

***In silico* Analysis of Natural Compounds as Modulators of Type I Collagen**

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Abstract

Collagen plays a vital role in the maintenance of structure and function of a human body. It has been widely applied in various fields including biomedical, cosmeceutical, food, pharmaceutical and tissue engineering. In the present study, the docking behaviour of type I collagen with 15 different ligands namely hydroxymethylfurfural, methylglyoxal, methylsyngate, *O*-methoxyacetophenone, 3-phenyllactic acid, 4-hydroxybenzoic acid, kojic acid, lumichrome, galangin, artoindonesianin F, caffeic acid, 4-coumaric acid, origanol A, thymoquinone and quercetin was evaluated along with their putative binding sites using Discovery Studio Version 3.1. Docking studies and binding free energy calculations revealed that origanol A has maximum interaction energy (-40.48 kcal/mol) and quercetin with the least interaction energy (-15.44 kcal/mol) as compared to the other investigated ligands. Three ligands which are galangin, methylsyngate and origanol A were shown to interact with Asp21 amino acid residue of chain B (type I collagen). Therefore, it is strongly suggested that the outcomes from the present study might provide new insight in understanding these 15 ligands as potential type I collagen modulators for the prevention of collagen associate disorders.

Key words: Collagen, 4-coumaric acid, Kojic acid, Origanol A, Quercetin; Docking

1. Introduction

Collagen is a ubiquitous extracellular protein, which plays a vital role in physiological and pathological processes in humans^[1]. Collagen is predominantly present in all connective tissues including blood vessels, bone, cartilage, tendon and skin. To date 26 different types of collagen have been reported of which Type-I collagen (2 α 1 and 1 α 2 chains) is the most abundant collagen in the human body. This type is mainly present in tendons, skin (reticular dermis), artery walls, endomysium of myofibrils, fibrocartilage, and the organic part of bones and teeth. Ramachandran and Kartha^[2] were the first to report triple helical structure of the collagen; thereafter

many studies have made progress on its structure and molecular properties. Miller and Scheraga^[3] were the pioneers in the computational modeling of collagen. Two years later, Piez and Truoz^[4] constructed three-dimensional energy-minimized model for calf-skin (Type-I collagen). Interestingly, Chen and co-workers^[5] performed molecular docking with micro fibril template based on the smith model and which was later followed by Qi and Brown^[6] who constructed the *N*-terminal telopeptides model.

Honey is one of the nutritious foods which have cultural and religious importance. For instance in the Quran, it has been recognized as a healthy and nutritious food. “Madhu Abhishekam” is a Hindu ritual wherein honey is offered to deities. Traditionally slices of apple dipped in honey are consumed by Jewish people as part of the welcoming event of the New Year called “Rosh Hashana”^[7]. Malaysia is one of the Asian countries that is well known for its varieties of honey types including Acacia, Belimbing, Coconut, Gelam, Kelulut, Nenas, Pineapple and Tualang. Gelam honey (*Melaleuca* spp.) was reported to have wound healing, anti-inflammatory and anti-oxidant activities^[8]. Honey

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produced by stingless bee (*Trigona* spp.) commonly known as Kelulut in Malay, has been reported to have antimicrobial^[9] and antibacterial activity against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Pseudomonas aeruginosa* organisms^[10]. Apart from this, metabolites of honey which are artoindonesianin F (*Artocarpus heterophyllus* Lam) and origanol A (*Origanum vulgare* Linn) were reported to have tyrosinase^[11] and elastase inhibition^[12] activities, respectively. Since honey is known to stimulate collagen synthesis and wound healing, nine selected constituents of honey namely hydroxymethylfurfural, methylglyoxal, methylsyringate, *O*-methoxyacetophenone, 3-phenyllactic acid, 4-hydroxybenzoic acid, kojic acid, lumichrome, galangin, along with artoindonesianin F, caffeic acid, 4-coumaric acid, origanol A, thymoquinone and quercetin were selected to be evaluated. Hence, these constituents were evaluated on the docking behaviour of type I collagen and investigation also carried out on their putative binding sites using Discovery Studio Version 3.1.

2. Materials and methods

2.1. Ligands Preparation

Chemical structures of the 13 ligands namely i) hydroxymethylfurfural [Chempid ID 207215]; ii) methylglyoxal [Chempid ID 857]; iii) methylsyringate [CID70164]; iv) *O*-methoxyacetophenone [CID68481]; v) 3-phenyllactic acid [CID3848]; vi) 4-hydroxybenzoic acid [CID135]; vii) kojic acid [CID3708]; viii) lumichrome [CID4483963]; ix) galangin [CID4444935]; x) caffeic acid [CID689043]; xi) 4-coumaric acid [Chempid ID553148]; xii) thymoquinone [CID no: 10281] and xiii) quercetin [Chempid ID: 4444051] were retrieved from Chempid (www.chemspider.com) and Pubmed (www.pubmed.com) compound databases. Unavailable three dimensional structures of artoindonesianin F and origanol A were generated as previously reported^[12].

2.2. Target Protein Preparation

In the present study, 24-mer collagen triple helix was constructed by Object Technology Framework (OTF) using the GENCOLLAGEN package (<http://www.cgl.ucsf.edu/cgi-bin/gencollagen.py>). The 24-residue long triple helix constructed corresponds to the residues 193 to 216 (2 α 1 and 1 α 2 chains) of the native type I col-

lagen except for the residue 204 of the α 1 chain. The alanine amino acid of native collagen was replaced by lysine (basic amino acid) in order to study the interaction of ligands with the side chains of basic amino acids^[13].

2.3. Docking Studies

Docking studies were carried out on the prepared protein (type I collagen) using the CDOCKER protocol under the protein-ligand interaction section in Discovery Studio[®] 3.1 (Accelrys, San Diego, USA). In general, CDOCKER is a grid-based molecular docking method that employs CHARMM force fields. This protein was firstly held rigid while the ligands were allowed to flex during the refinement. Two hundred random ligand conformations were then generated from the initial ligand structure through high temperature molecular dynamics followed by random rotations, refinement by grid-based (GRID I) simulated annealing, and a final grid-based or full force field minimisation^[14]. In this experiment, the ligand was heated to the temperature of 700 K in 2000 steps. The cooling steps were set to 5000 steps to 300 K cooling temperature. The grid extension was set to 10 Å, and hydrogen atoms were added to the structure and all ionisable residues were set at their default protonation state of a neutral pH. For each ligand, top ten ligand binding poses were ranked according to their CDOCKER energies, and the predicted binding interactions were analysed. The best among the ten ligand binding poses was chosen and carried out *in situ* ligand minimization using a standard protocol.

3. Results and Discussion

Collagen is the major structural protein which accounts for 30 % dry weight of the human body. Any defects in collagen^[15] will result in genetic disorders (such as Alport syndrome, Ehlers-danlos syndrome, Marfan syndrome, Stickler syndrome, Epidermolysis bullosa and Osteogenesis imperfect) and autoimmune disorders (oral submucous fibrosis and systemic sclerosis). In the present study 15 ligands were selected and the natural sources of these ligands are as depicted in the Table 1. 3-Phenyllactic acid, lumichrome, galangin and quercetin are flavonoids in the chemical group. Artoindonesianin F is a flavone which belongs to the prenylated flavones category. Methylsyringate, 4-

Table 1. The botanical source of the fifteen ligands

Ligand name	Nature of chemical class	Natural source
Hydroxymethylfurfural	Furaldehyde	<i>Cornus officinalis</i> Sieb. et Zucc., <i>Jaborosa magellanica</i> Griseb., <i>Laurenica undulate</i> , <i>Schisandra</i> spp., Honey, Fruit-juice and ultra high temperature processed milk.
Methylglyoxal	Aldehyde	Honey
Methylsyringate	Phenol	Honey
<i>O</i> -methoxyacetophenone	Acetophenone	Honey
3-phenyllactic acid	Flavonoid	Honey and Lactic acid bacteria fermented food.
4-hydroxybenzoic acid	Phenol	<i>Cocos nucifera</i> L., <i>Hypericum perforatum</i> Linn., <i>Macrotyloma uniflorum</i> Lam., <i>Phyllanthus acidus</i> L., <i>Vitex negundo</i> Linn., <i>Spongiochloris spongiosa</i> and honey.
Kojic acid	Pyrone	<i>Aspergillus</i> spp., <i>Acetobacter</i> spp., <i>Penicillium</i> spp., and honey.
Lumichrome	Flavonoid	Honey
Galangin	Flavonoid	<i>Alpinia officinarum</i> Hance, <i>Helichrysum aureonitens</i> Sch. Bip., Propolis and honey.
Artoindonesianin F	Flavones	<i>Artocarpus heterophyllus</i> Lam.
Caffeic acid	Phenol	<i>Coffea arabica</i> L., <i>Eucalyptus globulus</i> Labail, <i>Hordeum vulgare</i> L <i>Phellinus linteus</i> and <i>Salvinia molesta</i> D.S. Mitch.
4-coumaric acid	Phenol	<i>Arachis hypogaea</i> L., <i>Phaseolus vulgaris</i> L., <i>Allium sativum</i> L., <i>Solanum lycopersicum</i> L., <i>Coffea arabica</i> L., <i>Hordeum vulgare</i> L and honey.
Origanol A	Phenolic glucoside	<i>Origanum vulgare</i> L.
Thymoquinone	Quinone	<i>Nigella sativa</i> Linn.
Quercetin	Flavonoid	Many fruits, vegetables, grains and honey.

hydroxybenzoic acid, caffeic acid and 4-coumaric acid are phenolic compounds. The docking studies and binding free energy calculations as in Table 2 show origanol A of having the highest binding energy (-40.48 kcal/mol) while quercetrin gave the least binding energy (-15.44 kcal/mol). Three ligands namely galangin, methylsyringate and origanol A was shown to have interaction with Asp21 amino acid residue of chain B (type I collagen). Artoindonesianin F and quercetrin interacted with Asp21 and Gly22 amino acid residues of chain C. Four ligands which are methylglyoxal, *O*-methoxyacetophenone, kojic acid and thymoquinone did not interact with any of the amino acid residues (Table 2). The other four ligands, hydroxymethylfurfural, 3-phenyllactic acid, 4-hydroxybenzoic acid and 4-coumaric acid were shown to have π - π interaction with Tyr24 amino acid residue (Table 2). Suguna and co-workers^[16] reported that honey accelerates the synthesis

and maturation of collagen which thereby enhances the wound healing process. Many compounds in honey have been reported to have effect on collagen, such as 2, 5-dihydroxyphenyl acetic acid has ability to bind with collagen^[17], kojic acid shown to inhibit the human prolyl-4-hydroxylase enzyme activity which is responsible for collagen hydroxylation^[18] and alpha-hydroxy acids (glycolic acid, lactic acid and citric acid) increases density of collagen in photoaged skin^[19]. Catechin was reported to bind with collagen^[20], whereas asiaticoside induces type I collagen biosynthesis^[21]. Similarly, D (+) β -3, 4-dihydroxyphenyl lactic acid has been reported to stimulates type I collagen mRNA expression^[22]. Acetophenone was reported to induce collagen binding^[23], while alginic acid^[24] and embelin^[13] have been reported to bind with the collagen. The compound 4 hydroxy-3-methoxyacetophenone in honey was reported as an anti-arthritis agent^[25].

Table 2. Binding energy analysis of fifteen ligands with that of type I collagen using Discovery Studio® 3.1

Ligand name	-cDock interaction energy (kcal/mol)	Interaction amino acid residue	Bond distance (Å)
Hydroxymethylfurfural	19.72	TyrC24*	4.0
Methylglyoxal	17.62	No binding	-
Methylsyngate	21.52	AspB21	2.0
<i>O</i> -methoxyacetophenone	17.21	No binding	-
3-phenyllactic acid	24.30	TyrA24*	4.8
		TyrC24*	5.7
4-hydroxybenzoic acid	19.38	TyrC24	2.4
		TyrA24*	4.0
Kojic acid	17.69	No binding	-
Lumichrome	16.89	GlnB23	2.2
		LeuC23	2.4
Galangin	15.66	GlyB19	1.4
		AspB21	2.1
Artoindonesianin F	30.34	AspC21	1.2
		GlyC22	1.6
Caffeic acid	24.97	TyrC24	2.2
4-coumaric acid	20.18	TyrA24	2.4
		TyrA24*	4.7
Origanol A	40.48	AspB21	1.2
Thymoquinone	17.50	No binding	-
Quercetin	15.44	AspC21	1.6
		GlyC22	1.4

Note: * π - π interaction

4. Conclusion

In the present study, all of the tested ligands have shown to dock with type I collagen. However, four ligands which are methylglyoxal, *O*-methoxyacetophenone, kojic acid and thymoquinone did not interact with any amino acid residues of type I collagen. Origanol A exhibited the highest interaction energy (-40.48 kcal/mol) and quercetin in contrast showed the least interaction energy (-15.44 kcal/mol). The results from the present study provide a new insight in understanding these 15 ligands as the potential type I collagen modulators, in which the molecular docking studies could contribute for further development and understanding of the type I collagen modulators for the prevention of collagen associated disorders.

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