COMMUNICATION

ChemComm
www.rsc.org/chemcomm

May GSH and L-His contribute to intracellular binding of zinc? Thermodynamic and solution structural study of a ternary complex

Artur Krężel,[a] Jacek Wójcik,[b] Maciej Maciejczyk[b] and Wojciech Bal*[b]

[a] *Faculty of Chemistry, University of Wrocław, Joliot-Curie 14, 50-383 Wrocław, Poland. E-mail: arti@wchuwr.chem.uni.wroc.pl; Fax: +48-71-3282348; Tel: +48 71 3757264*

[b] *Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawińskiego 5a, 02-106 Warszawa, Poland. E-mail: wbal@ibb.waw.pl; Fax: +48-22-6584636; Tel: +48-22-6597072 x2353*

Received (in Cambridge, UK) 16th January 2003, Accepted 6th February 2003
First published as an Advance Article on the web 21st February 2003

GSH and L-His are abundant biomolecules and likely biological ligands for Zn(II) under certain conditions. Potentiometric titrations provide evidence of formation of ternary Zn(II) complexes with GSH and L-His or D-His with slight stereoselectivity in favour of L-His (*ca.* 1 log unit of stability constant). The solution structure of the ZnH(GSH)(L-His)(H_2O) complex at pH 6.8, determined by NMR, includes tridentate L-His, monodentate (sulfur) GSH, and weak interligand interactions. Calculations of competitivity of this complex for Zn(II) binding at pH 7.4 indicate that it is likely to be formed *in vivo* under conditions of GSH depletion. Otherwise, GSH alone emerges as a likely Zn(II) carrier.

Reduced glutathione (GSH) is one of the most abundant and ubiquitous molecules of life, at 1–20 mM intracellularly, with strong compartmentalisation and various functions in cellular metabolism and defenses, including detoxication of heavy metals.[1] Zn(II) is involved, among others, in DNA transcription (enzymes, zinc fingers) and intracellular signaling. O'Halloran *et al.* demonstrated the absence of free Zn(II) in *E. coli*.[2] On the other hand, estimates for free Zn(II) in the cytoplasm of eukaryotic cells range from 10^{-12} M to 10^{-9} M, depending on cell type and state, and up to 10^{-3} M in specific secretory vesicles.[3,4] Zn(II) is an emerging signalling ion, and thus its metallothionein (MT)-bound pool ought to be easily mobilisable. Glutathione is capable of releasing Zn(II) from MT in a redox reaction involving its oxidised form (GSSG).[5] A lack of consensus regarding interactions between GSH and Zn(II) ions *in vitro*,[6] and the absence of specific information on possible interactions *in vivo*, made it difficult to predict further steps in Zn(II) release. Also transport of Zn(II) outside and into the eukaryotic cell is not understood very well. Histidine, which is present ubiquitously in the body at *ca.* 10^{-4} M, has been implicated as a possible Zn(II) shuttle in some tissues.[7]

In order to provide a chemical basis for assessment of the possible participation of glutathione in Zn(II) transport, we have examined the acid–base chemistry and Zn(II) coordination of GSH, GSSG and many of their analogues, using potentiometry and NMR.[8,9] In the course of these studies we noted that GSH readily forms ternary complexes with Zn(II) and amino acids and peptides. Here we present the results for such complexes, involving histidine.

Protonation constants of GSH, L-His and D-His, as well as stability constants for their binary and ternary complexes with Zn(II), were obtained from potentiometric titrations and confirmed by one-dimensional ^{1}H-NMR spectra at 300 and 500 MHz. These constants are provided in Table 1. The values for major complexes of L-His and D-His, ZnA and ZnA_2, agree well with those determined previously.[10] The same can be stated for GSH complexes, where our model is practically identical with the previous one.[11] The only difference is our $ZnH_{-2}L_2^{6-}$ species instead of $ZnH_{-1}L^{2-}$, postulated previously, and the differences in values of constants can be ascribed to differences in ionic strengths of determinations.

Ternary Zn(II) complexes with GSH and L-His or D-His are novel. The analysis of 1D NMR spectra indicated that Zn(II) in these complexes is coordinated to all three donors of histidine (imidazole and amine nitrogens and carboxylate oxygen) and to the thiol sulfur of GSH. The deprotonation yielding $ZnLA^{2-}$ from $ZnHLA^-$ is, as indicated by the spectra, that of the uncoordinated amine. Its pK_a is the same in both diastereomers (8.2 ± 0.1), and lowered compared to free GSH by 1.5 log units, apparently due to the increased overall charge in the complex and altered electrostatics in bonded GSH compared to free GSH (neutralisation of the thiolate by Zn(II) and spatial shielding of the amine from the Gly carboxylate).[8] There is a significant difference in stabilities of diastereomers, *ca.* 1 order of magnitude in favour of the L-His containing species, which translates into $\Delta\Delta G$ of 2.8 kJ mol^{-1}. Fig. 1 presents a simplified species distribution diagram, calculated for the L-His system at the concentrations of the NMR experiments. The abundance of the $ZnHLA^-$ complex at weakly acidic to weakly basic pH allowed for the determination of its structure by 2D NMR at 500 MHz and molecular mechanics.[13] Fig. 2 presents the resultant lowest-energy structure and the overlap of ten low-energy conformers, allowed by NOE connectivities. The complex formation is primarily due to high enthalpies of Zn(II) bonding by thiol sulfur and histidine donors. Its structure is additionally stabilised by a local network of weak C–H···C, C–H···N and N–H···C type hydrogen bonds (Fig. 2, dashed lines), which is anchored on the β-methylene protons of His and Cys residues.[14] The differences in the involvement of individual protons of each pair in H-bonding are reflected in the spectacular differences of half-widths of their ^{1}H NMR signals, shown in Fig. 3.

Stability constants allow us to estimate the competitiveness of the system studied towards zinc proteins.[15] The competitivity

Table 1 Protonation and stability constants, I = 0.1 M, T = 25 °C[12]

	log β_{ijkl}[a]		log β_{ijkl}[a]
GSH[b]		**L-His**	
HL^{2-}	9.655(2)	HA	9.129(1)
H_2L^-	18.391(2)	H_2A^+	15.165(2)
H_3L	21.903(3)	H_3A^{2+}	16.85(5)
H_4L^+	24.029(7)		
		D-His	
Zn(GSH)		HA	9.129(2)
ZnHL	14.74(2)	H_2A^+	15.142(3)
ZnL^-	8.31(2)	H_3A^{2+}	16.83(6)
$ZnH_2L_2^{2-}$	29.50(4)		
$ZnHL_2^{3-}$	22.533(5)	**Zn(L-His)**	
ZnL_2^{4-}	13.617(5)	ZnA^+	6.567(5)
$ZnH_{-1}L_2^{5-}$	3.817(6)	ZnA_2	12.025(5)
$ZnH_{-2}L_2^{6-}$	−6.485(6)	$ZnH_{-1}A^{2-}$	1.18(2)
		$ZnH_{-2}A_2^{2-}$	−9.90(2)
Zn(L-His)(GSH)			
$ZnHLA^-$	21.46(5)	**Zn(D-His)**	
$ZnLA^{2-}$	13.26(8)	ZnA^+	6.61(1)
		ZnA_2	12.09(1)
Zn(D-His)(GSH)		$ZnH_{-1}A^{2-}$	1.15(4)
$ZnHLA^-$	20.37(10)	$ZnH_{-2}A_2^{2-}$	−9.83(3)
$ZnLA^{2-}$	12.24(5)		

[a] log β_{ijkl} = log($[M_iH_jL_kA_l]/[M]^i[H]^j[L]^k[A]^l$). [b] Ref. 8.

DOI: 10.1039/b300632h

index, at pH 7.4, for GSH is 8.05 at 10 mM and 6.1 at 1 mM. Inclusion of the ternary system with L-His increased these values to 8.1 and 6.25, respectively, while the value for L-His alone at 100 μM is 4.1. This indicates that GSH, rather than L-His, may be an important player in zinc homeostasis, due to a difference in normal intracellular concentrations of these molecules. The participation of the ternary complex is *ca.* 4% of total GSH-bonded Zn(II) at 10 mM GSH and *ca.* 20% at 1 mM GSH. Its overall charge of −1 is lower than those of major bis-complexes of GSH, therefore it may play a specific transport role in more hydrophobic compartments. Altogether, the binary and ternary complexes of GSH emerge as likely regulators of zinc homeostasis. It should be noted that other efficient Zn(II) chelators, abundant intracellularly, such as nucleotides (*e.g.* ATP), should also be considered as likely partners for the formation of ternary complexes with GSH.

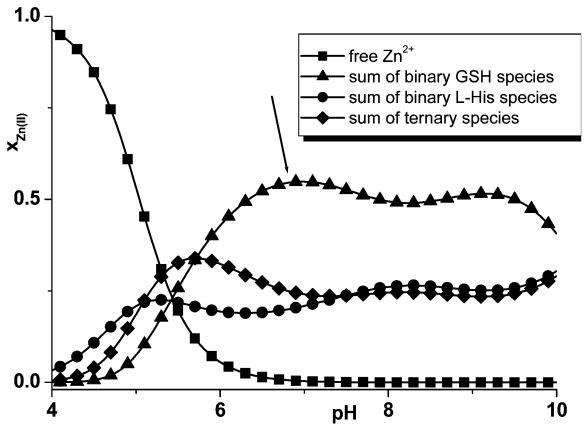

Fig. 1 Species distribution in the Zn(II)/GSH/L-His ternary system (each reactant at 20 mM); arrow marks the pH of the 2D NMR experiment.

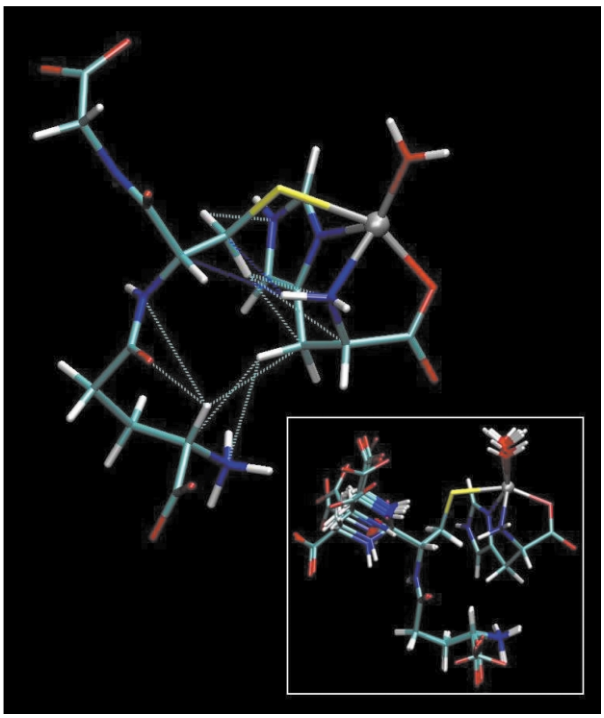

Fig. 2 Solution structure of ZnH(GSH)(L-His)$(H_2O)^-$ (lowest energy conformer); insert shows 10 low energy conformers to demonstrate the mobility of the Gly residue.

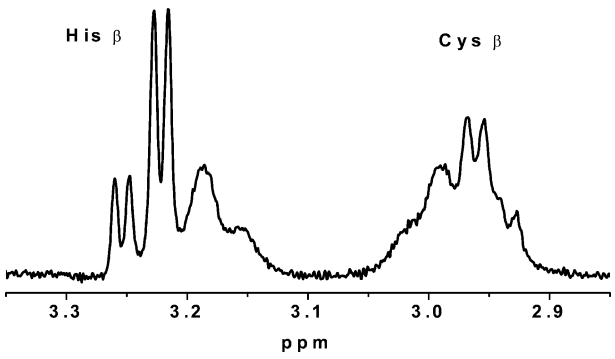

Fig. 3 Part of ^{1}H NMR spectrum (500 MHz), demonstrating differentiation of linewidths between β protons of Cys and His residues in the ternary complex.

This work was sponsored by the Polish State Committee for Scientific Research (KBN), grant 7 T09A 079 20.

Notes and references

1 H. Sies, *Free Rad. Biol. Med.*, 1999, **27**, 916–921.
2 C. E. Outten and T. V. O'Halloran, *Science*, 2001, **292**, 2488–2492 and references therein.
3 J. Benters, U. Flögel, T. Schäfer, D. Leibfritz, S. Hechtenberg and D. Beyersmann, *Biochem. J.*, 1997, **322**, 73–79 and references therein.
4 S. R. Kar, A. C. Adams, J. Lebowitz, K. B. Taylor and L. M. Hall, *Biochemistry*, 1997, **36**, 15343–15348.
5 Y. Chen and W. Maret, *Eur. J. Biochem.*, 2001, **268**, 3346–3353.
6 A. Krezel and W. Bal, *Acta Biochim. Polon.*, 1999, **46**, 567–580.
7 E. C. Tibaduiza and D. J. Bobilya, *J. Cell. Physiol.*, 1996, **167**, 539–547.
8 A. Krezel and W. Bal, *Eur. J. Biochem.*, manuscript in preparation.
9 W. Bal, A. Krezel, J. Wójcik and M. Maciejczyk, *J. Inorg. Biochem.*, 2001, **86**, 135, and manuscript in preparation.
10 L. D. Pettit and J. L. M. Swash, *J. Chem. Soc., Dalton Trans.*, 1976, 588–594.
11 A. M. Corrie, M. D. Walker and D. R. Williams, *J. Chem. Soc., Dalton Trans.*, 1976, 1012–1015.
12 Titrations performed as described recently (M. Sokolowska, A. Krezel, M. Dyba, Z. Szewczuk and W. Bal, *Eur. J. Biochem.*, 2002, **269**, 1323–1331); calculations performed using SUPERQUAD (P. Gans, A. Sabatini and A. Vacca, *J. Chem. Soc., Dalton Trans.*, 1985, 1195–1199).
13 Part of ^{1}H NMR spectrum (500 MHz), demonstrating differentiation of linewidths within β protons of Cys and His residues in the ternary complex.
14 The NOESY spectra (A. A. Bothner-By, R. L. Stephens, J.-M. Lee, C. D. Warren and R. W. Jeanloz, *J. Am. Chem. Soc.*, 1984, **106**, 811–813; A. Bax and D. G. Davis, *J. Magn. Reson.*, 1985, **63**, 207–213) were recorded on a Varian Unity+ 500 instrument at 500 MHz. 42 NOESY cross-peaks were calibrated with a CALIBA module from the DYANA package. The reference distance was histidine r(HA − HD1) = 5.20 Å. As a result, 20 inter-proton distance constraints were obtained. Conformations were calculated using the simulated annealing protocol with floating chirality module and square-well potential constraints. Electrostatic interactions were turned off to obtain better conformational sampling. Coordination of Zn^{2+} was based on potentiometric and 1D NMR titrations, in agreement with B. J. Fuhr and D. L. Rabenstein, *J. Am. Chem. Soc.*, 1973, **95**, 6944–6950. The conformation of L-His was fixed and distance restraints were put on Zn^{2+}–(coordinating atom) distances. The initial structure generation was followed by 50 ps of high-temperature (1000 K) molecular dynamics and a 30 ps cooling process, until the temperature reached 100 K. The final structure was minimised with electrostatic interactions turned on. A total of 90 structures were calculated and the 10 lowest energy structures were analysed. The average heavy-atom RMSD to mean structure is 0.8 Å. The origin of the relatively large RMSD comes from the flexibility of glycine, which can rotate around the Cys ψ dihedral. The rest of the complex is rigid. The complex attained the conformation which allows binding of a water molecule by Zn(II).
15 G. R. Desiraju and T. Steiner, *The Weak Hydrogen Bond in Structural Chemistry and Biology*, Oxford University Press Inc., New York, 2001; the cutoff distance for H bonds was taken as 3.6 Å.
16 The competitivity index of ligand L or the pair of ligands L, A towards metal ion M is defined here as the logarithm of the conditional stability constant of MZ, the metal complex of a hypothetical molecule Z, such that $\Sigma_{ijkl}([M_iH_jL_kA_l] = [MZ]$. Concentrations used: 0.2 mM for M and Z, were based on the discussion in ref. 2.