Axial Hydrophobic Fence in Highly-Stable Ni(II) Complex of Des-Angiotensinogen N-Terminal Peptide

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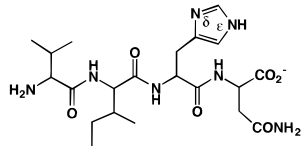
Received November 28, 1995

Angiotensinogen is a 66 kDa globular protein circulating in human blood with a concentration of ca. 1 μg mL^{-1} (15 nM).[1] The kidney protease renin cleaves the N-terminal decapeptide angiotensin I from angiotensinogen as an essential part of the blood pressure regulatory system. The remaining protein, des-angiotensin 1-angiotensinogen (des-angiotensinogen),[2] has the N-terminal sequence Val-Ile-His-Asn-Glu-Ser-Thr- (VIHNEST).[3] Peptides with an N-terminal amino group and His in position 3 often have a very strong affinity for square-planar metal ions such as Cu(II) and Ni(II),[4] forming two five-membered and one six-membered chelate rings via coordination to the terminal NH_2, deprotonated amide Ns of residues 2 and 3 and His Nδ.[5] Such a high affinity allows square-planar metal ions to play a role in the biological activity of this class of peptides. An example of this is Ni−albumin[6,7] (N-terminal sequence Asp-Ala-His-Lys), which is known to be the allergenic determinant in Ni-induced allergy.[8] We report here that the Ni(II) complex of the des-angiotensinogen tetrapeptide VIHN has the highest stability of known Ni(II) complexes in this class, attributable in part to the erection of an axial hydrophobic fence. This work suggests that metal-induced sequence-specific conformational ordering of amino acid side chains[9] can be a powerful feature for incorporation into the design of bioactive peptides.



Val-Ile-His-Asn = L$^-$

The formation constant of Ni−VIHN was first determined. It was not possible to do this by the usual potentiometric method[10] since the rate of reaction was very slow. Instead complexation was followed by CD spectroscopic changes in the region 300−700 nm. Complex formation (peptide:Ni 1.2:1, 4 mM, I = 0.1 KNO_3, 298 K) was monitored over the pH range 5−9 using solutions which had equilibrated overnight. Complete formation of yellow $[NiH_{-2}L]^-$ was observed at pH 8.23,[11] and measurements in the pH range 5.3−5.9, where the degree of complex formation was between 0.05 and 0.8, were used in the stability constant calculations.[12] It can be seen from Table 1 that $[NiH_{-2}L]^-$ is an extremely stable complex, more than 2 orders of magnitude more stable than other reported complexes in this class.[13] To investigate the reasons for the high stability, we determined the structure of the complex in solution by NMR methods.

^{1}H NMR spectra[14] of D_2O solutions containing VIHN and Ni(II) in a mole ratio 2:1 (8 mM) were studied over the pH* range 8−10, and assignments for free VIHN and diamagnetic $[NiH_{-2}L]^-$ (in slow exchange) were made by a combination of DQF-COSY[15] and TOCSY experiments. These data are listed in Table S1 of the supporting information. 2D transverse ROESY[16] experiments on this sample were carried out with six mixing times in the range 50−350 ms. These spectra did not contain any interchain cross peaks due to insufficient accumulation times (<8 h per spectrum) but were used to determine the cross-relaxation buildup rates for pairs of protons with known geometry such as methylene protons, to judge the accuracy of cross-peak volumes in distance measurements, and to determine the optimum mixing time to achieve the best signal-to-noise ratio. Subsequently, a 57 h acquisition on a sample containing a 1:1 mole ratio of VIHN:Ni(II) (5 mM, pH* 10) with a 300 ms mixing time provided enough interresidue cross peaks

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(1) Gould, A. B.; Skeggs, L. T.; Kahn, J. R. *Lab. Invest.* **1966**, *15*, 1802−1813.

(2) High concentrations of des-angiotensinogen have been reported to occur in some important human conditions, e.g. in patients with elevated plasma renin concentrations secondary to bacterial sepsis: Hilgenfeldt, U. *Mol. Cell. Endocrinol.* **1988**, *56*, 91−98.

(3) GenPept (Gene Bank Products), database distributed by National Cancer Institute, Frederick Biomedical Supercomputing Center. For GenBank, cf.: Burks, C.; Cassidy, M.; Cinkosky, M. J.; Cumella, K. E.; Gilna, P.; Hayden, J. E. D.; Kelley, T. A.; Kelly, M.; Kristofferson, D.; Ryals, J. *Nucleic Acids Res.* **1991**, *19* (*Suppl.*), 2221−2225.

(4) Hay, R. W.; Hassan, M. M.; You-Quan, C. *J. Inorg. Biochem.* **1993**, *52*, 17−25.

(5) E.g.: (a) Camerman, N.; Camerman, A.; Sarkar, B. *Can. J. Chem.* **1967**, *54*, 1309−1316. (b) Bal, W.; Djuran, M. I.; Margerum, D. E.; Gray, E. T., Jr.; Mazid, M. A.; Tom, R. T.; Nieboer, E.; Sadler, P. J. *J. Chem. Soc., Chem. Commun.* **1994**, 1889−1890.

(6) (a) Sigel, H.; Martin, B. *Chem. Rev.* **1982**, *82*, 385−426. (b) Glennon, J. D.; Sarkar, B. *Biochem. J.* **1982**, *203*, 15−23.

(7) (a) Patel, S. U.; Sadler, P. J.; Tucker, A.; Viles, J. H. *J. Am. Chem. Soc.* **1993**, *115*, 9285−9286. (b) Sadler, P. J.; Tucker, A.; Viles, J. H. *Eur. J. Biochem.* **1994**, *220*, 193−200.

(8) Dolovich, J.; Evans, S. L.; Nieboer, E. *Br. J. Ind. Med.* **1984**, *41*, 51−55.

(9) Bal, W.; Kozlowski, H.; Kupryszewski, G.; Mackiewicz, Z.; Pettit, L.; Robbins, R. *J. Inorg. Biochem.* **1993**, *52*, 79−87.

(10) The pK_a values of VIHN were calculated using SUPERQUAD (Gans, P.; Sabatini, A.; Vacca, A. *J. Chem. Soc., Dalton Trans.* **1985**, 1195−1199) from pH-metric titration curves obtained using a Molspin automatic titrator. The pH electrode was calibrated in terms of hydrogen ion concentration: Irving, H.; Miles, M. G.; Pettit, L. D. *Anal. Chim. Acta* **1967**, *38*, 475−488.

(11) Bands were observed at 468 nm (−1.73) and 400 nm (+0.80). These are typical of square-planar peptidic Ni(II)N_4 sites involving a His residue; see e.g., ref 6a, p 407.

(12) The equilibrium was monitored by checking the ellipticity drift at the spectral maximum. Values used in calculations were sums of max and min intensities, thereby minimizing the effects of base line drifts. Data were analyzed assuming formation of $[NiH_{-2}L]^-$ only, and a good fit was obtained (standard error < 0.1 log units). A systematic deviation at the acidic end of the pH range indicated formation of minor octahedral species, e.g. $[NiHL]^{2+}$, and $[NiL]^+$. No correction was made for the formation of these, but if they are assumed to have stabilites similar to GGH complexes, then the average stability constant for $[NiH_{-2}L]^-$ would be 0.2 log units higher.

(13) Pettit, G.; Pettit, L. D. *IUPAC Stability Constants Database*; IUPAC and Academic Software: Leeds, U.K., 1993.

(14) Spectra (500 MHz) were recorded on a Varian VXR500S spectrometer. Transmitter presaturation was used to suppress the residual HOD resonance; the "clean" TOCSY sequence (mixing time 50 ms) was used (Bax, A.; Davis, J. *J. Magn. Reson.* **1985**, *65*, 355−360. Levitt, M.; Freeman, R.; Frenkiel, T. A. *J. Magn. Reson.* **1982**, *47*, 328−330), with spin-lock 90° pulse width of 32 μs, with 32 μs delays between pulses. The Tr-ROESY sequence used a +180, −180 spin lock with 90° pulse of 37.5 μs, spectral width 3852 Hz, total recycle time 3.07 s. Peaks are referenced to TSP via internal acetate (1.92 ppm).

(15) Piantini, U.; Sorensen, O. W.; Ernst, R. R. *J. Am. Chem. Soc.* **1982**, *104*, 6800−6801.

(16) Hwang, T. L.; Shaka, A. J. *J. Am. Chem. Soc.* **1992**, *114*, 3157−3159.

S0002-7863(95)03988-6 CCC: $12.00

Table 1. Protonation and Complex Stability Constants in Aqueous Solution at 298 K, $I = 0.1$ M (KNO_3)

species	log β	pK	log $*K$[a]
H_3L	16.87(3)	2.51(3)[b]	
H_2L	14.36(1)	6.57(1)[c]	
HL	7.79(1)	7.79(1)[d]	
$[NiH_{-2}L]$	−5.39(9)		−19.75
$[Ni(H_{-2}GGH)]^{-}$ [e]	−6.93		−21.83

[a] Stability constant corrected for protonation, where $*K$ is the binding constant for the reaction Ni(II) + H_2L = Ni(II)$H_{-2}L$ + $4H^+$ and is numerically equal to log $*K$ = log β_{1-21} − log β_{021}. [b] C-terminal carboxyl. [c] His imidazole ring. [d] N-terminal amino group. [e] From ref 4.

(illustrated in Figures S1 and S2 of the supporting information) to allow a structure determination.

ROE volumes were converted to upper distance limits as follows. Two sets of calibration distances were used: the interproton distance for the methylene protons of His3, Asn4, and Ile2 and the methyl to methylene or methine distances for Val1 and Ile2. Volumes for each group were averaged giving a two point calibration of all other ROE volumes. The calculated interproton distances are listed in Table S2 of the supporting information. A total of 300 structures, based on 18 intraresidue, 5 interresidue, and 2 dihedral angle constraints, were generated using the metric matrix distance geometry and simulated annealing functions of X-PLOR.[17] The root-mean-square differences (rmsds) between all 300 structures were 0.029 Å for the backbone atoms, 0.23 Å for the non-hydrogen atoms, and 0.54 Å for all atoms (structures aligned on the backbone atoms, and rmsds calculated for residues 1−3 only). All structures satisfied all the NOE conditions. An average structure was computed, and the 10 structures with the lowest total energy are shown in Figure 1A. The structure with the lowest overall energy is shown as a space-filling model in Figure 1B.[18]

The most striking feature of the structure is the ordering of the side chains of Val1 and Ile2 with formation of a hydrophobic fence, shielding one side of the coordination plane from the bulk of the solution. The Asn side chain does not appear to interact with the metal nor with the rest of the peptide. The stabilities of Ni(II) peptide complexes depend on the rate of dissociation, and, in the case of XXH complexes, dissociation of Ni(II) from the ligand requires attack of water or H^+ on one of the Ni−amide nitrogens.[4,19] The structure reveals that the approach to both coordinated amide nitrogens by water molecules is hindered from above the plane of the complex due to the hydrophobic shielding from Val and Ile side chains. This could account for the decrease in dissociation rate and the increase in stability by over 2 orders of magnitude. It has been noted previously that hydrophobic shells around hydrophilic ligands appear to enhance metal binding to proteins.[20,21]

The ability of metal ions to promote organization of the side chains of peptides may be important in their biological activity, for example in the assembly of specific receptor binding sites,

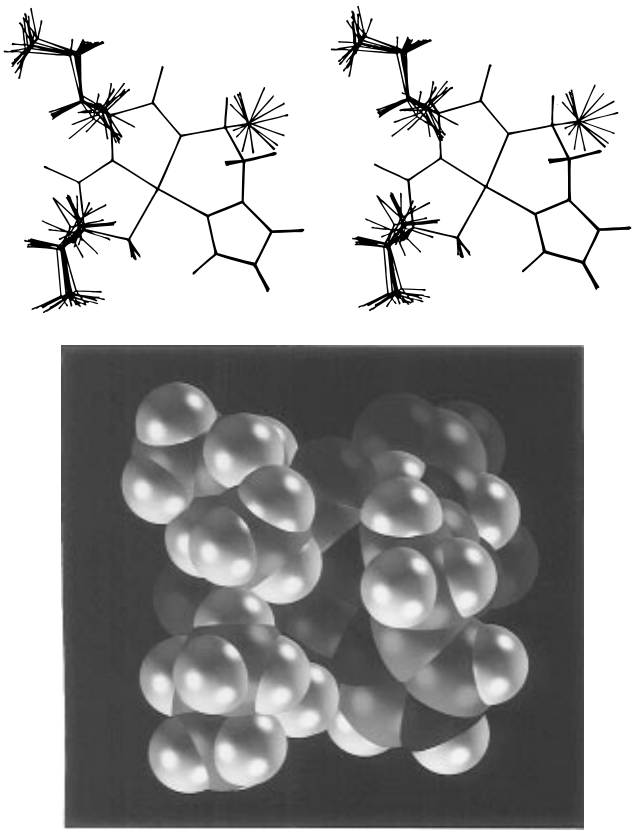

Figure 1. (A, top) Stereoview of the 10 structures of Ni(II)−VIHN with the lowest total energy, as determined from NMR data, showing all atoms but omitting residue 4 since it has no interresidue constraints. (B, bottom) Space-filling model of the structure with the lowest overall energy showing the amino acid side chains which hinder axial approach to Ni(II). Color code: C, light blue; H, white; N, dark blue; O, red; Ni(II), green.[18]

the production of antigenic sites, folding processes, and catalysis at the metal site. An intriguing possibility arising from this work is that Ni(II) or Cu(II) could play a role in the metabolism of des-angiotensinogen and/or *vice versa*. The affinities of these metal ions for des-angiotensinogen itself may be even higher than for its terminal tetrapeptide, since Asn4 is a glycosylation site and the carbohydrate chain may increase the shielding of the metal square plane and further decrease the dissociation rate of the complex.

Acknowledgment. We thank the Royal Society, Biomolecular Science Programme of the Biotechnology and Biological Sciences and Engineering and Physical Sciences Research Councils, and University of London Intercollegiate Research Service for their support for this work and Mr. John Klose (SAIC-Frederick) for recording the Tr-ROESY spectra. The contents of this publication do not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

Supporting Information Available: Figures S1 and S2 showing typical off-diagonal and near-diagonal sections, respectively, of Tr-ROESY spectra of Ni(II)−VIHN, Table S1 listing ^{1}H NMR assignments for free and Ni(II)-bound VIHN at pH 10, and Table S2 listing calculated interproton distances for Ni(II)−VIHN (7 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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(17) Structures were calculated from interproton distances and dihedral angle constraints (Table S2) using the program X-PLOR version 3.1, by A. T. Brünger, Yale University Press, 1992. Lower distance bounds were van der Waals radii, and upper distance bounds were set as 1.2 times the calculated interproton distances. Dihedral angles were constrained to be within ±30° of the ideal calculated angle. Parameters for Ni(II) were adapted from a force field previously developed for the program CHARMm, which reproduced known square-planar Ni(II) peptides well (Sadler, P. J.; Tucker, A. Unpublished results). A square-planar arrangement of the coordinating N atoms was obtained from an initial structure calculation even without the constraint imposed by insertion of a square-planar metal ion.

(18) Figure 1A,B was produced using Setor version 4.13.4: Evans, S. V. *J. Mol. Graphics* **1993**, *11*, 134−138.

(19) Raysheba, J. M. T.; Margerum, D. *Inorg. Chem.* **1980**, *19*, 837−843.

(20) Yamashita, M. M.; Wesson, L.; Eisenman, G.; Eisenberg, D. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 5648−5652.

(21) Regan, L. *Annu. Rev. Biophys. Biomol. Struct.* **1993**, *22*, 257−281.