Copper reduction and dioxygen activation in Cu-Amyloid Beta peptide complexes. Insight from molecular modelling – Supplementary informations

Federica Arrigoni, Tommaso Prosdocimi, Luca Mollica, Luca De Gioia, Giuseppe Zampella, Luca Bertini

Outline

1.	l	Redox state assigment on the basis of the spin NBO population						
2.	,	Validation of the level of theory						
3.		Structures and properties of the Cu(II/Cu(I) model coordinations	4					
	3.1	L Cu(II)-Aβ model coordinations	5					
	3.2	2 3-histidine Cu(II)-Aβ model coordinations	7					
	3.3	3 Cu(II)/Cu(I) Aβ-Asc ⁻ model adducts	8					
	3.4	Evaluation of the ligand dissociation propensity in 11	8					
	3.5 Cu	5 Final Cu(I) Asc coordination after geometry optimization from Asc- deprotonated adduct at the (II) geometry2	0					
	3.6	5 Final Cu(II) superoxide adducts optimized geometries and redox states2	1					
4.		Selected geometry optimization histories2	2					
5.		ΔG and Asc ⁻ ΔG binding plots2	6					
6.	I	Detail of the Cu(II)-Aβ(1-16) computations2	7					

1. Redox state assignment on the basis of the spin NBO population



Figure 1S. The Cu oxidation state has been evaluated by interpolation using a linear rule using NBO Cu spin population for **1** coordination (component I) and for any fully Cu(I) form with s(Cu)=0.

2. Validation of the level of theory

Validation of the level of theory. The choice of the DFT pure functional adopted was made on the basis of the previous experience on Cu2+ peptide interactions. Here we compare energy differences computed with other pure and hybrid DFT functional, namely PBEⁱ, PBEO,ⁱⁱ MO6ⁱⁱⁱ, TPSS and TPSSh.^{iv} Def2-TZVP basis set is adopted.

	1	2	6	H2O	lm	ΔE_2	ΔΕ_6
BP86	-2624,85445	-2701,3241	-2551,46114	-76,4775863	-226,3374	5,0	7,0
PBE	-2623,121799	-2699,50294	-2549,83835	-76,387013	-226,049439	3,7	5,0
PBE0	-2623,158807	-2699,5386	-2549,8656	-76,387731	-226,05949	5,0	5,8
TPSS	-2624,701562	-2701,1676	-2551,27976	-76,474255	-226,3607	5,2	6,0
TPSSh	-2624,55838	-2701,01554	-2551,1475	-76,465949	-226,33395	5,5	5,5
M06	-2623,8267	-2700,2535	-2550,5234	-76,4343454	-226,1585	4,7	8,5
Average						4,8	6,3
Standard							
deviation						0,6	1,2
BP86/D3			-2551,50988			1.2	8.9

3. Structures and properties of the Cu(II/Cu(I) model coordinations

In the following pictures are reported the Cu(II)/Cu(I) optimized coordinations computed in this paper. In each panel the distances are reported in Å, angles/dihedral in degree; q(Cu) and s(Cu) are the NBO copper atomic changes and spin population respectively.

Cu(II)-Aβ model coordinations 3.1





ΌH₂

ŃΗ

δ=1.8°





6



H₃C q(Cu)=1.26 HN-CH3 H₂O H₂N s(Cu)=0.70 q(Cu)=1.26 0 H_2 s(Cu)=0.67 H₂C 2.008 L ۰ ۲.986 کرد ۲.986 کرد 1.960 / 2.107 ---Cu^{II}-2.107 S / L H₂N COO 1,994 2.354 ŃΗ ὑH₂ δ=3.5° δ=0.9° 8 9



In the table below are reported the HOMO/LUMO gap for the Ci(II) coordination which are likely involved in the Cu(II)-to-Cu(I) reduction.

Cu(II)	HOMO-LUMO gap	НОМО	LUMO
Coordination	(eV)	(eV)	(eV)
1	1.07601	-5.33395	-4.25794
2	1.05487	-5.57927	-4.52439
6	1.07243	-5.82786	-4.75543
9	1.05382	-5.60697	-4.55315
11	1.08055	-5.94958	-4.86903

3.2 3-histidine Cu(II)-Aβ model coordinations

In this section are reported the Cu(II) minimum optimized geometries for the four 3-His models considered. These are:

- His₂·Im·Asp1(C=O) models in which carbonyl of the peptide bond of the His dimer is involved (12, pentacoordinated form) or not involved (12a, petracoordinated form) is the Cu(II) coordination;
- 2. Pentacoordinated $His_2 \cdot Im \cdot H_2O$ model coordination in which the carbonyl of Asp1 is involved (13) or not involved (13a) in the Cu(II) coordination sphere.

In the Figure below are reported the main interatomic distances of the Cu(II) coordination sphere . **12a** coordination results 5.1 kcal/mol higher in energy compared to **12**. Charge and spin population are compatible with Cu(II) redox state.







12a







13

3.3 Cu(II)/Cu(I) Aβ-Asc⁻ model adducts

















4 Asc⁻ AcO⁻ adduct





6 Asc⁻ adduct







6 Asc⁻⁻ AcOH adduct









11 Asc^{- -} adduct

11 Asc⁻⁻ adduct



11 AcO- adduct

11 Asc detach







12 Asc⁻⁻ adduct

3.4 Evaluation of the ligand dissociation propensity in 1.



The plot reports the scan of the potential energy surface (PES) of **1** along the 5 possible ligand dissociation coordinates



in order to evaluate numerically the energy first derivative. The exploration of the PES were carried out by considering a number of structures in which is the Cu-L bond is progressively lengthened simultaneously freezing the copper-ligand bond during geometry optimizations.

Table S2. For each ligands are reported the numerical evaluation of the first derivative $\Delta E/\Delta R$ in kcal/(mol Å) for a given ΔR (in Å) Cu-L bond elongation

Ligand	ΔE/ΔR (kcal/(mol Å))	ΔR (Å)	
СО	3,1	0,26	
lm	9,0	0,18	
	10,1	0,19	
Amino N-term	6,9	0,15	
CO0-	9,4	0,20	

3.5 Final Cu(I) Asc coordination after geometry optimization from Ascdeprotonated adduct at the Cu(II) geometry.

In the following scheme are resumed the fate of the Cu(I) coordination upon geometry optimization starting from the corresponding Cu(II) optimized geometries with deprotonated Asc-. The process is therefore the following

 $Cu(II)A\beta \cdot AscOH^{-} \rightarrow Cu(II)A\beta \cdot AscO^{-2} \rightarrow Cu(I)A\beta \cdot AscO^{--}$

For sake of clarity, Asc radical is not reported in the Cu(I) coordination.



In the table below are resumed the initial and final copper coordinations starting from the Cu(II) Asc⁻ adducts and then the Cu(I) Asc⁻ adducts. In the leaving ligand column are reported which are the ligands that dissociate upon reduction.

	Cu(II)·Aβ·Asc ⁻	Cu(I)·Aβ·Asc ⁻ *	
	final coordination	final coordination	Leaving ligand
2	2Im·COO ⁻ ·NH ₂ ·Asc ⁻	2Im ·NH2·Asc-●	COO-
1ac	2Im·COO ⁻ ·CO·Asc ⁻	2Im	CO, COO-
3	2Im·CO·NH₂·Asc ⁻	2Im·NH ₂ ·Asc ^{-•}	СО
4	2Im·CO·COO ⁻ ·Asc ⁻	2Im·COO⁻·Asc⁻•	СО
5	Im·CO·COO⁻·NH₂·Asc⁻	Im·COO⁻·NH₂·Asc⁻•	СО
6	Im·COO ⁻ ·NH ₂ ·H ₂ O·Asc ⁻	Im·COO⁻·NH₂·Asc⁻•	H ₂ O
7	Im·CO·NH ₂ ·H ₂ O·Asc ⁻	Im ·NH₂·Asc⁻•	CO, H ₂ O
8	Im·CO·COO ⁻ ·H ₂ O·Asc ⁻	Im·COO ⁻ ·Asc ^{-•}	CO, H ₂ O
9	2Im·COO ⁻ ·H ₂ O·Asc ⁻	2Im·COO ⁻ ·Asc ⁻	H ₂ O
10	2Im·CO·Asc ⁻	2Im·Asc⁻•	CO
11	2Im·NH ₂ ·H ₂ O·Asc ⁻	$2Im \cdot NH_2$	H₂O, Asc⁻•
12	His ₂ ·Im· Asc ⁻	His₂·Im· Asc⁻•	-

3.6 Final Cu(II) superoxide adducts optimized geometries and redox states.



4. Selected geometry optimization histories.



Figure 2S. Energy (in hartree) history of the geometry optimization of the **1** model starting from from the square pyramidal coordination with the carboxylated in apical position. After a first rapid energy decrease, a first plateau is reached in which the structure has still the carboxylate in apical position which successively it moves to equatorial position in the carbonyl coordination position. The two isomers differ by 4 kcal/mol. In the figure the bond distances are reported in Å.



Figure 3S. Energy (in hartree) history of the geometry optimization of the **1** Asc⁻ model starting from van del Waals adduct between Asc- and **1** (Cu-O initially fixed at 4.0; the carboxylated in apical position).During the geometry optimization, the Asc⁻ approaches the metal ion with the concomitant elongation of the Cu-O=C bond distance with the carbonyl that belong to the Asp1-Ala2 peptide bond. The energy difference (in kcal/mol) between the two structures is around 6 kcal/mol. The isomer in which the carbonyl is completely dissociated forming an H-bond interaction with the Asc⁻ is 1 kcal/mol lower is the energy.



Figure 4S. Energy (in hartree) history of the geometry optimization of the **2** Asc and **3** Asc⁻⁻ models starting from the Asc⁻ adducts geometry in which the OH- group that belong to Asc- is deprotonated. In both cases, the optimizations converge to the **11** Asc⁻⁻ coordination.



Figure 5S. Qualitative energetic profile obtained results of the PES scan of the **2** Asc⁻ CH₃COO⁻ adduct along the proton transfer coordinate (first part) and Asc radical dissociation coordinate. The copper redox states have been evaluated from the Cu spin populations using the linear equation reported in section 1.3. Distances in Å. Starting from the Cu(II) minimum (redox state Cu^{1.9}) along the elongation of the O-H bond distances between the hydrogen on the OH of the Asc⁻, we obtain a Cu^{1.7} structure 1.2 kcal/mol higher in energy. At this point the scan along the Cu-OAsc bond distance evidenced a Cu^{1.7} structure 2.0 kcal/mol higher in energy with might be considered as a guess structure of the electron transfer transition state. Keep going along this coordinate at Cu-O equal to 4.180 Å we found a Cu^{1.2} structure with can be considered as the **2** Asc CH₃COOH product of the electron transfer. This latter is 2.5 kcal/mol lower in energy with respect to the initial form. Finally the further ligand dissociation (COO- or NH2) can take place with a gain of energy and the formation of a genuine Cu(I) form (final spin population close to zero).



Figure 6S. Energy (in hartree) history of the geometry optimization of the **9** Asc⁻ models starting from the Asc⁻ adducts geometry in which the OH- group that belong to Asc- is deprotonated. Here the barrierless dissociation of the Asc⁻ radical and the water molecule is observed.

5. ΔG and Asc⁻ ΔG binding plots

In the two plots below are reported the ΔG for each coordination compared to **1** and ascorbate ΔG binding computed according to the process in box a and b respectively. Free energy in kcal/mol computed at 298 K. These values are those used to compute the sums reported in the Figure 9 (left) in the main text.



6. Detail of the Cu(II)-A β (1-16) computations

In this section we report some details regarding the simulation of the full 1-16 peptide with Cu(II) and one molecular of ascorbate.

10 A β (1-16) peptide conformation have been selected from a MD of 100ns from which 1000 structures were extracted. In the plot below is reported the RMSD computed using the first structure as reference



The average RMSD is 6.6± 1.4 Å.

In the Table below are reported the main features of the 10 structures extracted from MD after geometry optimization at the BP86/DZ level + implicit COSMO solvation at ϵ =80 introducing the Cu²⁺ ion.

	Type of structure	Residues	ID	E(BP86)	q(Cu)	s(Cu)	dE	Final Cu coordination
1	His2 Asp	HIs13,14, Asp1	6	-8492.0475	1.21	0.59	0.0	2Im, CO, COO-, NH2
2	His2 Asp	HIs13,14, Asp1	61	-8492.0360	1.08	0.42	7.2	2Im, COO-
3	His2 Asp	HIs13,14, Asp1	122	-8491.9959	1.22	0.57	32.4	2Im, CO, COO-, NH2
4	His2 Asp	sp His13,6,Asp1		-8492.0370	1.22	0.57	6.6	2Im, CO, COO-, NH2
5	His2 Asp	His14,6,Asp1	423	-8492.0356	1.24	0.59	7.5	lm, 3CO, NH2
6	His3 2CO	His6,13,14, CO Asp1	613	-8491.9904	1.25	0.54	35.9	lm, 3CO, NH2
7	His2 Asp	His13,6, Asp1	714	-8491.9941	1.24	0.57	33.5	2Im, CO, COO-, NH2
8	His3	His6,13,14	720	-8491.9989	1.00	0.25	30.5	Im3
9	His2 Asp	His14,6,Asp1	721	-8491.9915	1.22	0.56	35.2	lm, 3CO, NH2
10	His2 Asp	His14,6,Asp1	928	-8492.0382	1.25	0.6	5.8	2Im, CO, COO-, NH2
ave					1.19	0.53		

In particular we considered

- 1) 3 His13-His14-Asp1 coordinatios
- 2) 2 His13-His6-Asp1 coordinations
- 3) 3 His14-His6-Asp1 coordinations
- 4) 1 3·His coordination
- 5) 1 3·His-Asp1 coordination

After geometry fully optimization

- 1) The initial coordination have been confirmed; Cu(II) results penta-coordinated except for 3·His coordination (ID 720):
- The central ion is in a genuine Cu2+ redox state according to copper NBO atomic charge and spin population (on average the redox state is Cu^{+1.8} according to atom charge and Cu^{+1.9} according to spin population);
- 3) The ΔE computed against the first coordination (ID 6 in the table) range up to 35.2 kcal/mol. These ΔE values are only indicative since they strongly depend on the number of H-bonding interactions which can vary significantly in the various structures considered.

In the table below are reported the main feature of the 10 structure in which an ascorbate ligand has been added to the Cu coordination environment.

ID	E(Asc-) (hartree)	q(Cu)	s(Cu)	dE (kcal/mol)	Leaving ligand	Cu-OAsc- (Å)	Final Cu Coordination
6	-9175.8944	1.21	0.53	2.1	-CO	2.368	2Im,COO-,NH2,Asc-
61	-9175.9229	1.18	0.53	-23.0		2.0314	2Im, COO-, Asc-
122	-9175.8675	1.24	0.56	-13.4	-COO-	2.2064	2Im, CO,NH2,Asc-
324	-9175.9172	1.2	0.48	-18.8	-CO	2.1627	Im2,COO-,NH2,Asc-
423	-9175.9264	1.22	0.59	-25.5	-CO	2.0192	Im, CO,COO- NH2, Asc-
613	-9175.8566	1.2	0.42	-10.0	-CO	2.3753	21m, 2CO, Asc-
714	-9175.9001	1.2	0.53	-35.0	-Im	2.0014	Im, CO, COO-,NH2,Asc-
720	-9175.8765	1.13	0.38	-17.2		2.0564	His2 Im Asc-
721	-9175.8877	1.21	0.50	-28.9	-CO	2.0934	Im COO- NH2 Asc-
928	-9175.9133	1.14	0.42	-15.6	-CO	2.0111	Im,COO-,NH2,Asc-
ave		1.19	0.49	-18.5		2.133±0.143	

The procedure has been the following:

- 1) An Asc⁻ molecule was added to the initial five-coordinated Cu(II)-A β (1-16) in the free vacant Cu coordination position; the initial Cu(II)-A β (1-16) Asc⁻ coordination is therefore initially six-coordinated;
- 2) Starting from these latter, fully geometry optimization has been carried out.

In all the cases, upon geometry optimization we observed

- 1) Asc- ligand results always bound to the Cu(II);
- 2) The average Cu-O bond distances with the oxygen atom that bound Cu is 2.133 Å.
- 3) The Cu-O distance has an acceptable linear correlation with the Asc⁻ binding ΔE (the more Cu-O is shorter, the more Asc⁻ binding energy is negative; see plot below)



Finally starting from the Cu(II)-A β (1-16) Asc- energy minimized coordinations, we deprotonate the OH group of the Asc- inducing the electron transfer. These 10 deprotonated coordination have been successively optimized.

ID	Cu(I)	q(Cu)	s(Cu)	Cu(I)-OAsc	Final Cu(I) Coordination
6	-9175.466	1.09	0.40	1.986	lm,COO,NH2,Asc
61	-9175.451	1.04	0.31	2.105	2Im,COO,Asc
122	-9175.411	1.03	0.21	2.033	2Im,NH2,Asc
324	-9175.407	1.12	0.35	2.237	2Im,COO,NH2,Asc
423	-9175.417	1.07	0.33	2.079	lm,COO-,NH2,Asc
613	-9175.399	0.98	0.08	2.411	lm,2CO,Asc
714	-9175.423	1.07	0.28	2.033	Im,CO,COO,NH2,Asc
720	-9175.426	0.96	0.11	2.073	3Im,Asc
721	-9175.419	1.04	0.27	2.222	lm,COO-,NH2,Asc
928	-9175.476	1.03	0.25	1.967	lm,COO-,NH2,Asc
		1.04	0.26		

Upon geometry optimization we observed

- 1. According to the copper NBO atomic charges and spin population the redox state are Cu^{+1.6} and Cu^{+1.4} respectively;
- 2. 8 out of 10 cases we observe the spontaneous detach of one ligand except for ID 714 and ID 324, in which a general elongation of the Cu-L bond distances is observed;
- 3. The Cu-O distance in three cases results shortened compared to the corresponding Cu(II) form due to the new electrostatic interactions of the deprotonated OH.

ⁱ Perdew, J. P.; Burke, K.; Ernzerhof, M. *Phys. ReV. Lett.* **1996**, *77*, 3865.

ⁱⁱ Adamo, C.; Scuseria, G.; Barone, V. J. Chem. Phys., 1999, 111, 2889-2899.

^{III} Zhao, Y. Truhlar, D.G. Theor Chem Account (2008) 120: 215.

^{iv} Tao, J.; Perdew, J, P.; Staroverov, V. N.; Scuseria, G. E. Phys. Rev. Lett. 2003, 91, 146401; Staroverov, V. N.; Scuseria, G. E.; Tao, J.; Perdew, J, P.; J. Chem. Phys. 2003, 119, 12129