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Zn(II) dipicolylamine-based artificial receptor as a new entry for surface recognition of α-helical peptides in aqueous solution

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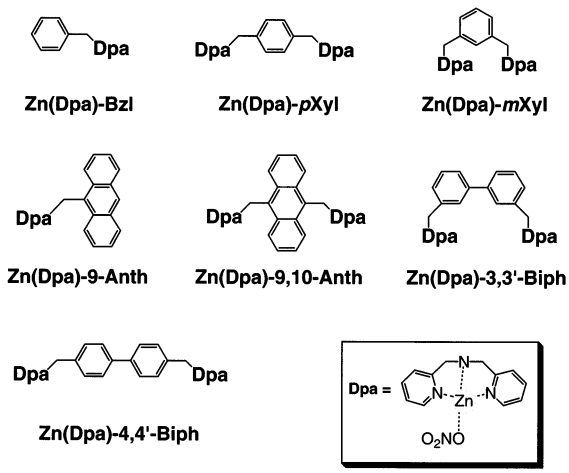
Abstract—It is clear by CD spectral titration that Zn(II)dipicolylamine-based dinuclear complexes selectively bind and stabilize the α-helix conformation of peptides having two histidine (His) residues at specific positions (*H-i* and *i*+4 or *i*+7 or *i*+11).

Artificial receptors for bioactive peptides are actively developed in the field of recent molecular recognition chemistry because of their importance for peptide sensing and pharmaceutical application.[1] However, recognition events have been exploited in organic solvents in most cases, which are still far from the biological prospects.[2] For the development of artificial receptors that can selectively bind a peptide/protein surface so as to inhibit or enhance the function, it is desirable to establish a design strategy for artificial receptors toward a peptide surface in aqueous solution.[3] We describe herein that zinc(II) dipicolylamine (Zn(Dpa))-based coordination chemistry is promising for design of artificial receptors in aqueous solution toward α-helical peptides displaying two histidine (His) on their surface (*H-i* and *i*+4, or *H-i* and *i*+7, or *H-i* and *i*+11).

Dipicolylamine Zn(II) complex (Zn(Dpa)) is employed as a binding module for histidine residues because of its moderate affinity in aqueous solution. Five dinuclear Zn(II)-Dpa complexes bearing *meta*- or *para*-xylene, 9,10-dimethylanthracene and 3,3′- or 4,4′-dimethylbiphenyl as a connector were prepared according to the literature.[4] Two mononuclear Zn complexes based on benzyl-Dpa or 9-anthrylmethyl-Dpa were used as control compounds. As target molecules, four model peptides in which one or two His are located at one side of the α-helix were employed (shown in Fig. 1).[5] These peptides were prepared using the Fmoc chemistry-based solid-phase peptide synthesis, subsequently purified by reverse-phase HPLC, and characterized by MALDI-TOF mass spectroscopy (Voyger RP).[6]



	1 5 10 15	
peptide (H-16)	Ac-AEAAAKEAAAKEAAA***H***A-NH$_2$	i
peptide (H-12,16)	Ac-AEAAAKEAAAK***H***AAA***H***A-NH$_2$	i,i+4
peptide (H-9,16)	Ac-AEAAAKEA***H***AKEAAA***H***A-NH$_2$	i,i+7
peptide (H-5,16)	Ac-AEAA***H***KEAAAKEAAA***H***A-NH$_2$	i,i+11

Figure 1. A series of Zn(Dpa)-based receptors and peptide sequences discussed here.

Keywords: peptide recognition; artificial receptor; Zn(II) dipicolylamine complex.

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For the rapid screening of the affinity of these Zn-Dpa based receptors to the four peptides, the receptor-induced conformational change of the peptides was examined by circular dichroism spectroscopy (CD, Jasco J-720w). Fig. 2 summarizes the changing ratio of the θ values monitored at 222 nm in the presence of the corresponding receptors (5 equiv.) based on the θ in the absence of the receptors. It is apparent that significant increase of the helix content upon addition of certain receptors occurs for every peptide having two His residues, but it does not occur for the mono-His peptide. The helical conformation of H-12,16 peptide, for instance, is selectively intensified by Zn(Dpa)-9,10-Anth. In contrast, Zn(Dpa)-*p*Xyl and Zn(Dpa)-*m*Xyl do not stabilize it. For H-9,16 peptide, Zn(Dpa)-*p*Xyl, Zn(Dpa)-9,10-Anth, and Zn(Dpa)-4,4′-Biph are effective to the same extent.[7] In the case of H-5,16 peptide, Zn(Dpa)-4,4′-Biph selectively induces the helical conformation. It should also be noted that neither the Zn(II) cation nor Zn(Dpa)-Bzl, a mononuclear receptor, effectively induce the conformational change for all peptides.

Based on the above screening data, we next conducted careful CD titration experiments for the following combinations: H-12,16 peptide to Zn(Dpa)-9,10-Anth, H-9,16 peptide to Zn(Dpa)-*p*Xyl, or Zn(Dpa)-9,10-Anth or Zn(Dpa)-4,4′-Biph, and H-5,16 peptide to Zn(Dpa)-4,4′-Biph. Fig. 3 shows the CD titration of H-12,16 peptide with Zn(Dpa)-9,10-Anth. Two negative Cotton peaks at 208 and 222 nm, characteristic of the α-helix conformation, were intensified with three isosbestic points (at 203, 240 and 265 nm) by addition of Zn(Dpa)-9,10-Anth, indicating that the α-helix content increased upon complexation with Zn(Dpa)-9,10-Anth. In addition to the helix region, a positive CD peak was observed at 250–270 nm, which may be ascribed to the induced CD (*i*-CD) of the Dpa moiety, suggestive that the Zn-Dpa moieties are positioned in the chiral microenvironment of the H-12,16 peptide. Other three negative Cotton peaks were newly detected at 363, 382 and 403 nm, characteristic of the anthracene chromophore (inset of Fig. 3). This *i*-CD implies that the anthracene moiety is exposed closely to the chiral peptide surface. The *i*-CD around 380 nm, in contrast, does not strongly appear by addition of the mononuclear Zn(Dpa)-9-Anth. Therefore, it may be concluded that a stable receptor/peptide complex forms through the two points interaction between two Zn(Dpa) sites and two His residues.

The Job's plot monitored by these CD peaks shows the maximum at 0.5 of the molar fraction of the receptor (inset of Fig. 4). Consistently, ESI-TOF mass spectroscopy (Mariner), under identical conditions, displayed a 1:1 complex between the receptor and the peptide as a major species (the observed peak at 806.0 (3+) corresponds to one-third of the sum of the peptide plus Zn(Dpa)-9,10-Anth($1NO_3$)).[8] Thus, the binding stoichiometry was determined to be 1:1 ratio and the CD titration curve, which obeys a typical saturation

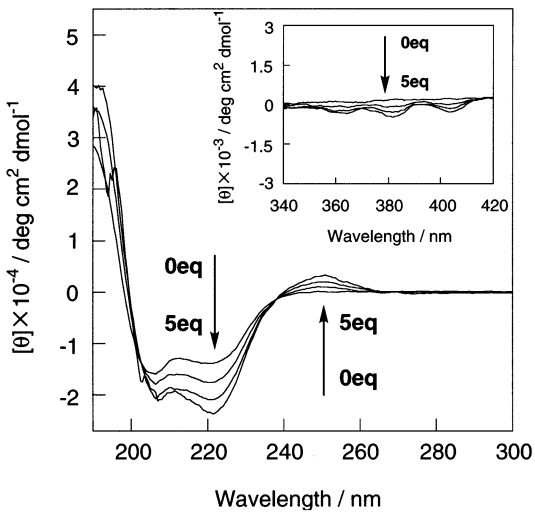

Figure 3. CD spectral change upon addition of Zn(Dpa)-9,10-Anth to H-12,16 peptide. Inset: the induced CD due to the anthracene moiety. [Peptide] = 50 μM in 10 mM borate buffer (pH 8.0) at 10°C.

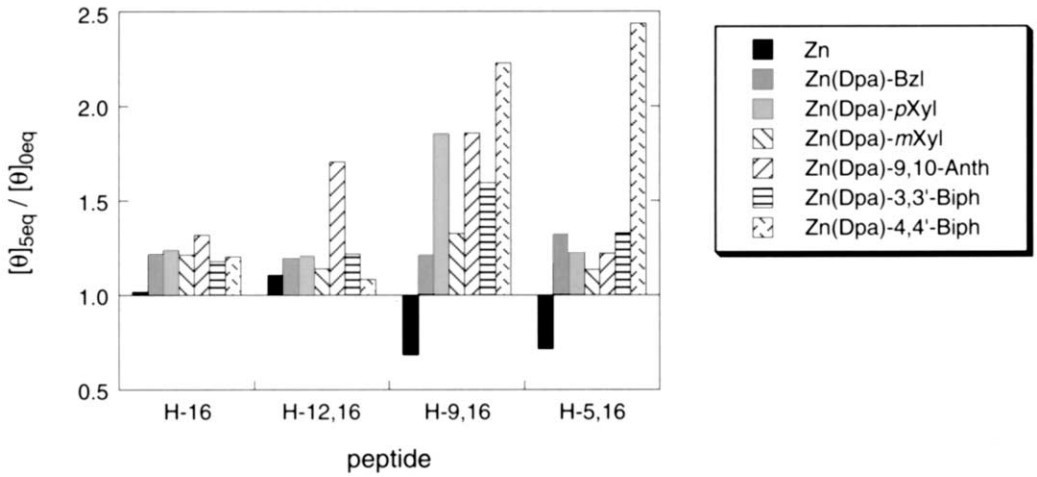

Figure 2. The variation ratio of θ values at 222 nm by addition of 5 equiv. of Zn(Dpa)-based receptors to the corresponding peptides. [Peptide] = 50 μM in 10 mM borate buffer (pH 8.0) at 10°C.

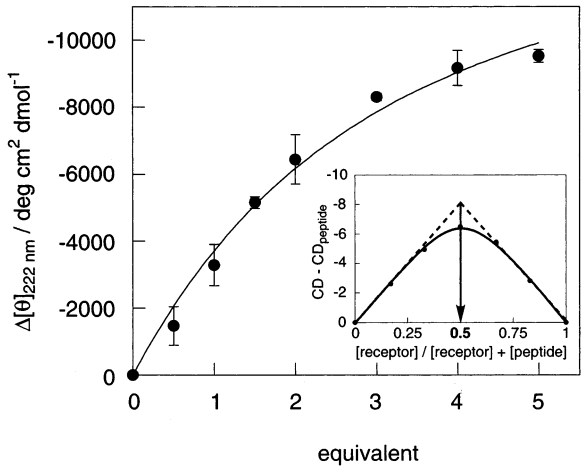

Figure 4. CD titration curve of H-12,16 peptide with Zn(Dpa)-9,10-Anth under the identical condition to Fig. 3. The error bars were estimated using the data of three titration experiments. Inset: Job's plot of this combination (the total concentration is 100 μM).

behavior (Fig. 4), plotted at 222 nm gave us the binding constant (K_{ass}) of $10^{3.9}$ M^{-1}. Almost the same values were estimated by the *i*-CD-titration curves monitored at 250 nm ($K_{ass}=10^{3.7}$ M^{-1}) and 385 nm ($K_{ass}=10^{4.3}$ M^{-1}). Such a good agreement supports the winding of the helix conformation concurrently taking place upon receptor binding (Fig. 5).

Similar titration curves and Job's plots were obtained for other combinations and the affinity constants are summarized in Table 1. It is clear that all the combination of receptors and peptides selected from the rough screening have the affinity in the range of $10^{4.0}$ to $10^{5.0}$ M^{-1}. Table 1 also displays the average distances between two His residues that are estimated by the molecular modeling of the corresponding peptides when their conformations are assumed to be α-helix in 100%. The nearest His (5.0 Å) of H-12,16 peptide suitably corresponds to the shortest distance between two Zn

Table 1. Binding constants of the Zn(Dpa) molecules with various peptides

Peptide	Receptor	K_{ass} (M^{-1})[a]	Average distance (Å)[b]
H-12,16	Zn(Dpa)-9,10-Anth	$8.5\pm2.7\times10^3$	5.0
H-9,16	Zn(Dpa)-9,10-Anth	$1.3\pm0.7\times10^4$	10.2
H-9,16	Zn(Dpa)-*p*Xyl	$2.5\pm0.7\times10^4$	10.2
H-9,16	Zn(Dpa)-4,4′-Biph	$2.5\pm0.4\times10^4$	10.2
H-5,16	Zn(Dpa)-4,4′-Biph	$3.9\pm0.6\times10^4$	18.0

[a] The errors were calculated using the data of at least three titration experiments.
[b] The distances between N^2 of the imidazole ring of two His were estimated by molecular mechanics with the restriction of the main chain to the α-helical conformation.

centers of Zn(Dpa)-9,10-Anth. In the case of the most distant His (18.0 Å) of H-5,16 peptide, Zn(Dpa)-4,4′-Biph, the longest receptor, fits well. In the case of H-9,16 peptide, the remaining freedom both in the side chain of His and the Dpa-receptors may result in this broad selectivity in the induced fit manner. Apparently, the coincidence of the distance between two Zn centers of the receptor with the two His distance is one of the most important factors for selective binding. The present results indicate that the 'measuring worm strategy' is promising for the design of artificial receptors toward protein/peptide surfaces.

In conclusion, we established that the dinuclear Zn(Dpa) complex is a versatile molecular motif for binding peptides bearing two His at specific positions. The spatial juxtaposition by a modular connector greatly influences the affinity to these peptides. To the best of our knowledge, this is the first example of artificial receptors that can selectively bind peptides bearing two His in the distance of two or three helix pitches in perfectly aqueous solution. This motif can be readily combined with other binding motifs, so that one can design the more selective and efficient artificial

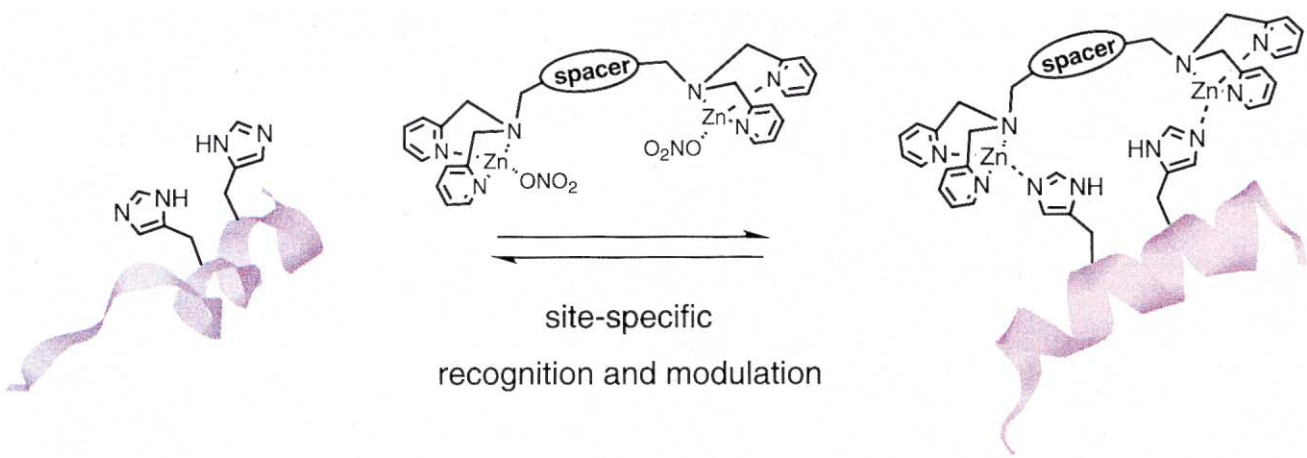

Figure 5. Schematic representation of peptide selective recognition by a Zn(Dpa)-based receptor.

receptors toward a peptide or protein. Our research is now under way in this area.[9]

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7. It is interesting that Zn(Dpa)-*p*Xyl is effective only to H-9,16 peptide, in contrast to Zn(Dpa)-9,10-Anth which can bind to H-9,16 peptide, as well as H-12,16 peptide. This may be due to the fact that the two methylene units of *p*Xyl favor the *trans* conformation rather than the *cis* conformation.
8. This peak is 2.9-fold in its intensity, relative to the peak at 543.1(+3) due to the peptide only.
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