

Naturally Occurring Enzyme Inhibitors: A Smart Way to Fight against Micro-Inflammation in Human Gut

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Abstract

Human body is a reservoir for microorganisms, the majority of which resides in the gastrointestinal tract. The diverse micro biota helps to speed up the metabolic processes such as digestion along with providing protection against infections. A balanced diet plays major role in maintaining the balance of gut flora, which could otherwise lead to Dysbiosis. Dysbiosis is an inflammatory condition caused by the over production of bacterial nitroreductase, azoreductase and beta-glucuronidase enzymes. In the following article the active sites of all three enzymes along with their inhibitors were studied. One inhibitor against nitroreductase, two against azoreductase and three against beta-glucuronidase were selected and docking was done. The inhibitors blocked the enzymes activity. These inhibitors can be obtained from natural food sources to attain a balanced microbial flora resulting in a better metabolism.

Keywords: Nitroreductase; Azoreductase; Beta-glucuronidase; Dysbiosis; Human gut microinflammation; Nicotinamide; Riboflavin; Silymarin

Introduction

The gastrointestinal tract consists of a set of microbes that is known as gut flora. These microorganisms consist of over 1000 of species that have millions of genes in the gastrointestinal tract of humans. The digestion of food somehow depends upon the presence of microbes that does the carbohydrate breakdown that leads to the fatty acid and vitamin production [1,2]. These microbes are also responsible in determining the susceptibility of the host to the gastrointestinal infection-also protecting the host from other pathogenic bacteria by limiting the nutrient source to them thus blocking their activity.

At lower taxonomic levels, the microbiota of mammals is highly variable. Four important phyla are: *Firmicutes*, *Actinobacteria*, *Proteobacteria* and *Bacteroidetes*. *Firmicutes* and *Bacteroidetes* are present in a greater amount in the colon as compared to *Actinobacteria* and *Proteobacteria* which are scarcely present [3,4].

Sometimes there is a microbial imbalance inside the body, mainly digestive tract, termed as dysbacteriosis or dysbiosis. This is linked to the inflammation in the bowel along with obesity and also cancer. Medically, the term 'dysbiosis' is described as "microbial/bacterial imbalance" [5]. So, ultimately dysbiosis occurs when the balance of flora and organisms in the GI tract becomes upset.

The changes that occur in the microbes have certain effects on the inflammatory and metabolic processes. The imbalanced diet can lead to the over production of the bacterial enzymes including nitroreductase, azoreductase and beta-glucuronidase. Dysbiosis can generally alter the functional processes of other microbes present in the gut region. So it depends upon the certain diet that is being taken, which either can be beneficial to the gut microbes to grow that have

certain effects on the host or could promote the beneficial microbes that does the food digestion.

The gram negative bacteria in the intestines secrete lipopolysaccharide (LPS) which is phagocytosed by macrophages and is then migrated to the peripheral tissue thus causing inflammation [6]. This induces the release of inflammatory cytokines whereas the macrophages follow decreased syntheses of adiponectin and increased release of leptin and resistin, which generally induce inflammation.

High intake of carbohydrates can elevate blood glucose level thus, causing increased production of insulin. This gives rise to insulin-like growth factors that stimulate the proliferation of cells and induce apoptosis consequently, affecting the protein expression in regulating the cell cycle. Low carbohydrate and high protein diet reduces the total fecal short chain fatty acids, which is associated with bacterial reduction involved in the production of butyrate [7,8]. The gut microbiota can readily mediate behavioral changes and physical activity, in fact higher weight loss is connected with larger changes occurring in the gut microbiota which were associated with the weight loss level.

Metabolic parameters can be improved by following a vegetarian diet which also modulates the gut microbiota thus reducing *Firmicutes* and *Bacteroidetes* ratio. Maintaining a balance in our daily diet is a promising strategy for a proper gastrointestinal function system as this helps keeping a check on bacterial translocation inhibition and decrease in inflammatory reactions.

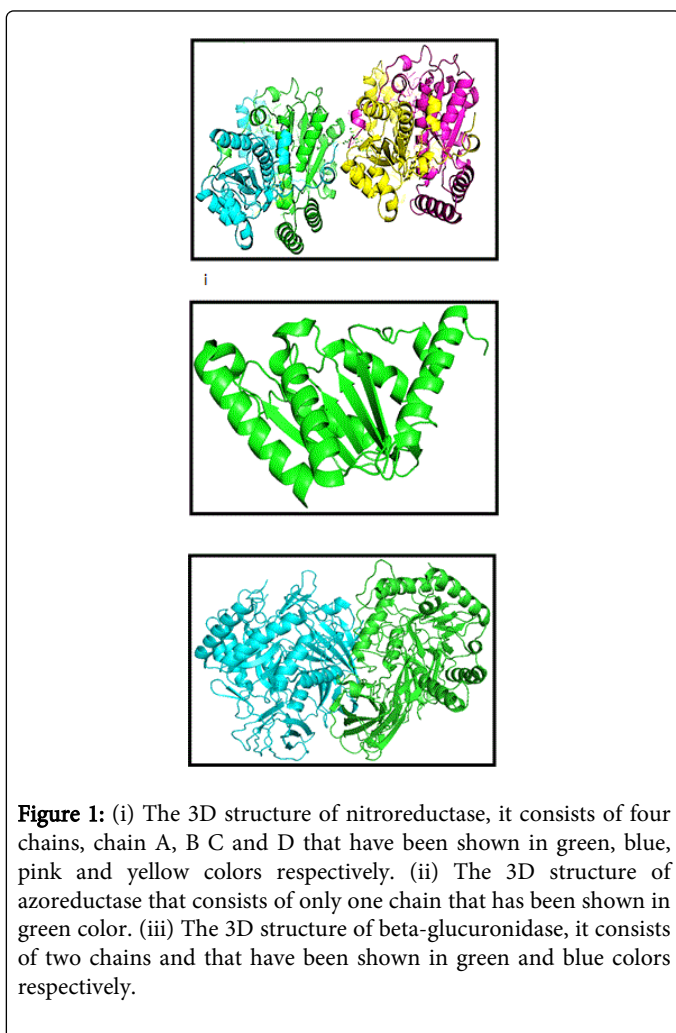
Materials and Methods

The structures of three enzymes nitroreductase, azoreductase and beta-glucuronidase were analyzed. Information regarding enzyme structures and their inhibitors was found from literature, Protein Data Bank (PDB) [9-11] and Chemspider [12-16]. The target structures were in pdb file format whereas the ligand structures obtained were in mol file format. These mol files were converted into pdb files using

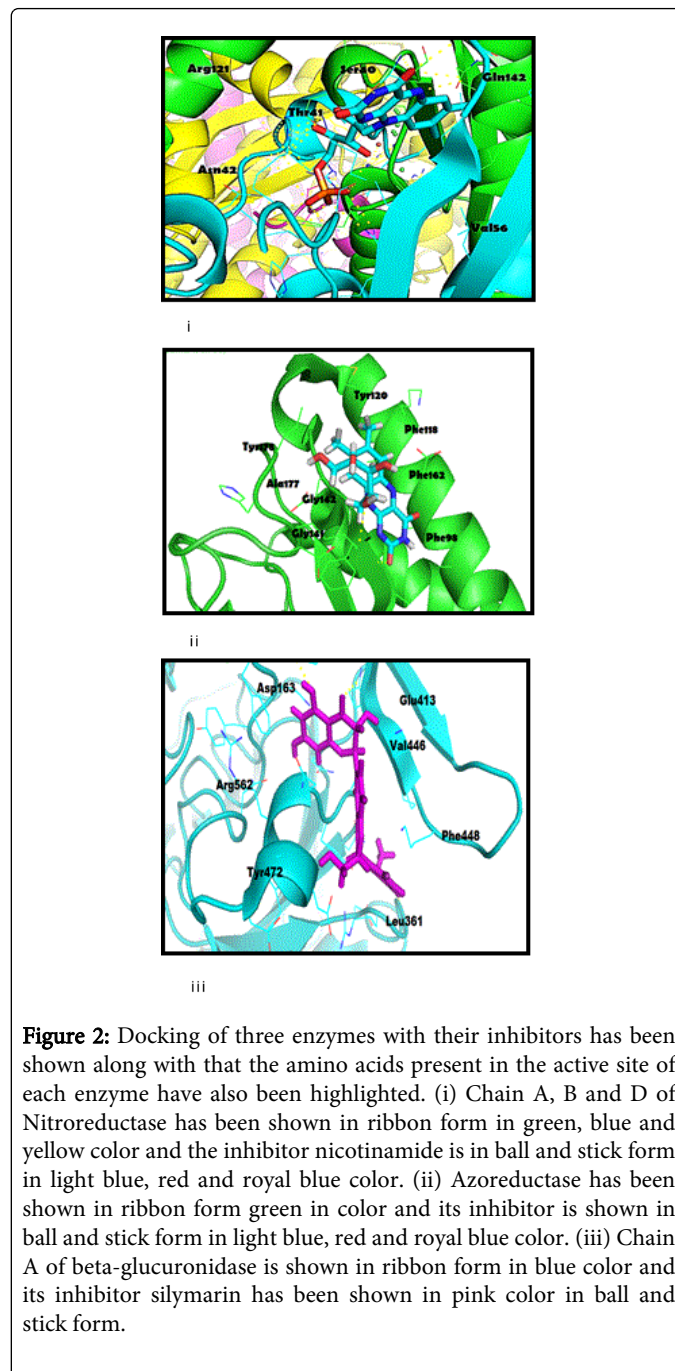
Online SMILES Translator and Structure File Generator [15]. Target sites of enzymes were then docked with the ligands using PatchDock [8,9]. After reviewing Dr. Duke's Phytochemical and Ethnobotanical Databases [14] and literature different plants were found to have these enzymes inhibitors [10]. The results for docking were analyzed using the software PyMOL [17].

Results

The structure obtained from PDB for nitroreductase was 1NEC [18], for azoreductase was 2Z9C [19] and for beta-glucuronidase was 3K46 [20]. One inhibitor against nitroreductase, two against azoreductase and three against beta-glucuronidase were selected and docking was done. The active site blocker nicotinamide for nitroreductase; riboflavin and allopurinol for azoreductase and aspartic acid, glucuric acid and silymarin for beta-glucuronidase were analyzed. It was found that the inhibitors blocked the active sites of their respective enzymes. The 3D structure of all three enzymes has been shown in the Figure 1.



The docking of enzymes with their inhibitors: nitroreductase with nicotinamide, azoreductase with riboflavin and beta-glucuronidase with silymarin has been shown in the figure. The amino acids present in the active sites have also been highlighted in the Figure 2.



Discussion

Results analysis revealed that these inhibitors bound to the active sites of the microbial enzymes, thus inhibiting their activity. The amino acids present in the active sites of the enzymes azoreductase and beta-glucuronidase interacted with the inhibitors as reported earlier [19,12]. However, in case of nitroreductase, in addition to Ser40 and Thr41 [13], some other amino acids (Asn42, Arg121, Gln142 and Val56) were also observed to be present in close proximity with the inhibitor nicotinamide. This suggests that nicotinamide might be a good inhibitor as it interacts efficiently with the amino acids that present in the active site. As a result of the inactivation of these

enzymes, dysbiosis can be prevented. These inhibitors are usually found naturally in certain plants.

Beta-glucuronidase inhibitors such as Aspartic acid, Glucaric acid and Silymarin are commonly present in vegetarian food as Aspartic acid is present in leaves of *Ipomoea aquatica* (Swamp Cabbage, Water Spinach); Sprout Seedling of *Phaseolus vulgaris subsp. var. vulgaris* (beans), *Vigna radiata* (L.) (Green Gram, Mungbean), and *Lens culinaris* (Lentil); Seeds of *Glycine max* (L.) (Soybean), *Arachis hypogaea* L. (Groundnut, Peanut), *Juglans cinerea* L (Butternut), *Vigna radiata* (L) (Green Gram, Mungbean), *Cucurbita pepo* L. (Pumpkin), *Citrullus lanatus* (Watermelon), *Helianthus annuus* L. (Sunflower) and *Prunus dulcis* (Almond); Roots of *Rehmannia glutinosa* (Chinese Foxglove); Tuber of *Solanum tuberosum* L. (Potato) and Whole plant of *Spinacia oleracea* L. (Spinach).

Glucaric acid can be found in vegetables and fruits like Brassica oleracea var. italica (broccoli), *Solanum tuberosum* L. (Potato) and *Citrus sinensis* (oranges). Similarly, seeds of *Silybum marianum* (L.) (Milk Thistle) contain Silymarin.

Azoreductase enzyme can be inhibited by riboflavin and allopurinol. To uptake Riboflavins a variety of plant parts can be used in the diet such as, leaves of *Justicia pectoralis* (Angel Of Death, Bolek Hena, Curia), *Chenopodium album* L. (Lambsquarter), *Momordica charantia* L. (Bitter Melon, Sorosi), *Malva sylvestris* L. (High Mallow), *Mentha x piperita subsp. nothosubsp. piperita* (Peppermint), *Colocasia esculenta* (L.) (Taro) and *Corchorus olitorius* L. (Jew's Mallow, Mulukiya, Nalta Jute); Silk, Stigma and Style of *Crocus sativus* L. (Saffron); Shoots of *Phytolacca americana* L. (Pokeweed) and *Asparagus officinalis* L.(Asparagus); Gum of *Sterculia urens* (Karaya) while whole plant of *Thymus vulgaris* L. (Thyme), *Spirulina pratensis* (Spirulina), *Triticum aestivum* L. (Wheat) and *Apium graveolens* L. (Celery) can be used for this purpose.

For another inhibitor of azoreductase i.e. *Allopurinol*, seeds of *Cowpea*, *Soybean*, and *Medicago truncatula* are useful.

Theobroma cacao L. (Cacao) and *Pisum sativum* L. (Pea) seeds play an important role in the inhibition of Nitroreductase as they contain nicotinamide (inhibitor of nitroreductase) in them.

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