

## MOLECULAR DOCKING OF ASTAXANTHIN TO $\beta$ -GLUCURONIDASE

N. Safarov<sup>1</sup>, R. Agalarov<sup>1</sup>, R. Gasanov<sup>2\*</sup>

<sup>1</sup>Center for Biotechnology, Baku State University, Baku, Azerbaijan

<sup>2</sup>Department of Biophysics and Molecular Biology, Faculty of Biology,  
Baku State University, Baku, Azerbaijan

**Abstract.** It is known that inhibitors of *Escherichia coli glucuronidase* (eGUS) significantly alleviate side effects of modern chemotherapeutics (such as irinotecan and topotecan) as well as side effects of nonsteroid anti-inflammatory drugs (NSAIDs) such as diclofenac and ketoprofen. Taking into account \$ 8 billion world market of NSAIDs only it becomes obvious that development of new efficient eGUS inhibitors should have a great importance. In the present work using molecular docking we studied interactions of eGUS with carotenoid astaxanthin, which could be obtained from diverse natural sources including microalgae. The molecular docking results show that relatively elongate molecule of astaxanthin neatly fits the active center of eGUS enzyme occupying practically the whole substrate binding site of the enzyme. Terminal parts of the astaxanthin molecule make hydrogen bonding with such amino acid residues as Val484, Ser557 and Gln558. Such amino acid residues as Glu413, Tyr472, Met447 and some other ensure tight binding of the central hydrophobic part of astaxanthin into the hydrophobic substrate -binding tunnel of eGUS. Comparison of molecular docking features of astaxanthin with other known eGUS inhibitors let us conclude that development of astaxanthin based natural eGUS inhibitors is highly feasible.

**Keywords:**  $\beta$ -glucuronidase, astaxanthin, molecular docking, *Escherichia coli*.

**Corresponding Author:** Ralphreed Gasanov, Prof., Department of Biophysics and Molecular Biology, Faculty of Biology, Baku State University, Baku, Azerbaijan, e-mail: [ra38hasan@gmail.com](mailto:ra38hasan@gmail.com)

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### 1. Introduction

Astaxanthin is naturally occurring carotenoid found in nature primarily in marine organisms such as microalgae, salmon, trout, krill, shrimp, crayfish, and crustaceans. It is a reddish pigment and causes the pink or red color in salmon, trout, lobster, shrimp, 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. It may also be found in the feathers of birds, such as quail, flamingo, and storks, as well as in propolis, the resinous substance collected by bees.

Carotenoids are well known for their therapeutic benefits in the aging process and various diseases, because of their potent antioxidant and anti-inflammatory effects (Fassett & Coombes, 2011). Astaxanthin is a xanthophyll carotenoid like lutein, zeaxanthin, and cryptoxanthin, which do not convert to vitamin A. Astaxanthin contains two oxygenated groups on each ring structure, which is responsible for its enhanced antioxidant features (Guerin *et al.*, 2003). Health-promoting effects of carotenoids and particularly astaxanthin from microalgae have been well documented (Yuan *et al.*, 2011). In modern medicine astaxanthin is used for treating Alzheimer's

disease, Parkinson's disease, “brainattack” (stroke), high cholesterol, and an eye condition called age-related macular degeneration (AMD). It is also used for preventing cancer. Astaxanthin is an antioxidant. This effect might protect cells from damage (Nakagawa *et al.*, 2011; Pashkow *et al.*, 2008). Astaxanthin might also improve the way the immune system functions. Experimental evidence suggests astaxanthin may have protective effects on cardiovascular disease. In addition, there is evidence that astaxanthin may decrease oxidative stress and inflammation which are known accompaniments of cardiovascular disease (Fassett & Coombes, 2011).

Dietary astaxanthin was found to help fight symptoms of ulcer disease from *Helicobacter pylori*. Astaxanthin reduced symptoms of gastric inflammation and was also associated with shifts in the inflammation response (Bennedsen *et al.*, 1999). Although it could be assumed that the antioxidant properties of astaxanthin explain its anti-inflammatory activity, further studies are needed to better understand the specific mode of action of astaxanthin in fighting inflammation. Several studies suggest protective effects of astaxanthin against non-steroidal anti-inflammatory drugs (NSAIDs), such as naproxen (Kamath *et al.*, 2008). Therapeutic effect of astaxanthin on gastric ulcer (Yang *et al.*, 2009; Kamath *et al.*, 2008) and functional dyspepsia (Andersen *et al.*, 2007; Kupcinskas *et al.*, 2008) has also been reported. Side effects of non-steroidal anti-inflammatory drugs causing ulceration and bleeding in the upper gastrointestinal tract are known since 1938 (Douthwaite & Lintott, 1938). NSAIDs can cause damage to the gastroduodenal mucosa via several mechanisms (Wallace, 2000). One of the possible mechanisms of intestinal mucosal injury could be the liberation of toxic NSAIDs intermediate in the gut upon *Escherichia coli* glucuronidase (eGUS). Such mechanism is supported by the findings that oral pretreatment with bacteria specific glucuronidase inhibitor significantly alleviates mucosal injury caused by administration of an ulcerogenic dose of diclofenac (NSAID) (LoGuidice *et al.*, 2012).

Taking into account that world market for NSAIDs is approximately \$8 billion the development of new effective and preferably natural and selective eGUS inhibitors would be of great importance to mitigate side effects of NSAIDs as well as camptothecin chemotherapeutics. Putting together the data regarding protective effects of astaxanthin and eGUS inhibitors on gastrointestinal damage caused by NSAIDs we have performed comparative molecular docking studies of astaxanthin on human and bacterial glucuronidases.

## 2. Experimental

Protein and ligand preparations for molecular docking.  $\beta$ -Glucuronidase 3D structures were retrieved from protein data bank at <http://www.rcsb.org> (pdb entries 3LPG for bacterial glucuronidase, and pdb 1BHG for human enzyme). Ligands bound to the enzyme as well as crystallographic waters were removed from the original .pdb file using PyMol. The obtained ligand free  $\beta$ -glucuronidase 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 (Krieger *et al.*, 2009). Molecular graphics and analyses were performed with the PyMol Molecular Graphics System, Version 1.5.0.4, Schrödinger, LLC. Molecular structures of new inhibitors in .pdb format were generated using Avogadro v.1.1.0 for Windows. The geometry of a molecule was optimized using Gchemical 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 (Safarov & Gasanov, 2015; Trott & Olson, 2009) software. Thanks to the natural origin inhibitors have to be considered as the most perspective from the point of view of their introduction in the range of the pharm industry. As a result of our research we create 2D and 3D models of a structure for corresponding sites. First the two dimensional mode of active center, interactions (polar and nonpolar) and distances between functional groups allow to present visually the role of that groups and the mechanism of catalysis which is carried out by E-coli  $\beta$ -glucuronidase.

The model of a structure of complex (substrates or inhibitors with enzyme active site) allowed to reveals also details of interaction of various inhibitors in binding sites to enzyme. Besides, our research allow to establish existence of two transport tunnels leaded to binding sites of glucones part of substrate. There are no such a tunnels in structure of human  $\beta$ -glucuronidase, and it is likely that existence of such two tunnels defines specificity of some inhibitors to bacterial but not to human enzyme. Existence of two tunnels in structure of E-coli  $\beta$ -glucuronidase also allow to explain detailed molecular mechanism of uncompetitive type of inhibition for some especially interesting to cancer therapy of a colon cancer inhibitors. As a result of our research we have identified new effective inhibitors of E-coli  $\beta$ -glucuronidase (Gasanov *et al.*, 2015).

Rectangular grid box for the ligand-binding site was of dimensions 10 x 10 x 10 Å. Due to the significant difference in quaternary structures of bacterial and human enzymes, the grid box center coordinates were defined differently for bacterial and human enzyme as it was described previously (Bankeu *et al.*, 2011). Binding energy (affinity) of a ligand was calculated by the Vina module. The predicted inhibition constants ( $K_i$ ) were calculated according to the equation:

$$K_i = K_d = \exp(\Delta G / RT)$$

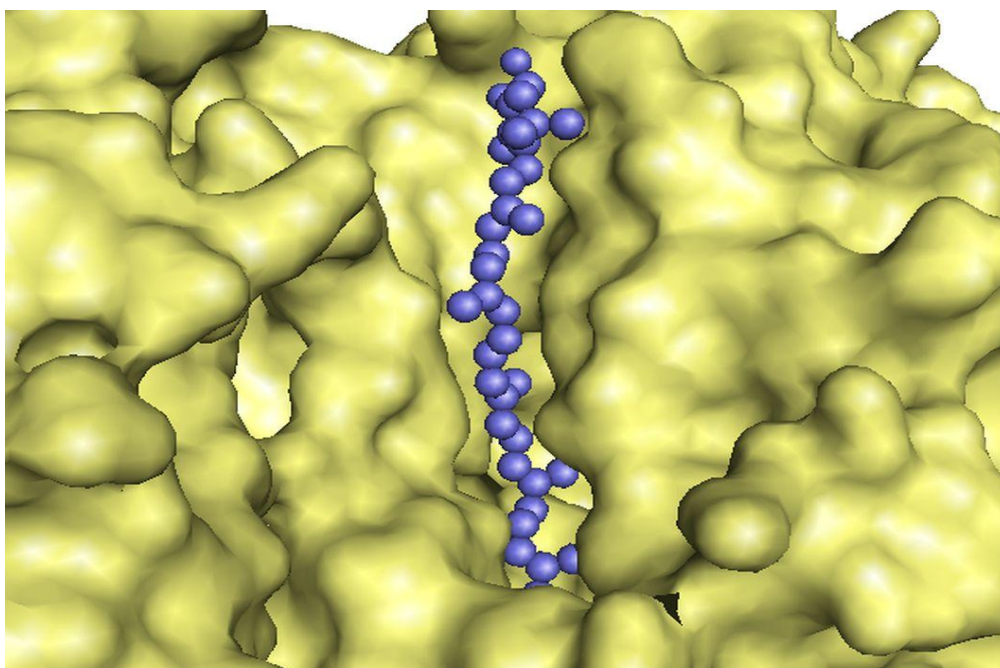
where  $\Delta G$  is a ligand-binding energy; R-gas constant equal to 1.986 cal/mol; T – temperature in Kelvin absolute temperature scale.

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 molecular dynamics simulations, visual inspection of a ligand orientation and its interactions in the substrate cavity environment, compliance with the known ligand - enzyme biological assembly models. Molecular dynamics simulations were carried out on Linux Ubuntu 3.5.0-21 system

with PyMol 1.4.1 version using GROMACS v. 4.5.5. Amber 3 force field and default water model were applied. PyMol was used to visualize ligand-enzyme 3D interactions. Crystal structure of *E. coli*  $\beta$ -glucuronidase complexed with the glucaro-D-lactam inhibitor bound (pdb code 3K4D) (Nakagawa et al., 2011) was used as a reference model.

The results of molecular docking studies of astaxanthin and some other known inhibitors are represented in the Table 1. It can be seen from the Table 1 that astaxanthin could be potentially more efficient inhibitors than other known natural inhibitors such as mucusisoflavone A (Schmeda-Hirschmann *et al.*, 2004), scoparic acid A (Mishra *et al.*, 2011), scopoletin (Schmeda-Hirschmann *et al.*, 2004), and standard inhibitor (Niwa *et al.*, 1972). Therefore, excluding baicalin and wogonoside, astaxanthin show the best potential inhibitor efficiency against eGUS.

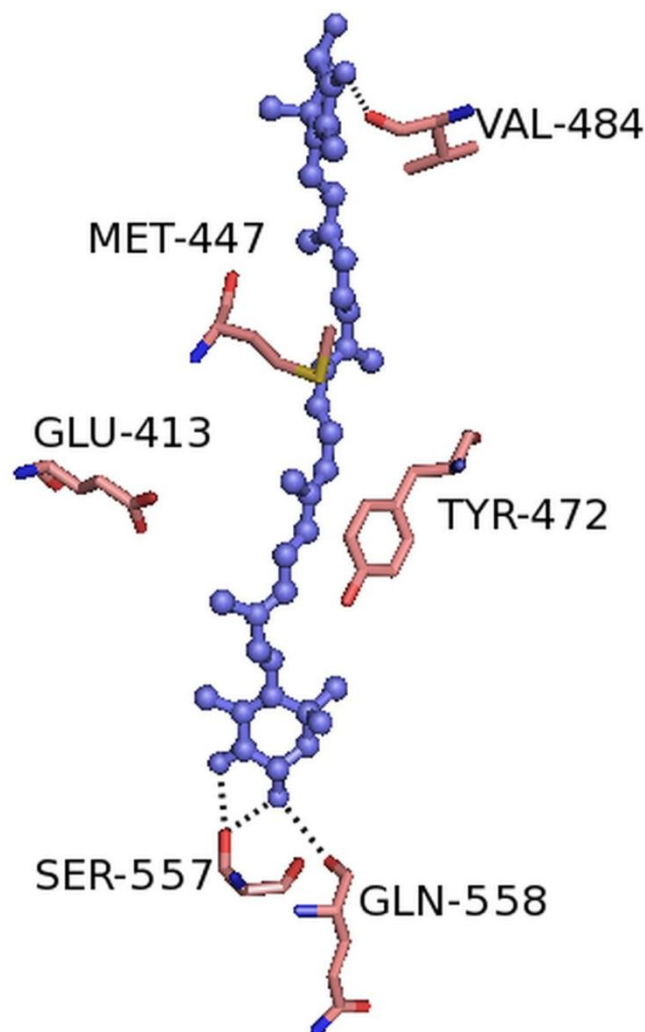


**Fig.1.** Pose of astaxanthin molecule (blue) in eGUS substrate binding cavity

**Table 1.** Comparison of dissociation constants of astaxanthin and some known GUS inhibitors

Compound	$K_D$ ( $\mu$ M)	
	eGUS	hGUS
Baicalin	0.014	0.18
Wogonoside	0.014	0.25
<b>Astaxanthin</b>	0.15	0.58
Mucusisoflavone A	0.15	4.39
Scoparic acid A	0.21	0.29
ST-I	0.81	3.71
Scopoletin	8.64	28.2

ST-I- standard Inhibitor (D-glucaro-1,4-lacton); hGUS - human  $\beta$ -glucuronidase;  
eGUS -*E.coli*  $\beta$ -glucuronidase



**Fig. 2.** eGUS amino acid residues making polar and hydrophobic interactions with astaxanthin

### 3. Conclusions

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, is well known for its therapeutic benefits in the aging process and various diseases, because of its potent antioxidant and anti-inflammatory effects. Structural differences of human and bacterial glucuronidases are found. The inhibition of bacterial glucuronidase is one of the approach to treatment of neoplastic changes or elimination of side effects of some chemotherapeutic means. The sum of the experimental data may form a basis for design of new effective *in silico* inhibitors.

In this paper we put forward the idea that astaxanthin may have additional benefits in medicine by inhibiting *E. coli*  $\beta$ -glucuronidase. Inhibitors of eGUS have found their use in cancer treatment for alleviating side effects of modern chemotherapeutics and NSAIDs (Wallace *et al.*, 2010). In the present paper we describe comparative molecular docking of microalgal astaxanthin to human and bacterial beta-

glucuronidases. The results suggest that astaxanthin in addition to its remarkable food and antioxidant properties might have potential effect on the inhibition of eGUS and, therefore, could be used as a relief for NSAIDs/chemotherapeutics side effects. Using molecular docking we show that astaxanthin could be potential eGUS inhibitor. Therefore, astaxanthin can be considered as promising preparation preventing enteropathies caused by some toxic compounds.

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