Dipeptides Made up Solely from Histidine: Solution Behaviour and Zinc Complexation[‡]

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The acid/base chemistry and the zinc complexation of the four dipeptides H-HisHis-OH, H-HisHis-OEt, Ac-HisHis-OH and Ac-HisHis-OEt were investigated by potentiometric methods. 1:1 (zinc:dipeptide) complexes prevail in solution, and those complexes that could be isolated on a preparative scale also have 1:1 compositions. The stability data confirm that exclusive zinc-imidazole(histidine) coordination is weak, and additional effects are essential to effect the high preference of zinc for histidine in proteins. A comparison of the stability data for zinc complexes of all dipeptides made up solely from histidine and/or cysteine reveals systematic trends and allows a parameterization of the log β values.

Introduction

Both catalytic and structural zinc in enzymes are always coordinated by at least one cysteine or histidine donor, and specifically in hydrolytic zinc enzymes a $(histidine)_3Zn-X$ coordination is typical.[2,3] This is the reason why we have focused heavily on peptides containing cysteine or histidine in our series of studies on zinc peptide complexes. Prior and parallel to our investigations there were relatively few reports in the literature on the zinc coordination of small peptides dominated by these two amino acids. The main reason for this is the great effort which is necessary for the synthesis of peptides that are rich in cysteine or histidine.

This paper completes our work on the zinc complexation of peptides containing two cysteine and/or histidine units. Previously we have reported on HisCys[4] and CysCys[1] dipeptides, on cyclic dipeptides,[5] and on tri- and tetrapeptides with cysteine and/or histidine at both termini and referenced the related literature.[6−8] The missing part — the HisHis dipeptides — is reported here.

In principle simple unprotected peptides are not suitable as representatives of the related proteins. They offer additional donor functions by their amino and carboxylate termini, and they lack the geometrical effects resulting from folding and helix formation. This disadvantage is compensated, however, by the basic information obtainable from them. When they have unprotected termini, the effect of those on complex formation can be quantified; when they offer only their side-chain donors the strength of the zinc−side-chain interactions can be evaluated free from the influences of protein preorganization. Being inorganic and not protein chemists we see our justification in providing this basic information.

For the present study the four possible HisHis dipeptides listed below were chosen. They represent the various stages of reduction of the donor functionality to exclusive imidazole donation. They were subjected to potentiometric titrations in the absence and presence of zinc nitrate, and they were treated with zinc salts on a preparative scale. The data obtained from them have enabled a comprehensive comparison of zinc complex stabilities for all small peptides with two closely spaced histidine and/or cysteine units.

H-HisHis-OH H-HisHis-OEt
Ac-HisHis-OH Ac-HisHis-OEt

Results and Discussion

Synthesis of the Peptides

The preparation of HisHis dipeptides requires the heavy use of protective groups due to the trifunctional nature of histidine itself. Such dipeptides have been reported in the literature,[9] and H-HisHis-OH is commercially available. We chose to prepare the four dipeptides by the methods used in our laboratory. As outlined in the Exp. Sect., Boc protection of the amino terminus and ethyl ester protection of the carboxylate terminus were used. The imidazole function was protected either by the Boc or the Trt groups. Peptide coupling was effected either by the CAIBE or by the DCC methods, and the standard procedures were applied to remove the protective groups as necessary. Final purification by preparative HPLC yielded the TFA adducts of the

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four peptides in gram quantities as analytically pure solids which are very hygroscopic.

Isolated Zinc Complexes

All four peptides were treated in a 1:1 and a 2:1 ratio with zinc perchlorate, nitrate, trifluoroacetate, chloride and iodide. Solid products containing zinc and the peptide could be obtained in all cases, but only some of the 1:1 reactions yielded complexes with simple compositions. It must be concluded that the formation of coordination polymers prevails. As observed by us before[1,4,5] the odd zinc:peptide ratios of these compounds make it difficult to assign constitutions or structures to them.

Those 1:1 reactions that were successful yielded 1:1 complexes in water or ethanol/water. The fully unprotected peptide could be combined with zinc trifluoroacetate to form **1**. Likewise, Ac-HisHis-OH yielded the trifluoroacetate **2**. Zinc chloride could be combined with Ac-HisHis-OH to form **3**, and with the fully protected peptide to form **4**. After slow crystallization these complexes were analytically pure but not suited for crystal structure determinations. Once crystallized they were not soluble enough for NMR measurements. Thus, although they seem to be mononuclear in solution (as is also evident from potentiometry, see below) they must be coordination polymers in the solid state. Counting the number of donor functions available for the zinc ions in the observed compositions allows the conclusion that zinc is tetrahedrally coordinated both in the monomers and the polymers. While none of our zinc complexes of bis(histidine) oligopeptides[1,6,8] has formed crystals suitable for X-ray analysis, we could confirm the tetrahedral coordination by an EXAFS analysis for $Zn(Ac\text{-}His\text{-}GlyGlyHis\text{-}NH_2)_2Zn(ClO_4)_2$.[6]

(H-HisHis-O)Zn-OCOCF$_3$ **1**

(Ac-HisHis-O)Zn-OCOCF$_3$ **2**

(Ac-HisHis-O)Zn-Cl **3**

(Ac-HisHis-OEt)ZnCl$_2$ **4**

Potentiometric Titrations

The acid/base titrations of the peptides themselves initially determined the amount of trifluoroacetic acid contained in them, as given in the Exp. Sect. Then they yielded the pK_a values which are listed in Table 1.

Table 1. Acid dissociation constants (pK_a) of the peptides (standard deviations in parentheses)

	COOH	ImH$^+$(1)	ImH$^+$(2)	NH_3^+
H-HisHis-OH	2.58(9)	5.72(1)	6.83(1)	8.04(4)
H-HisHis-OEt		5.47(1)	6.63(2)	7.86(3)
Ac-HisHis-OH	2.68(8)	6.40(6)	7.60(7)	
Ac-HisHis-OEt		5.96(11)	6.89(17)	

The carboxylate and amine pK_a's of the dipeptides can simply be referenced against those of histidine itself [pK(COOH) = 1.77, pK(NH_3^+) = 9.24].[9] The insertion of the second histidine unit between the carboxylate and ammonium functions, i.e. increasing their distance, eliminates completely their mutual influence. As a result the carboxylic pK_a rises and the ammonium pK_a decreases by about one unit. For histidine itself the imidazole pK_a (6.14) rises when the amino function is blocked and decreases when the carboxylate function is blocked;[10] these are basically charge effects. This can also be seen when comparing H-HisHis-OH with both H-HisHis-OEt and Ac-HisHis-OH in terms of the first imidazole pK_a. It seems that a mutual compensation of these two effects moves the first imidazole pK_a of Ac-HisHis-OEt back to the value of H-HisHis-OH.

In terms of exclusive histidine coordination, like in the proteins themselves the imidazole pK_a's of the fully protected histidine peptides are relevant. While the fully protected histidine Ac-His-NH_2 (pK = 7.07)[11] and isolated histidine in the peptide Ac-HisVal-OH (pK = 6.84)[12] have the typical pK_a for histidine near 7, the first histidine pK_a for all four dipeptides in this study is roughly one unit lower. This again is a charge effect: the positive charge on the second imidazolium function makes the deprotonation of the first one easier. The two pK_a values for Ac-HisHis-OEt are almost identical to those for the two histidine units in fully protected His-X-His tripeptides with X = Gly, Pro, Val and Apa.[8] This indicates that the distances between the two imidazolium functions has no pronounced effect. On the other hand, cyclo-HisHis shows pK_a's of 5.53 and 6.61[5] which are significantly lower than the others, and which may be correlated with the highly polar nature of this diketopiperazine derivative. One feature is common for all the bis(histidine) peptides mentioned here: their two imidazole pK_a's are all 1.1 ± 0.1 units apart.

The titrations in the presence of zinc nitrate did not suffer from the problem of precipitation which is a common one for cysteine-containing peptides. The rich species distributions exemplified by Figures 1 and 2 could be analyzed and correlated with the complex stabilities listed in Table 2.

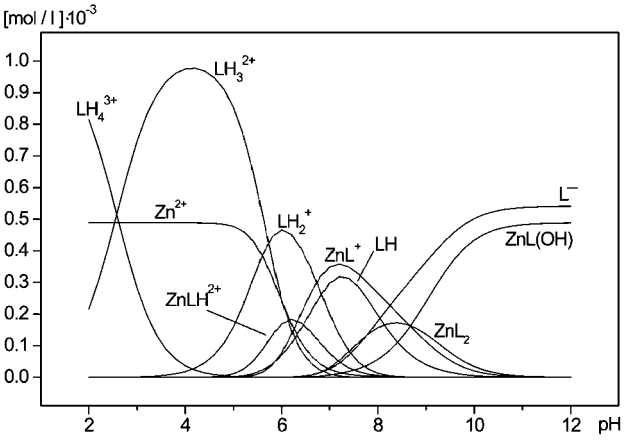

Figure 1. Species distribution in a solution of H-HisHis-OH and $Zn(NO_3)_2$ in a ratio of 2.1:1

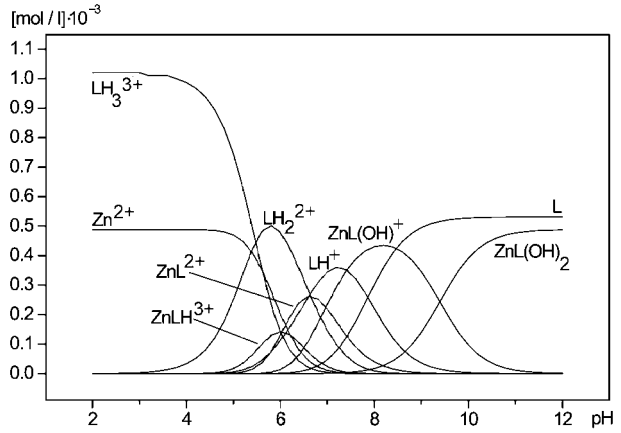

Figure 2. Species distribution in a solution of H-HisHis-OEt and $Zn(NO_3)_2$ in a ratio of 2:1

Table 2. Stability constants for the zinc complexes (log β) in solutions of the peptides (σ values in parentheses)

	ZnLH	ZnL	ZnL(OH)	$ZnL(OH)_2$	ZnL_2
H-HisHis-OH	12.02(6)	5.79(13)	−3.18(18)	−	9.18(6)
H-HisHis-OEt	11.70(2)	5.70(6)	−1.20(9)	−10.62(8)	−
Ac-HisHis-OH	−	3.35(15)	−3.74(18)	−	−
Ac-HisHis-OEt	10.05(4)	4.19(11)	−2.31(11)	−	−

As a rule only three complex species were necessary to fit the data: ZnLH, ZnL and ZnLOH, which are all 1:1 complexes. Of these, ZnLH represents a complex with a protonated peptide ligand, and ZnLOH represents a complex with a deprotonated water ligand, and hence ZnL is the only relevant species. Only in the case of H-HisHis-OH is a ZnL_2 complex observed, which must have to do with the fact that this ZnL_2 complex is uncharged and there is good chelation involving the imidazole and amino nitrogen donors.[10]

The stability constants for the four ZnL species have characteristic and very informative values. First and most important, they underline that the complex stability provided by exclusive Zn-imidazole(histidine) coordination is unusually small. Even two such interactions, as for Ac-HisHis-OEt, yield only a moderate stability of the ZnL species. This is also evident from the data for ZnL complexes of fully protected His-X-His peptides (X = Gly, Val, Apa), whose log β values are 3.4 ± 0.1.[8] Even the presumed preorganization of the two imidazole donors in cyclo-HisHis yields the very poor log β for ZnL of 2.55.[5] Reference compounds with a single and exclusive imidazole(histidine) donor are Ac-His-NH_2 (log β for ZnL = 1.96)[11] and cyclo-GlyHis (log β for ZnL = 1.71).[5]

This situation only gets better when additional donors of the dipeptides are involved. The best combination is that with the amino function, which forms a six-membered chelate ring. As Table 2 shows by the comparison of the H-HisHis with the Ac-HisHis dipeptides, this combination accounts for 2−2.5 log units in stability. This increase of stability is larger than the one resulting from the combination of one imidazole(histidine) donor with a second, neighbouring one (see above). The same observation also results from a comparison of the mono-histidine reference compounds H-His-OMe/Ac-His-NH_2 (log β = 4.45/1.96)[10] or H-ValHis-OEt/Ac-HisVal-OH (log β = 4.20/2.89).[12]

The effect of using the carboxylate function as an additional donor is small. The log β values for ZnL of H-HisHis-OH and H-HisHis-OEt are virtually identical, and that of Ac-HisHis-OH is even smaller than that of Ac-HisHis-OEt. For the simpler reference compounds the effect is noticeable: ZnL of Ac-His-OH (log β = 2.91)[10] is more stable than that of Ac-His-NH_2 (log β = 1.96),[11] and that of H-HisCys-OEt (log β = 8.36) is more stable than that of Ac-HisCys-OEt (log β = 6.17).[4]

The latter two values are again reminiscent of the low donor strength of the histidine-imidazole function relative to that of the cysteine-thiolate function. For all pairs of peptide derivatives X-His-Y/X-Cys-Y that form ZnL complexes in solution, the Cys compound is more stable than

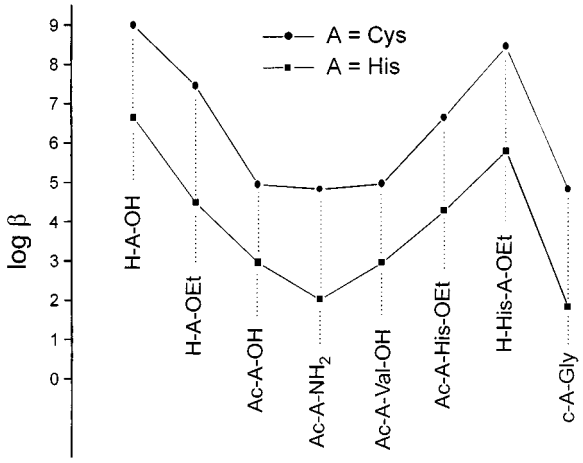

Figure 3. Stabilities of ZnL complexes for amino acid and peptide derivatives with one histidine (A = His) or cysteine (A = Cys) unit

the His compound by roughly 2.5 log β units. Figure 3 visualizes this for all available data for ZnL complexes. A similar relation holds for ZnL complexes of peptide ligands representing pairs of comparable His_2 and Cys_2 derivatives. As Figure 4 shows, the difference of the stabilities amounts to roughly six log β units, i.e. twice the amount of the mono-His/mono-Cys pairs.

This latter observation points to a further generalization, namely the possibility of expressing the stabilities of all ZnL complexes mentioned in this paper by parameters for the donors involved. It seems that this can be done with a satisfactory degree of precision in the ± 0.2 log β range of total stabilities for ZnL complexes with exclusive Cys or His coordination. Table 3 demonstrates this in section (a), based on log β parameters of 4.7 for Cys and 2.1 for His. Serious deviations from this approximation occur only for cyclo-HisHis, the unusually low log β value of which has already been discussed before,[5] and for Ac-CysCys-OEt for which the calculated log β is 0.8 units off.

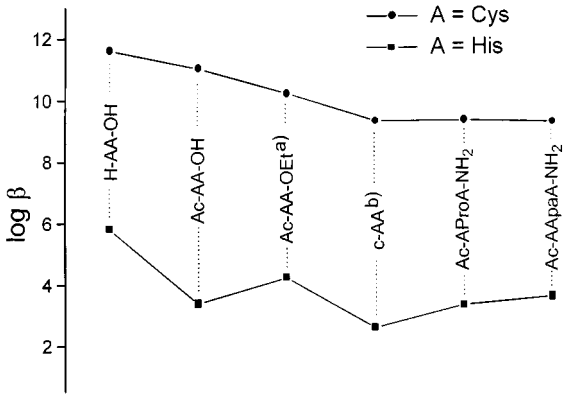

Figure 4. Stabilities of ZnL complexes for peptide derivatives with two histidine (A = His) or cysteine (A = Cys) units
[a] Data for Ac-LCys-DCys-OEt. [b] Estimated value for cyclo-CysCys[5]

Table 3. Comparison of observed log β values for ZnL complexes with calculated ones, using log β parameters of 2.1 for imidazole-(histidine), 4.7 for thiolate(cysteine), 2.2 for amine adjacent to Cys or His and 0.5 for carboxylate adjacent to Cys or His

L	log β (obs)	log β (calc)
(a) exclusive imidazole or thiolate coordination		
Ac-His-NH_2	2.0	2.1
Ac-Cys-NH_2	4.8	4.7
cyclo-GlyHis	1.7	2.1
cyclo-GlyCys	4.7	4.7
Ac-HisHis-OEt	4.2	4.2
Ac-HisCys-OEt	6.6	6.8
Ac-CysCys-OEt	10.2	9.4
cyclo-HisHis	2.5	4.2
cyclo-HisCys	6.6	6.8
cyclo-CysCys	9.3[a]	9.4
Ac-HisApaHis-NH_2	3.6	4.2
Ac-CysApaCys-NH_2	9.3	9.4
(b) with additional amine or carboxylate coordination		
H-His-OMe	4.5	4.3
H-Cys-OEt	7.4	6.9
Ac-His-OH	2.9	2.6
Ac-Cys-OH	4.9	5.2
H-HisHis-OEt	5.7	6.2
H-HisCys-OEt	8.4	8.8
H-CysCys-OEt	n.o.	11.4
Ac-HisHis-OH	3.4	4.7
Ac-CysCys-OH	11.0	9.9
(c) with additional amine and carboxylate coordination		
H-His-OH	6.6	4.8
H-Cys-OH	9.0	7.4
H-HisHis-OH	5.8	6.9
H-Cys-His-OH	9.4	9.5
H-CysCys-OH	11.6	12.1

[a] Estimated value from ref.[5]

The parameterization works less satisfactorily for the amine and carboxylate donors, cf. parts (b) and (c) of Table 3. As mentioned above the effect of chelation involving the amino function is strong, accounting for a log β contribution of more than two units. However, it is obviously variable, depending on whether the amino group is part of histidine or cysteine and on the number of amino acid building blocks. Likewise the contribution of the carboxylate function cannot be accounted for by a fixed parameter. Thus the agreement between observed and calculated log β values is not so good in section (b) of Table 3, and in section (c), where four parameters are involved, the disagreement amounts to 0.1−1.8 log β units. Nevertheless the given parameters should be good enough to estimate the stabilities of ZnL complexes of peptides containing two cysteine and/or histidine units, either in adjacent positions or separated by one or two nonligating amino acid constituents.

Conclusions

This paper has completed our studies of the acid/base chemistry and zinc complexation of small peptides rich in cysteine and histidine. The four possible His-His dipeptides (unprotected, partly and fully protected) were employed. Their 1:1 zinc complexes are of moderate stability. Even for two imidazole(histidine) donor functions there is no stabilization which could compete with that of a single thiolate-(cysteine) donor, and the complex stability for a chelating coordination of the imidazole and a terminal amino function surpasses that for a bis(imidazole) coordination. Furthermore, conformational effects due to the position of histidine in a peptide are not evident: compared to the ZnL complex of Ac-HisHis-OEt both the ZnL complexes of cyclo-HisHis or Ac-HisApaHis-NH_2, whose imidazole donors should be positioned more favourably, are less stable. All this underlines the notion that the frequent occurrence of Zn-histidine coordination in proteins reflects a very efficient tuning of preorganization of the donors by the polypeptide backbone.

Our accumulated data for zinc complexes of peptides with one or two cysteine and/or histidine constituents has allowed a comparative discussion of their stabilities. As Table 3 shows, this has enabled a parameterization of the contributions of the individual donors to the stability of 1:1 complexes in terms of log β increments. The agreement between calculated and observed complex stabilities is quite satisfying for the systems with exclusive histidine and/or cysteine coordination. When additional carboxylate or amine coordination is involved the parameterization for these donors works less satisfactorily. However, as cysteine and histidine are the natural donors and terminal amino or carboxylate functions do not coordinate in proteins, the stability parameters should be valuable for estimating the strength of zinc-peptide interactions for new or hitherto uninvestigated peptides containing cysteine and/or histidine.

Experimental Section

General: For general working and measuring procedures, see ref.[13] The histidine derivatives Boc-His(Boc)-OH, H-His-OEt, Ac-His-OH and Ac-His(Trt)-OH were obtained commercially. The detailed procedures of peptide coupling by the CAIBE and the DCC method as well as for the removal of the protective groups Boc and

Trt[8] have been described by us before. Purifications by HPLC were performed with a Merck−Hitachi system using Nucleosil 7C18 from Macherey−Nagel. The flow rate was 6 mL/min. The eluents always contained 0.1% of trifluoroacetic acid which thereby became a constituent of the isolated peptides. The specific HTFA content of each peptide was determined by potentiometric titration.

H-HisHis-OH: Boc-His(Boc)-OH (10.00 g, 28.14 mmol) and H-His-OEt·3*p*-TosOH (19.69 g, 28.14 mmol) were coupled by the CAIBE method resulting in 4.42 g (30%) of Boc-His(Boc)-His-OEt as a colourless solid. 2.15 g (4.13 mmol) of this in a mixture of 25 mL of methanol and 25 mL of 1 M NaOH was stirred at 50 °C for 30 min. After cooling to room temp. the solution was brought to pH 2 with 1 M HCl. All volatiles were removed in vacuo, the residue was suspended in 30 mL of dry methanol, filtered, and the filtrate evaporated to dryness again. The residue was washed three times with 15 mL of diethyl ether and dried in vacuo to give of Boc-His(Boc)-His-OH (1.99 g, 98%) as a pale yellow solid. This solid was treated with trifluoroacetic acid as described previously[8] to remove the Boc groups, giving H-HisHis-OH·4HTFA (2.10 g, 82%) as a yellow oil. Preparative HPLC with water and freeze-drying yielded the analytically pure product as a colourless, very hygroscopic solid, m.p. 63 °C. $C_{12}H_{16}N_6O_3·4C_2HF_3O_2$ (292.30 + 456.09): calcd. C 32.10, H 2.69, N 11.23; found C 31.82, H 2.71, N 11.27. IR (KBr): $\tilde{\nu}$ = 3436 bs, (OH), 3156 bs, (NH), 1676 vs (amide I), 1559 m, 1540 m (amide II), 1205 vs (CF). ^{1}H NMR: ([D$_6$]DMSO): δ = 2.92−3.31 (m, 4 H, H$_\beta$), 4.19−4.25 (m, 1 H, H$_\alpha$), 4.59−4.69 (m, 1 H, H$_\alpha$), 4.91−5.58 (m, 3 H, NH_3^+), 7.40 (s, 1 H, im), 7.42 (s, 1 H, im), 8.92−9.00 (m, 1 H, amide), 8.98 (s, 1 H, im), 8.99 (s, 1 H, im).

H-HisHis-OEt: Boc-His(Boc)-His-OEt (2.17 g, 4.17 mmol; see above) was treated with trifluoroacetic acid as described previously[8] to remove the Boc groups, giving H-HisHis-OEt·4HTFA (2.57 g, 93%) as a yellow oil. Preparative HPLC with water/acetonitrile (98:2) and subsequent freeze-drying yielded the analytically pure product as a colourless, very hygroscopic solid, m.p. 59 °C. $C_{14}H_{20}N_6O_3·4C_2HF_3O_2·2H_2O$ (320.35 + 456.09 + 36.03): calcd. C 32.52, H 3.47, N 10.34; found C 32.69, H 3.07, N 10.60. IR (KBr): $\tilde{\nu}$ = 3442 m, 3385 m (NH), 1672 vs (amide I), 1559 m (amide II), 1203 vs (CF). ^{1}H NMR ([D$_6$]DMSO): δ = 1.14 (t, *J* = 7.4 Hz, 3 H, Et), 3.00−3.29 (m, 4 H, H$_\beta$), 4.09 (q, *J* = 7.4 Hz, 2 H, Et), 4.20−4.26 (m, 1 H, H$_\alpha$), 4.65−4.76 (m, 1 H, H$_\alpha$), 7.40 (s, 1 H, im), 7.44 (s, 1 H, im), 8.99 (s, 1 H, im), 9.00 (s, 1 H, im), 9.12 (d, *J* = 6.3 Hz, 1 H, amide).

Ac-HisHis-OEt: Ac-His(Trt)-OH (8.79 g, 20.0 mmol) and H-His-OEt·3*p*-TosOH (14.00 g, 20.0 mmol) were coupled by the DCC method to give Ac-His(Trt)-His-OEtr (9.89 g, 82%) as a colourless solid. 3.98 g of this were treated with ethanethiol as described previously[8] to remove the Trt group, yielding Ac-HisHis-OEt·2.4HTFA (1.63 g, 42%) as a colourless solid. Preparative HPLC with water which was gradually replaced by acetonitrile, and subsequent freeze-drying, yielded the analytically pure product as a colourless, very hygroscopic solid, m.p. 48 °C. $C_{16}H_{22}N_6O_4·2.4C_2HF_3O_2$ (362.39 + 273.65): calcd. C 39.28, H 3.87, N 13.21; found C 39.18, H 3.85, N 13.41. IR (KBr): $\tilde{\nu}$ = 3424 bs, 3148 m (NH), 1672 vs (amide I), 1541 m (amide II), 1204 vs (CF). ^{1}H NMR ([D$_6$]DMSO): δ = 1.14 (t, *J* = 7.0 Hz, 3 H, Et), 1.82 (s, 3 H, Ac), 2.78−3.23 (m, 4 H, H$_\beta$), 4.08 (q, *J* = 7.0 Hz, 2 H, Et), 4.48−4.65 (m, 2 H, H$_\alpha$), 7.31 (s, 1 H, im), 7.38 (s, 1 H, im), 8.25 (d, *J* = 8.0 Hz, 1 H, amide), 8.58 (d, *J* = 7.6 Hz, 1 H, amide), 8.95 (s, 1 H, im), 8.97 (s, 1 H, im).

Ac-HisHis-OH: Ac-HisHis-OEt·2.4HTFA (2.01 g, 3.40 mmol; see above) was treated with NaOH as described above for H-HisHis-OH to remove the ethyl group, giving Ac-HisHis-OH·2HCl (1.28 g, 93%) as a colourless solid. Preparative HPLC with water/acetonitrile (95:5) and subsequent freeze-drying yielded the analytically pure product as a colourless, very hygroscopic solid, m.p. 144 °C. $C_{14}H_{18}N_6O_4·4C_2HF_3O_2·5H_2O$ (334.33 + 456.09 + 90.08): calcd. C 30.01, H 3.66, N 9.54; found C 29.63, H 3.72, N 9.87. IR (KBr): $\tilde{\nu}$ = 3437 vs, b (OH), 1659 s (amide I), 1641 s, 1548 s (amide II), 1206 s (CF). ^{1}H NMR ([D$_6$]DMSO): δ = 1.83 (s, 3 H, Ac), 2.76−3.33 (m, 4 H, H$_\beta$), 4.42−4.62 (m, 2 H, H$_\alpha$), 7.33 (s, 1 H, im), 7.43 (s, 1 H, im), 8.38 (d, *J* = 8.0 Hz, 1 H, amide), 8.61 (d, *J* = 7.8 Hz, 1 H, amide), 8.99 (s, 1 H, im), 9.01 (s, 1 H, im).

1: A solution of $Zn(TFA)_2$ (13.2 mg, 0.045 mmol) in 10 mL of water was added to a solution of H-HisHis-OH·4HTFA (34.0 mg, 0.045 mmol) in 10 mL of ethanol. Triethylamine (31.7 μL, 23.0 mg, 0.23 mmol) was then added with vigorous stirring, and the solution was reduced in vacuo to 5 mL. The resulting precipitate was filtered off, washed twice with 5 mL of dry ethanol and dried in vacuo to give **1**·EtOH (11.0 mg, 47%) as a colourless solid, m.p. 270 °C (dec.). $C_{16}H_{21}F_3N_6O_6Zn$ (515.76): calcd. C 37.26, H 4.10, N 16.29, Zn 12.68; found C 37.23, H 3.99, N 17.04, Zn 12.09. IR (KBr): $\tilde{\nu}$ = 3415 vs, b (OH), 3274 vs, b, 3133 s (NH), 1669 vs (amide I), 1507 s (amide II), 1204 s (CF).

2: A solution of $Zn(TFA)_2$ (55.0 mg, 0.19 mmol) in 10 mL of water was added to a solution of Ac-HisHis-OH·4HTFA·5H$_2$O (166 mg, 0.19 mmol) in 10 mL of water. The reaction flask was attached to a second flask containing triethylamine (132 μL, 96 mg, 0.95 mmol) which diffused into the reaction solution. After two days the resulting precipitate was filtered off, washed three times with 5 mL of dry ethanol and dried in vacuo to give **2**·2H$_2$O (22.5 mg, 43%) as a colourless solid, m.p. 280 °C (dec.). $C_{16}H_{21}F_3N_6O_8Zn$ (547.76): calcd. C 35.08, H 3.86, N 15.34, Zn 11.94; found C 35.21, H 3.61, N16.31, Zn 12.50. IR (KBr): $\tilde{\nu}$ = 3416 vs, b (OH), 3282 s, 3135 s (NH), 1646 vs (amide I), 1540 s (amide II), 1203 s (CF).

3: Prepared as for **2** from of $ZnCl_2$ (38.5 mg, 0.28 mmol), Ac-HisHis-OH·4HTFA·5H$_2$O (249 mg, 0.28 mmol) and triethylamine (197 μL, 143 mg, 1.41 mmol). Yield 81.0 mg (63%) of **3**·H$_2$O as a colourless solid, m.p. 280 °C (dec.). $C_{14}H_{19}ClN_6O_5Zn$ (452.18): calcd. C 37.19, H 4.24, N 18.59, Zn 14.46; found C 36.90, H 3.74, N 17.72, Zn 14.12. IR (KBr): $\tilde{\nu}$ = 3291 vs, b (OH), 3131 s (NH), 1650 vs (amide I), 1536 s (amide II).

4: Prepared as for **2** from $ZnCl_2$ (23.2 mg, 0.17 mmol), Ac-HisHis-OEt·2.4HTFA (108 mg, 0.17 mmol) and triethylamine (119 μL, 0.85 mmol). Yield 46.3 mg (55%) of **4** as a colourless solid, m.p. 260 °C (dec.). $C_{16}H_{22}Cl_2N_6O_4Zn$ (498.68): calcd. C 38.54, H 4.45, N 16.85, Zn 13.11; found C 38.82, H 4.23, N 17.21, Zn 13.65. IR (KBr): $\tilde{\nu}$ = 3378 bs, 3285 bs, 3134 s (NH), 1654 vs (amide I), 1539 s (amide II).

Potentiometric Titrations: The apparatus used, the experimental details, the calibration techniques and the titration procedure were as described before.[8,10] The common anion in all solutions was ni-

Table 4. Starting concentrations (× 10^{-3} M) of the regents for the potentiometric titrations

Peptide	c(peptide)	c(acid)	c(Zn)
H-HisHis-OH	0.890−1.030	0.602−1.082	0.235−0.940
H-HisHis-OEt	1.001−1.020	1.331−1.405	0.240−0.977
Ac-HisHis-OH	0.860−1.741	0.013−1.716	0.250−1.038
Ac-HisHis-OEt	0.707−0.778	0.827−1.573	0.309−0.865

trate [KNO_3, $Zn(NO_3)_2$, HNO_3]. Zuberbühler's TITFIT program[14] was used for the computations. The ionic strength was 0.1. All measurements were done at 25.0 ± 0.1 °C. The starting concentrations of peptides, acid and zinc nitrate are given in Table 4.

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