# A STUDY OF AN HYPOTHESIS LINKING ALUMINUM FLUORIDE TO ALZHEIMER DISEASE: THE AFFINITY OF AMINO ACIDS OCCURRING IN BETA-AMYLOID TO $[Al(H_2O)_6]^{3+}$

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**ABSTRACT: β-amyloid (Aβ) is produced from a precursor membrane protein by normal proteolytic processing as a soluble peptide of 39–43 amino acids. Under unknown conditions, Aβ aggregates and accumulates to form amyloid fibrils. Exposure to certain metal ions, including aluminum, has been proposed as a risk factor in developing Alzheimer disease. Until now the mode of aluminum binding has not been completely explained. In this study we focus on the binding affinities of $[Al(H_2O)_6]^{3+}$ to several compounds (X) mimicking amino acid side chains present in Aβ. Binding affinities were calculated as the free energy of exchange reaction replacing one water ligand in $[Al(H_20)6]^{3+}$ with X. The effects of hydration were studied by performing single point calculations within the Conductor Polarized Continuum Model (CPCM) at B3LYP/6-31G(d) level of theory. Calculations in the gas phase were performed with Gaussian 98 (G 98). In our computational study, we demonstrate that the aqueous solution $[Al(H_2O)]^{3+}$ preferentially binds to aspartic acid residues and less to lysine and histidine residues.**

Keywords: Aluminum; Alzheimer disease; β-amyloid; Fluoride.

## INTRODUCTION

Twenty years have passed since the first publication in *Fluoride* by Jensen et al. on the pathological changes in the brain and kidneys of rats exposed to $AlF_3$ and NaF.[1] Since then, $AlF_x$ complexes have been the subject of immense interest due to their effect on living organisms, especially on the human body.[2]

Aluminum-fluoride complexes form spontaneously in aqueous solutions where fluorides are accompanied by trace quantities of aluminum ions.[3,4] The occurrence of $AlF_x$ in the natural environment is observed mainly in industrialized countries, which is connected with the emission of gases and dusts containing these elements.[5] The $AlF_x$ complexes are more harmful than the individual aluminum or fluoride components, as bioassimilability of aluminum may increase after binding to fluoride.[6] Fluoride also changes some of its biochemical properties in the presence of aluminum, for example it begins to activate G proteins.[7] Considerable amounts of aluminum in the brains of patients with Alzheimer disease (AD) have resulted in an aluminum hypothesis[8] suggesting that aluminum is one of the risk factors in this disease.[9]

Long-term studies have shown that the impairment of brain tissues in AD is a result of characteristic changes—amyloid fibrils, known as senile plaques.[8] One of the

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components of the plaques is β-amyloid (Aβ), a peptide formed by 39–43 amino acids. It is released from a greater transmembrane protein known as the Aβ precursor (amyloid precursor protein – APP).[10] Many factors influence the aggregation or conformation changes in Aβ: the number and type of amino acids in the Aβ molecule, solution pH, temperature, and the presence of some metals.

Both Aβ and APP are proteins that bind metals via histidine residues, a factor increasing Aβ aggregation.[11] Additionally, Aβ aggregation is thought to be enhanced in the presence of copper, zinc, iron, or aluminum.[12] In an acid environment, aluminum forms $[Al(H_2O)_6]^{3+}$ (octahedral hexahydrate) and subsequent deprotonation results in the formation of $Al(OH)_3$,[7] abbreviated $Al^{3+}$.

Aluminum is responsible for the greatest affinities of Aβ to *in vitro* aggregation.[13] The exact position of metal-binding domains in Aβ is not exactly known. According to some researchers, they are situated in the hydrophilic N-terminal part of Aβ, i.e., between the amino acid residues 1–16,[14] and according to other reports between 1–28.[15] Based on our previous publications,[16,17] in this paper we attempt to identify the sites of strong affinity to aluminum in the Aβ amino acid sequence.

## METHODS

Binding affinities to $[Al(H_2O)_6]^{3+}$ for several model compounds (X), mimicking amino acid side chains present in Aβ, were calculated as the free energy of exchange reaction replacing one water ligand in $[Al(H_20)_6]^{3+}$ with X. The unconstrained geometry optimization of reactants were carried out at B3LYP (Becke's three-parameter hybrid functional – B3LYP)[18-20] level employing the 631G(d) polarized basis sets of double-ζ quality. In order to check for the basis set saturation effects, single point calculations were performed at the same level of theory with 6-311+G(2df,2p) polarized basis sets of triple-ζ quality. The electron energies in the gas phase were first corrected for unscaled zero-point vibration energies. Next, thermal corrections as well as entropy terms for T=298 K and p=1 atm were included in harmonic oscillator-rigid rotor approximation to derive the relevant Gibbs free energies.

The effects of hydration were studied by performing single point calculations within the Conductor Polarized Continuum Model (CPCM) (21) at B3LYP/6-31G(d) level of theory. In this approach, a solute occupies a cavity within the solvent. The charge distribution of the molecule will polarize the medium, and the electric field applied by the solvent's distribution of charge will in turn interact with the charge distribution of the molecule.

Calculations in the gas phase were performed with Gaussian 98 (G 98),[22] whereas those concerning solvent effects with performed with Gaussian 03 (G 03).[23] The codes were run on clusters of dual Intel/P4Xeon and dual Intel Itanium2 nodes with a 1GB Ethernet interconnection. The Molden program was used for the visualization of the singly occupied molecular orbital.[24]

## RESULTS

In order to determine the coordination number for aluminum, we performed calculations for the gas and aqueous phases (Table 1). Gibbs free energy values are given for individual reactions of dehydration of the aluminum complex with water: $Al(H_2O)_6$, $AL(H_2O)_5$, and $Al(H_2O)_4$. All the values are presented in kcal/mol.

**Table 1.** Values of the reaction free energies of $[Al(H_2O)X]^{3+}$ dissociation calculated in the gas phase (ΔGg) and water solution (ΔGaq) at the B3LYP/6-31G* level. All values given in kcal/mol.

| Reaction | ΔGg | ΔGaq |
|---|---|---|
| $Al.(H_2O)_6 \rightarrow Al.(H_2O)_5 + H_2O$ | 53.83784 | 35.93378 |
| $Al.(H_2O)_5 \rightarrow Al.(H_2O)_4 + H_2O$ | 57.84036 | 40.33102 |
| $Al.(H_2O)_4 \rightarrow Al.(H_2O)_3 + H_2O$ | 101.4829 | 74.70301 |

Full optimization of geometry in the gas phase was performed at B3LYP/6-31G, and in the aqueous phase we allowed for corrections allowing for the solvent area, using the CPCM solvent model. G98 denotes calculations with Gaussian 98,[22] and G03 with Gaussian 03.[23] The symbol ΔGg denotes calculation in the gas phase, and ΔGaq in aqueous solution. Table 2 presents the chemical affinity of a bond between $[Al(H_2O)_6]^{3+}$ and the selected amino acid residues marked as X, that mimic the amino acid composition of the Aβ chain. The values of free energy in the exchange reaction are given in kcal/mol. In the gas phase these values were negative for all the reactions.

**Table 2.** Values of BA for model compounds (X), mimicking amino acid side chains (X: Asp, His, Met, $NH_3$(Lys) or Ser) calculated at the B3LYP/6-31G(d)//B3LYP/6-311+G(2df,2p) level in a gas phase (ΔGg) and aqueous solution (ΔGaq). All values given in kcal/mol.

| Complex | ΔGg | ΔGaq |
|---|---|---|
| $Al(H_20)_5Asp$ | –335.5 | –25.0 |
| $Al(H_20)_5His$ | –49.4 | –1.4 |
| $Al(H_20)_5Met$ | –9.9 | 22.0 |
| $Al(H_20)_5NH_3(Lys)$ | –7.8 | –5.8 |
| $Al(H_20)_5Ser$ | –9.0 | 4.4 |
| $Al(H_20)_5Tyr$ | –25.8 | 11.1 |

Figure 1 presents the geometry of $Al(H_2O)_5X$ complexes, where X denotes the following amino acid residues: aspartate (Asp), histidine (His), methionine (Met), lysine (Lys), serine (Ser) and tyrosine (Tyr).

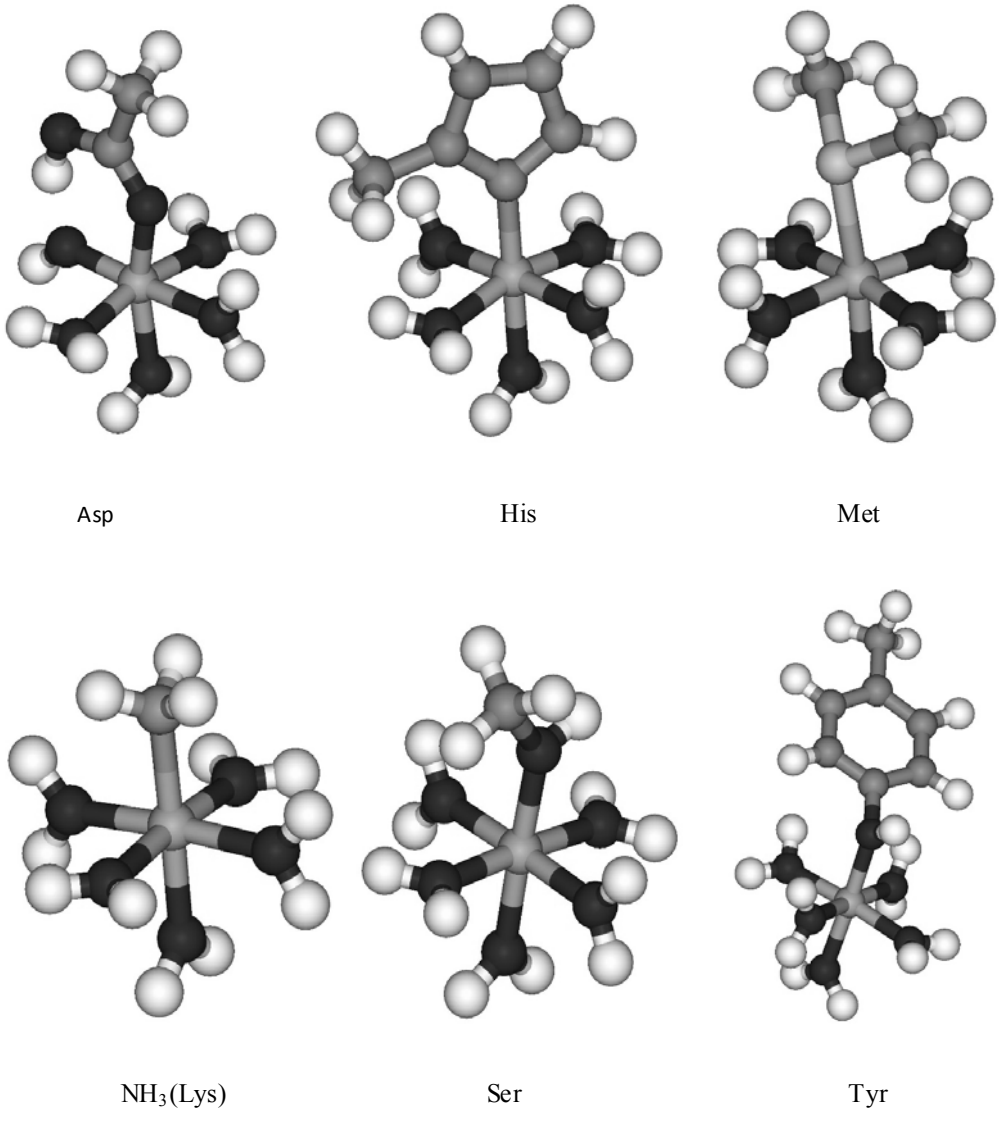


**Figure 1.** Geometries of the $Al(H_2O)_5X$ complexes obtained at the B3LYP/6-311+G(2df,2p) level of theory. X stands for Asp, His, Met, NH3(Lys), Ser, or Tyr.

Figure 2 presents the scale of differences between the values shown in Table 2.

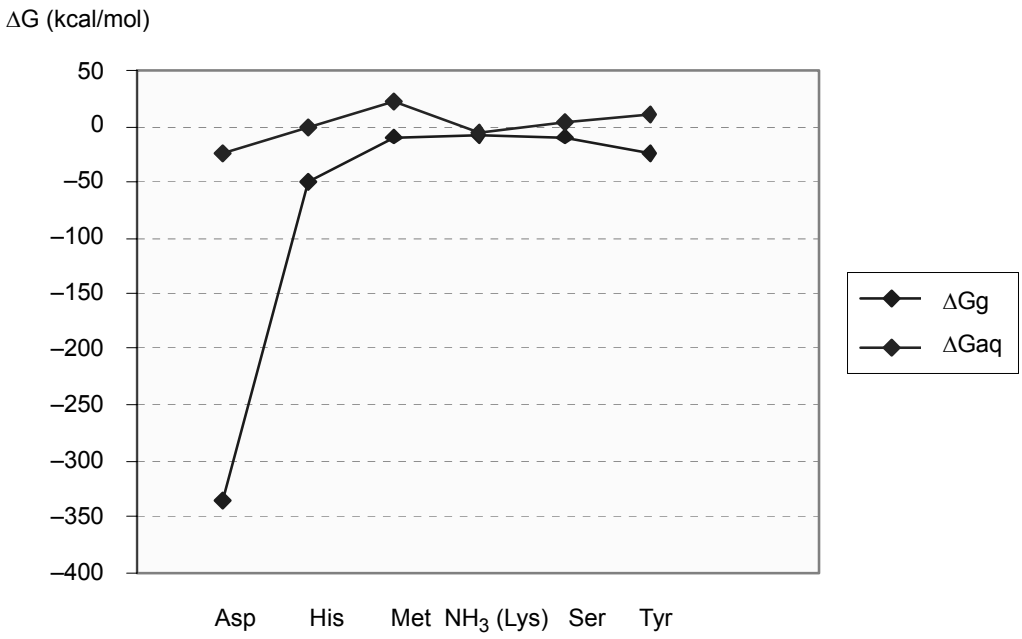


**Figure 2.** Plot of binding affinities (kcal/mol) in the gas phase (ΔG) and aqueous solution (ΔGaq) for model compounds, mimicking amino acid side chains: Asp, His, Met, $NH_3$ (Lys), Ser, and Tyr calculated at the B3LYP/6-311+G(2df,2p).

## DISCUSSION

In this study, we concentrated first on the determination of the coordination number for aluminum in two environments: in a gas phase and then in the aqueous environment for which a CPCM solvent model was used.[21] After the analysis of individual equations of the dehydration reaction, the calculations, and Gibbs free energy values obtained for the dehydration of aluminum complexes, it was ascertained that $[Al(H_2O)_6]^{3+}$ is stable in the aqueous phase. It has a special significance for biological systems.

Aluminum creates complexes with a coordination number 6 also in many other compounds, e.g., $AlF_6$ halogen complexes,[25] hexofluorosilicate,[26] and amino-complexes. Besides pH, the type of environment is also important, as confirmed by the results obtained in the gas and aqueous phase.[27,28] After the performance of the analyses, studies were performed to find the sites binding the aluminum in Aβ. Attention was drawn to previously discovered iron-binding sites in Aβ,[14] due to certain chemical similarities of iron and aluminum. Both of the elements are trivalent, they create relatively stable complexes with a coordination number 6, they increase Aβ aggregation,[29] and they bind to proteins: transferrin and ferritin.[30] Miura et al., using a Raman spectroscopy method, showed that iron is bound by the Tyr residue in

the position 10 Aβ, and to a lesser extent by carboxylic groups of glutamate (Glu) or Asp.[14] Also Chong believes that APP, a precursor of Aβ peptide contains binding sites rich in Glu.[31]

It is worth mentioning here that from position 222 through to 264 there are 21 Glu residues. The results by the aforementioned authors are confirmed by our results (Table 2 and Figure 1). Amino acid Asp residues that contain two carboxylic groups (similarly to Glu) show the greatest affinity to $[Al(H_2O)_5]^{2+}$. Lys and His bind Al to a lesser degree.

A diagram presenting the Lys binding by the aluminum-fluoride complex was presented by Li in 2003.[7] His is considered a key metal-binding amino acid.[11,15,28,32] Perhaps one or more amino acid residues is involved in the Aβ aggregation process.[27] It seems that ways of binding various metals by amino acid residues may differ.[29] There have been attempts to apply this knowledge in metal chelation using DPO (desferrioxamine) and EDTA (ethylenediaminetetraacetic acid).[12-13]

*In vitro* studies increasingly often use computational methods[27-28] based on modern software[22-23] which enables the determination of many physiochemical parameters of a reaction. However, despite the application of the latest technological achievements and detailed knowledge on AlFx, its direct relation with physiological processes is yet not entirely recognized.[7]

## CONCLUSIONS

To summarize, our computational study demonstrates that Asp/Glu seems to have the largest affinity to $[Al(H_2O)_6]^{3+}$ as indicated by the free energies of exchange reaction replacing one water ligand in the aluminum aqueous complex with the model of an amino acid side chain. This substantial Asp/Glu aqueous affinity of –25 kcal/mol is related to a spontaneous proton transfer from the water molecule interacting with $Al^{3+}$ to the carboxylic group of the amino acid residue. In contrast to the analogous studies carried out for the $Cu^{2+}$ ion,[27] we demonstrate that $Al(H_2O)_6$ preferentially binds to the Asp/Glu residue rather than to His.

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