7.0). Note the differences in the scales of time in (a) and (b).

**Table 1** $K_d$ values of peptide **2** for metal ions

| Ni(II) | Fe(II) | | Co(II) | | Zn(II) | |
|---|---|---|---|---|---|---|
| $K_d$ (nM) | $K_{d1}$ (nM) | $K_{d2}$ (nM) | $K_{d1}$ (nM) | $K_{d2}$ (nM) | $K_{d1}$ (nM) | $K_{d2}$ (nM) |
| 220 | 2000 | 10 000 | 41 | 8600 | 8.3 | 210 |

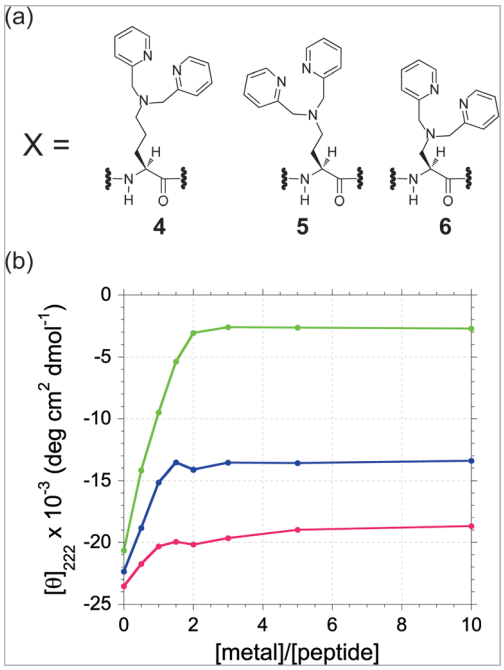



**Fig. 6** (a) Structures of Dpa derivatives with different side-chain lengths. (b) The molar ellipticities at 222 nm, $[\theta]_{222}$, of peptides **4** (blue), **5** (magenta), and **6** (green) as a function of the ratios of Ni(II) to peptides. The sample contained 30 µM peptide in 10 mM MOPS containing 100 mM NaCl (pH 7.0).

peptide main-chain to the Dpa moieties (peptides **4**–**6**) were synthesised; ornithine (Orn), (2*S*)-2,4-diaminobutyric acid (Dab), and (2*S*)-2,3-diaminopropionic acid (Dap) were respectively employed instead of lysine in the preparation of these peptides (Fig. 6a). Synthesis of each peptide was carried out without difficulty and in the same way as peptide **2**. The effect of Ni(II) addition to each peptide was analysed by CD spectroscopy (Fig. 6b). While no significant change in helical content was observed by the addition of Ni(II) to peptide **2** on the basis of $[\theta]_{222}$, addition of 1 equivalent of Ni(II) to peptide **4** yielded a considerable decrease in helicity and no significant decrease in helicity was observed by further addition of Ni(II) to peptide **4**. Similarly, addition of Ni(II) to peptide **5** yielded a decrease in helicity, leading to saturation upon addition of 1 equivalent of Ni(II). However, the extent of the decrease in helicity was much smaller than in the case of peptide **4**. Although we do not currently have a satisfactory explanation for the difference in the extent of helical decrease, these results suggest that both peptides **4** and **5** formed 1 : 1 complexes with Ni(II); these peptides have shorter alkyl side-chains than peptide **3** and the formation of the Dpa–Ni(II) complexes and the resulting cross-links may simultaneously inhibit helix formation of these peptides, yielding a decrease in the helicity of the peptides. A significant decrease in helicity was also observed by the addition of Ni(II) to peptide **6**. However, 2 equivalents of Ni(II) was needed to obtain a plateau in the decrease in the helical content, suggesting that length of the side chain was too short to form a stable intra-molecular cross-linking.

The stabilisation of the helical structures by cross-link formation between the Dpa groups in the presence of Ni(II) was assessed *via* the thermal stability of peptide **2** in the presence

**Table 2** $T_m$ values of the peptides in the presence or absence of Ni(II) (1.2 eq.)

| | $T_m$ (°C) | |
|---|---|---|
| | No metal | Ni(II) |
| Peptide **1** | 30.4 | 30.9 |
| Peptide **2** | 31.5 | 36.9 |

and absence of Ni(II) (Table 2 and Fig. S4a‡). The $T_m$ value of peptide **2** in the absence of Ni(II) analysed by alteration of $[\theta]_{222}$ was 31.5 °C, and the addition of Ni(II) yielded a slight increase in $T_m$ (~5 °C). These results suggested that the thermal stability of the helical structure of peptide **2** by Ni(II) coordination with Dpa moieties would be comparable or slightly higher than that of the possible interaction of intra-molecular Dpa moieties in the absence of Ni(II). No significant stabilisation by the addition of Ni(II) was observed for peptide **1** (Table 2 and Fig. S4b‡).

This unique binding between Ni(II) and peptide **2** may lead to the development of novel structural and functional switching systems. The results shown in Fig. 4 suggest the possibility of creating a reversible structural switch, considering that Ni(II) binds to the peptide **2** more strongly than Fe(II) (Table 1). To evaluate this possibility, we examined the effects of the addition of Ni(II) to a Fe(II) complex of peptide **2** (Fig. 7a).

After peptide **2** was allowed to form a complex with Fe(II) (two equivalents of peptide), Ni(II) was added and the effects on the helical content were assessed by CD spectrometry. The addition of five equivalents of Ni(II) led to almost complete recovery of the helical structure, approaching the structure that exists in the absence of metal ions (Fig. 7a and b). The formation of a maximum at ~260 nm in the CD spectra was also indicative of Dpa–Ni(II) complexation (Fig. 7a inset). These results suggest the possible substitution of Fe(II) by Ni(II). These changes in the helicity of the Fe(II)–peptide **2** complex were prominently observed only after the addition of Ni(II). In contrast with the case of Ni(II), slight decreases in helicity were observed following the addition of Cu(II), Co(II), Zn(II) or Cd(II). No significant changes were observed with the addition of other metal ions (Fig. 7c and Fig. S5‡). These results demonstrated the potential applicability of the Fe(II)–peptide **2** system for detection of Ni(II). Note that the plot of helicity as a function of Ni(II) concentration was pseudo-linear from 0 to 5 equivalents of Ni(II) per peptide (Fig. 7b) and could be used a calibration curve for the detection of Ni(II).

Similarly, metal substitution of the Ni(II)–peptide **2** complex was made possible by the addition of Cu(II), Co(II) or Zn(II). Addition of any of these ions led to a decrease in helicity (see Fig. S6a in ESI‡) and dissociation of Ni(II) (deduced from the decreased molecular ellipticity at ~260 nm as shown in the inset of Fig. S6b‡).

Therefore, we examined whether successive repetitions of the above procedures would continuously switch the peptide structure (Fig. 8). A sharp switch in helical content was observed for peptide **2** with the addition of increased concentrations of Fe(II), Ni(II) or Zn(II) based on the difference in the affinity as shown in Table 1. Removal of the metals by addition of EDTA returned peptide **2** to its initial state or home position.

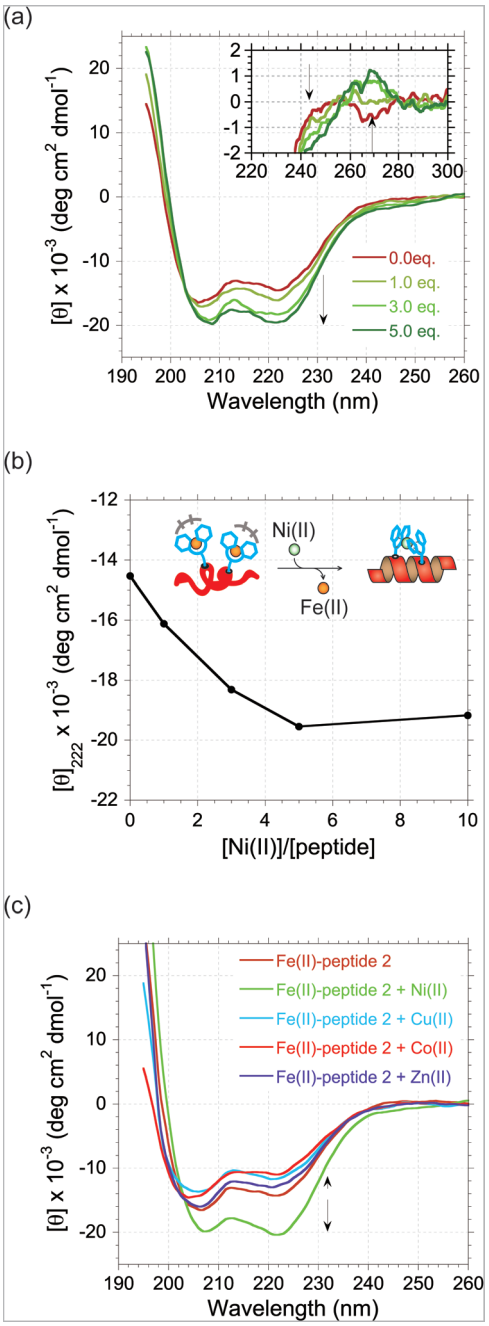



**Fig. 7** (a) Recovery of the helical structure of the Fe(II)–peptide **2** complex by addition of Ni(II). The Fe(II)–peptide **2** complex was formed by 30 μM peptide **2** in the presence of 60 μM Fe(II). (b) The molar ellipticity of the Fe(II)–peptide **2** complex is shown at 222 nm, $[\theta]_{222}$, as a function of the ratio of Ni(II) to peptide. (c) The helicity of the Fe(II)–peptide **2** complex is shown following the addition of other metal ions (20 equivalents per peptide).

## Conclusions

In this study, we developed a simple method for introducing Dpa moieties into peptides using reductive amination. We found that peptide **2** bearing a pair of Dpa moieties at $i$ and $i + 4$ positions forms 1 : 1 complex with Ni(II) while this peptide forms 1 : 2 complexes with other metal ions. We also demonstrated the

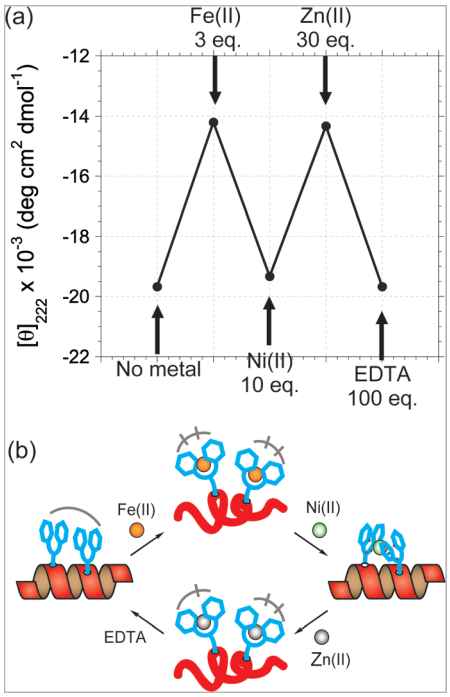



**Fig. 8** (a) Reversible and continuous helix switching of peptide **2**. (b) A possible mechanism of bidirectional helix switching of peptide **2** is shown in response to metal ions and EDTA.

potential applicability of Fe(II)–peptide **2** complex as a Ni(II)-selective detector. Moreover, continuous and bidirectional control of peptide structure was accomplished using a suitable selection of metal ions. Considering the unique and attractive features of Dpa-modified peptides, our strategy of Dpa-modification can facilitate novel concepts for the control of structure and function of designed peptides.

## Experimental section

### General

Fmoc-Ala, Fmoc-Arg(Pbf) (Pbf = 2,2,4,6,7-pentamethyldihydrobezofuran-5-sulfonyl), Fmoc-Glu(O*t*Bu) and Fmoc-Trp(Boc) were purchased from Peptide Institute. Fmoc-Lys(Mtt) and Fmoc-Dap(Mtt) were purchased from Novabiochem. Fmoc-Orn(Mtt), Fmoc-Dab(Alloc) (Alloc = allyloxycarbonyl) were purchased from Watanabe Chemical Industries. The TGS-RAM resin was purchased from Shimadzu. Matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOFMS) was measured using an Applied Biosystems-Voyager-DE STR or Bruker Daltonics-Microflex. CD spectra were obtained using a Jasco J-820 spectrometer. ITC experiments were carried out on a MicroCal $ITC_{200}$ (GE Healthcare). As metal ions, $Fe(NH_4)_2(SO_4)_2{\cdot}4H_2O$, $CuSO_4{\cdot}5H_2O$, $NiCl_2{\cdot}6H_2O$, $CoCl_2{\cdot}6H_2O$, $ZnCl_2$, $AlCl_3{\cdot}6H_2O$, $MnCl_2$, $Pb(NO_3)_2$, $CdCl_2{\cdot}2.5H_2O$, $AgNO_3$, $CaCl_2$ and $MgCl_2$ were used. These were purchased from Wako Pure Chemical. $Fe(NH_4)_2(SO_4)_2$ was dissolved in the de-gassed buffer containing 5% $HONH_4Cl$ to prevent oxidation of Fe(II).

### Syntheses of the peptides

The peptide chain **2** was constructed by Fmoc-solid-phase peptide synthesis on a TGS-RAM resin (50.4 mg, 0.11 mmol) using a Shimadzu PSSM8 peptide synthesiser with its standard protocol. A 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU)/1-hydroxybenzotriazole (HOBt)/diisopropylethylamine (DIEA) coupling system was employed. As amino acid derivatives for the preparation of peptide **2**, Fmoc-Ala, Fmoc-Glu(O*t*Bu), Fmoc-Arg(Pbf), Fmoc-Trp(Boc) and Fmoc-Lys(Mtt) (for Lys that is converted to Dpa) were used. The N-terminal of the peptide resin was acetylated using acetic anhydride in the presence of 4-methylmorpholine (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 25 °C for 3 h. After the peptide resin was treated with 2-pyridinecarboxaldehyde (Tokyo Chemical Industry) (20 eq.) in DCM for 1 h at 25 °C, suspension of NaBH$(AcO)_3$ (Kanto Chemical) (20 eq.) in DCM was added to the resin.[9] The reaction mixture was shaken at 25 °C for 4 h in the reaction vessel of SPPS. After the result peptide resin was washed with DCM, additional overnight reaction was conducted with same agents at 25 °C to yield the peptide resin bearing Dpa moieties. The final deprotection of the peptide resin using trifluoroacetic acid (TFA)–ethanedithiol (EDT) (95 : 5) at 25 °C for 3 h followed by reverse-phase HPLC purification [column, Cosmosil 5C18-AR-II (10 × 250 mm); eluents, A = $H_2O$ containing 0.1% TFA, B = $CH_3CN$ containing 0.1% TFA; gradient, 20–35% B in A over 15 min] gave the desired sample **2** (7.4 mg, 30%). Peptides **4** and **6** were synthesised using Fmoc-Orn(Mtt) and Fmoc-Dap(Mtt) instead of Lys(Mtt), which was used for the preparation of peptide **2**. Fmoc-Dab(Alloc) was used for the preparation of peptide **5** instead of the corresponding Mtt derivative due to difficulty in introducing Fmoc-Dab(Mtt) on the peptide chain under the above coupling conditions. Deprotection of the alloc group was accomplished by treatment with a $Pd(0)(PPh_3)_4$ (Wako Pure Chemical) solution in de-gased $CHCl_3$–AcOH–NMM (37 : 2 : 1) at room temperature for 2 h under an Ar atmosphere.[10] Peptide **1** was synthesised using standard Fmoc-SPPS. Peptide **3** was prepared following previously reported protocols.[3a] All peptides were obtained with >95% purity (HPLC). The fidelity of the purified peptides were confirmed by MALDI-TOFMS: Peptide **1** 1812.67 [calcd for $(M + H)^+$ 1812.97]; Peptide **2** 2147.97 [calcd for $(M + H)^+$ 2148.44]; Peptide **3** 2015.47 [calcd for $(M + H)^+$ 2016.15]; Peptide **4** 2119.55 [calcd for $(M + H)^+$ 2119.38]; Peptide **5** 2091.69 [calcd for $(M + H)^+$ 2091.33]; Peptide **6** 2063.51 [calcd for $(M + H)^+$ 2063.28]. Concentrations of the peptides were determined by UV absorption at 290 nm following Pace *et al.*[11]

### CD spectroscopy

The metal was added in a stepwise manner to a 30 μM solution of peptide in 10 mM MOPS (3-(*N*-morpholino)propanesulfonic acid) containing 100 mM NaCl (pH 7.0). Each spectrum was recorded after incubation at 25 °C for 5 min.

### Thermal denaturing curve

Temperature-dependent changes in the ellipticity at 222 nm were monitored using 30 μM peptide solutions in 10 mM MOPS containing 100 mM NaCl and 36 μM Ni(II) (pH 7.0) with a gradient of 1.0 °C $min^{-1}$. Each $T_m$ value was estimated by fitting the experimentally obtained values to the equilibrium eqn (1) of the helix to coil transition model and van't Hoff eqn (2)[12] using the Kaleida Graph program (Abelbeck software).

$$K = [\mathrm{H}]/[\mathrm{C}] = ([\theta] - [\theta]_c/[\theta]_h - [\theta]) \quad (1)$$

$$K = \exp\,[-\Delta H_m/R\ (1/T - 1/T_m)] \quad (2)$$

where [H] and [C] are concentration of the helical form and random coil of the peptide, respectively. $[\theta]_h$ and $[\theta]_c$ are the ellipticity of the helical form and random coil of the peptide, respectively. $[\theta]$ is observed ellipticity. $T_m$ is the temperature at the midpoint of the transition and $\Delta H_m$ is enthalpy change at $T_m$. $R$ is molar gas constant. The unknown parameters, $[\theta]_c$, $[\theta]_h$, $\Delta H_m$ and $T_m$ were directly evaluated by curve fitting analyses.

### ITC measurement

The metal solution was titrated into the peptide solution (30 μM for Ni(II), Co(II) or Zn(II) and 150 μM for Fe(II)) at 25 °C. All samples were in 10 mM MOPS containing 100 mM NaCl and the buffer was degassed under vacuum before use. The cell volume was 200 μL and the syringe volume was 40 μL. Each titration consisted of 19 injections (2.0 μL each) at 120 s (600 s for Ni(II)) intervals. The stirring rate was 1000 rpm throughout the experiments. Data were analysed using the Origin software supplied by MicroCal.

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