Zinc Complexes of Amino Acids and Peptides, 6[1]

Zinc Complexes of Oligopeptides Containing Histidine at Both Termini

Martin Förster[a], Ingo Brasack[a], Anne-Kathrin Duhme[b], Hans-Friedrich Nolting[b], and Heinrich Vahrenkamp*[a]

Institut für Anorganische und Analytische Chemie der Universität Freiburg[a],
Albertstraße 21, D-79104 Freiburg, Germany

EMBL c/o DESY[b],
Notkestraße 85, 22603 Hamburg, Germany

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One tripeptide, five tetrapeptides, and one pentapeptide, all containing His−X_n−His sequences and being blocked at the N and C termini with acyl and amide functions, respectively, were synthesized by solid-phase methods. With one exception their reaction with various zinc salts led to the precipitation of 1:1 (zinc/peptide) complexes. Analytically pure compounds were obtained from zinc tetrafluoroborate and His−Gly−His (**1**), from zinc chloride (resp. bromide) and His−Gly−Gly−His (**2**), His−Ala−Gly−His (**3**), His−Leu−Gly−His (**4**), His−Pro−Gly−His (**5**), and His−Pro−Asn−His (**6**) as well as from zinc sulfate and His−Leu−Gly−His (**4**) and His−Ala−Pro−Gly−His (**7**). ^{1}H-NMR data, when available, indicate the coordination of both histidine units to zinc in all cases. The low solubility of the complexes points to their polymeric nature. The only 1:2 (zinc/peptide) complex in this series was obtained from zinc perchlorate and His−Gly−Gly−His (**2**). An EXAFS study revealed that it contains zinc symmetrically coordinated by four histidine imidazole ligands. Based on the available information it is proposed that all complexes are one-dimensional polymers containg [−Zn−His−X_n−His−Zn−]$_x$ backbones.

Our investigations into the chemistry of the coordination of zinc with amino acids and peptides aim at two goals. Firstly, we want to gain basic knowledge of ligation patterns and complex stabilities in comparison with the natural coordination in zinc-containing proteins. Secondly, we try to find functional zinc complexes in which the zinc ion is encapsulated and electronically suitable for biomimetic reactions. Both aims, in order be realized successfully, require the use of larger peptides.

Of the metal-ligating amino acids bearing N, O, and S containing donors in their side chains, cysteine and histidine are the most prominent ones for zinc[2,3], and quite often the zinc ion in a protein is bound to two cysteine or histidine residues. Thus the challenge of preparing peptides containing two such residues and using them for zinc complexes arises. After our previous work on ligating peptides with one cysteine[4] or histine[1] unit we are now taking up this challenge. This paper reports on zinc complexes of peptides containing two histidine building blocks.

To our knowledge such complexes have not been obtained on a preparative scale yet. Potentiometric investigations of larger peptides have in some cases included their interaction with zinc salts[2], and examples of NMR studies of zinc complexes of larger peptides involve the antibiotic bacitracin A[5] and zinc finger fragments[6]. In general, the larger peptides investigated for such purposes were available in sufficient quantities from natural sources, i.e. they did not have to be prepared by the cumbersume procedures of peptide synthesis.

His−X_n−His Peptides

In order to obtain the required peptides in sufficient quantities for a preparative coordination chemistry (0.2−1.0 g) we chose to prepare them manually by solid-phase techniques. As the solid phase we used a commercial ADPV resin consisting of polystyrene crosslinked with divinylbenzene and covered by the ADPV anchoring groups (ADPV = Fmoc-protected 4-(aminomethyl)(3,5-dimethoxy)phenoxyvalerianyl[7]). The amino acid components were also Fmoc-protected, and histidine functions were protected by trityl groups. The amino acids were activated at the carboxyl terminus by the DCC/HOBT technique[8] (DCC = dicyclohexylcarbodiimide, HOBT = 1-hydroxybenzotriazole). The Fmoc groups were removed by treatment with piperidine, and coupling was achieved by NEM (*N*-ethylmorpholine). After attachment of the final amino acid the amino terminus of the peptide chain was acyl-protected by adding acetic anhydride or benzoyl chloride. The peptide was released from the resin by cleavage with TFA/TA (trifluoroacetic acid/thioanisole), leaving it amide-protected at the carboxyl terminus and unprotected at the histidine functions. The raw peptides were purified by HPLC using a reversed-phase adsorbent.

Using these techniques, we obtained the tri-, tetra-, and pentapeptides **1**−**7**. The abbreviated notation for **1**−**7** implies that all peptides have a CO−NH_2 terminus, that **1**, **4**, and **7** contain a benzoyl-NH terminus, and **2**, **3**, **5**, and **6** an acetyl-NH terminus. The choice of the "inner" amino acids in **1**−**7** was dictated mainly by reasons of simplicity,

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i.e. only amino acids with simple aliphatic substituents were used in **1**–**4**. Proline was used in **5**–**7** because it can induce a β-turn, especially in the Pro–Gly sequence[10]. This was hoped to improve the ligating properties of the peptides by bringing the histidine donors at both ends closer together.

The identity and purity of all seven peptides were confirmed by ^{1}H-NMR spectroscopy (see Experimental). Mass spectra of **1**, **4**, and **6** were obtained by using the electrospray ionization technique. In all three cases the parent ions MH^+ and MH_2^{2+} produced dominating peaks in the spectra. Due to the chromatographic procedure the peptides are free from side products but contain varying amounts of TFA (as evidenced by ^{19}F-NMR analysis) which is likely to be bound by protonation of the peptide NH functions[9]. Accordingly, elemental analyses did not serve the purpose of determining the purity of the peptides but rather of estimating the amount of TFA contained.

His–Gly–His **1**

His–Gly–Gly–His **2**

His–Ala–Gly–His **3**

His–Leu–Gly–His **4**

His–Pro–Gly–His **5**

His–Pro–Asn–His **6**

His–Ala–Pro–Gly–His **7a**

Complexes

Numerous reactions of the peptides **1**–**7** with zinc salts were performed in various solvents and under various pH conditions. As a rule, there was a precipitation in neutral or weakly basic solution, but in most cases the precipitate was neither a single compound nor a mixture of compounds of integer ratio as evidenced by elemental analyses. This points to the fact that besides the preferred histidine imidazole donors all other donor functions in the reaction system (solvent, anions, peptide NH and CO functions) have a comparable chance of being ligated to zinc and that the onset of precipitation of coordination polymers inhibits the "finding" of the thermodynamically preferred compositions and constitutions.

Those compounds that could be identified as having a simple composition are listed below as complexes **K1**–**K8**, the numbers 1–7 relating to the corresponding peptides. They were obtained from aqueous or methanolic solution and their precipitation was achieved by carefully adding a base to the slightly acidic solutions. All compounds were obtained as amorphous powders, and attempts to crystallize those which have a low solubility in water or dimethyl sulfoxide remained unsuccessful.

His–Gly–His · $Zn(BF_4)_2$ · 2 TFA **K1**

His–Gly–Gly–His · $ZnCl_2$ **K2**

His–Ala–Gly–His · $ZnBr_2$ **K3a**

His–Leu–Gly–His · $ZnCl_2$ · TFA **K4a**

His–Leu–Gly–His · $ZnSO_4$ · TFA **K4b**

His–Pro–Gly–His · $ZnCl_2$ **K5**

His–Pro–Asn–His · $ZnCl_2$ **K6**

His–Ala–Pro–Gly–His · $ZnSO_4$ · TFA **K7**

$(His–Gly–Gly–His)_2Zn(ClO_4)_2$ **K8**

Except for **K8** all complexes have a 1:1 (zinc:peptide) composition, i.e. there are less than the ideal number of four histidine imidazole donors per zinc ion. This is compensated by the presence of two halide donors each in **K2**, **K3**, **K4a**, **K5**, and **K6**. In **K1**, **K4b**, and **K7** the anions present in the complexes (SO_4^{2-}, BF_4^-) are unlikely to be ligands, which in this case is compensated by the presence of TFA providing possibly coordinating trifluoroacetate anions. Of the nine complexes obtained only six were soluble enough for ^{1}H-NMR spectroscopy (see below).

Constitutional Assignments

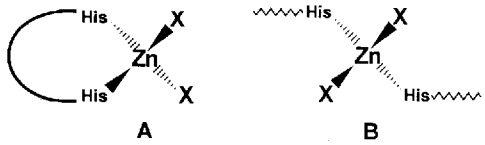
The ^{1}H-NMR data of the soluble complexes and the IR spectra (see Experimental) furnish two important pieces of information. The NMR data give clear evidence that both histidine units of the peptides are coordinated to zinc in all cases. This is obvious from the significant high-field shifts of the imidazole 2-H and (except for **K5** and **K6**) 4-H resonances upon complexation which are an order of magnitude larger than all other complexation shifts in **K2**–**K6**. Table 1 lists the relevant values. Just as there are no systematic complexation shifts of the NMR signals of any other groups in the peptides, there are no systematic movements of the NH bands and only negligible movements of the amide bands in the IR spectra of the peptides upon complexation. This is puzzling when one considers that further coordination positions on the zinc ions have to be occupied and that protonation by TFA has occurred in four cases. It seems to indicate, however, that each peptide ligand offers only its two imidazole donors for ligation.

Table 1. ^{1}H-NMR data relating to the histidine coordination in the complexes

Peptide	2-H	4-H	Complex	2-H	4-H
2 (DMSO)	7.94	7.31/7.36	**K2** (DMSO)	6.91/7.06	6.42/6.55
3 (DMSO)	8.17/8.21	7.04/7.08	**K3** (DMSO)	7.18/7.21	6.28/6.33
4 (D_2O)	8.60/8.62	7.27/7.32	**K4a** (D_2O)	7.86/7.91	6.92/6.97
			K4b (CD_3CN/D_2O)	8.18/8.20	7.11/7.12
5 (DMSO)	7.78/7.92	6.88/7.01	**K5** (DMSO)	7.23/7.34	7.04/7.15
6 (DMSO)	7.99/8.05	6.91/6.98	**K6** (DMSO)	7.40/8.10	7.03/7.23

The possible coordination of the zinc ions by the bis-histidine peptides can therefore be outlined as in **A** and **B**. Formula **A** relates to monomeric complexes, formula **B** to coordination polymers. We favor formula **B** due to the low solubility of all compounds. Support for this comes from our experience with similar bis-cysteine peptide complexes of zinc which, when mononuclear, have a good solubility[11]. This implies that a possible β-turn of the peptides **5**, **6**, and **7** is not sufficient to bring the two imidazole donors in these peptides into close enough a proximity to ligate the same zinc ion in their respective complexes **K5**, **K6**, and **K7**. It

also implies that all complexes, including **K8**, are one-dimensional polymers.



Assuming that zinc is tetrahedrally coordinated in all those complexes it remains to be proposed which donors occupy the third and fourth coordination positions in **K1**–**K7**. If one considers the high tendency of halide ions to bind to zinc and the propensity of $ZnHal_2(N\text{-ligand})_2$ complexes[12] it is likely that **K2**, **K3**, **K4a**, **K5**, and **K6** have a $ZnHal_2(histidine)_2$ coordination. Along the same line one would assume a $Zn(trifluoroacetate)_2(histidine)_2$ coordination for **K1**. Unfortunately, **K4b** and **K7** contain only one equivalent of TFA, and **K4a** contains one equivalent of TFA in addition to the two halide ligands. This renders trifluoroacetate coordination somewhat uncertain and, since SO_4^{2-} and BF_4^- are unlikely to coordinate, brings back the possibility of coordination of other peptide backbone constituents.

EXAFS Analysis of $(His-Gly-Gly-His)_2Zn(ClO_4)_2$ (K8)

The lack of conclusive evidence concerning the constitutions of complexes **K1**–**K8** prompted us to perform an EXAFS study of one of them. **K8** was chosen for this purpose because it promised a simple coordination of zinc with histidine imidazole ligands and because the analysis could not be impaired by the presence of coordinating anions. The results of the analysis revealed that our expectations were correct.

Figure 1 shows the normalized XANES spectra of **K8** and the model compound $[Zn(imidazole)_4](ClO_4)_2$[13]. The edge structures are very similar, indicating that the absorbing Zn atom is situated in a similar tetrahedral coordination environment, consisting of four histidine residues. Consequently, the analysis of the first coordination shell contribution to the EXAFS spectrum, shown in Figure 2a, was performed with a fixed coordination number of four by keeping the Zn–N distance and the Debye-Waller factor variable.

Then the imidazole rings were refined as rigid groups, allowing the Zn–N(3) distance and the angle between Zn, N(3), and the remaining imidazole atoms to vary. The Debye-Waller factors for the higher shell atoms of the imidazole rings were assumed to be constant and thus refined together. Initial values for the Zn imidazole unit were taken from ref.[14]. The result of the best fit is reported in Table 2. The quality of the fits was assessed by using the fit index as defined within EXCURVE88 (see Experimental). Figure 2b shows that all the features of the EXAFS spectrum and of its Fourier transform could be reproduced quite well by introducing the contributions of the remaining outer imidazole shells into the calculation in addition to the first shell N atoms.

Figure 1. Normalized edge spectra of $[(His-Gly-Gly-His)_2Zn](ClO_4)_2$ (——) and $[Zn(imidazole)_4](ClO_4)_2$[13] (-----); energy-zero at 9660 eV

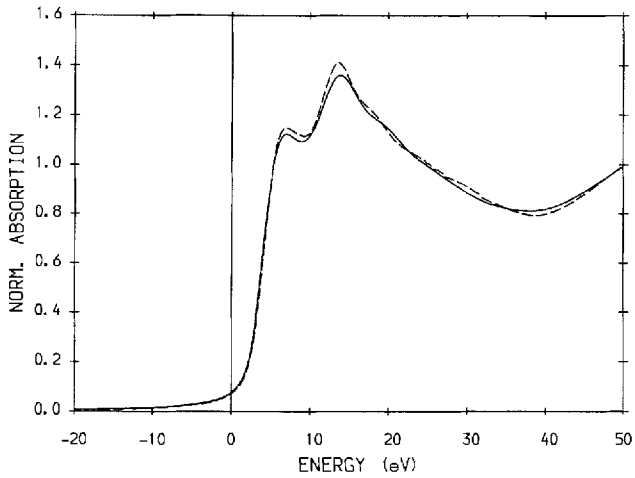
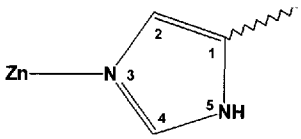


Table 2. Parameters used to calculate the k^3 weighted EXAFS spectrum in Figure 2

Backscatterer	N	r [Å]	$2\sigma^2$ [Å^2]	β [°]
N(3)	4	1.96	0.007	–
C(4)	4	2.93	0.020	123
C(2)	4	3.05	0.020	–129
N(5)	4	4.09	0.020	158
C(1)	4	4.16	0.020	–165

N: coordination number; r: distance; $2\sigma^2$: Debye-Waller factor; β: angle between Zn, N(3), and imidazole backend atom C(4), C(2), N(5), and C(1), respectively. E.s.d. on distances = ±0.02 Å.



The environment of the zinc ion in **K8**, as resulting from the EXAFS analysis, is depicted in Figure 3. A tetrahedral coordination of zinc by the amino acid donor functions is the normal case in zinc-containing proteins[2,3,15]. We observed, however, that coordination numbers larger than four are not unusual in zinc complexes of small histidine-containing peptides[1,16], and imidazole itself forms tetrahedral[14] as well as octahedral[17] zinc complexes.

The Zn–N distance of 1.96 Å in **K8** compares favorably with those in $[Zn(imidazole)_4](ClO_4)_2$[14] (av. 2.00 Å) and $[Zn(NH_3)_4](ClO_4)_2$[18] (av. 2.01 Å). The only known Zn–N distance in a tetrahedral zinc complex of a histidine-containing peptide is that of 2.01 Å (av.) in [Zn(*cyclo*-Met–His)$_4$]SO_4[19]. A homoleptic $Zn(histidine)_4$ coordination in proteins has, to our knowledge, not been observed yet.

On the basis of the tetrahedral ZnN_4 coordination resulting from the EXAFS analysis of **K8** a tentative structural model for this complex can be derived. Again the choice is between a mononuclear bis(ligand) complex analogous to formula **A** or a coordination polymer analogous to formula **B**. We disfavor the mononuclear complex not

Figure 2. Experimental (——) and calculated (-----) k^3 weighted EXAFS spectra (top: Fig. 2a) and their Fourier transforms (bottom: Fig. 2b) for $[(His-Gly-Gly-His)_2Zn](ClO_4)_2$

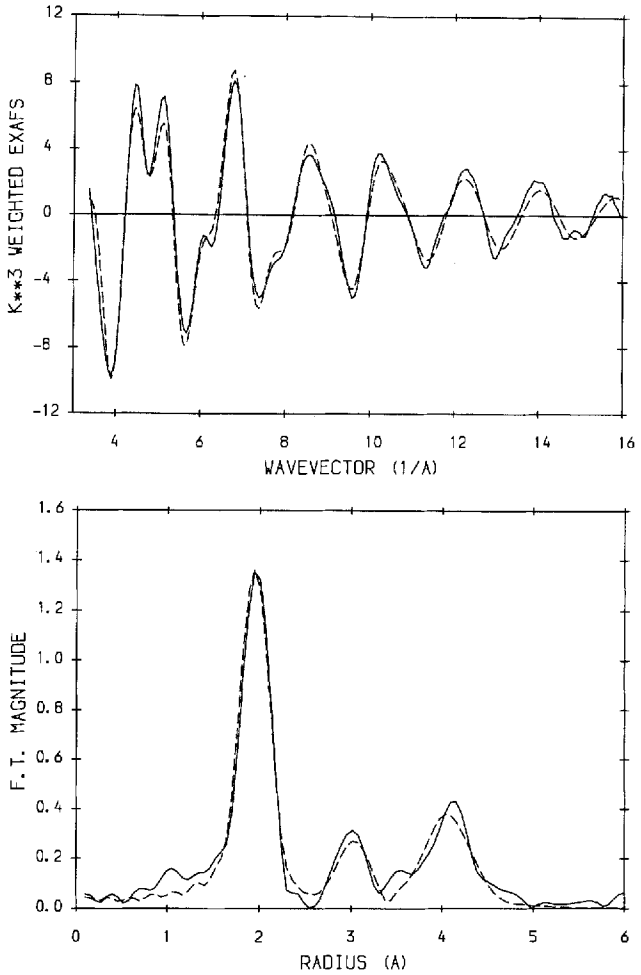
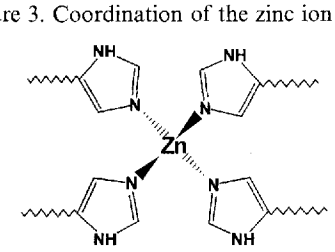


Figure 3. Coordination of the zinc ion in **K8**

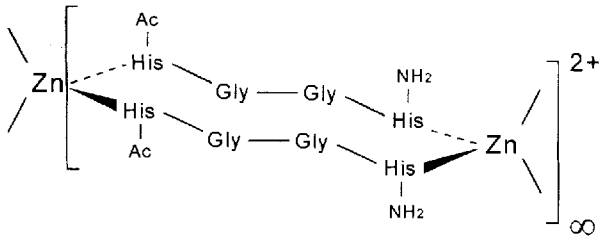


only because of the insolubility of the compound but also because it would contain two seventeen-membered chelate rings of considerable strain. Among the coordination polymers a choice has to be made between the one-dimensional chain and the two- or three-dimensional network type. We have a slight preference for the one-dimensional model because in our opinion a network of zinc ions and tetrapeptide units would have too many large cavities. We therefore suggest the structure outlined in Figure 4 for complex **K8**.

Conclusions

The seven peptides investigated in this study which bear histidine units at both ends prefer to form zinc complexes with a 1:1 (zinc:peptide) composition. The available structural data for these complexes as well as for the one complex with a 1:2 (zinc/peptide) composition confirm that all available histidine imidazole groups are bound to the metal in all cases. While this provides a closed coordination sphere of the ZnN_4 type for the bis(peptide)complex, there is some ambiguity concerning the remaining donor ligands in the mono(peptide)complexes. For these a $ZnN_2(anion)_2$ coordination seems to be the most likely one. Despite some provisions to have the peptide ligands fold such that their two histidine units get close to one another it seems that mononuclear complexes are not formed and that one-dimensional coordination polymers are present in all cases.

Figure 4. Proposed structure for $(His-Gly-Gly-His)Zn(ClO_4)_2$ (**K8**)



By the attachment of protecting groups to both ends of the peptide chain the coordinative abilities of the peptides were limited to those of the histidine donor functions, i.e. they were made analogous to histidine-containing proteins. The fact that mononuclear complexes don't form allows the interpretation that the mutual preferences between zinc and histidine are not strong enough to shape a His–X–His or His–X–X–His sequence in a peptide into a folding which conforms to coordination to a single zinc ion. This corresponds to the fact that the natural His–X–His or His–X–X–His sequences binding to zinc (e.g. in carbonic anhydrase, carboxypeptidases, or zinc fingers[2,3]) are all part of a large protein and thereby preorganized. It does not correspond, however, to the situation with Cys–X–Cys or Cys–X–X–Cys sequences in small proteins like the metallothioneins[15]. For these systems, and according to our experiences with zinc complexes of the corresponding small peptides[11], it can be assumed that there is a significant tendency of the zinc ion to bind to both cysteine units of the same sequence and thus to exert a stabilizing and structuring effect on the peptide. Thus, although histidine and cysteine are equally common as zinc-binding units in large proteins, the large stability difference between the Zn–thiolate and the Zn–imidazole bonds is the reason for qualitative differences which are evident in small proteins and which cause a completely different coordination chemistry with small peptides.

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Experimental

The general working and measuring methods are described in ref.[20], the HPLC procedures are described in ref.[1]. – MS: Finnigan TSQ 700, electrospray ion source and a triple quadrupole MS detector. The peptides were injected as solutions in acetonitrile/water, acidified with TFA, the complexes were dissolved in dimethyl formamide.

All amino acid derivatives and all zinc salts were obtained commercially. The following abbreviations are used: HOBT: hydroxybenzotriazole, DCC: dicyclohexylcarbodiimide, NEM: *N*-ethylmorpholine, TFA: trifluoroacetic acid. The Fmoc-protected ADPV resin which had a bonding capacity of 0.85 mmol/g was obtained from Rapp Polymere, Tübingen.

Solid-State Peptide Synthesis: The following procedure was strictly adhered by using 50-ml reaction vessels being charged through the top opening and being emptied laterally through a G3 frit with a teflon stopcock. The resin was soaked in DMF for 2 h and then put into the reaction vessel. The Fmoc groups were removed from the resin by treating it twice with 10 ml of methanol/piperidine (1:1) for 5 min. Then the resin was washed three times with 5 ml of DMF for 2 min, once with 5 ml of methanol for 3 min, twice with 5 ml of DMF for 2 min, twice with 5 ml of methanol for 5 min, and three times with 5 ml of DMF for 2 min. The first amino acid component was activated with stoichiometric amounts of HOBT and DCC in 5 ml of DMF at 0 °C and then filtered into the reaction vessel. One equivalent of NEM per equivalent of resin was added and the mixture was shaken overnight. Then two further equivalents of NEM were added, and after 30 min the liquid phase was separated from the resin by filtration. The remaining resin, now bearing a peptide component, was washed three times with 5 ml of DMF for 2 min, twice with 5 ml of methanol for 5 min, and three times with 5 ml of DMF for 2 min. After this completion of the first amino acid attachment step any free amino groups which possibly remained on the resin were acetylated by adding five equivalents of acetic anhydride and two equivalents of NEM per equivalent of resin in 10 ml of DMF. After shaking for 15 min all liquids were filtered off again from the resin. Thus the first coupling cycle was finished.

All subsequent coupling cycles started with the first washing sequence described above followed by the activation and coupling of the next amino acid component and ending with the second washing cycle.

When the final amino acid was attached and the resin washed again the Fmoc groups were removed from the end of the peptide chain as described above followed again by the first washing cycle. Acetylation of the N-terminus was achieved by adding ten equivalents of acetic anhydride and five equivalents of NEM per equivalent of resin in 10 ml of DMF, shaking for 30 min, filtering, performing the first washing cycle, and finally washing three times with 5 ml of methanol for 5 min and three times with 5 ml of ether for 5 min. Benzoylation of the N-terminus was achieved by the same procedure starting with five equivalents of benzoyl chloride and five equivalents of NEM per equivalent of resin.

For the final step, the removal of the peptide from the resin, the loaded resin was first dried in vacuo. Then 10 ml of TFA and 1 ml of thioanisole were added, the mixture was shaken overnight, then the liquids were filtered off, and the remaining resin was washed twice with 10 ml of TFA and twice with 10 ml of acetonitrile. The combined filtrates were evaporated to dryness at temperatures below 50 °C to afford the peptide (mostly colored and sometimes oily). This was dissolved in a small amount of TFA or glacial acetic acid and the solution was poured dropwise into 100 ml of ether cooled to 0 °C. The peptide formed a colorless precipitate. After centrifugation the precipitate was dissolved in a minimum amount of water and freeze-dried. The remaining colorless powder was subjected to preparative HPLC for the final purification.

1: From 1.20 g (1.00 mmol) of resin, 1.60 g (2.50 mmol) of Fmoc–His(Trt)–OH, 0.76 g (2.50 mmol) of Fmoc–Gly–OH, and 1.60 g (2.50 mmol) of Fmoc–His(Trt)–OH. Benzoylation with 1.41 g (10.0 mmol) of benzoyl chloride. HPLC: acetonitrile/water (12:88) with 0.1% TFA; flow rate 6.0 ml/min. Yield 176 mg (39%) of *N*-Bz–His–Gly–His–NH_2 · 3 TFA abbreviated as **1**.

2: From 1.00 g (0.85 mmol) of resin, 1.60 g (2.50 mmol) of Fmoc–His(Trt)–OH, 0.76 g (2.50 mmol) of Fmoc–Gly–OH, another 0.76 g (2.50 mmol) of Fmoc–Gly–OH, and 1.60 g (2.50 mmol) of Fmoc-His(Trt)–OH. Acetylation with 0.80 g (8.5 mmol) of acetic anhydride. HPLC: acetonitrile/water (4:96), containing 0.1% TFA, flow rate 7.0 ml/min. Yield 328 mg (57%) of *N*-Ac–His–Gly–Gly–His–NH_2 · 2 TFA abbreviated as **2**.

3: From 0.52 g (0.43 mmol) of resin, 0.62 g (1.00 mmol) of Fmoc–His(Trt)–OH, 0.30 g (1.00 mmol) of Fmoc–Gly–OH, 0.31 g (1.00 mmol) of Fmoc–Ala–OH, and 0.62 g (1.00 mmol) of Fmoc–His(Trt)–OH. Acetylation with 0.40 g (4.3 mmol) of acetic anhydride. HPLC: acetonitrile/water (1:99) with 0.1% TFA, flow rate 8.0/min. Yield 161 mg (53%) of *N*-Ac–His–Ala–Gly–His–NH_2 · 2 TFA abbreviated as **3**.

4: From 1.81 g (1.50 mmol) of resin, 2.32 g (3.75 mmol) of Fmoc–His(Trt)–OH, 1.34 g (3.75 mmol) of Fmoc–Gly–OH, 1.59 g (4.50 mmol) of Fmoc–Leu–OH, and 3.10 g (5.00 mmol) of Fmoc–His(Trt)–OH. Benzoylation with 1.41 g (10.0 mmol) of benzoyl chloride. HPLC: acetonitrile/water (22:78) with 0.1% TFA, flow rate 6.0 ml/min. Yield 444 mg (52%) of *N*-Bz–His–Leu–Gly–His–NH_2 · 4 TFA abbreviated as **4**.

5: From 2.00 g of (1.70 mmol) resin, 3.16 g (5.10 mmol) of Fmoc–His(Trt)–OH, 1.51 g (5.10 mmol) of Fmoc–Gly–OH, 1.72 g (5.10 mmol) of Fmoc–Pro–OH, and 3.16 g (5.10 mmol) of Fmoc–His(Trt)–OH. Acetylation with 1.60 g (17.0 mmol) of acetic anhydride. HPLC: acetonitrile/water (6:94) with 0.1% TFA, flow rate 7 ml/min. Yield 701 mg (57%) of *N*-Ac–His–Pro–Gly–His–NH_2 · 2 TFA abbreviated as **5**.

6: From 2.0 g (1.70 mmol) of resin, 3.16 g (5.10 mmol) of Fmoc–His(Trt)–OH, 1.80 g (5.10 mmol) of Fmoc–Asn–OH, 1.72 g (5.10 mmol) of Fmoc–Pro–OH, and 3.16 g (5.10 mmol) of Fmoc–His(Trt)–OH. Acetylation with 1.60 g (17.0 mmol) of acetic anhydride with 0.1% TFA. HPLC: acetonitrile/water (7:93), flow rate 8 ml/min. Yield 925 mg (73%) of *N*-Ac–His–Pro–Asn–His–NH_2 · 2 TFA abbreviated as **6**.

7: From 1.73 g (1.4 mmol) of resin, 2.23 g (3.60 mmol) of Fmoc–His(Trt)–OH, 1.07 g (3.60 mmol) of Fmoc–Gly–OH, 1.59 g (4.32 mmol) of Fmoc–Pro–OH, 1.19 g (3.60 mmol) of Fmoc–Ala–OH, and 2.23 g (3.60 mmol) of Fmoc–His(Trt)–OH. Benzoylation with 1.41 g (10.0 mmol) of benzoyl chloride. HPLC: acetonitrile/water (18:82) with 0.1% TFA, flow rate 1.5 ml/min. Yield 446 mg (50%) of *N*-Bz–His–Ala–Pro–Gly–His–NH_2 · TFA abbreviated as **7**.

Relevant IR data of the peptides [ν(NH), amide I, amide II, in KBr, $\tilde{\nu}$ in cm^{-1}]: **1**: 3307, 3157, 1675, 1539. **2**: 3410, 3287, 1671, 1540. **3**: 3440, 1673, 1538. **4**: 3288, 1672, 1535. **5**: 3432, 1670, 1536. **6**: 3443, 3380, 1670, 1535. **7**: 3278, 1671, 1541.

^{1}H-NMR data of the peptides (calibrated on the residual proton resonance of the deuterated solvent, δ in ppm): **1** (D_2O): δ = 3.17–3.49 (m, 4H, $C_\beta H_2$ His), 3.97 (dd, ^2J = 12.8 Hz, ^3J = 8.5 Hz, 2H, $C_\alpha H_2$ Gly), 4.85 (m, 2H, $C_\alpha H$ His), 7.27 (s, 1H, 4-H), 7.75–7.50 (m, 5H, CH Bz), 7.35 (s, 1H, 4-H), 8.54 (s, 1H, 2-H), 8.64 (s, 1H, 2-H). **2** (DMSO): δ = 1.83 (s, CO-CH_3), 2.9–3.2 (m, 4H, $C_\beta H_2$, His), 3.73 (m, 4H, Gly), 4.4–4.7 (m, 2H, $C_\alpha H$, His),

7.31 (s, 1H, 4-H), 7.36 (s, 1H, 4-H), 7.94 (s, 2H, 2-H). **3** (DMSO): δ = 1.31 (d, ^3J = 7.2 Hz, 3H, $C_\beta H_3$, Ala), 1.99 (s, 3H, CO-CH_3), 3.1–3.4 (m, 4H, $C_\beta H_2$, His), 3.93 (s, 2H, Gly), 4.05 (t, ^3J = 7.2 Hz, 1H, $C_\alpha H$ Ala), 4.27 (m, 2H, $C_\alpha H$, His), 7.04 (s, 1H, 4-H), 7.08 (s, 1H, 4-H), 8.17 (s, 1H, 2-H), 8.21 (s, 1H, 2-H). **4** (D_2O): δ = 0.87 (m, 6H, $C_\delta H_3$ Leu), 1.45 (m, 3H, $C_\beta H_2$ Leu, $C_\gamma H$ Leu), 3.08–3.39 (m, 4H, $C_\beta H_2$ His), 3.92 (m, 2H, Gly), 4.40 (m, 1H, $C_\alpha H$ Leu), 4.65–4.94 (m, 2H, $C_\alpha H$ His), 7.27 (s, 1H, 4-H), 7.32 (s, 1H, 4-H), 7.47–7.70 (m, 5H, CH Bz), 8.60 (s, 1H, 2-H), 8.62 (s, 1H, 2-H). **5** (DMSO): δ = 1.78 (m, 6H, $COCH_3$, Pro β/γ), 2.03 (m, 1H, Pro γ), 2.7–3.1 (m, 4H, $C_\beta H_2$, His), 3.49 (s, 2H, Gly), 3.5–3.7 (m, 2H, Pro δ), 4.25 (m, 1H, $C_\alpha H$, His), 4.41 (m, 1H, $C_\alpha H$, His), 4.61 (m, 1H, $C_\alpha H$, Pro), 6.88 (s, 1H, 4-H), 7.01 (s, 1H, 4-H), 7.78 (s, 1H, 2-H), 7.92 (s, 1H, 2-H). **6** (DMSO): δ = 1.7–2.1 (m, 7H, $COCH_3$, Pro β/γ), 2.7–3.1 (m, 4H, $C_\beta H_2$, His), 3.3–3.5 (m, 4H, Pro δ, Asn β), 4.2–4.5 (m, 4H, $C_\alpha H$, His/Pro/Asn), 6.91 (s, 1H, 4-H), 6.98 (s, 1H, 4-H), 7.99 (s, 1H, 2-H), 8.05 (s, 1H, 2-H). **7** (D_2O): δ = 1.65 (m, 3H, $C_\beta H_3$ Ala), 2.13 (m, 4H, $C_\beta H_2$ Pro, $C_\gamma H_2$ Pro), 2.93–3.38 (m, 6H, $C_\beta H_2$ His, $C_\delta H_2$ Pro), 4.02 (m, 2H, Gly), 4.42 (m, 2H, $C_\alpha H$ Ala, $C_\alpha H$ Pro), 4.66 (m, 1H, $C_\alpha H$ His1), 4.90 (m, 1H, $C_\alpha H$ His2), 7.11 (s, 1H, 4-H), 7.18 (s, 1H, 4-H), 7.51–7.78 (m, 5H, CH Bz), 8.05 (s, 1H, 2-H), 8.12 (s, 1H, 2-H).

Zinc complexes. **K1**: 51.1 mg (64.3 μmol) of **1** was dissolved in 1 ml of water. 22.3 mg (64.3 μmol) of $Zn(BF_4)_2 \cdot 6\ H_2O$ and 4 ml of water were added to the solution. This was treated dropwise with stirring with a 0.1 M NaOH solution until the mixture had pH 7. The resulting precipitate was removed by centrifugation and washed with ether to furnish 29 mg (49%) of colorless **K1**, m.p. 230°C (dec.). $C_{21}H_{24}B_2F_8N_8O_4Zn \cdot 2\ C_2HF_3O_2$ (919.5): calcd. C 32.66, H 2.85, N 12.19, Zn 7.11; found C 31.99, H 2.77, N 12.50, Zn 6.85.

K4a: Likewise from 10.2 mg (18.1 μmol) of **4** and 2.45 mg (18.0 μmol) of $ZnCl_2$. Yield 6.9 mg (47%) of colorless **K4a**, m.p. 220°C (dec.). $C_{27}H_{35}Cl_2N_9O_5Zn \cdot C_2HF_3O_2$ (815.9): calcd. C 42.69, H 4.45, N 15.45, Zn 8.01; found C 43.15, H 4.63, N 15.84, Zn 7.95.

K4b: Likewise from 49.0 mg (86.5 μmol) of **4** and 19.7 mg (86.5 μmol) of $ZnSO_4 \cdot 7\ H_2O$. Yield 15 mg (21%) of colorless **K4b**, m.p. 230°C (dec.) $C_{27}H_{35}N_9O_9SZn \cdot C_2HF_3O_2$ (841.1): calcd. C 41.41, H 4.31, N 14.99, Zn 7.77; found C 42.08, H 4.46, N 14.90, Zn 7.44.

K7: Likewise from 22.0 mg (35.5 μmol) of **7** and 10.2 mg (35.5 μmol) of $ZnSO_4 \cdot 7\ H_2O$. Yield 5 mg (14%) of colorless **K7**, m.p. 245°C (dec.). $C_{29}H_{36}N_{10}O_{10}SZn \cdot C_2HF_3O_2$ (896.2): calcd. C 41.55, H 4.16, N 15.63, Zn 7.30; found C 41.66, H 4.07, N 14.76, Zn 7.43.

K2: To a solution of 100 mg (0.13 mmol) of **2** in 5 ml of methanol a solution of 18 mg (0.13 mmol) of $ZnCl_2$ in 5 ml of methanol was added dropwise. The mixture was heated at reflux for 1 h, then filtered through a membrane filter. The flask containing the filtrate was connected via a stopcock to a second flask containing a 2 M aqueous ammonia solution. Precipitation started and was complete after about 3 d. The precipitate was removed by centrifugation and washed with methanol and ether to afford 64 mg (84%) of colorless **K2**, m.p. 365°C (dec.). $C_{18}H_{25}Cl_2N_9O_5Zn$ (583.8): calcd. C 37.03, H 4.32, N 21.60, Zn 11.20; found C 37.24, H 4.25, N 21.57, Zn 11.30.

K3: Likewise from 70 mg (0.095 mmol) of **3** and 22.5 mg (0.095 mmol) of $ZnBr_2$. Yield 35 mg (56%) of colorless **K3**, m.p. 335°C (dec.). $C_{19}H_{27}Br_2N_9O_5Zn$ (686.7): calcd. C 33.23, H 3.96, N 18.36, Zn 9.52; found C 32.34, H 3.50, N 16.48, Zn 9.75.

K5: Likewise from 86 mg (0.12 mmol) of **5** and 17 mg (0.12 mmol) of $ZnCl_2$. Yield 44 mg (58%) of colorless **K5**, m.p. 295°C (dec.). $C_{21}H_{32}Cl_2N_9O_5Zn$ (626.8): calcd. C 40.24, H 5.15, N 20.12, Zn 10.43; found C 40.47, H 4.79, N 19.89, Zn 10.30.

K6: Likewise from 77 mg (0.10 mmol) of **6** and 14 mg (0.10 mmol) of $ZnCl_2$. Yield 37 mg (54%) of colorless **K6**, m.p. 305°C (dec.). $C_{23}H_{35}Cl_2N_{10}O_6Zn$ (683.8): calcd. C 40.39, H 5.16, N 20.49, Zn 9.56; found C 39.76, H 4.98, N 19.76, Zn 9.25.

K8: Likewise from 100 mg (0.13 mmol) of **2** and 20 mg (0.06 mmol) of $Zn(ClO_4)_2 \cdot 6\ H_2O$. Yield 59 mg (58%) of colorless **K8**, m.p. 310°C (dec.). $C_{36}H_{50}Cl_2N_{18}O_{18}Zn$ (1159.3): calcd. C 37.30, H 4.35, N 21.75, Zn 5.64; found C 39.17, H 4.52, N 22.56, Zn 5.50.

Relevant IR data of the complexes [ν(NH), amide I, amide II, in KBr, $\tilde{\nu}$ in cm^{-1}]: **K1**: 3404, 1673, 1529. **K2**: 3385, 3304, 1656, 1538. **K3**: 3410, 3134, 1667, 1538. **K4a**: 3306, 1672, 1532. **K4b**: 3306, 1672, 1532. **K5**: 3446, 3184, 1683, 1646, 1539. **K6**: 3470, 3414, 1650, 1618, 1537. **K7**: 3304, 1643, 1536. **K8**: 3388, 3308, 1674, 1536.

^{1}H-NMR data of the complexes (calibrated on the residual proton resonance of the deuterated solvent, δ in ppm): **K2** (DMSO): δ = 1.82 (s, 3H, CO-CH_3), 2.9–3.2 (m, 4H, $C_\beta H_2$, His), 3.67 (s, br., 4H, Gly), 4.47 (s, br., 2H, $C_\alpha H$, His), 6.42 (s, 1H, 4-H), 6.55 (s, 1H, 4-H), 6.91 (s, br., 1H, 2-H), 7.06 (s, br., 1H, 2-H). **K3** (DMSO): δ = 1.40 (d, ^3J = 5.6 Hz, 3H, $C_\beta H_3$, Ala), 1.93 (s, 3H, CO-CH_3), 2.9 (m, 4H, $C_\beta H_2$, His), 3.49 (d, br., ^3J = 4.2 Hz, 2H, Gly), 3.88 (t, ^3J = 5.6 Hz, 1H, $C_\alpha H$, Ala), 4.12 (m, 2H, $C_\alpha H$, His), 6.28 (s, br., 1H, 4-H), 6.33 (s, br., 1H, 4-H), 7.18 (s, br., 1H, 2-H), 7.21 (s, br., 1H, 2-H). **K4a** (D_2O): δ = 0.77 (m, 6H, $C_\delta H_3$-Leu), 1.48 (m, 3H, $C_\beta H_2$ Leu, $C_\gamma H$ Leu), 3.00–3.14 (m, 4H, $C_\beta H_2$ His), 3.59–3.85 (m, 2H, Gly), 4.20 (m, 1H, $C_\alpha H$ Leu), 4.53–4.73 (m, 2H, $C_\alpha H$ His), 6.92 (s, 1H, 4-H), 6.97 (s, 1H, 4-H), 7.39–7.59 (m, 5H, CH Bz), 7.86 (s, 1H, 2-H), 7.91 (s, 1H, 2-H). **K4b** (CD_3CN, 10% D_2O): δ = 0.82 (d, ^3J = 8.7 Hz, 3H, $C_\delta H_3$ Leu), 0.82 (d, ^3J = 8.0 Hz, 3H, $C_\delta H_3$ Leu), 1.48 (ddd, ^3J = 7.6 Hz, ^3J = 7.1 Hz, ^2J = 10.7 Hz, 1H, $C_\beta H_2$ Leu), 1.58 (m, 1H, $C_\gamma H$ Leu), 1.61 (ddd, ^3J = 8.0 Hz, ^3J = 7.6 Hz, ^2J = 10.7 Hz, 1H, $C_\beta H_2$ Leu), 3.08 (dd, ^3J = 5.1 Hz, ^2J = 15.3 Hz, 1H, $C_\beta H_2$ His2), 3.17 (dd, ^3J = 7.6 Hz, ^2J = 14.3 Hz, 1H, $C_\beta H_2$ His1), 3.22 (dd, ^3J = 7.6 Hz, ^2J = 15.3 Hz, 1H, $C_\beta H_2$ His2), 3.28 (dd, ^2J = 14.3 Hz, ^2J = 7.6 Hz, 1H, $C_\beta H_2$ His1), 3.78 (dd, ^3J = 7.6 Hz, ^2J = 17.8 Hz, 1H, $C_\alpha H_2$ Gly), 3.91 (dd, ^3J = 7.6 Hz, ^2J = 17.8 Hz, 1H, $C_\alpha H_2$ Gly), 4.25 (dd, ^3J = 7.6 Hz, ^3J = 7.4 Hz, 1H, $C_\alpha H$ Leu), 4.58 (ddd, ^3J = 5.1 Hz, ^3J = 7.6 Hz, ^3J = 10.2 Hz, 1H, $C_\alpha H$ His2), 4.78 (ddd, ^3J = 7.6 Hz, ^3J = 7.6 Hz, ^3J = 7.6 Hz, 1H, $C_\alpha H$ His1), 7.11 (s, 1H, 4-H His2), 7.12 (s, 1H, 4-H His1), 7.46–7.78 (m, 5H, CH Bz), 7.56 (d, ^3J = 10.2 Hz, 1H, NH His), 7.77 (d, ^3J = 7.6 Hz, 1H, NH Leu), 7.92 (dd, ^3J = 7.6 Hz, ^3J = 7.6 Hz, 1H, NH Gly), 8.04 (d, ^3J = 7.6 Hz, 1H, NH His1), 8.18 (s, 1H, 2-H His2), 8.20 (s, 1H, 2-H His1). **K5** (DMSO): δ = 1.79 (m, 6H, $COCH_3$, Pro β/γ), 2.0 (m, 1H, Pro γ), 2.7–3.0 (m, br., 4H, $C_\beta H_2$, His), 3.49 (m, br., 2H, Gly), 3.5–3.7 (m, br., 2H, Pro δ), 4.25 (m, br., 1H, $C_\alpha H$, His), 4.49 (m, br., 1H, $C_\alpha H$, His), 4.74 (m, br., 1H, $C_\alpha H$, Pro), 7.04 (s, br., 1H, 4-H), 7.15 (s, br., 1H, 4-H), 7.23 (s, br., 1H, 2-H), 7.34 (s, br., 1H, 2-H). **K6** (DMSO): δ = 1.6–2.2 (m, 7H, $COCH_3$, Pro β/γ), 2.8–3.6 (m, 8H, $C_\beta H_2$, His, Pro δ, Asn β), 4.2–4.7 (m, 4H, $C_\alpha H$, His/Pro/Asn), 7.03 (s, br., 1H, 4-H), 7.23 (s, br., 1H, 4-H), 7.40 (s, br., 1H, 2-H), 8.10 (s, br., 1H, 2-H).

XAS-Measurements and Data Analysis: A finely powdered sample of **K8** was introduced into a 1 mm thick sample cell (approx. 50 μl) and covered with kapton windows. The X-ray absorption data (transmission, 20 K) were recorded at the EMBL EXAFS beam line[21,22] at HASYLAB (DESY, Hamburg) by using ionization chambers. During data collection DORIS III was operated at 4.5 GeV in the dedicated mode with ring currents between 80 and 40 mA.

A Si(111) double crystal monochromator with an energy resolution of 1.8 eV at 9670 eV was used. The first monochromator crystal was detuned to 50% in order to reject higher-order harmon-

ics. No focussing mirror was used. The monochromator angle was converted to absolute energy by using the calibration technique described in ref.[23] The spectra were taken from 9200 eV to 10700 eV with variable step widths. In the XANES and EXAFS regions, steps of 0.3 and 0.7–1.5 eV respectively, were used. Absorption values μx of 0.5 below the edge and 1.6 above the edge were obtained from the experimental spectrum. The data reduction was performed by using the program set EXPROG[24]. Five spectra of the sample were averaged after individual energy calibration. A four-segmented cubic spline routine was used for background subtraction. The extracted EXAFS spectrum was converted to k space and weighted by k^3 to compensate for the reduced amplitude at high k values. The program package EXCURVE88[25] was used to derive structural parameters by using the small-atom approximation and applying multiple scattering calculations to model the imidazole ligands. No Fourier filtering was applied to the data.

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