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MOLECULAR DOCKING OF ASTAXANTHIN TO MONOAMINE OXIDASE

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Abstract: To comprehensively investigate the biological activity (positive or negative) of astaxanthin, a reddish food colorant, *in silico* studies have been performed. Molecular docking experiments have shown that astaxanthin can bind to the monoamine oxidase A (MAO-A) active center with predicted Kd=4.4 $x10^{-6}$ M. Distinguishly, monoamine oxidase B (MAO-B) does not, practically, bind astaxanthin; the very negligible binding with predicted Kd > 10^{9} M might occur on the enzyme surface apart from its active center. Hence, astaxanthin may act as a selective and reversible MAO-A inhibitor. Selective and reversible MAO-A inhibitors are widely used as antidepressant and anxiolytic drugs whereas non-selective and irreversible inhibitors have been withdrawn due to toxic side effects caused by their interactions with other drugs and some food products. The present study has been performed using molecular docking and computer modeling methods.

Keywords: monoamine oxidase, astaxanthin, molecular docking, MAO inhibitors.

1. Introduction

Astaxanthin is naturally occurring carotenoid found in nature primarily in marine organisms. It is a reddish pigment and causes the pink or red color in salmon, trout, lobster, prawn, and other seafood. The green microalga *Haematococcus pluvialis* is considered the richest source of astaxanthin. Other microalgae, such as *Chlorella zofingiensis*, *Chlorococcum spp*, and *Botryococcus braunii*, also contain astaxanthin. Health-promoting effects of carotenoids and particularly astaxanthin from microalgae have been well documented [1,6].

Monoamine oxidase, EC 1.4.3.4, (MAO), also known as FAD–dependent amine oxidase, the enzyme that catalyze the oxidation of monoamines, exists in the two isoforms, MAO-A and MAO-B [2]. MAOs are mitochondria outer membrane-bound flavoproteids and play significant role in neurotransmitters (such as serotonin, adrenaline, noradrenaline, melatonin, dopamine, tyramine, etc.) metabolism. Accordingly, the inhibitors of MAO are of special interest for the pharmacological therapy of anxiety disorders and depression (MAO-A inhibitors) and such neurodegenerative diseases as Alzheimer's and Parkinson's (MAO-B inhibitors) [3 - 5].

A number of studies indicating the prominent health benefits of astaxanthin [6] including its neuroprotective properties [7-10] caused a burst in the nutraceutical market of astaxanthin containing supplements. The supplement advertisers claim that their products possess potent effects in treating Alzheimer's disease, Parkinson's disease, "brain attack" (stroke) etc. In order to investigate the

relevance of MAO inhibition to the neuroprotective properties of astaxanthin we performed the molecular docking studies of MAOs and astaxanthin.

2. Experimental

Protein and ligand preparations for molecular docking. Monoamine oxidase 3D structures were retrieved from protein data bank at http: //www.rcsb.org (pdb entries 1oja for MAO-A, and pdb 2v5z for MAO-B). Ligands bound to the enzyme as well as crystallographic waters were removed from the original **.pdb** file using PyMol. The obtained ligand free enzyme structures were checked for missing atoms, bonds and contacts using PyMol and AutoDock Tools v.4.2 (© 1999-2011 Molecular Graphics Laboratory, The Scripps Research Institute) and energy minimization was performed using Yasara force field and Yasara minimization server [11]. Molecular graphics and analyses were performed with the PyMol Molecular Graphics System, Version 1.5.0.4, Schrödinger, LLC. Molecular structures of ligands in .pdb format were generated using Avogadro v.1.1.0 for Windows. The geometry of a molecule was optimized using Ghemical force field. The optimization was achieved by Conjugate gradient algorithm (number of steps 500). The minimization termination condition was set to the convergence criteria of RMS gradient of 0.0001 kcal/Å· mol *in vacuo*. Protein structure files were prepared for docking procedure by assigning Kollman charges, merging nonpolar hydrogens and saved as .pdbqt files using AutoDock Tools. The similar procedure was applied to create ligand.pdbqt files. Ligand preparation procedure included Gasteiger charges addition instead of Kollman charges and defining the rotatable bonds of a ligand.

Docking protocol and computational details. Flexible ligand docks were performed by AutoDock Vina [12] software. Rectangular grid box covering the ligand-binding sites were of dimensions 40 x 40 x 40 Å for MAO-A and 20x 20 x 20 Å for MAO-B. The center of the gridbox was defined by the bound inhibitor using respective ligand - enzyme biological assembly pdb1 file. Binding energy (affinity) of a ligand was calculated by the Vina module. The predicted inhibition constants (Ki) were calculated according to the equation:

$$Ki = Kd = \exp(\Delta G / RT),$$

where ΔG is a ligand-binding energy; R-gas constant equal to 1.986 cal/mol; T-Kelvin absolute temperature (298 K). All calculations were performed on Intel Core2 Duo CPU 2.60 GHz running Microsoft Windows XP Professional, version 2002, service pack 3 OS.

Postdocking analysis. A number of approaches were applied for docking outputs post processing in order to rank the inhibitory potential of studied compounds. High-ranking vina outputs were analyzed by compliance with the known ligand - enzyme biological assembly models, visual inspection of a ligand orientation and its interactions in the substrate cavity environment. PyMol was used to visualize ligand-enzyme 3D interactions. Enzyme – ligand interactions were also analyzed by LigPlot^{+} v.1.4.5.

3. Results and Discussion

The results of molecular docking studies of astaxanthin and the two known selective inhibitors, isatin and labazemide, are represented in the Table 1. Calculated Kd values for astaxanthin – MAO-A complex is 4.4 μM, which is comparable to 0.96 μM for isatin-MAO-A complex. Binding energy and predicted Kd (>10^9 M) of astaxanthin to MAO-B are too high to expect significant binding. Therefore, one can conclude that astaxanthin is potentially selective MAO-A inhibitor.

Table 1. Comparison the binding energy of astaxanthin and known MAO inhibitors

Compound	Ref.	Binding energy, E, kcal/mol	
		MAO-A	MAO-B
Isatin	[14]	-8.2	-7.1
lazabemide	[13]	-4.8	-7.0
astaxanthin		-7,3	+14,2

The results of Table 1 have been verified and explained by 3D molecular modeling. Molecular docking to MAO_A shows that astaxanthin binds to substrate binding site on the same place as isatin, a well-known endogenous MAO-A inhibitor. Astaxanthin is highly hydrophobic molecule and, as it was expected, makes multiple hydrophobic interactions with the enzyme. Such amino acid residues as Pro105, Trp107, Tyr112 and other participate in hydrophobic bonding (Figure 2).

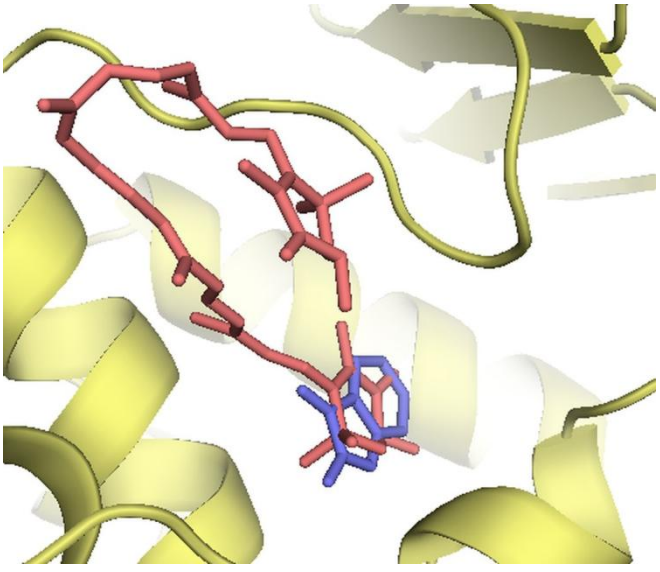

Fig.1. The poses of isatin (blue) and astaxanthin (pink) molecules in MAO-A substrate binding site

Quite different picture is observed with astaxanthin binding to MAO-B (Figure 3). The known reversible MAO-B inhibitors, safinamide and labazemide, bind to the substrate binding site entering substrate binding cavity. Distinquishly, astaxanthin cannot enter the substrate binding cavity due to steric restrictions. It could bind very weakly on the enzyme surface apart from the active center. Obviously, astaxanthin being outside of the binding site would not inhibit MAO-B.

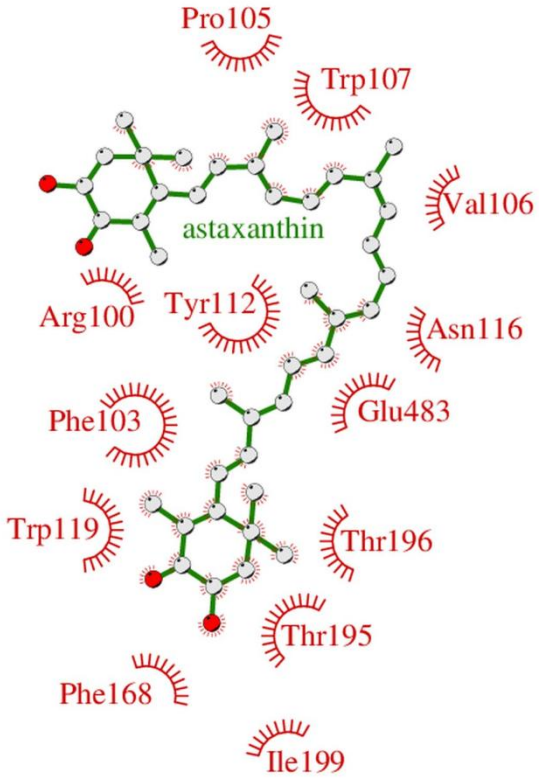

Fig.2. LigPlot outputs of MAO-A – astaxanthin interactions

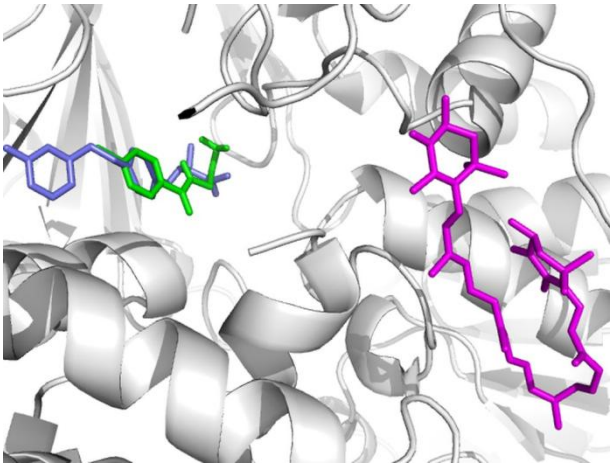

Fig. 3. The poses of safinamide (blue), astaxanthin (pink) and labazemide (green) molecules in MAO-B binding sites

3. Conclusion

Microalgae are a source of high value substances. Most of microalgae species produce unique products like carotenoids, antioxidants, fatty acids, enzymes, polymers, peptides, toxins and sterols. Carotenoids, particularly astaxanthin, are well known for their therapeutic benefits in the aging process and various diseases because of its potent antioxidant and anti-inflammatory effects. The research has been performed in continuation of our ongoing research on the comprehensive evaluation of the bioactivity of microalgal carotenoid astaxanthin. In this paper we have performed molecular docking studies of astaxanthin to monoamine oxidase A and B. The present in *silico* studies show that astaxanthin binds to the MAO-A active center with the predicted Kd of micromolar order. Diverse hydrophobic interactions are involved in astaxanthin – MAO-A binding. Distinquishly, MAO-B does not bind astaxanthin in the substrate binding cavity, however a weak binding may occur on the enzyme surface outside of the active center. The obtained data suggest that astaxanthin may serve as a selective mild MAO-A inhibitor, which will not affect the activity of the MAO-B isoform of the enzyme.

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References

1. Yuan J.P., Peng J., Yin K., Wang J.H., Potential health-promoting effects of astaxanthin: a high-value carotenoid mostly from microalgae, Mol. Nutr. Food Res., Vol.55, No.1, 2011, pp.150-165.
2. Slotkin T.A., Mary Bernheim and the discovery of monoamine oxidase, Brain Research Bulletin, Vol.50, No.5-6, 1999, p.373.
3. Yamada M., Yasuhara H., Clinical Pharmacology of MAO Inhibitors: Safety and Future, Neurooxicology, Vol.25, 2004, pp.215-221.
4. Fariello R.G., Lieberman A., Present and Future Approaches to Parkinson Disease: From Molecular Insights to New Therapeutic Avenues, Neurology, Vol.67, 2006, S1-S4,
5. Youdim M.B., Edmondson D.E., Tipton K.F., The Therapeutic Potential of Monoamine Oxidase Inhibitors, Nat. Rev. Neurosci., Vol.7, 2006, pp.295–309.
6. Dhankhar J., Kadian S.S., Sharma A., Astaxanthin: a potential carotenoid, Int. J. Pharma. Res., Vol.3, No.5, 2012, pp.1246-1259.
7. Guerin M., Huntley M.E., Olaizola M., Haematococcus astaxanthin: applications for human health and nutrition, Trends in Biotechnology, Vol.21 No.5, 2003, pp.210-216.

8. Barros M.P., Poppe S.C., Bondan E.F., Neuroprotective Properties of the Marine Carotenoid Astaxanthin and Omega-3 Fatty Acids, and Perspectives for the Natural Combination of Both in Krill Oil, Nutrients, Vol.6, 2014, pp.1293-1317.
9. Hussein G., Nakamura M., Zhao Q., Iguchi T., Goto H., Sankawa U., Watanabe H., Antihypertensive and neuroprotective effects of astaxanthin in experimental animals, Biol. Pharm. Bull, Vol.28, No.1, 2005, pp.47-52.
10. Liu X., Yamada N., Osawa T., Assessing the neuroprotective effect of antioxidant food factors by application of lipid-derived dopamine modification adducts, Methods Mol.Biol., Vol.594, 2010, pp.263-273.
11. Krieger K., Joo J., Lee S., Raman J., Thompson M., Tyka D., Baker D., Karplus K., Improving physical realism, stereochemistry, and side-chain accuracy in homology modeling: Four approaches that performed well in CASP8, Proteins, Vol.77, Suppl. 9, 2009, pp.114-122.
12. Trott O., Olson A., AutoDock Vina: improving the speed and accuracy of docking with a new scoring fun ction, efficient optimization and multithreading, J. Journal of Comp. Chemistry, Vol.31, 2010, pp.455-461.
13. Henriot S., Kuhn C., Kettler R., Da Prada M. Lazabemide (Ro 19-6327), a reversible and highly sensitive MAO-B inhibitor: preclinical and clinical findings, In: Amine Oxidases: Function and Dysfunction, series Journal of Neural Transmission, Vol.41, 1994, pp. 321-325.
14. Hamaue N., Minami M., Hirafuji M. et al., Isatin, an Endogenous MAO Inhibitor, as a New Biological Modulator, CNS Drug Reviews, Vol.5, No.4, 1999, pp.331–346.