Journal of the Serbian Chemical Society
JSCS-info@shd.org.rs • www.shd.org.rs/JSCS

ACCEPTED MANUSCRIPT

Journal of the Serbian Chemical Society
JSCS-info@shd.org.rs • www.shd.org.rs/JSCS

J. Serb. Chem. Soc. **00(0)** 1-15 (2022)
JSCS–11964

Original scientific paper
Published DD MM, YYY

Deeper inside the use of chitooligosaccharides in wound healing process. A computational approach

MARIANA A. MATICA[1], DIANA L. ROMAN[2,3], VASILE OSTAFE[2,3] and ADRIANA ISVORAN[2,3]*

[1]Department of Chemistry-Biology, Institute for Advanced Environmental Research, West University of Timisoara, Oituz 4C, 300086 Timisoara, Romania; [2]Department of Biology-Chemistry, Faculty of Chemistry, Biology, Geography, West University of Timisoara, 16 Pestalozzi, 300115 Timisoara, Romania and [3]Advanced Environmental Research Laboratories (AERL), West University of Timisoara, Oituz 4A, 300086 Timisoara, Romania

(Received 2 July; Revised 13 November; Accepted 20 November 2022)

Abstract: Chitooligosaccharides (COs) containing up to 10 monomeric units of N-acetyl D-glucosamine and/or D-glucosamine are water-soluble molecules revealing numerous biological activities and low toxicological profiles. Within this study, a computational approach has been used to predict the involvement of the COs having distinct chemical properties (molecular weight, deacetylation degree and acetylation pattern) in all the four wound healing phases: hemostasis, inflamemation, proliferation and tissue remodeling. There are predictions, for the investigated COs, regarding their molecular targets and the biological activities that are reliant to the wound healing process. Furthermore, a molecular docking approach was used to assess the interactions of the investigated COs with the myeloid differentiation factor 2 (MD-2), a protein involved in the inflammatory processes. The investigation confirms the functional roles of the investigated COs in wound healing. The molecular targets predicted for the COs containing totally and partially acetylated units are galectins and selectins and those predicted for COs containing totally deacetylated units are fibroblast growing factors, the COs containing 3 units revealing the higher number of molecular targets. All these proteins are involved in mediating immune response, inducing cell division, growth and cell adhesion during the process of wound healing. All the COs containing from 2 to 8 monomeric units are able to interact with the MD-2 protein, the interactions being stronger for the COs containing 6 and 8 monomeric units. The interaction energies increase with the increasing molecular weight and with decreasing deacetylation degree and are reliant on acetylation patterns. Among the investigated COs, the totally acetylated COs containing 6 and 8 N-acetyl glucosamine units can be better inhibitors of the LPS binding to MD-2 protein. Consequently, mixtures of COs with distinct properties should be considered suitable candidates as adjuvants in developing scaffolds for the wound healing process.

*Corresponding author E-mail: adriana.isvoran@e-uvt.ro
https://doi.org/10.2298/JSC220702081M

Keywords: immune response; myeloid differentiation factor 2 (MD-2); inflammatory process; cell adhesion; molecular docking

INTRODUCTION

Chitin, the second most abundant biopolymer on Earth, consists of N-acetyl-D-glucosamine units. Chitin is difficult to use due to its poor solubility in most organic solvents and water. Its partial deacetylated form, the chitosan, is soluble in acidic environment, which makes it more easy to process and use.[1] Chitosan consists of D-glucosamine and N-acetyl-D-glucosamine, with at least a 60 % deacetylation degree (percent of deacetylated units in the polymer).[1] Both chitin and chitosan are biodegradable, biocompatible and non-toxic[2] polymers of a great medicinal and economical interest. Chitooligosaccharide is the term used in specific literature to describe chitosan oligosaccharides with a polymerization degree below 20 and an average molecular weight of up to 3.9 kDa.[3] Chitooligosaccharides (COs) are obtained by chitosan enzymatic or chemical degradation and are composed by oligomers of D-glucosamine (GlcN or D) and N-acetyl-glucosamine (GlcNAc or A).

COs with a maximum of 10 monomeric units are considered water-soluble molecules, with enhanced biological activities such as antimicrobial, anti-cancer, anti-inflammatory, stimulation of the immune system, blood pressure control and so on.[4] The chemical characteristics of the COs, based on the molar fraction of D-glucosamine in the molecule (deacetylation degree, DaD) and the pattern of N-acetylation (PA), have a great impact on their biological activities.[3] Furthermore, the position of the acetyl groups along the glycan chain strongly influences their biological activities.[5] A computational study exposed that COs, regardless of their physicochemical properties, revealed promising pharmacological profiles and few toxicological effects on humans: the inhibition of the organic anion transporting peptides OATP1B1 and/or OATP1B3, low potential of cardiotoxicity, and the COs with high DaD exposed the potential of producing phospholipidosis.[6] A molecular docking study revealed favorable interactions of COs with plasma proteins, the interaction energies increasing with the molecular weight (MW), decreasing with increasing DaD and being reliant on the PA.[7] Furthermore, other studies emphasized that the COs characteristics conducted to distinct affinities for chitin deacetylases[8] and lysozyme.[9]

The wound healing process depends on four wound healing phases: hemostasis, inflammation, proliferation and tissue remodeling. Scientific literature has shown that COs can be useful in all stages of wound healing having a hemostatic effect, being able to protect the wound from infections, to have anti-inflammatory and immunostimulatory activities, to stimulate healing by enhancing the permeability of air and moisture, to support cell adhesion and promote cell proliferation. The chemical characteristics of COs influence their wound healing effects.[10] COs used in the mentioned study are not well characterized in terms of their chemical properties, especially in terms of the acetylation pattern.

This study aims to predict the molecular targets and biological activities of the COs having various chemical properties, with a focus on their anti-inflammatory properties. One of the molecules responsible for triggering the inflammatory response is the lipopolysaccharide (LPS), present in the outer membrane of Gram-negative bacteria. If a wound gets infected by a Gram-negative pathogen, a very small amount of LPS is sufficient to initiate an immune response and the inflammatory process will begin.[11] Normally, the inflammatory process will help the human body to overcome the damage produced by the foreign pathogens, but when the inflammatory response is excessive, this can cause severe damage to tissues and organs. LPS are crucial for the pathogenesis of inflammation, and septic shock syndrome is often related to the uncontrolled inflammatory response to LPS.[11] Various LPS receptors and accessory proteins, like LPS binding protein (LBP), CD14 and the Toll-like receptor 4 (TLR4) - myeloid differentiation factor 2 (MD-2) complex, are involved in helping the immune system to recognize LPS. It was revealed that LPS has an analogous affinity for MD-2 as for the TLR4-MD-2 complex indicating that MD-2 is the component assuring the LPS binding.[12] COs have been proven to inhibit the inflammatory process triggered by LPS[10]. Literature data reveal that COs, usually containing between 2 and 8 monomeric units and characterized by a deacetylation degree of 95 % and uncharacterized acetylation pattern, inhibit the binding of LPS to the TLR4–MD-2 receptor complex reducing the production of pro-inflammatory mediators.[13]

Within this study, a computational approach is considered in order to enhance the knowledge regarding the influence of the chemical properties of COs on the wound healing process and to emphasize the best composition of COs to increase the wound healing effect. COs having various MW (but no more than 8 monomeric units), DaD and AP are considered, their molecular targets and biological activities related to the wound healing process are predicted, respectively their anti-inflammatory effect through interactions with MD-2 protein using a molecular docking approach is assessed.

EXPERIMENTAL

Within the present study, COs having between 2 and 8 monomeric units were considered as it was specified in the scientific literature that they are efficient for wound healing and especially for the anti-inflammatory effect.[13] As open wounds usually have a neutral to alkaline pH and chronic wounds exist at alkaline pH[14], COs with the amino groups that are not protonated have been considered. The simplified molecular-input line-entry system (SMILES) formulas of the COs under investigation (TABLE I) were built using ACD/ChemSketch software (https://chemicalize.com). The COs 3D structures were obtained and minimized using Chimera software.[15]

In order to predict the COs molecular targets, the Swiss Target Prediction tool has been considered.[16] It allows predictions of the macromolecular targets of bioactive small molecules and is based on the similarity principle, meaning that two similar bioactive molecules are probable to share their molecular targets. In this study we have considered human targets in the

top 15 predictions being known that the level of predictive performance is usually higher than 70 % in this case. Only the molecular targets that can be involved in the wound healing process have been listed.

TABLE I. Chitooligosaccharides considered in this study (A – unit of N-acetyl-glucosamine, D – unit of glucosamine, DaD – deacetylation degree)

DaD = 0	DaD = 33 %	DaD = 50 %	DaD = 67 %	DaD = 100 %
2A, 3A, 4A, 5A, 6A, 8A	ADA	DA, AADD, ADAD, ADDA, DAAD, DDAA, DADA, ADADAD, DADADA, DADADADA	DDA, DDDADA ADDDAD	2D, 3D, 4D, 5D, 6D, 8D

Prediction of Activity Spectra of Substances (PASS) computational tool[17] has been considered for predicting the biological activities of the investigated COs. It estimates the probability that the query molecule belongs to the particular class of active (Pa) or inactive (Pi) compounds based on the analysis of structure activity-relationships for more than 250,000 biologically active substances. The values of the two probabilities (Pa and Pi) vary from 0 to 1 and are independent. Only those activities with Pa > Pi are considered promising for a particular compound and a good accuracy of prediction is obtained when Pa > 0.7. The average accuracy of prediction estimated for the whole PASS training set is about 95 %.[17] Only the biological activities related to the wound healing process have been listed in this article.

The molecular docking approach has been used for assessing the possible inhibitory effect of COs having various MW, DaD and AP against MD-2 protein. The crystallographic structure of the TLR4-MD-2 complex with the eritoran as a ligand bound to MD2 has been extracted from Protein Data Bank (PDB ID 2Z65). Eritoran is an analog of LPS antagonizing its activity by binding to the TLR4-MD-2 complex, this binding being mediated by the hydrophobic internal pocket in MD-2 and the opening region of the pocket containing positively charged residues,[18] as visualized in Fig 1 using Chimera software.[15] Eritoran occupies almost all the volume of the binding pocket.

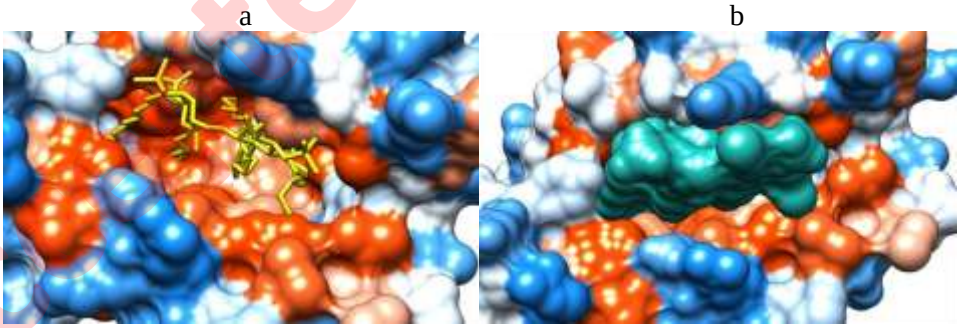

Fig 1. Visualization of the hydrophobicity surface (blue regions are hydrophilic and orange regions are hydrophobic) of the MD-2 protein in complex with eritoran (PDB ID 2Z65) revealed as yellow sticks (**a**) and green surface (**b**).

The MD-2 structure has been considered as target molecule and was prepared for docking using Chimera software. Molecular docking has been implemented using the SwissDock facility[19] that proposes binding modes for the ligand to the target and computes the energy of

the interactions. A blind and accurate docking protocol has been considered. Chimera software has been used for the analysis of docking results. To evaluate the docking results and characterize the molecular interactions in the complexes between COs and MD-2 protein that were obtained by molecular docking, the PLIP (protein-ligand interaction profiler) software has been considered with default settings.[20] This software yields the list of detected interactions on a single atom level and enables analyses of specific binding characteristics. Seven interaction types can be detected by using this software: hydrophobic contacts, hydrogen bonds, salt bridges, water bridges, pi-stacking, pi-cation interactions, and halogen bonds.

RESULTS AND DISCUSSION

The molecular targets predicted for the investigated COs using Swiss Target Prediction tool and that are relevant for wound healing are revealed in TABLE II. The probability of every prediction is also presented. Preceding studies demonstrated that any value above 0 for the probability was considered to be reasonable.[16] The molecular targets for COs containing more than 6 monomeric acetylated units were not predicted due to their high molecular weight.

Data presented in TABLE II reveal distinct molecular targets for the totally deacetylated COs by comparison to totally and partially acetylated COs. Moreover, there are distinct values for the probabilities of the predicted molecular targets for COs with various chemical properties. COs containing 3 monomeric units, regardless of their acetylated status, reveal the higher values for the probabilities. Among the COs containing 3 monomeric units totally or partially acetylated, the totally acetylated COs reveal the wider spectra of the molecular targets.

In the case of totally and partially acetylated COs, galectins are the main group of proteins considered molecular targets. Galectins are β-galactoside-binding proteins that modulate re-epithelialization, an important stage in wound healing, via a carbohydrate-based recognition system.[21] From the group of galectins, totally and partially deacetylated COs target galectin-3, galectin-4, and galectin-8. Galectin-3 is involved in the acute inflammatory response, triggering neutrophil activation and adhesion being also responsible for macrophage and monocytes chemotaxis. Galectin-4 is linked with gastro-intestinal tract wound healing, being expressed particularly in GI tissue, and it promotes both cell migration and cell proliferation. Galectin-8 mediates the cell adhesion process through interactions with integrins, other cell-surface proteins.[21]

Selectins, other targeted proteins by totally and partially acetylated COs, are adhesion molecules that regulate the leukocyte migration, from the circulatory system to inflammatory sites.[22] Is it is well known that the lack of adhesion molecules lead to suppressed angiogenesis, keratinocyte migration, granulation tissue formation and inhibits early wound healing, decreased the growth factor expression and inflammatory cell infiltration.[23]

TABLE II. The predicted molecular targets that are involved in wound healing of the investigated chitooligosaccharides. Between parentheses are the probability value for every predicted target.

COs	Predicted molecular targets and the probability of the predictions
	Totally acetylated COs
2A	galectin-3 (0.297), galectin-4 (0.104), galectin-8 (0.104)
3A	galectin-3 (0.219), galectin-4 (0.152), galectin-8 (0.152), myelin-associated glycoprotein (0.066), selectin E (0.066), adenosine A1 receptor (0.066), adenosine A2a receptor (0.066), adenosine A3 receptor (0.066), galectin-9 (0.066)
4A	galectin-3 (0.020), adenosine A1 receptor (0.012)
5A	galectin-4 (0.122), galectin-8 (0.122), galectin-3 (0.064), leukocyte adhesion molecule-1 (0.064), selectin E (0.064), P-selectin (0.064)
	Partially acetylated COs
DA	galectin-4 (0.166), galectin-8 (0.166), galectin-3 (0.109), selectin E (0.109)
ADA	galectin-4 (0.170), galectin-8 (0.170), galectin-3 (0.105)
DDA	galectin-4 (0.208), galectin-8 (0.208), galectin-3 (0.175), myelin-associated glycoprotein (0.126), selectin E (0.126)
ADAD, DADA, AADD, DAAD, DDAA	galectin-4 (0.118), galectin-3 (0.118), galectin-8 (0.118), leukocyte adhesion molecule-1 (0.050), selectin E (0.050), P-selectin (0.050)
ADDA	galectin-4 (0.118), galectin-8 (0.118), galectin-3 (0.060), leukocyte adhesion molecule-1 (0.050), selectin E (0.050)
ADADAD, ADDDAD, DDDADA	galectin-4 (0.122), galectin-3 (0.122), galectin-8 (0.122), myelin-associated glycoprotein (0.064), leukocyte adhesion molecule-1 (0.064), selectin E (0.064), P-selectin (0.064)
	Totally deacetylated COs
2D	vanilloid receptor (0.127), acidic fibroblast growth factor (0.102), vascular endothelial growth factor A (0.102), heparanase (0.102), heat shock protein HSP 90-alpha (0.102)
3D	vanilloid receptor (0.128), vascular endothelial growth factor A (0.120), basic fibroblast growth factor (0.120), heparanase (0.120), acidic fibroblast growth factor (0.120), heat shock protein HSP 90-alpha (0.120)
4D	vanilloid receptor (0.084), vascular endothelial growth factor A (0.056), basic fibroblast growth factor (0.056), heparanase (0.056), acidic fibroblast growth factor (0.056), Hheat shock protein HSP 90-alpha (0.056)
5D	vanilloid receptor (0.022), vascular endothelial growth factor A (0.022), basic fibroblast growth factor (0.022), heparanase (0.022), acidic fibroblast growth factor (0.022), heat shock protein HSP 90-alpha (0.022)
6D	vanilloid receptor (0.083), vascular endothelial growth factor A (0.064), basic fibroblast growth factor (0.064), heparanase (0.064), acidic fibroblast growth factor (0.064), heat shock protein HSP 90-alpha (0.064)

Adenosine receptors, which are considered molecular targets of the COs containing 3 acetylated units, are linked to the accelerated wound healing process. They are initiators of the first stage of the wound healing process and can promote

cell migration, cell proliferation, growth factor secretion and angiogenesis.[24] The leukocyte adhesion molecules play important roles in hemostasis, wound healing, morphogenesis, maintenance of tissue architecture.[25] The myelin-associated glycoproteins are involved in axon regeneration and cell adhesion.[26]

Growth factors are the main group of targets for totally deacetylated COs. They are signaling molecules characterized by chemotactic activities that attract fibroblast and inflammatory cell to the wound site, can stimulate angiogenesis and cell proliferation.[27]

Heat shock protein-90α (Hsp-90α), another target of the totally deacetylated COs, is a possible enhancer of wound closure by promoting cell survival and cell motility.[28] Keratinocyte-secreted Hsp90α is involved in wound closure[29], its expression being induced by heat stress and wounding of the epidermis. Studies have shown the great potential of Hsp-90α in treating different types of skin wounds and topical application of Hsp90α improved the wound healing time.[28]

Heparanase and vanilloid receptor are other molecules targeted by totally acetylated COs. Heparanase is responsible for mediating cell adhesion and migration[30] and the vanilloid receptor is linked to pain management involving different types of wounds.[31] Similar molecular targets have been predicted for a totally deacetylated chitooligosaccharide containing 9 units, chondroitin sulphate and agar.[32]

Several of these predicted molecular targets have been already noticed in specific literature and it underlines the validity of the predictions. The interactions between oligosaccharides and various galectins have been revealed, the preferred ligands of galectins being N-acetyl lactosamine and related disaccharides.[33] Literature data also reveal that chitosan was able to interact with fibroblast growth factor-2 and consequently protect it from inactivation[34] and the inhibition of heparanase by COs has been already emphasized.[35] Experimental studies demonstrated that the COs having molecular weights of 800 Da and prepared by degradation of chitosan with a DaD of 92.3 % promoted peripheral nerve regeneration with functional recovery in rats having sciatic nerve crush injury.[36] Similar results were obtained in other studies, therefore COs accelerated peripheral nerve regeneration, improved the number of myelinated nerve fibers and increased the thickness of regenerated myelin sheaths in rabbits, promoted Schwann cell proliferation, enhanced the axonal myelination, increased the expression of cell adhesion proteins and increased cell survival in a dose-dependent manner.[37]

The outcomes obtained using PASS software and regarding the wound healing of investigated COs are revealed in TABLE III. Only the activities predicted with a probability of being active (Pa) higher than 0.7 are revealed, being known that a good accuracy of prediction is obtained when Pa > 0.7. Because of the molecular weight limit of the PASS software, there are no predictions for the COs containing 8 monomeric units.

TABLE III. Predicted wound healing activities of investigated COs using PASS software. Pa is the probability to be active and is shown between parentheses for every predicted activity.

COs	Predicted wound healing activities
2A – 6A	hyaluronic acid agonist (0.894), angiogenesis stimulant (0.799), membrane integrity agonist (0.793), macrophage stimulant (0.781)
DA, ADA, DDA, AADD, DADA, ADAD, DAAD, DDAA, ADDA, ADADAD, DADADA, ADDDAD, DDDADA	macrophage stimulant (0.872), hyaluronic acid agonist (0.859), angiogenesis stimulant (0.772)
2D – 6D	macrophage stimulant (0.916), hyaluronic acid agonist (0.795), angiogenesis stimulant (0,781), membrane integrity agonist (0,719), transcription factor stimulant (0.711)

Prediction obtained using PASS shows that all investigated COs, regardless of their chemical properties, exhibit a variety of biological activities regarding the wound healing process. Aspects such as membrane integrity, hyaluronic acid agonists, angiogenesis, growth factor, and macrophage stimulants, are among those biological processes that are very important in wound healing. Membrane integrity is an essential condition for maintaining the cell viability and normal functions. Hyaluronic acid promotes wound healing and accelerates wound healing even in chronic wounds.[38] The totally deacetylated COs act as transcription factor stimulants as well. Transcription factor NF kappa B stimulant for example, mediates the wound healing through anti-inflammatory and anti-oxidant effects.[39]

Among these predicted wound healing activities, macrophage and angiogenesis stimulant activity have been reported. An experimental *in vitro* study revealed that COs having distinct polymerization degrees could promote the proliferation of macrophages cells and stimulate angiogenesis, the best results being obtained for chitopentaose hydrochloride.[40] COs also revealed anti-angiogenic effects [35]

The outcomes of the molecular docking study reveal that investigated COs are able to bind to the active site of the MD-2 protein. These results are in strong correlation with experimental data revealing that COs significantly inhibited binding of LPS to TLR4/MD-2 complex.[13] Fig. 2 present the best binding position of the 6A and respectively 6D chitooligosaccharides to MD-2 protein and the Fig. 3 illustrates the registered binding energies for the best binding poses of all investigated COs to MD-2 protein.

Fig. 2 shows that both 6A and 6D COs bind to the active site of MD-2 protein, but 6A chitooligosaccharide occupies almost the entire internal surface of the cavity and 6D chitooligosaccharide occupies only a region of the cavity. This is consistent with the values of the Gibbs energy revealing a stronger interaction between the 6A and MD-2, ΔG = -46339 J mol^{-1}, compared with ΔG = - 35246.12 J mol^{-1} for binding of 6D to MD-2.

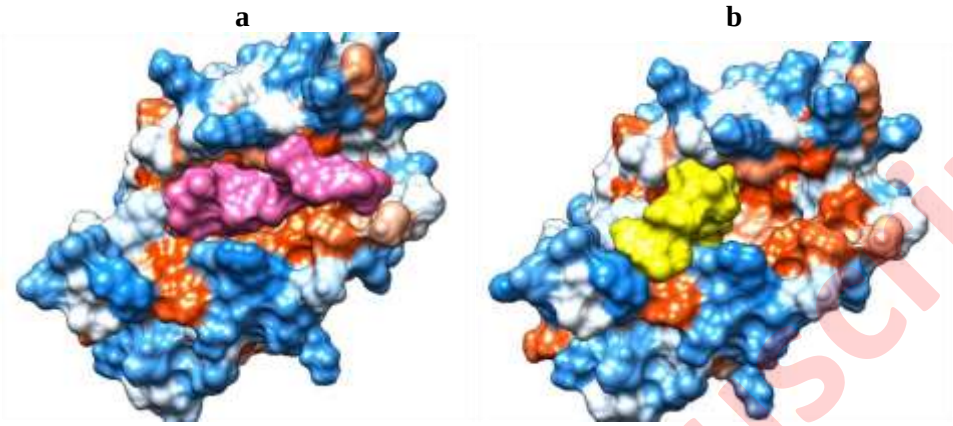

Fig 2. Visualization of the best docked binding poses to MD-2 protein of:
(a) the chitooligosacharide containing 6 acetylated units (6A) visualized as magenta surface;
(b) the chitooligosaccharide containing 6 deacetylated units (6D) visualized as yellow surface.
MD-2 protein is visualized as hydrophobicity surface, blue regions are hydrophilic and orange regions are hydrophobic

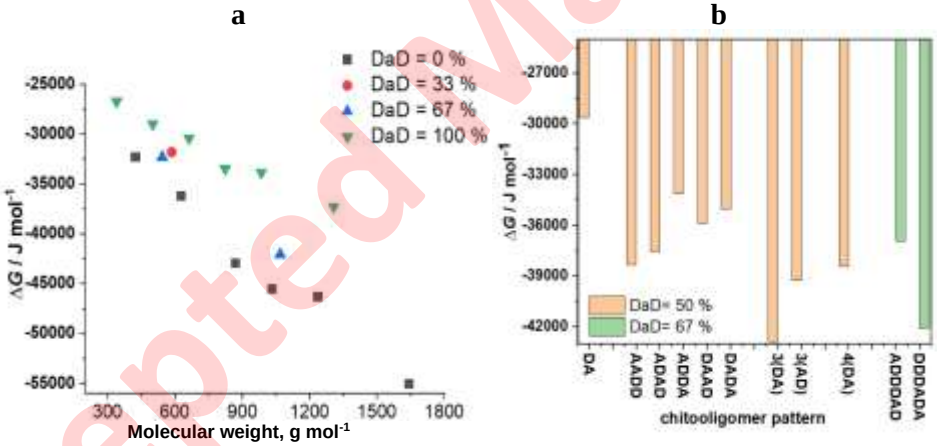

Fig 3. The interacting energies obtained for the best binding poses of COs to MD-2 protein taking into account: (**a**) the molecular weight and deacetylation degrees (DaD) and (**b**) the deacetylation degree and acetylation pattern

Data presented in Fig 3a reveal that interacting energy between COs and MD-2 protein increases with the molecular weight, chitooligosaccharides containing 6 and 8 monomeric units reveal stronger interactions. The interaction energies also increase with decreasing deacetylation degree, the totally acetylated chitooligosaccharides proving to be better inhibitors of the LPS binding to MD-2 protein. This result is in good agreement with the hydrophobic nature of the active site cavity of the MD-2 protein, as the acetylated COs have a higher hydrophobicity

than deacetylated ones. Fig 3b emphasizes that, for the same DaD and MW, the acetylation pattern also influences the interactions of COs with MD-2 protein. Quite similar results have been obtained when assessing the interactions of COs having various chemical properties with human and hen egg-white lysozymes. Therefore, COs containing only GlcNAc units revealing higher interaction energies with the two proteins compared with COs containing only GlcN units, and the selectivity of the interactions was dependent on the MW, DaD and AP of COs.[9] It was also true for the interactions between the COs and human plasma proteins (alpha-1 acid glycoprotein and human serum albumin), the interacting energies increased with the molecular weight and with decreasing of deacetylation degree and were dependent on the AP.[7] In addition, a molecular docking study suggested that chitin deacetylases from fungi and marine bacteria were able to bind both totally acetylated and partially acetylated COs, but the binding energy was usually higher in the cases of the interactions with totally acetylated COs.[8] Not at last, COs with different chemical properties revealed quite distinct pharmacological profiles.[6]

Results obtained using PLIP software and regarding the types of the interaction between the investigated COs and MD-2 and the amino acids involved in these interactions are presented in TABLE IV.

Data presented in TABLE IV reveal that the interactions between totally deacetylated COs and MD-2 protein are based on hydrogen bonds, whereas partially and totally acetylated COs form both hydrophobic interactions and hydrogen bonds with MD-2. The amino acids identified as being involved in the COs interactions with MD-2 usually are among those involved in the interaction of MD-2 with eritoran, the ligand that is present in the crystallographic structure. It underlines that COs are able to interact with MD-2 protein.

The limitations of this study are common to all *in sillico* studies, the predictions of the molecular targets and biological activities are totally dependent on the models used by the computational programs and do not allow to take into consideration the concentration of the query molecule. The computational tools considered in this study are widely used in the fields of cheminformatics and/or bioinformatics and have good accuracies for the predictions. Furthermore, the predictions were in the applicability domain for both used predictions tools. In the case of molecular docking study, the main limitation is due to the lack of flexibility of both protein and ligand, also a limitation that is specific to these types of studies. However, further experimental studies are necessary to assess the predicted activities of the COs related to wound healing and their interactions with MD-2 protein, but the experimental design should consider COs with better characterized composition in terms of molecular weight, acetylation degree and acetylation pattern.

TABLE IV. The types of the interaction between the investigated COs and MD-2 and the amino acids involved in these interactions. The interactions between MD-2 and the ligand (eritoran, E55) present in the crystallographic structure (PDB ID 2Z65) are also characterized for comparison purposes: A means N-acetyl glucosamine, D means glucosamine.

Complex MD-2-COs	Amino acids involved in the interactions	
	Hydrophobic interactions	Hydrogen bonds
Complex made with the ligand present in the crystallographic structure (eritoran)		
Complex MD-2-E55	ILE46, LEU61, ILE63, TYR65, PHE76, PHE104 ILE117, PHE119, PHE121, PHE151	SER120
Complexes made with totally acetylated COs		
Complex MD-2-2A	PHE151, ILE153	-
Complex MD-2-3A	PHE76, GLU92, PHE121, VAL135, PHE151	TYR102, SER120
Complex MD-2-4A	PHE76, PHE121, PHE151	TYR102, SER120
Complex MD-2-5A	VAL48, ILE52, PHE 76, ILE117, PHE119	TYR102, SER120
Complex MD-2-6A	LEU54, ILE63, PHE76, PHE121, ILE153	TYR102, SER120
Complex MD-2-8A	GLU92, ILE117, PHE119, TYR131, ILE153	ARG96, TYR102, LYS125, SER127
Complexes made with totally deacetylated COs		
Complex MD-2-2D	-	GLU92
Complex MD-2-3D	-	TYR102
Complex MD-2-4D	-	VAL93
Complex MD-2-5D	-	GLU92, VAL93
Complex MD-2-6D		ARG96, TYR102(2)
Complex MD-2-8D	-	GLU92, VAL93, TYR102, SER120, LYS122, GLY123
Complexes made with partially deacetylated COs		
Complex MD-2-DA	PHE119	-
Complex MD-2-ADA	ILE63, PHE76, ILE117	TYR102, SER120
Complex MD-2-DDA	ILE32, PHE151, ILE153	TYR102(2), SER120
Complex MD-2-AADD	VAL48, PHE119, PHE121	GLU92, TYR102
Complex-MD-2-ADAD	ILE52, LEU54, PHE121, ILE153	GLU92, TYR102
Complex MD-2-DAAD	LEU61, PHE76, ILE117, PHE119	TYR102(2)
Complex MD-2-DADA	ILE46, LEU61, PHE121	TYR102
Complex MD-2-DDAA	TYR131, PHE151	GLU92, TYR102(2)
Complex-MD-2-ADDA	ILE80, TYR131	GLU92, TYR102, CYS122
Complex-MD-2-DADADA	ILE52, LEU54, PHE121, ILE153	GLU92, ARG96, TYR102
Complex-MD-2-ADADAD	LEU78, PHE119	GLU92, VAL93, CYS95, ARG96, ASP100, ASP101, TYR102, SER120
Complex-MD2-ADDDAD	LEU87, PHE119	GLU92, TYR102, SER120
Complex-MD-2-DDDADA	ILE153	VAL93, ARG96, TYR102(2)
Complex-MD-2-DADADADA	VAL48, LEU61, PHE121, PHE151	ARG90, GLU92, VAL93, TYR102, GLY123, LYS132

CONCLUSION

In the present study, chitooligosaccharides with distinct chemical properties have been analyzed using a computational approach to evaluate their role in the wound healing process. Because it is still not straightforward to obtain COs with well-defined chemical characteristics (length, deacetylation degree and acetylation pattern), the computational approaches offer an advantage in such cases and their results may guide further experimental studies. This investigation confirms the functional role of COs in wound healing. Regardless of their chemical properties, all investigated COs reveal various wound healing activities. However, there are several distinct activities that are revealed by COs with dissimilar chemical properties. The molecular targets for the totally deacetylated COs are different by comparison to totally and partially acetylated COs and COs containing 3 totally acetylated, respectively totally deacetylated monomeric units reveal the wider spectra of the molecular targets. The main molecular targets predicted for the totally and partially acetylated COs are galectins and selectins, proteins that mediate immune response and respectively the cell adhesion during the process of wound healing. Other molecular targets, the myelin-associated glycoproteins are involved in the development of neural network in the damaged region. Fibroblast growth factors are the main class of molecular targets for the totally deacetylated COs and they are involved in inducing cell division and growth by promoting angiogenesis for the regrowth of damaged tissues. Besides the macrophage stimulant, hyaluronic acid agonist, angiogenesis stimulant, and membrane integrity agonist, which are predicted activities related to wound healing for all COs regardless of their chemical properties, totally deacetylated COs also act as transcription factors stimulant. The totally acetylated chitooligosaccharides proved to be better inhibitors of the LPS binding to MD-2 protein.

Taking into account that COs with dissimilar chemical properties may have distinct activities related to the wound healing process, mixtures of COs with distinct properties can be considered suitable candidates as adjuvants in developing scaffolds for the wound healing process.

ИЗВОД

ДУБЉИ УВИД У УПОТРЕБУ ХИТООЛИГОСАХАРИДА У ПРОЦЕСУ ЗАРАСТАЊА РАНА. РАЧУНАРСКИ ПРИСТУП

MARIANA A. MATICA[1], DIANA L. ROMAN[2,3], VASILE OSTAFE[2,3] и ADRIANA ISVORAN[2,3]

[1]Department of Chemistry-Biology, Institute for Advanced Environmental Research, West University of Timisoara, Oituz 4C, 300086 Timisoara, Romania; [2]Department of Biology-Chemistry, Faculty of Chemistry, Biology, Geography, West University of Timisoara, 16 Pestalozzi, 300115 Timisoara, Romania и [3]Advanced Environmental Research Laboratories (AERL), West University of Timisoara, Oituz 4A, 300086 Timisoara, Romania

Хитоолигосахариди (COs) који садрже до 10 мономерих јединица N-ацетил-D-глукозамина и/или D-глукозамина су у води растворни молекули који показују бројне биолошке активности и ниске токсиколошке профиле. У овој студији коришћен је

рачунарски приступ за предвиђање укључености хитоолигосахарида који имају посебне хемијске особине (молекулска маса, степен деацетилације и образац ацетилације) у све четири фазе зарастања рана: хемостази, упали, пролиферациј и формирању ткива. Има предвиђања, за изучаване хитоолигосахариде, у погледу њихових молекулских мета и биолошких активности на које се ослања процес зарастања рана. Даље, приступ молекулског доковања коришћен је за процењивање интеракција проучаваних хитоолигосахарида са мијелоидним диференцијацијским фактором 2 (MD-2), протеином укљученим у запаљењским процесима. Истраживање потврђује функционалне улоге проучаваних хитоологосахарида у зарастању рана. Молекулске мете претсказане за хитоолигосахариде што садрже потпуно и делимично ацетиловане јединице су галектини и селектини, а они претсказани за хитоолигосахариде што садрже потпуно деацетиловане јединице су фактори раста фибробласта, хитоолигосахариди који садрже 3 јединице показујући већи број молекулских мета. Сви ови протеини су укључени у посредовање имуног одговора, индукујући деобу ћелија и лепљење ћелија током процеса зарастања рана. Сви хитоолигосахариди који садрже од 2 до 8 мономерних јединица у стању су да интерагују са MD-2 протеином, стим да су интеракције јаче за хитоологосахариде који садрже 6 и 8 мономерних јединица. Енергије интеракције расту са порастом молекулске масе и са опадајућим степеном деацетиловања и зависи од обрасца ацетиловања. Међу проучаваним хитоологосахаридима, потпуно ацетиловани хитоологосахариди који садрже 6 и 8 N-ацетил глукозаминских јединица могу бити бољи инхибитори за LPS везивање на MD-2 протеин. Према томе, смесе хитоолигосахарида са различитим особинама треба сматрати погодним кандидатима за катализаторе у развијању скелета за процес зарастања рана.

(Примљено 2. јула; ревидирано 13. новембра; прихваћено 20. новембра 2022.)

REFERENCES

1. I. Aranaz, A. R. Alcántara, M. C. Civera, C. Arias, B. Elorza, A. Heras Caballero, N. Acosta, *Polymers* **13** (2021) 3256 (https://doi.org/10.3390/polym13193256)
2. B. Moghadas, A. Solouk, D. Sadeghi, *Polym. Bull.* **78** (2021) 4919 (https://doi.org/10.1007/s00289-020-03352-8)
3. L. A. M. van den Broek, C. G. Boeriu, C. V. Stevens, *Chitin and Chitosan: Properties and Applications*, Wiley, 2020, pp. 232-238 (https://doi.org/10.1002/9781119450467)
4. X. Guo, T. Sun, R. Zhong, L. Ma, C. You, M. Tian, H. Li, C. Wang, *Front. Pharmacol.* **9** (2018) (https://doi.org/10.3389/fphar.2018.01412)
5. J. Li, D. Wang, S.-C. Chang, P.-H. Liang, V. Srivastava, S.-Y. Guu, J.-J. Shie, K.-H. Khoo, V. Bulone, Y. S. Y. Hsieh, *J. Agric. Food. Chem.* **69** (2021) 3371 (https://doi.org/10.1021/acs.jafc.0c06804)
6. D. L. Roman, M. Roman, C. Som, M. Schmutz, E. Hernandez, P. Wick, T. Casalini, G. Perale, V. Ostafe, A. Isvoran, *Front. Bioeng. Biotechnol.* **7** (2019) (https://doi.org/10.3389/fbioe.2019.00214)
7. D. L. Roman, V. Ostafe, A. Isvoran, *Mar. Drugs.* **19** (2021) (https://doi.org/10.3390/md19030120)
8. D. L. Roman, M. Roman, H. Sletta, V. Ostafe, A. Isvoran, *J. Mol. Graph. Model.* **88** (2019) 41 (https://doi.org/10.1016/j.jmgm.2019.01.002)
9. D. L. Roman, V. Ostafe, A. Isvoran, *J. Mol. Graph. Model.* **100** (2020) 107676 (https://doi.org/10.1016/j.jmgm.2020.107676)

10. H. Jafari, K. V. Bernaerts, G. Dodi, A. Shavandi, *Mater. Sci. Eng. C.* **117** (2020) 111266 (https://doi.org/10.1016/j.msec.2020.111266)
11. B. S. Park, J. O. Lee, *Exp. Mol. Med.* **45** (2013) e66 (https://doi.org/10.1038/emm.2013.97)
12. S. Viriyakosol, P. S. Tobias, R. L. Kitchens, T. N. Kirkland, *J. Biol. Chem.* **276** (2001) 38044 (https://doi.org/10.1074/jbc.M105228200)
13. Y. Qiao, Y. Ruan, C. Xiong, Q. Xu, P. Wei, P. Ma, X. Bai, Y. Du, *Carbohyd. Polym.* **82** (2010) 405 (https://doi.org/10.1016/j.carbpol.2010.04.079)
14. E. M. Jones, C. A. Cochrane, S. L. Percival, *Adv. Wound. Care.* **4** (2015) 431 (https://doi.org/10.1089/wound.2014.0538)
15. E. F. Pettersen, T. D. Goddard, C. C. Huang, G. S. Couch, D. M. Greenblatt, E. C. Meng, T. E. Ferrin, *J. Comput. Chem.* **25** (2004) 1605 (https://doi.org/10.1002/jcc.20084)
16. D. Gfeller, A. Grosdidier, M. Wirth, A. Daina, O. Michielin, V. Zoete, *Nucleic Acids Res.* **42** (2014) W32 (https://doi.org/10.1093/nar/gku293)
17. D. A. Filimonov, A. A. Lagunin, T. A. Gloriozova, A. V. Rudik, D. S. Druzhilovskii, P. V. Pogodin, V. V. Poroikov, *Chem. Heterocycl. Comp.* **50** (2014) 444 (https://doi.org/10.1007/s10593-014-1496-1)
18. H. M. Kim, B. S. Park, J. I. Kim, S. E. Kim, J. Lee, S. C. Oh, P. Enkhbayar, N. Matsushima, H. Lee, O. J. Yoo, J. O. Lee, *Cell* **130** (2007) 906 (https://doi.org/10.1016/j.cell.2007.08.002)
19. A. Grosdidier, V. Zoete, O. Michielin, *Nucleic. Acids. Res.* **39** (2011) W270 (https://doi.org/10.1093/nar/gkr366)
20. 2S. Salentin, S. Schreiber, V. J. Haupt, M. F. Adasme, M. Schroeder, *Nucleic Acids Res.* **43** (2015) W443 (https://doi.org/10.1093/nar/gkv315)
21. N. Panjwani, *Ann. Transl. Med.* **2** (2014) 89 (https://doi.org/10.3978/j.issn.2305-5839.2014.09.09)
22. M. Subramaniam, S. Saffaripour, L. Van De Water, P. S. Frenette, T. N. Mayadas, R. O. Hynes, D. D. Wagner, *Am. J. Pathol.* **150** (1997) 1701 (https://pubmed.ncbi.nlm.nih.gov/9137094/)
23. H. Tomita, Y. Iwata, F. Ogawa, K. Komura, K. Shimizu, A. Yoshizaki, T. Hara, E. Muroi, K. Yanaba, S. Bae, M. Takenaka, M. Hasegawa, M. Fujimoto, S. Sato, *J. Invest. Dermatol.* **129** (2009) 2059 (https://doi.org/10.1038/jid.2008.446)
24. D. S. Allen-Gipson, J. Wong, J. R. Spurzem, J. H. Sisson, T. A. Wyatt, *Am. J. Physiol. Lung. Cell. Mol. Physiol.* **290** (2006) L849 (https://doi.org/10.1152/ajplung.00373.2005)
25. A. Ala, A. P. Dhillon, H. J. Hodgson, *Int. J. Exp. Pathol.* **84** (2003) 1 (https://doi.org/10.1046/j.1365-2613.2003.00235.x)
26. R. H. Quarles, *J. Neurochem.* **100** (2007) 1431 (https://doi.org/10.1111/j.1471-4159.2006.04319.x)
27. S. Yamakawa, K. Hayashida, *Burns & Trauma* **7** (2019) 10 (https://doi.org/10.1186/s41038-019-0148-1)
28. W. Li, Y. Li, S. Guan, J. Fan, C.-F. Cheng, A. M. Bright, C. Chinn, M. Chen, D. T. Woodley, *EMBO J.* **26** (2007) 1221 (https://doi.org/10.1038/sj.emboj.7601579)
29. J. Guo, C. Chang, W. Li, *Expert. Rev. Proteomics* **14** (2017) 665 (https://doi.org/10.1080/14789450.2017.1355244)
30. S. Gingis-Velitski, A. Zetser, M. Y. Flugelman, I. Vlodavsky, N. Ilan, *J. Biol. Chem.* **279** (2004) 23536 (https://doi.org/10.1074/jbc.M400554200)

31. M. D. Bagood, R. R. Isseroff, *Int. J. Mol. Sci.* **22** (2021) (https://doi.org/10.3390/ijms22116135)
32. L. Thomas, S. Mathew, S. Johnson, *Inform. Med. Unlocked.* **20** (2020) 100406 (https://doi.org/10.1016/j.imu.2020.100406)
33. G. R. Vasta, *Nat. Rev. Microbiol.* **7** (2009) 424 (https://doi.org/10.1038/nrmicro2146)
34. K. Masuoka, M. Ishihara, T. Asazuma, H. Hattori, T. Matsui, B. Takase, Y. Kanatani, M. Fujita, Y. Saito, H. Yura, K. Fujikawa, K. Nemoto, *Biomaterials* **26** (2005) 3277 (https://doi.org/10.1016/j.biomaterials.2004.07.061)
35. H. Quan, F. Zhu, X. Han, Z. Xu, Y. Zhao, Z. Miao, *Med. Hypotheses* **73** (2009) 205 (https://doi.org/10.1016/j.mehy.2009.02.018)
36. M. Jiang, X. Zhuge, Y. Yang, X. Gu, F. Ding, *Neurosci. Lett.* **454** (2009) 239 (https://doi.org/10.1016/j.neulet.2009.03.042)
37. M. Jiang, Q. Cheng, W. Su, C. Wang, Y. Yang, Z. Cao, F. Ding, *Neurochem. Res.* **39** (2014) 2047 (https://doi.org/10.1007/s11064-014-1387-y)
38. V. Voinchet, P. Vasseur, J. Kern, *Am. J. Clin. Dermatol.* **7** (2006) 353 (https://doi.org/10.2165/00128071-200607060-00003)
39. T. Liu, L. Zhang, D. Joo, S.-C. Sun, *Signal. Transduct. Target.* **2** (2017) 17023 (https://doi.org/10.1038/sigtrans.2017.23)
40. H. Huang, Y. Zou, H. Chi, *Drug. Des. Devel. Ther.* **12** (2017) 67 (https://doi.org/10.2147/DDDT.S148064).