DALTON

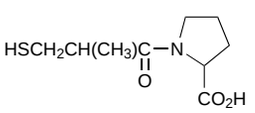
Metal complexes of the angiotensin-converting enzyme inhibitor, lisinopril. Solution studies and the crystal and molecular structure of a dimeric copper(II)–lisinopril complex

Elena Bermejo Gonzalez,[a] Etelka Farkas,*[,b] Ali A. Soudi,[a] Terence Tan,[a] Alexander I. Yanovsky[c] and Kevin B. Nolan*[,a]

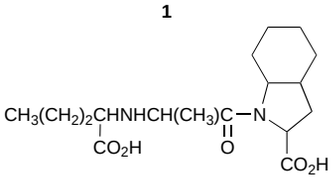
[a] *Department of Chemistry, Royal College of Surgeons in Ireland, St. Stephen's Green, Dublin 2, Ireland*
[b] *Department of Inorganic and Analytical Chemistry, Lajos Kossuth University, Debrecen, H-4010, Hungary*
[c] *X-Ray Structural Centre of the Russian Academy of Sciences, Institute of Organoelement Compounds, 28 Vavilov St., Moscow, 117813, Russia*

The binding of the angiotensin-converting enzyme inhibitor lisinopril to zinc(II), copper(II) and nickel(II) has been investigated in solution by pH-metric methods and the crystal structure of the dimeric copper(II)–lisinopril complex, $[Cu_2(HA)_2(H_2O)_2][ClO_4]_2$ (H_4A^{2+} = fully protonated lisinopril), has been determined. In the case of the metal ions investigated a major species present in neutral or weakly acidic solution is $M(HA)^+$, the formation constants of which suggest that co-ordination to the metal ions occurs through the amino nitrogen, carboxylate oxygen and the amide oxygen atoms. The crystal structure of the dimeric copper complex shows that each copper is in a distorted square-pyramidal environment in which the basal plane is occupied by carboxylate (Cu–O 1.944 Å) and carbonyl (Cu–O 1.996 Å) oxygens, and an amino group nitrogen (Cu–N 1.989 Å) from one ligand as well as the prolyl carboxylate of another ligand (Cu–O 1.909 Å). An aqua ligand Cu–O (2.355 Å) is axially bonded to each copper.

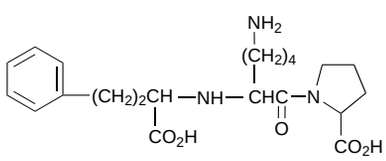
Hypertension is a serious health problem in both developed and developing countries,[1] leading to complications such as cardiovascular disease, stroke and renal failure.[2] Antihypertensive drug therapy is therefore an area of major importance in medicine and among the groups of drugs in current use are inhibitors of the angiotensin-converting enzyme (ACE). This is a zinc metalloenzyme which is responsible for the hydrolysis of the decapeptide angiotensin I to angiotensin II, a vasoconstrictive octapeptide.[3] The ACE inhibitors of which captopril (**1**), enalapril, perindoprilat (**2**) and lisinopril (**3**) are examples,[2] compete with the natural substrate by binding to Zn^{II} at the active site of the enzyme and also by hydrogen bonding and hydrophobic interactions.[3–5] While captopril contains a thiol group which binds to the zinc ion of ACE, the other drugs contain instead a carboxylate group (or carboxylate ester which is metabolised by esterase enzymes to carboxylates) which according to the proposed models for site recognition of ACE co-ordinates to the zinc in a bidentate manner.[5] However the above non-thiol-containing drugs also contain a secondary amino group which with the carboxylate group could form a much more stable five-membered ring chelate with the zinc. Despite this and the recognised ability of catalytic zinc sites in enzymes to exhibit flexibility in co-ordination number and geometry,[6] none of the proposed models for enzyme–inhibitor interactions implicates the amino group in metal-ion binding. It is surprising that although the above drugs owe their activity to complex formation no structures of complexes of any of them have yet been reported. We report herein the structure of a copper(II)–lisinopril complex in the solid state as well as structures of complexes in solution inferred from pH-metric titration data, and contend that the aminocarboxylate moiety may well act as a binding locus for the metal ion *in vivo*.



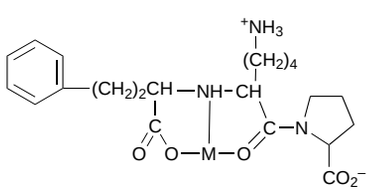
1



2



3



4 $M(HA)^+$

Results and Discussion

The pK_a values of lisinopril, H_4A^{2+}, at 25 °C, $I = 0.2$ mol dm^{-3} KCl are 1.4 ± 0.1, 3.00 ± 0.01, 7.10 ± 0.01 and 10.78 ± 0.01. These were assigned as follows: 10.78 to the lysyl $^+NH_3$ by comparison with lysine,[7] 7.10 to the secondary ^+NH group which is more acidic than the lysyl $^+NH_3$ due to the proximity of the electron-withdrawing amide group, 3.00 to the prolyl CO_2H[8] and 1.4 to the central CO_2H which is more acidic than the prolyl CO_2H due to the proximity of the ^+NH group. Species distribution curves for zinc(II)–, copper(II)– and nickel(II)–lisinopril solutions are shown in Fig. 1 with formation con-

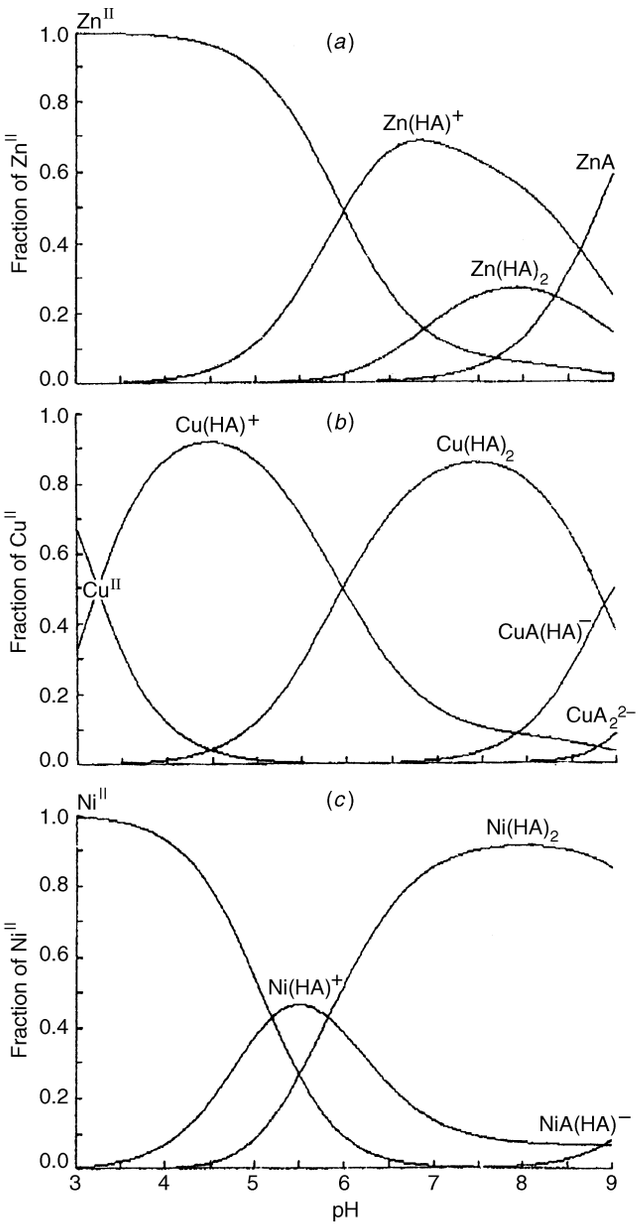


Fig. 1 Species distribution curves for (*a*) zinc(II)–, (*b*) copper(II)– and (*c*) nickel(II)–lisinopril systems at [lisinopril] = 5.00×10^{-3} mol dm^{-3}, [metal ion] = 1.65×10^{-3} mol dm^{-3}, I = 0.2 mol dm^{-3} at 25 °C

Table 1 Complex-formation constants (log β) for metal–lisinopril complexes at 25 °C, I = 0.2 mol dm^{-3} KCl

Metal Ion	$M(HA)^+$*	$M(HA)_2$	$MA(HA)^-$
Zn^{II}	14.34(1)	27.38(1)	—
Cu^{II}	17.28(1)	31.85(2)	22.97(2)
Ni^{II}	15.14(1)	29.79(1)	19.77(2)

* From these values $[M^{2+} + H^+ + A^{2-} \overset{\beta}{\rightleftharpoons} M(HA)^+]$ and the value of pK_{a4} for lisinopril ($HA^- \overset{K_{a4}}{\rightleftharpoons} A^{2-} + H^+$), log K values for the equilibrium $M^{2+} + HA^- \overset{K}{\rightleftharpoons} M(HA)^+$ were calculated using the equation $\log K = \log \beta - pK_{a4}$: Zn^{II}, 3.57(1); Cu^{II}, 6.52(1); Ni^{II}, 4.36(1).

stants (log β) summarised in Table 1. In the presence of these metal ions a major complex species in neutral or weakly acidic solution is $M(HA)^+$. From the formation constants, log K values for the equilibria $M + HA \rightleftharpoons M(HA)$ of 3.57(1) in the case of Zn^{II}, 6.52(1) in the case of Cu^{II} and 4.36(1) in the case of Ni^{II} have been calculated (Table 1, footnote). Since these values are similar to those for complexes of α-amino acidates,[7] the most likely co-ordination site for metal ions involves the secondary amino group and the adjacent carboxylate group. The values may be compared with those of analogous sarcosine, MeNH-CH_2CO_2H, complexes which have similar donor sites and for which log K_{MA^+} values are 4.31 for Zn^{II}, 8.83 for Cu^{II} and 5.95 for Ni^{II}.[9] Since the amino and the carboxylate groups in sarcosine are more basic than the corresponding groups in lisinopril (ΔpK_a = 2.81 and 0.5 respectively), the stability constants of the lisinopril complexes are higher than expected and indicate that the carbonyl oxygen may also be involved in co-ordination, as shown.

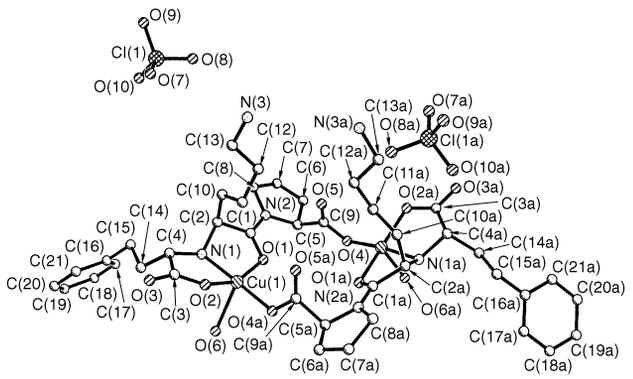


Fig. 2 Molecular structure of $[Cu_2(HA)_2(H_2O)_2][ClO_4]_2$ showing the atom numbering scheme

Table 2 Selected bond lengths (Å) and angles (°) with estimated standard deviations for $[Cu_2(HA)_2(H_2O)_2][ClO_4]_2$

Cu(1)–O(1)	1.996(7)	Cu(1)–O(2)	1.944(8)
Cu(1)–O(6)	2.355(10)	Cu(1)–N(1)	1.989(8)
Cu(1)–O(4a)	1.909(8)	O(1)–C(1)	1.260(14)
O(2)–C(3)	1.272(13)	O(3)–C(3)	1.226(13)
O(4)–C(9)	1.285(15)	O(5)–C(9)	1.190(19)
N(1)–C(2)	1.490(15)	N(1)–C(4)	1.527(13)
N(2)–C(1)	1.312(12)		
O(1)–Cu(1)–O(2)	157.0(4)	O(1)–Cu(1)–O(6)	103.9(4)
O(2)–Cu(1)–O(6)	93.6(4)	O(1)–Cu(1)–N(1)	80.2(3)
O(2)–Cu(1)–N(1)	85.0(3)	O(6)–Cu(1)–N(1)	90.4(4)
O(1)–Cu(1)–O(4a)	96.8(3)	O(2)–Cu(1)–O(4a)	99.2(3)
O(6)–Cu(1)–O(4a)	85.8(4)	N(1)–Cu(1)–O(4a)	174.4(4)
Cu(1)–O(1)–C(1)	111.9(7)	Cu(1)–O(2)–C(3)	115.3(7)
C(9)–O(4)–Cu(1a)	119.7(7)	Cu(1)–N(1)–C(2)	107.4(6)
Cu(1)–N(1)–C(4)	110.3(6)	C(2)–N(1)–C(4)	118.9(10)
O(1)–C(1)–C(2)	119.9(9)	O(1)–C(1)–N(2)	120.0(10)
N(1)–C(2)–C(1)	103.0(10)	O(2)–C(3)–O(3)	122.1(10)
O(2)–C(3)–C(4)	118.6(9)	O(3)–C(3)–C(4)	119.2(10)
N(1)–C(4)–C(3)	108.8(8)	O(4)–C(9)–O(5)	126.4(11)

Dark blue crystals of the copper(II)–lisinopril complex $[Cu_2(HA)_2(H_2O)_2][ClO_4]_2$ suitable for structure determination were obtained by adding a solution of $Cu(ClO_4)_2 \cdot 6H_2O$ in methanol to a solution of lisinopril dihydrate–triethylamine in methanol and recrystallising the resulting precipitate from acetone–water (1:1). The molecular structure of the complex is shown in Fig. 2 with selected bond lengths and angles in Table 2. The structure confirms that the complex is dimeric with each lisinopril acting as a bridging ligand. The geometry around each copper is a distorted square pyramid for which binuclear complexes of copper(II) exhibit propensity.[10] In this complex oxygen atoms of carboxylate (1.944 Å) and carbonyl (1.996 Å) groups as well as the secondary amino nitrogen (1.989 Å) of one lisinopril ligand occupy three positions in the basal plane while the fourth is occupied by the prolyl carboxylate of a second lisinopril ligand (1.909 Å). An oxygen atom of water is located at the apex at a distance of 2.355 Å from the copper. Square-pyramidal geometry surrounding copper(II) has previously been observed in many copper(II)–peptide and other

complexes. In the dimer $[Cu_2(L\text{-Leu-}L\text{-Tyr})_2]\cdot 8H_2O\cdot Et_2O$ (L-Leu-L-Tyr = L-leucyl-L-tyrosinate) for example the basal plane around each metal contains two oxygen atoms (carbonyl and carboxylate) and two nitrogen atoms (amino and amido) at distances of 1.92–2.02 Å from the metal with oxygen atoms from H_2O and bridging carboxylate occupying the axial sites at distances of 2.57 and 2.32 Å respectively from the two copper ions.[11]

Although the current models for site recognition of ACE by non-thiol containing inhibitors such as perindoprilat and lisinopril do not implicate the secondary amino group in co-ordination to the zinc ion, the results of our solution studies show that this may be a realistic model and is confirmed by the crystal structure, albeit of a dimeric copper complex, whereas the inhibitor–ACE interaction is monomeric and involves zinc(II). Moreover the flexibility in co-ordination number and geometry shown by catalytic zinc(II) sites in many enzymes lends further weight to this possibility.[6]

Experimental

Solution studies

Lisinopril dihydrate was kindly provided by Zeneca Pharmaceuticals. Stock copper(II) and nickel(II) solutions were prepared from $CuCl_2\cdot 2H_2O$ and $NiCl_2\cdot 2H_2O$ and standardised with ethylenedinitrilotetraacetate (edta).[12] The stock zinc(II) solution was prepared by dissolving ZnO in an excess of 0.1 mol dm^{-3} HCl and was also standardised with edta.[12] In order to obtain pK_a values a 5.0×10^{-3} mol dm^{-3} solution (25.0 cm^3) of lisinopril dihydrate in 0.015 mol dm^{-3} HCl–0.2 mol dm^{-3} KCl was titrated with 0.20 mol dm^{-3} NaOH. To obtain formation constants of the metal complexes, solutions (25.0 cm^3) containing 5.0×10^{-3} mol dm^{-3} lisinopril and 1.65×10^{-3} mol dm^{-3} metal ion in 0.20 mol dm^{-3} KCl–0.015 mol dm^{-3} HCl were titrated with 0.20 mol dm^{-3} NaOH.

The pH-metric titrations were carried out on a Mettler DL 25 Automatic Titrator fitted with a Mettler DG III combined electrode. Electrode calibration was carried out as previously described,[13] by a strong acid *vs*. strong base titration at the same ionic strength as above. Concentration stability constants were calculated from pH-metric data using the PSEQUAD computer program.[13,14]

Crystallography

Crystals of $[Cu_2(HA)_2(H_2O)_2][ClO_4]_2$ suitable for structure determination were obtained as follows. The addition, with stirring, of a solution of $Cu(ClO_4)_2\cdot 6H_2O$ (0.426 g, 1.15 mmol) in methanol (2 cm^3) to a solution of lisinopril dihydrate (0.51 g, 1.15 mmol) and triethylamine (0.116 g, 1.15 mmol) in methanol (15 cm^3) at room temperature gave, on standing overnight, a blue precipitate which was filtered off, dried and recrystallised from acetone–water (1:1). This gave dark blue crystals of $[Cu_2(HA)_2(H_2O)_2][ClO_4]_2$ (0.48 g, 71%) (Found: C, 42.4; H, 5.5; Cu, 11.0; N, 7.0. $C_{42}H_{64}Cl_2Cu_2N_6O_{20}$ requires C, 43.1; H, 5.5; Cu, 10.9; N, 7.2%); $\tilde{\nu}_{max}/cm^{-1}$ 3410 (OH), 3190 (NH), 1610 (CO), 1135, 1120, 1105, 1090 (ClO).

CAUTION: as this preparation involves reaction of a metal perchlorate with an organic ligand due care must be taken.

Crystal data and data-collection parameters. $C_{42}H_{64}Cl_2Cu_2N_6O_{20}$, $M = 1170.6$, orthorhombic, space group $C222_1$, $a = 10.412(4)$, $b = 15.630(5)$, $c = 32.074(12)$ Å, $U = 5220(3)$ Å^3, $Z = 4$, $F(000) = 2440$, $D_c = 1.490$ Mg m^{-3}, blue plates, dimensions $0.3 \times 0.2 \times 0.2$ mm, μ(Mo-Kα) = 9.97 cm^{-1}.

2834 Independent reflections were collected on a Siemens P3/PC diffractometer ($T = 293$ K, graphite-monochromated Mo-Kα radiation, $\lambda = 0.710\,73$ Å, ω-scan technique, $2\theta < 52°$, two standards measured every 98 reflections). The structure was solved by direct methods and refined by full-matrix least squares (based on F) using 1697 reflections with $I > 3\sigma(I)$. The H atoms of the water molecule were located in the Fourier-difference synthesis and refined isotropically; all other H atoms were included in the final refinement in the riding model approximation. A weighting scheme $w^{-1} = \sigma^2(F) + 0.0005F^2$ was employed. Final R and R' factors were 0.0730 and 0.0786 respectively. The absolute structure was determined using the Hamilton test: the R factor for the inverted structure was 0.0780. All calculations were carried out on an IBM personal computer using the SHELXTL PLUS program package.[15]

Atomic coordinates, thermal parameters, and bond lengths and angles have been deposited at the Cambridge Crystallographic Data Centre (CCDC). See Instructions for Authors, *J. Chem. Soc.*, *Dalton Trans.*, 1997, Issue 1. Any request to the CCDC for this material should quote the full literature citation and the reference number 186/486.

Acknowledgements

We thank the Research Committee of the Royal College of Surgeons in Ireland, the Royal Irish Academy, the Hungarian Academy of Sciences and the Xunta de Galicia, Spain for supporting this work. We thank the University of Santiago de Compostela, Spain, for granting sabbatical leave to E. B. G. and the University of Zanjan, Iran for granting sabbatical leave to A. A. S. We thank Zeneca Pharmaceuticals, Macclesfield for supplying lisinopril.

References

1 R. Beaglehole, R. Bonita and T. Kjellstrom, in *Basic Epidemiology*, World Health Organisation, Geneva, 1993, p. 8.
2 S. Oparil, in *Cecil Textbook of Medicine*, eds. J. C. Bennett and F. Plum, W. B. Saunders, Philadelphia, 20th edn., 1996, pp. 256–271.
3 W. G. J. Hol, *Angew. Chem., Int. Ed. Engl.*, 1986, **25**, 767.
4 R. J. Hansin and P. W. Codding, *J. Med. Chem.*, 1990, **33**, 1940.
5 C. Pascard, J. Guilhem, M. Vincent, G. Remond, B. Porteven and M. Laubie, *J. Med. Chem.*, 1991, **34**, 663.
6 B. L. Vallee and D. S. Auld, *Proc. Natl. Acad. Sci. USA*, 1990, **87**, 225.
7 T. Kiss, in *Biocoordination Chemistry*, ed. K. Burger, Ellis Horwood, Chichester, 1990, ch. III, Table 3.2.
8 I. Sovago, in *Biocoordination Chemistry*, ed. K. Burger, Ellis Horwood, Chichester, 1990, ch. IV.
9 P. Daniele and G. Ostacoli, *Ann. Chim.* (*Rome*), 1977, **67**, 311.
10 B. J. Hathaway, in *Comprehensive Coordination Chemistry*, eds. G. Wilkinson, R. D. Gillard and J. A. McCleverty, Pergamon, Oxford, 1987, vol. 5, pp. 619–635.
11 D. Van der Helm and W. A. Franks, *J. Am. Chem. Soc.*, 1968, **90**, 5627.
12 A. I. Vogel, *A Textbook of Quantitative Chemical Analysis*, Longmans, Essex, 5th edn., 1989, ch. 10.
13 E. Farkas, E. Kozma, T. Kiss, I. Toth and B. Kurzak, *J. Chem. Soc., Dalton Trans.*, 1995, 447.
14 L. Zekany and I. Nagypal, in *Computational Methods for the Determination of Formation Constants*, ed. D. J. Leggett, Plenum, New York, 1985.
15 G. M. Sheldrick, SHELXTL PLUS, Siemens Analytical X-Ray Instruments, Madison, WI, 1989.

Received 4th *March* 1997; *Paper* 7/01500C