QUANTUM MECHANICS / MOLECULAR MECHANICS STUDY ON SELECTED NUTRACEUTICALS TARGETING MITOCHONDRIAL DYSFUNCTION-RELATED PROTEINS IN ALZHEIMER'S DISEASE

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Several pathways are related to Alzheimer's Disease (AD) progression, and previous studies have confirmed mitochondrial dysfunction as a potential therapeutic target for AD. The objective of this study was to solidify the results obtained from a previous *in-vitro* study targeting AD. This study is focused on four major proteins, namely Sirtuin-1, Sirtuin-3 (SIRT1/3), Adenosine monophosphate-activated kinase (AMPK), and PTEN-induced kinase 1 (PINK1) related to mitochondrial dysfunction pathways and the activity of the nutraceuticals, Urolithin-A, Luteolin, Docosahexaenoic acid (DHA) and Resveratrol. Quantum Mechanics / Molecular Mechanics (QM/MM) ONIOM (our own N-layered Integrated molecular Orbital and Molecular mechanics) calculations are used to assess the binding affinity of ligands with the target proteins. ONIOM calculation enables the application of Density Functional Theory (DFT) and molecular mechanics to be applied to different parts of the ligand-protein complex. Initially, the ligands were docked to the target proteins using the Schrodinger software. The docked poses were used to perform the ONIOM calculation using the two-layer method of Gaussian software. The QM/MM ONIOM calculations on SIRT1, SIRT3, and AMPK proteins selectively had a high binding affinity to ligands, while the PINK1 protein had a low affinity to all ligands. The binding interaction energy of SIRT1 with DHA is -19.39 kcal/mol, SIRT3 with Resveratrol is -18.14 kcal/mol, and AMPK with Urolithin A is -1.87 kcal/mol, also related to the *in-vitro* cell viability results of the human neuroblastoma cell line pre-treated with the specific ligand. The proteins have shown a binding affinity trend of SIRT1 > SIRT3 > AMPK, specifically binding to selective ligands and the possible activation of Sirtuin family-related mitochondrial biogenesis and mitophagy pathways. Therefore, further *in-silico* analyses together with *in-vitro* and *in-vivo* are needed to ensure the efficacy of these nutraceuticals, which may be improved as potential AD-related therapeutic approaches to enhance the functional mitochondrial mass in diseased neurons.

Keywords: Alzheimer's Disease, Mitophagy, ONIOM calculation, QM/MM, Density Functional Theory (DFT)

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INTRODUCTION

Alzheimer's Disease (AD) is a progressive neurodegenerative disorder, which is one of the common forms of dementia characterized by episodic memory loss, cognitive decline, and ultimate complete dependence on caregivers. Alzheimer's disease-related histopathological characteristics include extracellular deposition of amyloid-β (Aβ) protein aggregates as senile plaques, intracellular neurofibril tangles (NFT) and hyper-phosphorylation of microtubule protein Tau (Möller & Graeber, 1998). With the advancement of new knowledge, it is becoming increasingly recognized that mitochondrial dysfunction plays a key role in AD pathogenesis. Mitochondrial respiration produces Reactive Oxygen Species (ROS), which, when produced in high quantities, can react with biomolecules exerting harmful effects on the cells. Impaired mitochondria can further result in higher ROS production. The human brain is the consumer of 20% of the total oxygen usage in the body, making mitochondrial quality control and maintaining a healthy population of mitochondria vital to this organ (Fischer et al., 2012, Mink et al., 1981).

Due to the lack of effective treatments for AD, novel strategies have been implemented to prevent mitochondrial impairments using dietary interventions, nutritional supplements, and natural compounds (Nasri et al., 2014). Nutraceuticals are an emerging alternative approach due to their neuroprotective properties. The objective of this study was to solidify the results obtained from the study conducted by Jayatunga et al., 2022b. This was achieved through the *in-silico* analysis of the activity of nutraceuticals, Urolithin-A, Luteolin, Docosahexaenoic acid (DHA) and Resveratrol on the proteins related to mitochondrial dysfunction pathways, namely: SIRT1, SIRT3, AMPK and PINK1 (Jayatunga et al., 2020; Muhammed Khairujjaman Mazumder et al., 2019).

METHODOLOGY

The crystal structures of SIRT1 (PDB ID: 4KXQ), SIRT3 (PDB ID: 4BN4), AMPK (PDB ID: 6C9H) were downloaded from the protein data bank (https://www.rcsb.org). The crystal structure of PINK1 was homology modelled using SWISS-MODEL software of the Expasy server (https://swissmodel.expasy.org). This structure was based on the crystal structure of the cytosolic domain of *Tribolium castaneum* PINK1 protein in non-phosphorylated state which had 44.91% sequence identity. The structures of target proteins by protein preparation wizard workflow and the optimized ligands by Gaussian 09 were docked using the virtual screening workflow of the Glide module of Schrodinger software.

Quantum Mechanics / Molecular Mechanics (QM/MM) calculations were performed to obtain the ligands' and target proteins' electronic interaction energies. The docked structures of the protein-ligands complexes were used to perform the 'our own N-layered Integrated molecular Orbital and Molecular mechanics' (ONIOM) calculations using Gaussian software. The docked ligand was considered the high layer and the protein was the low layer. The Molecular Mechanics (MM) method was selected for the low layer with a universal force field (UFF), while the high layer was modelled at the B3LYP/6-31(d) level of theory. The total energy of the docked complexes (E_{Total}) was obtained using the following equation (Dushanan et al., 2022).

$$E_{Total} = E_{Low,\ Real} + E_{High,\ Model} - E_{Low,\ Model}$$

$E_{Low,\ Real}$ is the MM energy of the ligand-protein complex. $E_{High,\ Model}$ is the Quantum Mechanics (QM) energy of the ligand and some residues surrounding it in the complex. $E_{Low,\ Model}$ is the MM energy

of the rest of the protein. Then using the ligand complex's total energy (E_{Total}), the ligand's electronic interaction energy with the protein ($E_{Binding}$) was calculated using the equation below (Dushanan et al., 2022).

$$E_{Binding} = E_{Total} - [E_{High\ Ligand} + E_{Low\ Protein}]$$

Here, $E_{High\ Ligand}$ is the QM energy of the ligand and $E_{Low\ Protein}$ is the MM energy of the protein calculated separately.

RESULTS AND DISCUSSION

This study targeted the ligands to the protein pocket to which a previous ligand was already bound in the crystal structure. Then ONIOM calculations were performed on these docked structures to obtain the total energy of the complex, the ligand's energy, and the target protein's energy to find the binding energy between the ligand and the target protein. The ONIOM results are given in Table 01.

Table 01: Binding energy of the ligand-protein complex using the QM/MM method.

Protein	Ligand (a.u.)	E_{Real}^{Low} (a.u.)	E_{Model}^{High} (a.u.)	E_{Model}^{Low} (a.u.)	E_{Total} (a.u.)	E_{High} (a.u.)	E_{Low} (a.u.)	$E_{Binding}$ (a.u.)
SIRT1	Urolithin A	17.594	-809.258	0.086	-791.750	-809.258	17.502	0.005
	Luteolin	17.813	-1038.246	0.150	-1020.583	-1038.246	17.502	0.161
	DHA	17.642	-1016.207	0.171	-998.736	-1016.207	17.502	-0.031
	Resveratrol	17.683	-774.576	0.081	-756.974	-774.576	17.502	0.100
SIRT3	Urolithin A	14.628	-809.249	0.092	-794.713	-809.249	14.386	0.150
	Luteolin	14.624	-1038.218	0.154	-1023.748	-1038.218	14.386	0.084
	DHA	14.567	-1016.218	0.088	-1001.739	-1016.218	14.386	0.093
	Resveratrol	14.436	-774.580	0.079	-760.223	-774.580	14.386	-0.029
AMPK	Urolithin A	19.790	-809.251	0.113	-789.574	-809.251	19.680	-0.003
	Luteolin	19.823	-1038.218	0.141	-1018.536	-1038.218	19.680	0.002
	DHA	19.786	-1016.233	0.076	-996.523	-1016.233	19.680	0.030
	Resveratrol	19.783	-774.580	0.069	-754.866	-774.580	19.680	0.034
PINK1	Urolithin A	25.146	-809.251	0.092	-784.197	-809.251	24.930	0.124
	Luteolin	25.190	-1038.237	0.135	-1013.182	-1038.237	24.930	0.125
	DHA	25.050	-1016.223	0.091	-991.264	-1016.223	24.930	0.029
	Resveratrol	25.238	-774.584	0.065	-749.411	-774.584	24.930	0.243

The binding energy values obtained through QM/MM ONIOM calculations can be positive or negative, depending on the ligand-protein complex. Negative binding energy indicates that the interactions between the ligand and the protein at the binding site are attractive and likely to bind together, while positive binding energy indicates the presence of repulsive forces and that the ligand, and the protein are unlikely to bind at the selected binding site.

According to results from Table 01, SIRT1, SIRT3, and AMPK have shown high binding affinity to ligands selectively, while PINK1 protein has shown low affinity to all of the ligands. The binding interaction energy of SIRT1 with DHA is -0.031 a.u. (-19.39 kcal/mol), SIRT3 with resveratrol -0.029 a.u. (-18.14 kcal/mol) and AMPK with urolithin A -0.003 a.u. (-1.87 kcal/mol). It is interesting

to note that a previously conducted *in-vitro* assay of the human neuroblastoma cell line pre-treated with urolithin A, luteolin, DHA, and Resveratrol had shown 50% cell viability upon Aβ-induced toxicity at concentrations of 30 μM, 20 μM, 30 μM and 25 μM, respectively (Jayatunga et al., 2022b, Han et al., 2004). This further suggests the possible affinity of the ligands on selective pathways. Activation of SIRT1 and SIRT3 through the binding of DHA and Resveratrol can activate mitochondrial biogenesis pathways and mitophagy related to Sirtuin family of proteins. Activation of AMPK protein is related to autophagy pathways in addition to mitophagy. The activation of these proteins by specific ligands suggests an explanation for the synergistic mechanism of the ligand neuroprotective ability against AD (Jayatunga et al., 2022a).

CONCLUSIONS

The QM/MM ONIOM calculations on the SIRT1, SIRT3 and AMPK proteins have shown selectively high binding affinity to ligands while the PINK1 protein has shown low affinity to all the ligands. DHA's low binding energy and high binding affinity to SIRT1 and Resveratrol to SIRT3 suggest that these ligands form stable ligand-protein complexes. The binding interaction energy of SIRT1 with DHA is -19.39 kcal/mol, SIRT3 with resveratrol -18.14 kcal/mol and AMPK with urolithin A -1.87 kcal/mol also relates to the *in-vitro* cell viability results of the Aβ-induced toxicity of human neuroblastoma cell line pre-treated with the specific ligands. The current results suggest that the activity of the ligands on the target proteins might have restored the cell viability of the neuroblastoma cells by maintaining mitochondrial quality control. The proteins have shown a binding affinity trend of SIRT1 > SIRT3 > AMPK, specifically binding to selective ligands and the possible activation of Sirtuin family-related mitochondrial biogenesis and mitophagy pathways. Three of the four ligands under study have demonstrated distinct binding affinities with the target proteins, and this can be viewed as a mechanism for the synergistic effect of DHA and urolithin A to exert mitoprotective effects, which has been demonstrated in prior research (Jayatunga et al., 2022 (b)). Accurate prediction of protein-ligand interactions is important to reduce the cost of drug discovery. The docking aspect of the study focussed on targeting the binding site to previously bound ligands. Therefore, it can be recommended that further *in-silico* studies are required as a blind dock or a docking study focussing on other druggable sites on the target protein to predict more accurate ligand-proteins interactions. Further, analyses together with *in-vitro* and *in-vivo* studies are needed to ensure the efficacy of these nutraceuticals, which may be improved as potential AD-related therapeutic approaches to enhance the functional mitochondrial mass in the diseased neurons.

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