Unusual gain in the coordination ability of vasopressin-like peptides towards Cu^{2+} ions by insertion of the highly hydrophobic side chain

Elżbieta Chruścinska,[a] Izabela Derdowska,[b] Henryk Kozłowski,*[c] Bernard Lammek,[b] Marek Łuczkowski,[c] Stanislaw Ołdziej[b] and Jolanta Świątek-Kozłowska[d]

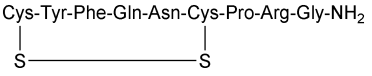
[a] *Institute of General Food Chemistry, Technical University of Łódź, 90-924, Łódź, Poland*
[b] *Faculty of Chemistry, University of Gdańsk, Gdańsk, Sobieskiego 18, Poland*
[c] *Faculty of Chemistry, University of Wrocław, F.Joliot-Curie 14, 50-383, Wrocław, Poland. E-mail: henrykoz@wchuwr.chem.uni.wroc.pl*
[d] *Department of Basic Medical Sciences, Wrocław Medical University, J. Kochanowskiego 14, 51-601, Wrocław, Poland*

Received (in London, UK) 11th July 2002, Accepted 5th November 2002
First published as an Advance Article on the web 2nd December 2002

Potentiometric and spectroscopic data show an unusual gain in the stability constants of Cu^{2+} complexes with vasopressin analogues having the highly hydrophobic naphthalene-alanine residue inserted in position three (Nal^3). The naphthalene derivative is a much more powerful ligand for binding Cu^{2+} ions that the parent peptide. Theoretical calculations indicate the effective hydrophobic protection of the metal site by Tyr^2 and Nal^3 aromatic side-chains. The interaction of the guanidine moiety of Arg^4 with naphthalene can also increase distinctly the stability of the respective 4N complex.

Introduction

Arginine vasopressin (AVP), a naturally occurring neurohormone, has a very specific structure with a 20-membered ring linked by a disulfide bridge between Cys-1 and Cys-6 with a tripeptide side chain:



Earlier works have shown that this peptide structure has a very critical influence on interactions with Cu^{2+} ions.[1–3] The pre-organization of the peptide back-bone into the ring structure results in the formation of extremely strong metal complexes with $\{NH_2, 3 \times N^-\}$ or (4N) binding mode.[1,2] Although the involvement of the disulfide donors in the binding of Cu^{2+} was suggested for some intermediate species,[3] neither of the sulfur atoms is involved in metal ion coordination in the final 4N complex. Thus, the high efficacy in the binding of vasopressin and vasopressin-like peptides is induced by a favorable arrangement of the donor atoms due to the ring formation. The strebgth of the metal ion binding is very sensitive to even slight modifications in the peptide sequence.[1–3] Especially effective are modifications within the sequence of the ring. The exchange of the L-amino acid residue in AVP to the D-enantiomer at position 4 decreases the stability by two orders of magnitude, while the binding ability of native AVP is more than four orders of magnitude more effective than oligoalanine.[1,2]

The metal ion binding may influence peptide conformation quite considerably and as a result it can affect the biological activity of this class of peptides.[4]

More detailed studies including theoretical calculations showing the impact of AVP modifications on the ability to form complexes with Cu^{2+} ions are presented in this work.

Experimental

Peptide synthesis

All peptides were synthesized manually by a solid phase method, *i.e.* by the stepwise coupling of Boc-amino acids to the growing peptide chain on a 4-methylbenzhydrylamine resin (MBHA resin, Senn Chemicals AG, 1% divinylbenzene (DVB), 200–400 mesh, 0.67 mmol g^{-1}). Fully protected peptide resins were synthesized according to standard procedures involving (i) deprotection steps using 33% trifluoroacetic acid (TFA) in the presence of anisole (1%), 5 and 25 min; (ii) neutralization with 10% triethanolamine (TEA)/dichloromethane (DCM), 3 and 7 min (iii), couplings in DCM or DCM/dimethylformamide (DMF) (1:1, v/v) carried out using *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU) and *N*-hydroxybenzotriazole (HOBt) in the presence of *N*,*N*-(diisopropyl)ethylamine (DIEA). The couplings of Boc-1-Nal, Boc-D-1-Nal, Boc-2-Nal, Boc-Tyr(2Br-Z), Boc-Cys(Mob), were mediated by *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU) and *N*-hydroxy-7-azabenzotriazole (HOAt) in the presence of DIEA in a mixture of DMF, *N*-methylpyrrolidone (NMP) and DCM (1:1:1 v/v) containing 1% triton. The completeness of each coupling reaction was monitored by the Kaiser[5] or chloranil test.[6] Re-coupling was performed when the test was positive. After completion of the synthesis, the protected nonapeptidyl resins were treated with 10 ml of liquid hydrogen fluoride (HF) containing 0.5 ml of anisole at −70 °C and stirred for 60 min at 0 °C.[7] After the removal of HF and anisole *in vacuo*, the mixture was washed with anhydrous diethyl ether, then with acetic acid and the solution diluted with methanol. The resulting dithiols were oxidatively cyclized with 0.1 M I_2 in methanol using the normal procedure. The solvents were evaporated under reduced pressure and the resulting materials dissolved in water and lyophilized. The crude products were desalted on a Sephadex G-15 column eluted with aqueous

acetic acid (30%) at a flow rate of 3.0 ml h^{-1}. After freeze-drying, the fractions comprising the major peak were purified by HPLC. Preparative HPLC of analogues was carried out in a gradient running from 10 to 50%[6] for 120 min. The peptides were eluted as single peaks. The purity and identity of each peptide was determined by HPLC and FAB mass spectrometry (molecular ion). The values of the molecular ions were as expected. The physicochemical properties of the peptides are presented in Table 1.

Potentiometric measurements

Stability constants for protonation and Cu(II) complexes were calculated from titrations curves carried out at 25 °C using a total volume of 1.5 cm^3. NaOH was added from a 0.250 cm^3 micrometer syringe which was calibrated by both weight titration and the titration of standard materials. The metal ion concentration was 1×10^{-3} mol dm^{-3} and the metal to ligand ratio was 1:1.2. The pH-metric titrations were performed at 25 °C in 0.1 mol dm^{-3} KNO_3 on a MOLSPIN pH-meter system using a Russel CMAW 711 semi-combined electrode calibrated for hydrogen concentrations using HNO_3.[8] The SUPERQUAD program was used for stability constant calculations.[9] Standard deviations were computed by SUPERQUAD and refer to random errors only. They are, however, a good indication of the importance of a particular species in the equilibrium.

Table 1 Potentiometric and spectroscopic properties for copper(II) complexes of AVP analogues

			UV-vis		CD		EPR	
	Log β	pK	λ/nm	ε	λ/nm	$\Delta\varepsilon$	A_{II}/G	g_{II}
[(2-Nal)^3, Arg^4]AVP								
HL	9.65(1)							
H_2L	15.46(2)	5.81						
CuHL	13.62(3)		699	36	284	−0.31	135	2.37
CuL	8.11(1)	5.51	538	72	549	−0.772	163	2.31
					328	0.225		
					289	−0.345		
$CuH_{-1}L$	1.91(3)	6.2 minor						
$CuH_{-2}L$	−4.08(1)	5.99	515	215	547	−3.112	208	208
					325	0.447		
					290	−0.719		
$CuH_{-3}L$	−15.21(2)	11.13	516	205	542	−2.999	208	208
					361	−0.333		
					306	−0.93		
					283 sh	2.245		
[(1-Nal)^3, Arg^4]AVP								
HL	9.66(1)							
H_2L	15.79(1)	6.13						
CuHL	13.83(4)		752	88	313	−0.058	135	2.38
					288	0.281		
CuL	8.41(3)	5.42	543	139	554	−0.397	177	2.29
					329	0.229		
					292	0.205		
					267	−0.159		
$CuH_{-1}L$	1.98(3)	6.43 minor						
$CuH_{-2}L$	−4.28(4)	6.26	507	284	543	−2.291	206	2.17
					326 sh	0.371		
					295	0.803		
$CuH_{-3}L$	−15.17(4)	10.89	510	278	533	−2.175	206	2.17
					319	−0.629		
					293	0.915		
					275	−0.539		
[(D-1-Nal)^3]AVP								
HL	9.77(1)							
H_2L	16.29(2)	6.52						
CuHL	14.42(2)		673	43	760	−0.193	133	2.37
					318	−0.061		
					277	−2.448		
CuL	7.67(2)	6.75 minor						
$CuH_{-1}L$	1.11(3)	6.56	517	104	713	−0.054	156	2.31
					583	0.078		
					489	−0.389		
					321	0.283		
					283	−4.075		
$CuH_{-2}L$	−5.73(2)	6.84	508	174	603	0.036	207	2.17
					489	−0.992		
					320	0.403		
					282	−7.478		
$CuH_{-3}L$	−16.64(2)	10.91	512	170	490	−0.977	207	2.17
					334	−0.045		
					280	−6.557		

Spectroscopic measurements

Solutions were of similar concentrations to those used in the potentiometric studies. Electron paramagnetic resonance (EPR) spectra were recorded on a Bruker ESP 300E spectrometer at X-band frequency (9.3 GHz) at 120 K. The EPR parameters were calculated for the spectra obtained at the maximum concentration of the particular species for which well-resolved separations were observed. The absorption spectra were recorded on a Beckman DU 650 spectrophotometer. Circular dichroism (CD) spectra were recorded on Jasco J 715 spectropolarimeter in the 800–230 nm range. The values of $\Delta\varepsilon$ (*i.e.* $\varepsilon_l - \varepsilon_r$) and ε were calculated at the maximum concentration of particular species obtained from the potentiometric data.

Computational studies

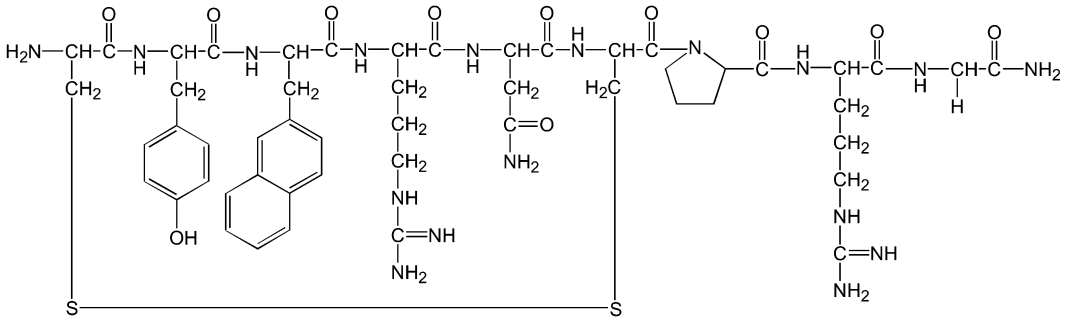
All semi-empirical calculations were carried out using the MOPAC2002 program.[10] The geometries of all AVP analogue–copper ion complexes were fully optimized using AM1 molecular orbital methods[11] in the unrestricted Hartree–Fock approximation. The AM1d method[12] and parametrization[13] were used for copper ion. In our previous papers we show that the AM1d method reproduced very well the geometries of complexes formed by copper ions with peptide or peptide-like ligands.[13,14] In all calculations the electronic state of the copper complex was assumed as a doublet and solvent (water) was simulated by employing the COSMO method.[15] The systematic search of the conformational space of the copper(II) complexes of AVP analogues was performed, about 500 different conformations were generated and fully geometry-optimized for every complex and the nature of the stationary points was determined by normal mode analysis. A typical geometry optimization plus frequency calculation run took from 3 to 5 hours of computation time on PIII XEON 700 MHz CPU.

Results and discussion

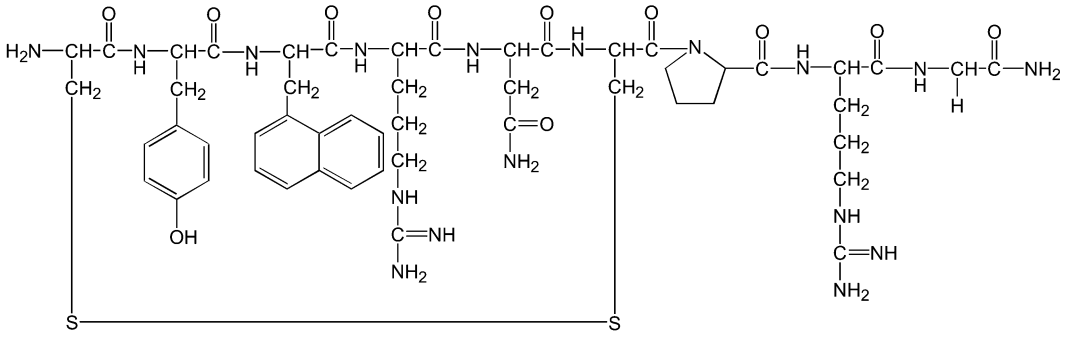
Protonation constants

Protonation constants of three vasopressin analogues (Scheme 1) are collected in Table 1. All three peptides behave

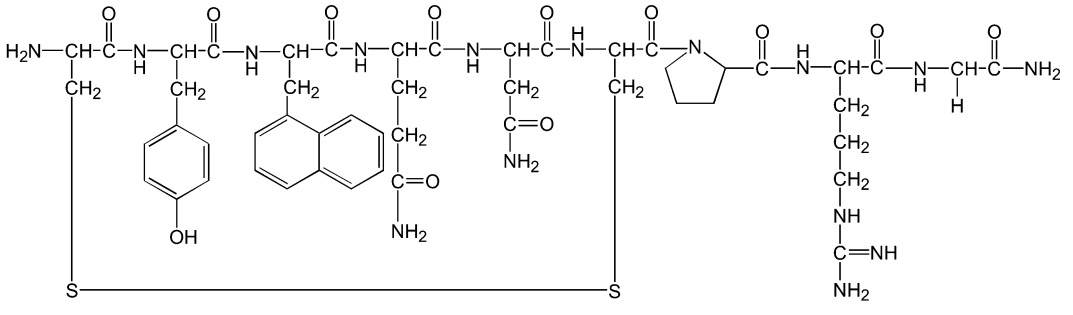
[(2-Nal)^3, Arg^4]AVP



[(1-Nal)^3, Arg^4]AVP



[(D-1-Nal)^3]AVP



Scheme 1

as H_2L acids with protonation sites at the N-terminal amino group and the Tyr side chain phenolate. Some effect on protonation constants is observed when the bulky (L-1-Nal)[3] residue is substituted by its D-enantiomer (Table 1). In the latter case the N-terminal amino group increases its basicity by one log unit, while Tyr phenolate becomes more acidic in comparison to the parent molecule.

Cu(II) complexes

According to the potentiometric data calculations all three vasopressin analogues give the same set of complex species as that obtained for the native hormone (Table 1).[1] Although the complex stability constants differ from each other the dominant species in the physiologically relevant pH is the $CuH_{-2}L$ complex. The spectroscopic parameters with d–d band around 510 nm and EPR parameters shown in Table 1 are typical for 4N coordination involving $\{NH_2,3\times N^-\}$ donor set.[1,2,15,16] The same coordination mode occurs also in the $CuH_{-3}L$ species in which the Tyr side chain becomes deprotonated. The formation of the 4N ($CuH_{-2}L$) complex for all analogues of vasopressin studied here begins at pH around 5 (Fig. 1). The complexes CuHL (1N), CuL (2N) and $CuH_{-1}L$ (3N) with $\{NH_2,CO\}$, $\{NH_2,N^-\}$ and $\{NH_2,2\times N^-\}$ coordination modes, respectively are only the minor species with concentrations below 40% of the total Cu(II) (Fig. 1).[1,2,15] The $\{NH_2,3\times N^-\}$ or 4N binding mode is usually a dominant coordination for simple oligopeptides (*e.g.* Ala_4) above pH 10.[1,15] The very high gain in the 4N complex stability (up to five orders of magnitude) derives, as suggested earlier, from the formation of the disulfide bridge and favorable positioning of the amide nitrogens to bind metal ion.[1–3] In this work we have used α- and β-naphthalene-alanine residues (1-Nal and 2-Nal) instead of Phe[3] to check the effect of the bulky naphthalene substituent on the complex formation. The effect of the L- to D-enantiomer change is also checked. The comparison of the binding ability of the [(2-Nal)[3], Arg[4]]AVP analogue with the native AVP (Fig. 2) clearly indicates that between pH 4 and 9 ($CuH_{-2}L$ species domination, Fig. 1) the naphthalene analogue is an even more powerful ligand than AVP itself. The plot showing competition between two ligands towards Cu(II) ions (Fig. 2) shows that in the pH region 6–9 AVP coordinates only about 20% of the total copper. This rather unusual stabilization effect of the $CuH_{-2}L$ species by the bulky side chain strongly suggests the hydrophobic impact of the naphthalene rings on the complex stability. The stabilizing effect derived from the hydrophobic amino acid residues was suggested earlier.[17–19] The comparison of the coordination ability of [(2-Nal)[3]] and [(1-Nal)[3]] derivatives indicates some difference between the two types of substitution of the naphthalene ring (Fig. 3) for the pH range 6–10 ($CuH_{-2}L$ species). The [(2-Nal)[3],Arg[4]]AVP binds around 60% of the total copper, while the [(1-Nal)] analogue binds around 40% (Fig. 3). At pH above 10, when $CuH_{-3}L$ dominates the coordination equilibrium the binding abilities of both analogues are almost identical. This suggests that the position of the naphthalene ring also has an influence on the complex stability. The additional hydrophobic effect derives certainly from the adjacent Tyr[2] residue, which in the $CuH_{-2}L$ complex is protonated. Deprotonation of the Tyr[2] residue above pH 9 destabilizes thr $CuH_{-3}L$ complex and the naphthalene analogue becomes less effective in Cu(II) ion binding than native AVP (Fig. 2). The negatively charged Tyr residue is less able to act efficiently together with Nal as the hydrophobic protection of the metal ion bound to AVP back-bone. The lack of the mutual hydrophobic interactions between negatively charged Tyr[2] and Nal[3] results in the complex destabilization due to the bulkiness of the side chains of both residues.

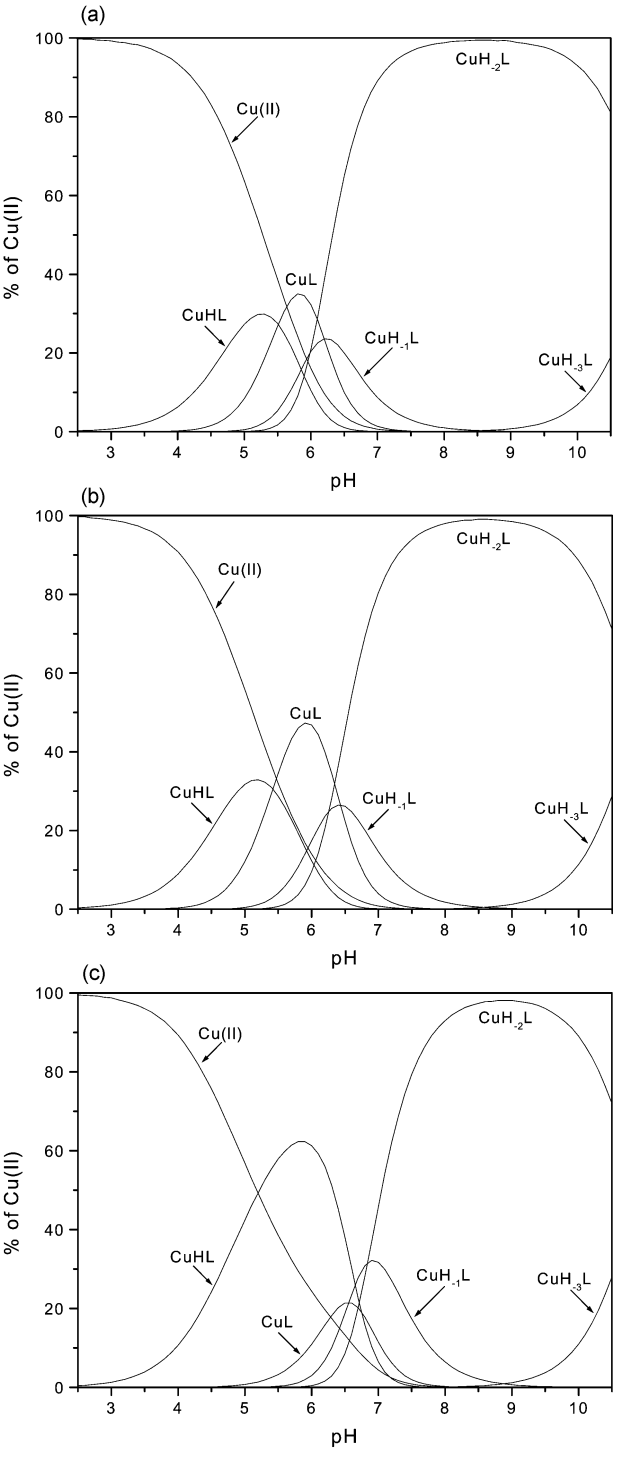


Fig. 1 Species distribution curves for Cu(II)-[(2-Nal)[3], Arg[4]]AVP (a), Cu(II)-[(1-Nal)[3], Arg[4]]AVP (b) and Cu(II)-[(D-1-Nal)[3]]AVP (c) complexes at 25 °C and $I = 0.1$ M KNO_3. Metal concentration 1×10^{-3} M.

A very drastic decrease in the coordination ability of the AVP analogue is observed when the L-(1-Nal) residue is changed for its D-enantiomer (Fig. 4). Above pH 6, when 4N complex ($CuH_{-2}L$) is formed the L-(1-Nal) containing AVP analogue binds more than 95% of the total copper (Fig. 4). Thus, insertion of the D-amino acid residue changes completely the binding power of vasopressin-like ligands.

Theoretical calculations

As it was shown in the experimental section, all AVP analogues form planar 4N complexes with Cu(II) ions. For all

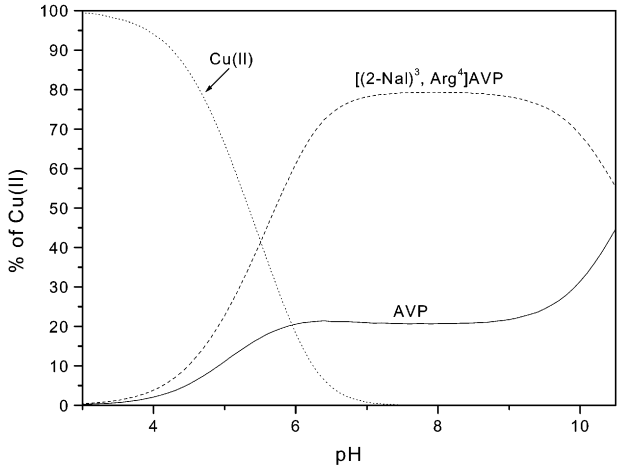


Fig. 2 Competition diagram for AVP (solid line) and [(2-Nal)^3, Arg^4]AVP (dashed line) in binding of Cu(II) ions as a function of pH for 1:1:1 (ligand:metal:ligand) molar ratio and metal concentration 1×10^{-3} M.

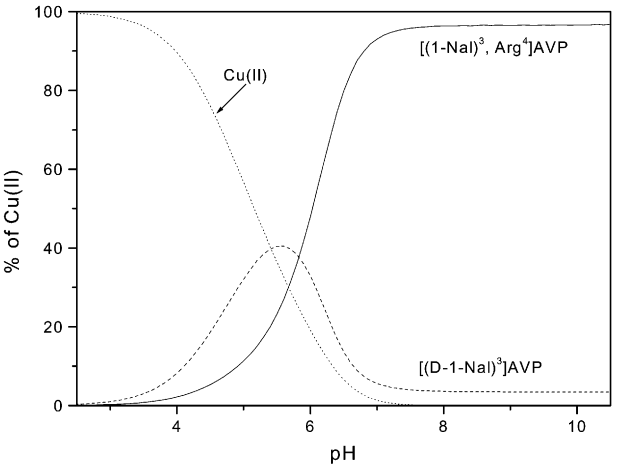


Fig. 4 Competition diagram for [(1-Nal)^3, Arg^4]AVP (solid line) and [(D-1-Nal)^3, Arg^4]AVP (dashed line) in binding of Cu(II) ions as a function of pH for 1:1:1 (ligand:metal:ligand) molar ratio and metal concentration 1×10^{-3} M.

theoretically investigated complexes the coordination sphere of the Cu(II) ion was composed by four nitrogen atoms and the coordination geometry was always square planar. The observed Cu–N distances varied from 1.9 to 2.13 Å which is in very good agreement with distances observed in X-ray structures of similar complexes found in the CSD database[20] and with our previous calculations on peptide and peptide-like complexes with Cu(II) ions.[14,15] In Fig. 5a the lowest energy structure of the Cu(II)-AVP complex obtained from the calculations is shown. As was mentioned above the coordination sphere of the Cu(II) ion is square planar and all four coordination sites are occupied by nitrogen atoms. The out of plane coordination sites remain empty but side chains of the hydrophobic residues Tyr^2, Phe^3 and hydrophilic Gln^4 shield one of them. The very similar shielding of a coordination site by the side chains of hydrophobic residues of valine and isoleucine and the hydrophilic side chain of asparagines was observed by NMR spectroscopy in the Ni(II)-Val-Ile-His-Asn-NH_2 complex.[21] Similar structures were observed in the case of both AVP analogues with L-amino acid substitution in positions 3 and 4 (see Figs. 5b, c). In both structures interactions between π-electrons from the naphthalene ring and the protonated guanidyl moiety of Arg^4 are observed. The latter interactions may have an impact on the stabilities of the described complexes.

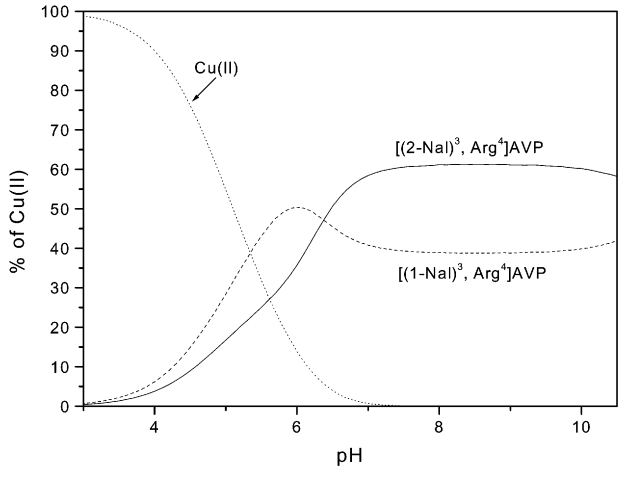


Fig. 3 Competition diagram for [(2-Nal)^3, Arg^4]AVP (solid line) and [(1-Nal)^3, Arg^4]AVP (dashed line) in binding of Cu(II) ions as a function of pH for 1:1:1 (ligand:metal:ligand) molar ratio and metal concentration 1×10^{-3} M.

The increase of the complex stability is clearly seen in Fig. 2 where the binding abilities of AVP and its analogue [(2-Nal)^3,Arg^4]AVP are compared. The introduction of a bulky hydrophobic residue in position three and basic substitution in position four leads to very strong binding. The coordination ability of the AVP analogue, however, decreases above pH 10. This suggests that the observed interactions between the naphthalene ring and the charged guanidyl moiety strongly contribute to the stability of the complex and de-charging of guanidine at high pH leads to a break up of these interactions resulting in the complex destabilization. It is also clear that the 2-Nal residue, for steric reasons, can better protect the central ion from interactions with the solvent. This may explain the experimental evidence that the AVP analogue with 2-Nal^3 residues is a more efficient ligand than those with 1-Nal residues in the same position (see Fig. 3). In Fig. 5d the lowest energy conformation of the AVP analogue with substitution with the D-1-Nal^3 residue is shown. In this case a hydrophobic side chain of the Nal residue is located on the other side of the complex plane from the hydrophobic side chain of Tyr^2. Such a location of the naphthalene ring makes the hydrophobic interactions with the side chain of Tyr^2 impossible. However, some unusual behavior of the [(D-1-Nal)^3]AVP analogue should be pointed out. Fig. 4 shows that the AVP analogue with D-amino acid in position three better coordinates Cu(II) ions in the pH range 4–6 when compared to all L-amino acid ligands. The explanation of this effect can be found in the analysis of the data concerning the structure of AVP and its analogues. NMR, X-ray and theoretical studies have shown that the favorable conformation of AVP and its analogues in the cyclic region is the formation of a β-turn structure on residues 2–3 or 3–4. The formation of this β-turn is basic for the biological activity of this family of peptides.[22–24] The probability of β-turn formation may increase with the introduction of a D-amino acid residue in position 2 or 3. The β-turn structure always brings the side chains of both amino acids participating in the turn formation close to each other. Taking into account this structural feature one can propose that in the case of all L-amino acid–AVP ligands the binding of the metal increases the β-turn formation. This leads to more energetically favorable interactions between the side chains which we can observe as the increase in the complex stability. In the case of the introduction of a D-amino acid in position 4 we can observe the reverse situation.[1] At the beginning of complex formation a ligand molecule possesses already a conformation which is favorable for the metal binding turn induced by the D-amino acid residue. In such case the formation of the initial complex at low pH is very efficient.

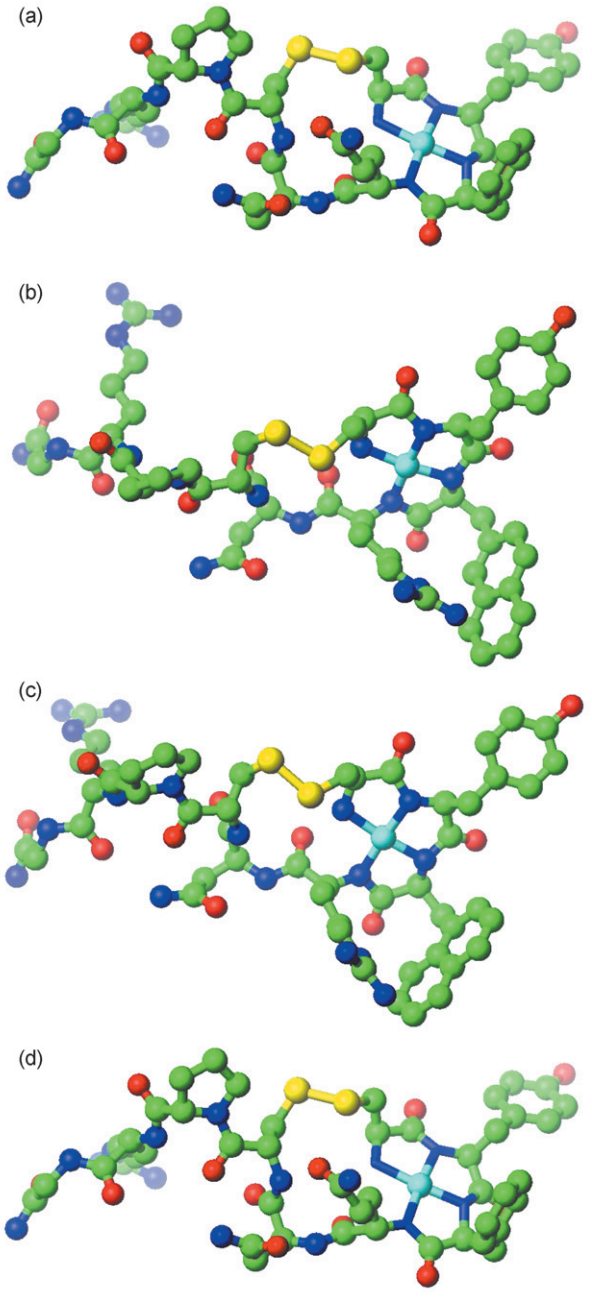


Fig. 5 The lowest energy conformations of copper(II) and AVP and its analogous complexes obtained in our theoretical study for AVP (a), [(2-Nal)^3,Arg^4]AVP (b), [(1-Nal)^3,Arg^4]AVP (c), [(D-1-Nal)^3]AVP (d). All hydrogen atoms are omitted for clarity, the copper ion is shown in cyan, carbon atoms in green, nitrogen atoms in blue and oxygen atoms in red.

However, when pH increases (see Fig. 4) the dissociation of the proton and the formation of $CuH_{-1}L$ species requires some reorganization of the peptide backbone, which moves the side chains of the amino acids from positions two and three far away from each other. The energy necessary to break up the naphthalene–phenol interactions is around 3.5 kcal mol^{-1} (estimation from indole–phenol interactions)[25–26] and this leads to the reduced metal ion binding efficiency by the [(D-1-Nal)^3]AVP analogue above pH 6.5 when compared to the [(1-Nal)^3,Arg^4]AVP analogue (see Fig. 4).

Conclusions

Potentiometric, spectroscopic and theoretical studies have shown that the very high stabilities of the 4N complexes of vasopressin-like peptides with Cu^{2+} ions result from two major structural features: i) the favorable arrangement of the nitrogen donor set realized by formation of the 20-membered ring linked by a disulfide bridge between Cys^1 and Cys^6,[1–3] and ii) the protection of the metal ion site by hydrophobic residues of Tyr^2 and Nal^3. The insertion of the D-residue into the N-terminal fragment of a peptide sequence may have an effect on the stabilities of complexes formed in the pH range 4–6.

References

1 H. Kozlowski, B. Radomska, G. Kupryszewski, B. Lammek, C. Livera, L. D. Pettit and S. Pyburn, *J. Chem. Soc., Dalton Trans.*, 1989, 173.
2 W. Bal, H. Kozlowski, B. Lammek, L. D. Pettit and K. Rolka, *J. Inorg. Biochem.*, 1992, **45**, 193.
3 P. Danyi, K. Várnagy, I. Sóvágó, I. Schön, D. Sanna and G. Micera, *J. Inorg. Biochem.*, 1995, **60**, 69.
4 G. Valensin, A. Maccotta, E. Gaggelli, Z. Grzonka, F. Kasprzykowski and H. Kozlowski, *Eur. J. Biochem.*, 1996, **240**, 118.
5 E. Kaiser, R. l. Colescott, C. D. Bossinger and P. Cook, *Anal. Biochem.*, 1970, **34**, 595–598.
6 T. C. Christensen, in *Peptides-Structure and Biological Function*, eds. E. Gross and J. Meienhofer, Pierce Chemical Company, Rockford, Illinois, USA, 1979, p. 385.
7 J. M. Stewart, *Solid Phase Peptide Synthesis*, Pierce Chem. Corp., Rockford, IL, USA, 1984.
8 H. Irving, M. G. Miles and L. D. Pettit, *Anal. Chim. Acta*, 1967, **38**, 475.
9 P. Gans, A. Vacca and A. Sabatini, *J. Chem. Soc., Dalton Trans.*, 1985, 1196.
10 MOPAC 2002, Fujitsu, Ltd, 2002.
11 M. J. S. Dewar, G. E. Zoebisch, F. H. Healy and J. P. P. Stewart, *J. Am. Chem. Soc.*, 1985, **107**, 3902.
12 A. A. Voityuk and N. Rosch, *J. Phys. Chem.*, 2000, **104**, 4089.
13 A. A. Voityuk, unpublished results.
14 J. Brasuń, P. Ciapetti, H. Kozłowski, S. Ołdziej, M. Taddei, D. Valensin, G. Valensin and N. Gaggelli, *J. Chem. Soc., Dalton Trans.*, 2000, 2639.
15 J. Brasuń, S. Ołdziej, M. Taddei and H. Kozłowski, *J. Inorg. Biochem.*, 2001, **85**, 79.
16 A. Klamt and G. Schurman, *J. Chem. Soc., Perkin Trans. 2*, 1993, 799.
17 H. Kozłowski, W. Bal, M. Dyba and T. Kowalik-Jankowska, *Coord. Chem. Rev.*, 1999, **184**, 319.
18 L. D. Pettit, J. E. Gregor and H. Kozlowski, *Perspectives on Bioinorganic Chemistry*, eds. R. W. Hay, J. R. Dilworth and K. B. Nolan, JAI Press, London, 1999, vol. 1, p. 1.
19 W. Bal, M. I. Djuran, D. W. Margerum, E. T. Gray, Jr., M. A. Mazid, R. T. Tom, E. Nieboer and P. Sadler, *J. Chem. Soc., Chem. Commun.*, 1994, 1889.
20 F. H. Allen, O. Kennar and R. Taylor, *Acc. Chem. Res.*, 1983, **16**, 395.
21 W. Bal, G. N. Chmurny, B. D. Hilton, P. J. Sadler and A. Tucker, *J. Am. Chem. Soc.*, 1996, **118**, 4727.
22 M. D. Shenderowich, F. Kasprzykowski, A. Liwo, I. Sekacis, J. Salutis and G. V. Nikiforowich, *Int. J. Peptide Protein Res.*, 1991, **38**, 528.
23 A. Liwo, A. Tempczyk, S. Ołdziej, M. D. Schenderovich, S. Talluri, J. Ciarkowski, F. Kasprzykowski, L. Łankiewicz and Z. Grzonka, *Biopolymers*, 1996, **38**, 157.
24 M. Tarnowska, A. Liwo, Z. Grzonka and A. Tempczyk, in *QSAR in Design of Bioactive Compounds*, ed. M. Kuchar, J. R. Prous Science Publishers, Barcelona, 1991, vol. 2, pp. 361–436.
25 J. B. O Michell, C. L. Nandi, I. K. McDonald, J. M. Thorton and S. L. Price, *J. Mol. Biol.*, 1996, **239**, 315.
26 A. Liwo, S. Ołdziej, M. R. Pinkus, R. J. Wawak, S. Rackovski and H. A. Scheraga, *J. Comput. Chem.*, 1997, **18**, 849.