Copper(II) Ion Binding to Cellular Prion Protein

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Received June 27, 2007

Prion diseases are fatal neurodegenerative diseases thought to arise from the post-translational conversion of normal cellular prion protein to a scrapie isoform. Experimental data suggest a role for copper(II) ions in the process. An ab initio QM/MM approach and available experimental data were combined in order to identify and evaluate three potential copper(II) ion binding sites in the C-terminal portion of the normal cellular prion protein. Our results suggest that copper(II) ion binds to His 187 but not to His 140 and His 177 of the binding site in the cellular prion protein.

INTRODUCTION

Prion diseases are fatal neurodegenerative disorders caused by a post-translational conversion of normal cellular prion protein (PrP^C) into a pathological isoform (PrP^{Sc}). During the conversion PrP^{Sc} species acquire infectivity, partial protease resistance, insolubility in nondenaturating reagents, and the ability to aggregate in affected brains.[1] PrP^C and PrP^{Sc} differ only in their secondary and tertiary structures with PrP^{Sc} having increased β-sheet content and decreased α-helix content.[2] The mechanism of PrP^C to PrP^{Sc} conversion is at present still unknown.

The nuclear magnetic resonance (NMR) structure determination of recombinant mouse,[3] hamster,[4] bovine,[5] and human[6] PrP^C has provided a large body of structural information on this 210 residue protein. The common feature of PrP^C from all these species is the presence of a globular, well structured C-terminal domain extending from residues 125−228 and a flexibly disordered N-terminal segment (residues 23−124) characterized by a highly conserved repeat of four identical octapeptide units with the consensus sequence PHGGGWGQ. The globular C-terminal region contains three α-helices and a short antiparallel β-sheet. The X-ray or NMR structure of complete PrP^C is still to be determined.

PrP^C has been shown to be a copper binding protein, and a number of reports[7−15] indicate its role in copper(II) metabolism. The stoichiometry of copper(II) binding to PrP^C is heavily buffer- and pH-dependent. Whittal[16] found that the binding stoichiometry of copper(II) ions to PrP^C is pH-dependent, with two copper(II) ions bound to the octapeptide repeat region at pH 6 and four Cu^{2+} ions bound at pH 7.4. It has been proposed that between two and ten copper(II) ions can bind to PrP^C. The binding is thought to be cooperative in the four octapeptide repeat units in the N-terminal regions spanning residues 58−91. The first coordination sphere is expected to have a square-planar geometry with three nitrogen atoms and one oxygen atom as ligands. Results from Arnoff-Spencer[13] using electronic paramagnetic resonance (EPR) and circular dichroism (CD) confirmed the binding of four copper(II) ions per binding unit (residue 57−91).

Recent findings indicate additional copper(II) binding sites are present in the C-terminal segment of the protein. By using fluorescence titration some high affinity Cu^{2+} binding sites located in the region around His 96 and His 123 and some weaker binding upstream of this region were discovered in recombinant human PrP (91−231).[14] Results from X-ray absorption fine structure spectrometry indicated Cu (II) ion binding at pH 8 at sites near residues His 96 and His 111.[15] Three pH-dependent Cu^{2+}coordination sites were also observed in isolated murine PrP (121−231). Binding of one Cu^{2+} ion to the C-terminal terminal region of PrP at physiological pH has been confirmed by Kramer.[17−19] The C-terminal part of the prion protein contains three histidine residues: His 140, His 177, and His 187.

Cereghetti's[20] results exclude the involvement of His 140 in the copper(II) binding, while aggregation of the polypeptide mutants did not allow capturing of spectroscopic data on Cu^{2+} interaction with His 177. Brown[21] characterized Cu^{2+} complexes with prion protein peptide fragment analogues and the results obtained using EPR, ESI-MS, CD, and UV−vis measurements indicated the involvement of His 187 residue. However their results come from work with small protein segments, whose behavior may not be typical of the whole protein.

Histidine residues outside the octarepeat region have been previously shown to be important for infection by exogenous prions. The structured C-terminal region of the prion protein is able to bind copper(II) ions, and this, hypothetically, may be its most important biological role.[20,21]

Copper(II) binding is essential for normal cellular prion protein function. Such binding is found at the octapeptide repeat sequences located in the N-terminal region of the protein. Little is known about C-terminal copper(II) binding as these sites have little affinity for copper(II) ions. Interest in C-terminal copper(II) binding is fueled by the possibility that the conversion from cellular to scrapie isoform is in some way affected by copper(II) binding at

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10.1021/ci700226c CCC: $40.75
Published on Web 02/02/2008

the C-terminal.

So far the structure determination of PrP^{C} and PrP^{Sc} has met with partial success as incomplete structures, all lacking bound copper(II) ions, are available. In these cases, molecular modeling can be used to evaluate the potential copper(II) binding sites, especially if data from molecular simulations are compared with existing experimental data.

Molecular simulations of biomolecules and bioinorganic complexes are proving to be an invaluable tool in biochemistry.[22,23] In many cases ligand (e.g., metal ion, small molecule, etc.) binding causes a major change in the local conformation of the macromolecule but only a minor one in the overall conformation of said macromolecule. This property is exploited in molecular simulations to simplify the system one is dealing with. This simplification allows for a substantial decrease in needed CPU time and resources and allows one to use better and more efficient computational methods, which consequently improve the result of the simulation.

There are many ways of simplifying the molecular system at hand. One possibility is to simply partition the system (i.e., biomolecule and ligand, two interacting biomolecules, etc.) in two parts—a quantum mechanics (QM) and a molecular mechanics (MM) one.[24,25] The quantum mechanics' part is the part of the system where major conformational changes caused by ligand binding are expected and is treated quantum mechanically. The molecular mechanics' part is the part of the system where little or no conformational changes are expected and is computed with classical mechanics. This approach allows for simulations to be performed on systems that are normally out of reach for ordinary quantum mechanics' simulations—because these systems are too big and contain too many atoms.

In the present study we report two possible copper(II) ion binding sites located in the C-terminal portion of the normal isoform of the cellular prion protein. Both reported binding sites have in common a histidine (His 177 and His 187, respectively) ligand and some water molecules coordinately bound to a copper(II) ion. The reported binding sites were found by a combined mechanical (QM)/molecular mechanical (MM) approach and taking available experimental data into account.

METHODS

Simulations were performed at the National Institute of Chemistry, Ljubljana, Slovenia, on computers members of the CROW[26] clusters. The CHARMM[28] molecular modeling package was used. For the initial coordinates we used the coordinates retrieved from the Protein Data Bank, entry 1I4M. Two cadmium atoms resulting from the crystallization process were deleted. The coordinates used do not represent the whole normal isoform of the cellular prion protein. The CHARMM parameter and topology files (version 22) for proteins[27] were used to specify the force field of the protein.

Building the starting set of coordinates was a multistep process. In the first step the protein moiety from the 1I4M PDB entry was imported into CHARMM, and its energy was minimized for 200 steps using the adopted Basis Newton−Raphson method (ABNR method).[28] Afterward one copper(II) ion was added, followed by a translation and rotation that gave us a dimer (two protein chains and two copper(II) ions). The parameters for translation and rotation were taken from the used PDB entry. For the purpose of this study we positioned the copper(II) ion in the vicinity of the following residues: His 140 (one Cu^{2+} position assessed), His 177 (one Cu^{2+} position assessed), and His 187 (five different Cu^{2+} positions assessed).

Table 1. Starting Distances for the Five Systems around His 187

atom label	distance to Cu before QM/MM calculation [Å]				
	1	2	3	4	5
ND1	3.4653	6.6284	6.0217	2.3257	4.1112
NE1	2.8643	7.0612	4.8621	1.9754	2.3599

Table 2. End Distances for the Five Systems around His 187 and Corresponding Binding Energies with Relative Energy Differences in Parentheses

atom label	distance to Cu after QM/MM calculation [Å], energies in [kcal/mol]				
	1	2	3	4	5
ND1	4.0431	6.7633	5.8802	4.0613	4.1225
NE1	1.9567	7.6831	4.9905	1.9754	2.1001
binding energy	650.5(0)	617.7(32.8)	603.1(47.4)	650.5(0)	-

After another 200-step minimization using the ABNR method the whole system was immersed in a cubic box (size 100 Å × 100 Å × 100 Å) of TIP3[29] water molecules. Thus, a water shell around the protein system was created. The water molecules overlapping with our system were deleted. Electrostatic interactions were computed with the particle-mesh-Ewald method.[30] By this step the system was composed of the following: two prion protein units, two copper(II) ions, and approximately 2000 water molecules. The whole system was then minimized for 200 steps using the ABNR method and then equilibrated for 50 ps, and afterward the molecular dynamics simulation was run for another 500 ps without the temperature scaling. The structure obtained from this dynamics calculation was then used in all further models.

In the next step the link atoms were added when breaking the covalent bond between CB and CG atoms dividing QM and MM regions. In general the QM region was composed of a copper(II) ion, the atoms from histidine's imidazole ring, and some water molecules. The basis set for the QM part of the system was 6-31G*, and we used HF Hamiltonian for the ab initio part of the system. The QM/MM system was minimized using the ABNR method as implemented in the CHARMM program for 1000 steps.

During the minimization a subsequently analyzed trajectory was written. Distances between the copper(II) ion and the possible ligands in the QM region were measured before, during, and after the minimization. All structures were visualized using the program VMD.[31] Charts were created using Gnuplot.[32]

RESULTS AND DISCUSSION

The aim of this study was to evaluate some potential copper(II) ion binding sites in the C-terminal portion of the human PrP^{C} with a combined QM/MM approach. The binding sites were in general composed of a copper(II) ion, a histidine residue, and some water molecules.

In the case of His 140 the simulations failed because the SCF did not converge. In the absence of further data we can assume that copper(II) does not bind to His 140. This is supported by the fact that the EPR spectra of the H140S

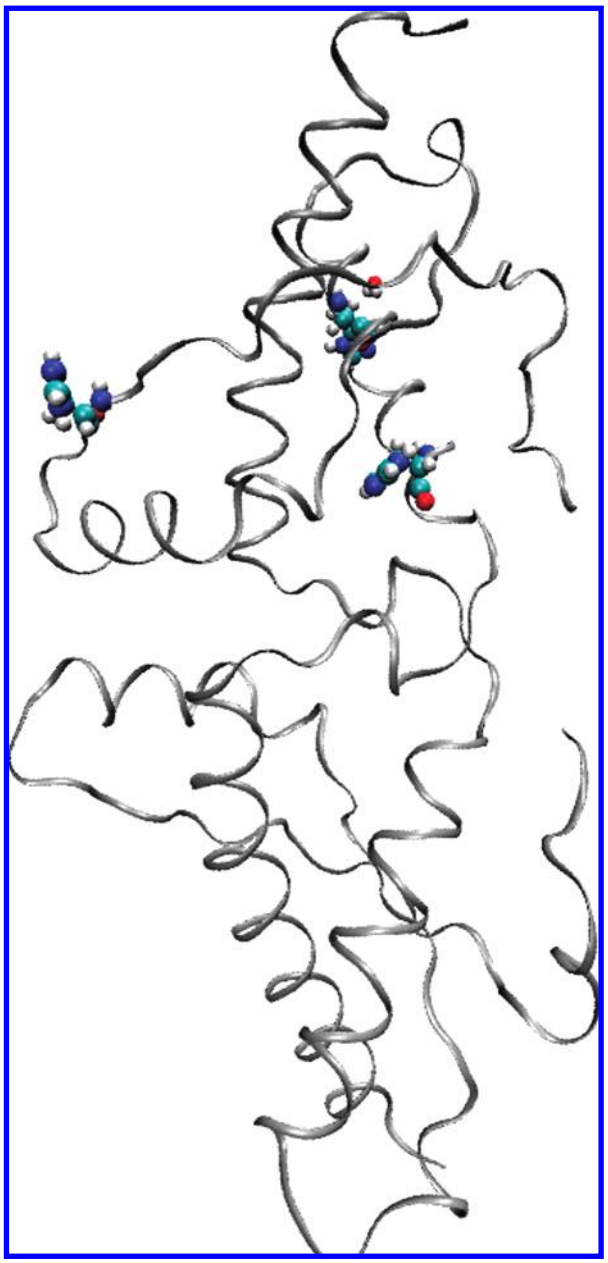

Figure 1. Prion protein dimer displayed as a ribbon with positions of evaluated histidines. The water molecules are omitted for reasons of clarity.

mutant fail to provide any evidence of the involvement of the imidazole residue in the position 140 in the copper(II) binding.[21]

For His 177 the simulations were successful but indicated that the copper(II) ion binds to a neighboring Asp residue rather than to His 177. The main reason for this is probably the large distance separating the copper(II) ion from the histidine imidazole ring at the start of the simulation. The copper(II) ion preferably binds to the neighboring aspartate residue, namely to atom OD1 of the two carboxylate oxygen atoms OD1 and OD2. After minimization, the distance between the copper(II) ion OD1 is approximately 2 Å, whereas the distance between the copper(II) ion OD2 atom is ~2.8 Å. Comparison with experimental data is not possible because the fragments at neutral pH aggregate before any data can be collected.[20,21]

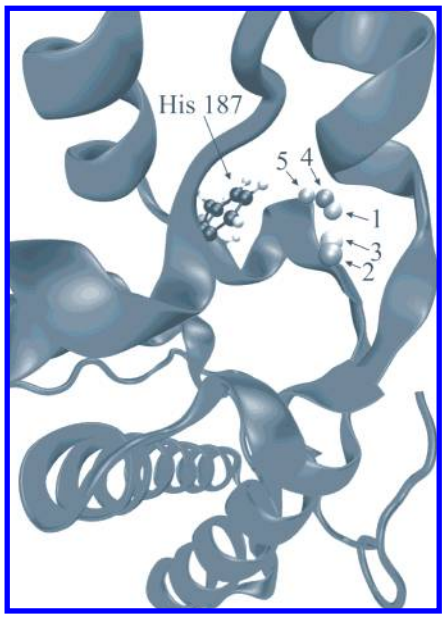

Figure 2. Different positions (labels: 1−5) of the copper(II) ion. See Tables 1 and 2 for related data.

Tables 1 and 2 summarize the initial and final distances between the copper(II) ion and the two nitrogen atoms ND1 and NE1 of His 187. Columns 1−5 represent the five systems we built around His 187. These differ mainly in the initial position of the copper(II) ion relative to the imidazole ring. The different positions were chosen after the results of the QM/MM run on the first His 187-related system (column 1 in Tables 1 and 2, respectively) indicated a possible binding.

Comparison of the data from Table 1 show that in all systems the copper(II) ion was pulled toward the NE1 atom of the imidazole ring. The only exceptions are systems 2 and 3, where the initial distance between the nitrogen atoms in the imidazole ring and the copper(II) ion was excessively small leading to significant steric hindrance of HE1 and expulsion of the copper(II) ion from the imidazole ring during the minimization.

Our results are in good agreement with experimental data by Cereghetti[20] and Brown.[21] The spectroscopic parameters of the copper(II) complex with NH−PrP−Ac at near neutral pH indicate that His 187 is involved in copper(II) ion binding.[20,21] From Table 2 it is evident that the copper(II) ion preferably binds to NE1 rather than ND1, which is also supported by the largest binding energy presented in Table 2.

The most striking change observed was in the case of the system built around His 187, in which the copper(II) ion was shifted ~3.8 Å toward an imidazole nitrogen atom (NE1) in the histidine residue. The same behavior was observed in all five His 187-related systems, albeit to a lesser extent, supporting the conclusion that copper(II) ion does indeed bind to His 187.

The coordination sphere around this copper(II) ion was composed of imidazole nitrogen atoms and water molecules. There are no other potential residues that could interact with copper(II) ion. We assume the complex with copper(II) ion is further stabilized by hydrogen bonding. In all figures the water molecules were omitted for clarity reasons.

In biological systems, however, the copper(II) ion has interactions beyond those with the two nitrogen atoms. The most common situation is one in which a copper(II) ion interacts with four atoms (e.g., the four sulfur atoms in a zinc finger protein) from the neighboring residues and two or more water molecules.[33]

CONCLUSION

The aim of our work was to examine some potential copper(II) ion binding sites around the histidine residues in the C-terminal portion of human PrP.

Our results indicate copper(II) ion (1) binds to His 187, (2) does not bind to His 140, and (3) probably does not bind to His 177.

Our results confirm previous experimental data from other research groups suggesting there is copper(II) ion binding to His 187 but not in the case of His 140 or His 177.

In all the systems with His 187, copper(II) ion binds to one specific imidazole nitrogen atom. The question is why the copper(II) ion coordinates only the imidazole ring and some water molecules when more ligands would be expected. This unusual behavior could be further investigated by longer (nanoseconds) molecular dynamics simulations which might unravel the structural changes in PrP^C that occur upon copper(II) binding and might include a partial conversion to some other PrP-like form. In addition proper calculation of free energy of binding[34−36] at the ab initio level is too demanding for current computer power. However we constructed empirical forcefield from our ab initio calculations and performed the classical free energy perturbation calculations for the case between structures 1 and 2 in Table 2. The free energy difference correction was negligible in comparison to the binding energy difference of 32.8 kcal/mol for that case.

ACKNOWLEDGMENT

The presented work was supported by grant P1 0134 0103 form Ministry of Higher Education, Science and Technology of the Republic of Slovenia.

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CI700226C