Eur Biophys J (2007) 36:841–845
DOI 10.1007/s00249-007-0162-2

ORIGINAL PAPER

Studying the Cu binding sites in the PrP N-terminal region: a test case for ab initio simulations

S. Furlan · G. La Penna · F. Guerrieri · S. Morante · G. C. Rossi

Received: 22 December 2006 / Revised: 9 March 2007 / Accepted: 20 March 2007 / Published online: 10 May 2007

Abstract First principle ab initio molecular dynamics simulations of the Car–Parrinello type have proved to be of invaluable help in understanding the microscopic mechanisms of chemical bonding both in solid state physics and in structural biophysics. In this work we present as a test case a study of the Cu coordination mode at the Prion Protein binding sites localized in the N-terminal octarepeat region. Using medium size PC-clusters, we are able to deal with systems with up to about 350 atoms and 10^3 electrons for as long as ~2 ps. With a foreseeable forthcoming scaling up of the available CPU times by a factor 10^3, one can hope to be soon able to simulate systems of biological interest of realistic size and for physical times of the order of the nanosecond

Keywords Ab initio · Simulations · PrP · Copper

Proceedings of the XVIII Congress of the Italian Society of Pure and Applied Biophysics (SIBPA), Palermo, Sicily, September 2006.

S. Furlan
CNR, ISMAC, Via De Marini 6, 16149 Genova, Italy
e-mail: furlan@ge.cnr.it

G. La Penna
Institute for Chemistry of Organo-metallic Compounds (ICCOM), National Research Council (NRC), Via madonna del Piano 10, Sesto Fiorentino, I-50019 Firenze, Italy
e-mail: glapenna@iccom.cnr.it

F. Guerrieri · S. Morante · G. C. Rossi (✉)
Dipartimento di Fisica, Università di Roma Tor Vergata, INFN, Sezione di Roma Tor Vergata, Via della Ricerca Scientifica, 00133 Rome, Italy
e-mail: rossig@roma2.infn.it

F. Guerrieri
e-mail: guerrieri@roma2.infn.it

S. Morante
CRS, SOFT, Dipartimento di Fisica, Università di Roma La Sapienza, P.le A. Moro, 00185 Rome, Italy
e-mail: morante@roma2.infn.it

Introduction

Owing to the recent technical and architectural advances in the design of new high performance computers, extremely powerful platforms are today available to the scientific community which allow to attack problems of unprecedented complexity in many research fields ranging from high energy particle physics to disordered systems, from material science to systems of biological interest.

In this short contribution we would like to present as a test case a ''first principle'' study of the Cu coordination mode at the Prion Protein (PrP) binding sites located in the N-terminal octarepeat region. For this purpose ab initio simulations of the Car–Parrinello type[1] have been carried out on rather large model systems comprising up to almost 350 atoms and 10^3 electrons.

The alternative road of using DFT for the study of fragments of PrP in the presence of copper ions has been followed by various authors. A non-exhaustive list of papers is given in the references (Pushie and Rauk 2003; Franzini et al. 2003; Cox et al. 2006).

[1] Good reviews on the subject, as well as references to the original papers can be found in Payne et al. (1992), Mark and Hutter (2000).

The biological system and the model

PrP is a cell surface glycolipid protein, highly expressed in the central nervous system of many mammals. Its physiological rôle is still unclear, but it has been shown that it can selectively bind Cu^{2+}. A number of Cu binding sites (not less than four) have been identified along the protein (Chattopadhyay et al. 2005; Gaggelli et al. 2006).

Mature PrP (comprising a.a.'s 23–231) has a flexible, disordered, N-terminal (a.a.'s 23–120) and a globular C-terminal (a.a.'s 121–231) domain. The N-terminal domain of human PrP contains four repeats of the eight residue peptide PHGGGWGQ (a.a.'s 60-91), usually referred to as *octarepeat*[2]. It has been shown that each octarepeat is able to bind one Cu^{2+} ion. Other possible binding sites have been reported to lie along the protein.

Cu-octarepeat interaction is cooperative in nature, and could possibly have a rôle in disease related PrP aggregation. Several techniques have been used to study the octarepeat structural arrangements, including X-ray crystallography (Burns et al. 2002), NMR (Zahn 2003) and XAS spectroscopy (Morante et al. 2004). Experiments have shown that at physiological pH the dominant Cu^{2+} coordination mode involves directly only the oligopeptide HGGGW.

Crystallographic data show that the Cu^{2+} ion is pentacoordinated, displaying a square planar equatorial coordination with three nitrogens and one oxygen, all belonging to HGGGW, and an axial oxygen from a nearby water molecule. In particular it turns out that the N3O1 equatorial coordination is realized with the $\delta 1$ nitrogen of the His, the deprotonated amide nitrogens of the two following Gly residues and the carbonyl oxygen from the second Gly of the sequence. The crystallographic structure suggests that the axially bound water molecule is kept in position by a hydrogen bond to the $\varepsilon 1$ hydrogen of the Trp indole ring.

The simulated model systems

Because of CPU limitations we could not afford simulating the whole octarepeat, but only smaller portions of it, complexed with Cu and water in various combinations. The downsizing of the system we will consider in this work is consistent with the experimental finding that apparently only the HGGGW portion of the octarepeat is directly involved in the copper coordination (Burns et al. 2002; Zahn 2003).

We have carried out a detailed simulation study of the following model systems: the two solvated complexes Cu^{2+} ($HG^{-}G^{-}GW$) + 65 H_2 O and Cu^{2+}(HGGG) + 41 H_2O as well as a system composed by two HGGG oligopeptides, both in the presence and in the absence of an associated pair of copper ions (Furlan et al. 2007). Throughout the text by G^{-} is for the amide deprotonated Gly residue.

Motivated by the results of recent EPR (Chattopadhyay et al. 2005) and XAS (Morante et al. 2004) experiments, we also present preliminary studies aimed at clarifying the possible existence of a configuration where a single Cu^{2+} ion is coordinated to more than one HGGG oligopeptide. In fact, experimental data collected at various pH and at different $[Cu^{2+}]$/[oligopeptide] concentration ratios, have brought to the conclusion that more than one His, and thus more than one oligopeptide, may be coordinated by a single Cu^{2+} ion. Generally speaking this is not an unexpected behaviour when more than one His is available for binding.

In order to investigate this issue we have performed simulations on a system composed by the Cu^{2+}(HGGG) complex (with the Gly's in various protonation states) plus a single imidazole ring, where the latter is intended to mimic the presence of a second His. Such a simplification is consistent with the experimental observation that the detected coordination geometries only involve the His side-chain (i.e. the imidazole ring) and not the His backbone.

Methods

In all cases we have considered, Car–Parrinello molecular dynamics (CPMD) simulations have been carried out employing the freely available Quantum-ESPRESSO package (Baroni et al. 2004), in the version which makes use of Vanderbilt's ultrasoft pseudo-potentials and PBE exchange-correlation functional (Perdew et al. 1996). Periodic boundary conditions have been imposed on the super-cell containing the system, with a minimum separation of five and eight Å between replica for neutral and charged systems, respectively. The small energy cutoff was fixed at 25 Ry, while the hard cutoff for the augmented charge density (required by the use of the ultrasoft pseudopotentials) was taken to be 250 Ry. Most of the simulations were spin-restricted. However, in some particularly interesting cases, namely the monomer $Cu^{2+}(HG^{-}G^{-}G)$ and the dimer $[Cu^{2+}(HG^{-}G^{-}G)]_2$, we have also performed spin unrestricted simulations with $S = 1/2$ and $S = 1$, respectively.

Every simulation consisted of the following successive steps.

1. Electronic energy minimization with fixed atomic positions.
2. Energy minimization with respect to both atomic and electronic degrees of freedom in order to attain a minimally strained initial geometry.

[2] In other species this number may be different. For instance, *Bos Taurus* has five octarepeats.

3. A sequence of molecular dynamics simulations of 0.25 ps each at fixed increasing temperature from $T = 50$ to room temperature. A Nosé-Hoover thermostat at the required temperature was coupled to the atomic degrees of freedom.
4. CPMD simulations about 2 ps long at $T = 300$ K, using the same thermostat as in Step 3.

Thermalization is necessary to slowly approach room temperature and avoid that uncontrolled temperature oscillations obscure the electronic properties of the ground state. Atomic equations of motion have been integrated using the usual velocity-Verlet algorithm with a time step of 0.12 fs.

Simulations have been carried out on Linux-clusters using different numbers of processors, depending on the size of the systems (see Table 1).

CPMD simulations are computationally extremely demanding, both in terms of CPU time and memory. As an example, we report in Table 1 the CPU times required for one 0.12 fs step for some of the systems we have studied. Even if the hardware set-up is an important issue, a large increase of CPU time is found when the box size is increased. For instance, when we move from the monomer to the dimer and the box side is increased by 5 Å (from ~14 to ~19 Å), the code performance becomes five times worse. This is related to the increase in the number of electronic plane waves necessary in a larger box.

Results

In this section we briefly report the structural results we can extract from the simulations of the systems listed in the first three rows of Table 1, while the results we have collected up to now about the systems in the last two rows are described in section ''Results on possible multiple His coordination''.

Table 1 CPU time per step of 0.12 fs (t_{CPU}) for a CPMD simulation run on a Linux-cluster with N_{CPU} processors

System	Machine	N_{CPU}	t_{CPU} (s)	N_{atoms}	N_{elect}
$Cu^{2+}(HG^-G^-GW) + 65H_2O$	Fermi	14	125	265	741
$Cu^{2+}(HG^-G^-G)$	BEN	16	12	49	157
$Cu^{2+}(HGGG)+41\ H_2O$	BEN	16	25	174	485
$[Cu^{2+}(HG^-G^-G)]_2$	Fermi	10	70	98	314
$Cu^{2+}(HG^-GG)$+imid+92 H_2O	Fermi	14	220	338	925
$Cu^{2+}(HGGG)$+imid+92 H_2O	BEN	32	99	339	925

The Fermi cluster (at the Fermi Institute, Rome) is based on 1.7 GHz Pentium IV processors. The BEN cluster (at the ITC*, Trento) is based on Xeon 2.8 GHz processors. N_{atoms} and N_{elect} are the numbers of atoms and electrons of the simulated systems

$Cu^{2+}(HG^-G^-GW)$ + water

We started our investigation with some preliminary simulations of $Cu^{2+}(HG^-G^-GW)$ in water in order to study the rôle of Trp in the crystallographic Cu penta-coordination mode. These studies have led us to the conclusion that the axially coordinated water molecule is not directly bonded to Cu, but rather it is hydrogen-bonded to the Trp side chain. ''Packing'' effects can be invoked to explain the presence of a coordinated water as seen in the crystal. In order to clarify this important issue we have performed two suitably designed sets of simulations. First we have simulated the reduced system $Cu^{2+}(HG^-G^-GW)\ H_2O$ in supercells of different size, finding that the volatility of the water molecule actually increases with the cell size. Secondly a 1.2 ps long CP trajectory was generated (Furlan et al. 2007) at $T = 300$ K for the $Cu^{2+}(HG^-G^-GW) + 65\ H_2O$ system with the purpose of carefully studying the dynamics of the Cu–water distance, d_{Cu-wat}. An analysis of the radial distribution function (Furlan et al. 2007) shows that the latter is zero for $d_{Cu-wat} < 3$ Å, thus confirming the weakness of the Cu–water interaction. Our finding is not in contradiction with the interpretation of NMR results given in Zahn (2003), where the presence of an axially Cu-coordinated water is not directly observed but it is rather inferred from the position of the Trp residue.

From the overall emerging pattern we tentatively conclude that Trp is not involved in the Cu coordination. We have thus decided to discard it from our further simulations.

$Cu^{2+}(HG^-G^-G)$

Analysis of several trajectories of the Cu^{2+} (HG^-G^-G) complex at $T = 300$ K shows that the N3O1-coordination is stable, with the bond between Cu and amide nitrogens from deprotonated Gly residues stronger than that between Cu and the $\delta 1$ nitrogen of the His. This result is somewhat surprising (Gaggelli et al. 2006) and has been the object of further studies. We briefly describe in section ''Results on possible multiple His coordination'' a few preliminary results that may shed some light on this interesting feature (Guerrieri 2007). The Cu–O bond with the carbonyl group of the second Gly is weak, and affected by the greater mobility of the peptide C-terminal.

$Cu^{2+}(HGGG) + 41\ H_2O$

Simulations of $Cu^{2+}(HGGG)$ solvated in a box with 41 water molecules confirm the picture in which deprotonation of Gly residues is favoured by the greater stability of the bond with Cu. This is confirmed by the lowering of the potential energy seen when a proton from a Gly is released

into water (data not shown). As for the relevance of this feature in vivo, it should be observed that at physiological pH and in the absence of Cu all Gly residues are expected to be protonated. NMR data (Zahn 2003) support this statement.

$[Cu^{2+}(HG^{-}G^{-}G)]_2$

Perhaps the most interesting result we have obtained is that the simulation of the $[Cu^{2+}(HG^{-}G^{-}G)]_2$ system (in vacuum) shows that, on the time scale of the ps, formation of a dimer is possible. Exchange of ligands between the two Cu ions (already visible after 0.86 ps at $T = 300$ K) is responsible for keeping the two tetra-peptides close to each other. Also for the dimer we observe a preference for an amidic nitrogen binding, while the bond with the imidazole ring of the His is easily broken.

In spin restricted simulations we observe a rather small Cu–Cu average distance (from 2.1 to 3 Å) which, in spin unrestricted simulations, becomes slightly larger (2.4–3.4 Å), but still pretty much smaller than what is experimentally observed (from 4.4 to 6.4 Å), see (Chattopadhyay et al. 2005).

In the absence of Cu, long-range electrostatic and dispersive interactions are unable to keep the two HGGG peptides close together. We have not yet studied the effects of hydrophobic interactions.

Results on possible multiple His coordination

From the analysis of a number of preliminary simulations on the [Cu^{2+}(HGGG) + imidazole] system carried out both in vacuum and in the presence of explicit water molecules (not reported), we confirm the observed preference (see section ''$Cu^{2+}(HG^{-}G^{-}G)$'') for Cu to bind amidic nitrogens rather than the (His) imidazolic nitrogen. In fact, when both Gly residues are deprotonated, we have never been able to observe Cu coordinating two imidazole rings at the same time.

In order to better understand this interesting feature we have decided to start a systematic study of the [Cu^{2+}(HGGG) + imidazole] system with the first two Gly's in different protonation configurations. This is done by adding one proton at the time to the two Gly's. Note that by adding protons to $Cu^{2+}(HG^{-}G^{-}G)$, the system becomes positively charged. The procedure we have described is the microscopic equivalent of lowering the pH of the solution.

In our 2 ps long simulations we have observed that, as long as there is an available amidic nitrogen from a Gly, only a single imidazole can be coordinated to copper at room temperature. Moreover, we find that, of the two available imidazoles, the one which finally binds to Cu^{2+} is the one which, with respect to Cu^{2+}, lies on the opposite side of the bonded negatively charged amidic nitrogen.

These results have led us to put forward the hypothesis that the coordination mode of Cu^{2+} can be understood in terms of a sort of ''*trans*'' effect (Pushie and Rauk 2003) by which the Cu ion is able to coordinate a His only if the imidazole nitrogen lies on the opposite side of the deprotonated amidic nitrogen, in a *trans*-configuration. This hypothesis can be tested by a careful study of the probability distribution of the electronic wave functions around the metal. Investigations in this direction are under way.

Conclusions

Although we are still far from a clear understanding of the rôle of copper in PrP misfolding and/or aggregation, theoretical studies seem to point to a rather complex structural scenario, where Cu binding to the octarepeat region seems to favour aggregation owing to a possible dimer formation mechanism (Furlan et al. 2007), with the opposite beeing true when Cu binding occurs in the PrP core region (Cox et al. 2006).

Obviously much longer and more accurate first principle calculations are necessary before we can have a satisfactory interpretation of the many existing experimental data. We are at this moment in the very lucky situation in which, thanks to the spectacular development in computer design and the ongoing continuous progresses in the implementation of innovative algorithmic softwares, reliable ab initio studies of structural properties of macromolecules are really within our reach in a few years from now.

Acknowledgments Use of *Cineca* cluster in Bologna, Fermi cluster at E. Fermi Research Center in Roma, BEN cluster at *ECT** in Trento and *CERM* cluster in Firenze is gratefully acknowledged. We also wish to thank the Organizers of the SIBPA Conference for the lively atmosphere of the meeting.

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