Journal of the Serbian Chemical Society
JSCS@tmf.bg.ac.rs • www.shd.org.rs/JSCS
J. Serb. Chem. Soc. 90 (2) 137–148 (2025)
JSCS–5825
Original scientific paper
Published 17 February, 2025

Theoretical evaluation of pectin therapeutic potential in relation to degree of methylation

JELENA B. MARTINOV NESTOROV[1], GORAN V. JANJIĆ[2] and MARIJA M. PETKOVIĆ BENAZZOUZ[3]*

[1]*University Clinical Centre of Serbia, Clinic for Gastroenterology and Hepatology, School of Medicine, University of Belgrade, Belgrade, Serbia,* [2]*University of Belgrade – Institute of Chemistry, Technology and Metallurgy, National Institute of the Republic of Serbia, Njegoševa 12, Belgrade, Serbia and* [3]*University of Belgrade – Faculty of Physics, Studentski trg 12–16, Belgrade, Serbia*

(Received 22 April, revised 16 May, accepted 2 June 2024)

Abstract: Pectin is the focus of scientific interest due to both its physicochemical and biochemical properties, as well as its non-toxic nature. Methylation of pectin is a natural process that exists as part of the cell wall defence system against various pathogens. In this study the docking analysis was conducted to predict if methylation o affects the anticancer and antimicrobial properties of pectin and what extent. Four pectin derivatives with varying degrees of methylation and two sets of biomolecules were used. The first set included enzymes responsible for anticancer activity (HMGR, the AGE receptors, tumour protein p53 and oncogenic phosphatase SHP2), while the second set included those for antimicrobial activity (*Salmonella Typhi* TtsA, *Pseudomonas aeruginosa* Earp, *Streptococcus mutans* MetE and *Staphylococcus aureus* Cas9). The results indicated that the degree of methylation does not play a decisive role in the mentioned activities. because all bind to the same sites with similar binding energies. Additionally, it was shown that pectin derivatives have a higher binding affinity towards DNA than towards enzymes. Only the fully methylated derivative exhibited different behaviour, binding to a different binding site in the case of *Streptococcus mutans* MetE.

Keywords: docking study; anticancer properties; antimicrobial properties.

INTRODUCTION

More recently, numerous studies have showed significant health benefits of pectin. Pectin represents soluble dietary fibre, have potential prebiotic, hypoglycemic, hypolipidemic, immunostimulating and anticancer properties.[1] It is believed that part of the positive effect of pectin is due to its influence in modul-

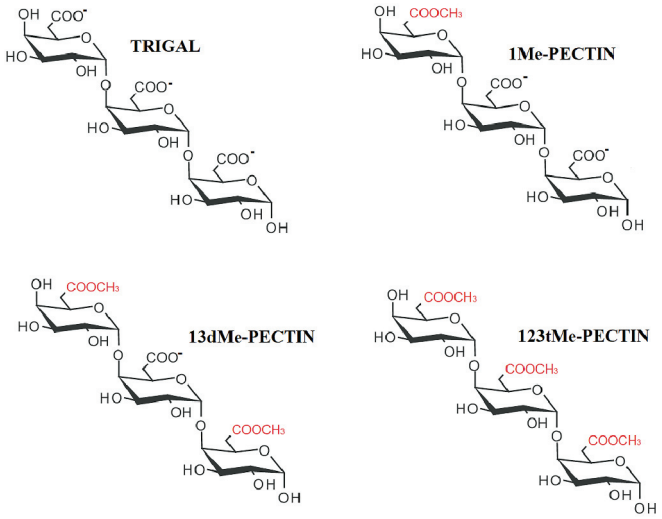
* Corresponding author. E-mail: marijapetkovic@ff.bg.ac.rs
https://doi.org/10.2298/JSC240422056P

ating the composition and activity of the intestinal microbiota. Pectin inhibits the growth of pathogenic bacteria and stimulates the growth of beneficial bacteria and act as potential prebiotic.[1–3] Pectin is hydrolysed in colon by intestinal bacteria[3] and most important products of its fermentation are short-chain fatty acids (SCFA). The prebiotic properties of pectin-oligosaccharides (POS) from apple, citrus and sugar beet have been evaluated using fecal fermented cultures and these sugars are able to increase the number of Bifidobacteria and Lactobacillus and reduce the number of Bacteroides and Clostridies.[2] The anti-infective properties of pectin are mainly associated with the improvement of the composition of intestinal microbiota in the colon, inhibiting the adhesion of pathogens to epithelial cells, inhibiting bacterial colonization and binding bacterial toxins.[4] Citrus oligogalacturonides exhibited antibacterial activity and bactericidal effect against selected food pathogens including *S. typhimurium*, *S. aureus*, *L. monocytogenes* and *P. aeruginosa*.[5] Pectin derivatives, prepared by the chemical modification of polysaccharides with natural fatty acids, are promising and effective antimicrobial agents against the two most common food pathogens, *E. coli* and *S. aureus*, which can find further application in the field of food packaging.[6] Pectin is the most promising biocompatible natural anticarcinogenic product, because many *in vitro* and *in v*ivo studies have demonstrated that pectin-derived compounds affect cancer progression. It inhibits cell growth and cancerous cell proliferation and promotes apoptosis.[7] Modified pectin, especially citrus pectin, is highly effective in preventing the growth and spread of cancers such as breast and colon cancer. Studies suggest that low molecular weight pectin fragments, rich in galectins, may bind to carbohydrate recognition domains (CRD) on the pro-metastatic protein Gal-3 (galectin-3) and thus inhibits cell-cellular tumour interaction, aggregation of cancer cells with each other and with healthy cells and inhibit metastatic lesions.[8] Pectin derivatives (modified with a maleoyl group) are much more effective than pure or unmodified pectin in inhibiting colon cell cancer growth.[9] The development of foods enriched with pectin might open new avenues regarding the management of colorectal cancer. Pectin has been reported to exhibit antioxidant and anti-inflammatory properties, making it a potentially interesting candidate in the prevention and management of carcinogenesis. In this study, the theoretical impact of pectin esterification (methylation of carboxyl groups) on their antimicrobial and antitumor (anticancer) activity will be predicted through the inhibition of respective biomolecules.

METHODOLOGY

For the purpose of investigating the effect of pectin methylation on its biological activities, four pectin derivatives (Scheme 1) were selected as model compounds, each containing three sugar units (α-1,4-linked D-galacturonic acid). In the first derivative, all three units are D-galacturonic acid (TRIGAL). The other three derivatives have one methylated carboxyl group (1Me-pectin), two methylated groups (13dMe-pectin), or all three groups methylated

(123tMe-pectin). The examined pectin derivatives are negatively charged (pK_a ≈ 3.5) due to their negatively charged carboxyl groups, except for the fourth derivative (123tMe-pectin), which lacks a carboxyl group as all three groups are esterified, rendering this derivative neutral. The structures of the investigated compounds were optimized at the wb97xd-def2tzvp level of theory.



Scheme 1. Illustration of the structures of the investigated pectin derivatives.

The anti-tumour efficacy of pectin derivatives was evaluated *via* docking analyses employing crystallographic structures obtained from the Protein Data Bank (PDB). For these purposes, the structures of encompassing HMG-CoA reductase (HMGR, pdb code: 1DQ8),[10] receptor for the advanced glycation endproducts (RAGE, pdb code: 6XQ1),[11] oncogenic phosphatase SHP2 (pdb code: 5IBS)[12] and human p53 DNA-binding domain (PDB code: 6GGB)[13] were extracted.

To assess antimicrobial potential, docking studies were performed on various enzymes, the crystal structures of which were also retrieved from the PDB. These enzymes include the crystal structures of *Pseudomonas aeruginosa* Earp in complex with TDP (pdb code: 6J7L),[14] *Salmonella typhi* TtsA (pdb code: 6V40),[15] *Staphylococcus aureus* Cas9 (pdb code: 5CZZ),[16] and *Streptococcus mutans* MetE (pdb code: 3T0C).[17]

The structures of ligands (pectin derivatives) and targets were prepared in AutoDockTools program, while the docking calculations were performed in the AutoDock program.[18] A grid box, encompassing the rigid structure of targets, was employed to accommodate the investigated pectin derivatives. The Lamarckian genetic algorithm served as the search method, applying 100 runs for each virtual screening. The analysis and graphical presentation of the docking study results were performed using the Discovery Studio software (BIOVIA Software product).[19]

RESULTS AND DISCUSSION

Pectin is the focus of increasing attention as a potential antioxidant because of its unique physicochemical properties and low toxicity. Pectins have a notable

ability to scavenge free radicals and their efficiency depends on the D-galacturonic acid (GalA) content.[20] In addition to radical mechanisms, it is known that pectins also possess inhibitory capabilities. To ascertain whether esterification (methylation in this case) significantly impacts the inhibitory potential of pectins, the docking studies were conducted on two sets of target proteins. For the first set of targets, the anticancer potential of pectin derivatives was investigated, while for the second set of targets, the antimicrobial activity of the tested pectin derivatives is predicted. The following four enzymes were selected as targets for testing anticancer potential.

Docking study on HMG-CoA reductase (HMGR)

HMGR serves as a catalyst in the initial step of cholesterol biosynthesis, thereby regulating a pivotal factor in cardiovascular diseases. Pectin is used as an agent to mitigate total blood cholesterol levels.[21] Moreover, HMGR inhibitors are explored as potential anticancer agents against malignant neoplasms in women.[22] To ascertain whether the investigated pectin derivatives possess inhibitory potential against HMGR, a docking study was conducted. The natural ligand of the enzyme (coenzyme A) and the drug atorvastatin were employed as control compounds (further detailed in Supplementary material to this paper). Docking results demonstrated that coenzyme A binds to the active sites of enzyme with binding energies of –30.12 kJ/mol (Fig. 1a). Atorvastatin also binds to both active sites, albeit with slightly higher binding energy (–30.96 kJ/mol), affirming its inhibitory activity. None of the four pectin derivatives bind to the active sites but instead occupy a nearby binding pocket, exhibiting similar binding energies (ranging from –30.96 to –31.38 kJ/mol) and significant conformational flexibility. By binding to this adjacent binding pocket, predominantly through hydrogen bonds with Glu559, Gly560, Asn658, Gly756, Leu862 or Ser865 (Fig. S-1 of the Supplementary material), pectin derivative impedes the approach of coenzyme A to the active site, indicative of noncompetitive inhibition. Furthermore, as all four derivatives bind to the same pocket with similar binding energies, it can be inferred that methylation does not influence the inhibitory activity of pectin.

Docking study on AGE receptor (RAGE)

The receptor for the advanced glycation end products (RAGE) is a ubiquitously expressed transmembrane immunoglobulin-like receptor with multiple isoforms, engaging in binding with a diverse array of endogenous extracellular ligands and intracellular effectors. Due to its involvement in various pathological conditions such as cancer, diabetes, cardiovascular diseases and neurodegeneration, RAGE has emerged as an appealing therapeutic target for inhibitors targeting both its extracellular and intracellular domains.[23] The detailed description

of the receptor and its binding sites are provided in the Supplementary material. To ascertain the inhibitory capabilities of the investigated pectin derivatives against the AGE receptor, a docking study was conducted, employing tranilast as a test compound. The receptor structure encompasses only the V and C1 domains from the extracellular portion and docking was performed on both domains. The results of docking revealed that the control compound (tranilast) binds to site 2 located on the V domain (Fig. 1b), with a binding energy of –27.20 kJ/mol. All four investigated pectin derivatives bind to the same binding site (site 3) on the V domain. Their binding to the same site indicates that methylation does not influence the activity of these derivatives. Additionally, besides sharing the same binding site, their binding energies are highly similar (ranging from –21.76 to –22.59 kJ/mol). The binding site of pectin derivatives aligns with that of galacturonic acid, as determined from the docking study.[24] Discrepancies in the binding site between the control compound and pectin derivatives result from the presence of aromatic rings in the control compound, as well as a significantly higher number of donor and acceptor groups for the conventional hydrogen bonding in pectin derivatives. Specifically, tranilast forms classical hydrogen bonds with Lys110 and Asn112, as well as π–aromatic interactions with Ala21, Ala23 and Arg98. Conversely, pectin derivatives predominantly form a larger number of hydrogen bonds in the most stable binding site, involving residues Gln24, Thr27, Glu32, Pro33, Val35 or Tyr118 (Fig. S2). The results of docking suggest the inhibitory capabilities of pectin derivatives, consistent with some previous experimental findings on this subject. It is noteworthy that pectic oligosaccharides from orange peel (OPOs) and pectin polysaccharides from Arabica coffee husks have been reported to exhibit inhibitory capacities against AGEs.[24,25]

Docking results on tumor protein p53

Pectin exhibits anticancer properties, which have been demonstrated to impede tumor development and proliferation across a diverse range of cancer cell types.[26] Notably, modified pectin (MP) shows promise in mitigating the progression and metastasis of colon and breast cancers. Furthermore, beyond its role as a soluble dietary fibre, MP demonstrates beneficial effects on malignancy by activating tumour-suppressor protein p53.[27] This activation subsequently triggers apoptosis pathways and induces cell cycle arrest, contributing to the suppression of tumour progression. The extended description of the structure and function of the tumour protein p53 is provided in the Supplementary material (SM3). The docking results indicate that all four derivatives of pectin bind to the same site (Fig. 1c), which does not correspond to the DNA-binding site. The binding energies are highly similar (ranging from –24.27 to –25.52 kJ/mol), suggesting that methylation does not affect the binding site and thus, the mechanism of enzyme inhibition. All four derivatives bind to the beta-sheet structure of the

enzyme, primarily through hydrogen bonds with amino acid residues. The derivatives predominantly form conventional hydrogen bonds with Phe113, Tyr126 and Asn131. In addition to the conventional and weak hydrogen bonds of the C–H/O type, derivatives also engage in π-aromatic interactions with Arg110 and Trp146 (Fig. S-3 of the Supplementary material). It is evident that a competitive mechanism is not at play and the binding of pectin to the region of the protein with a beta-sheet structure induces conformational changes, resulting in alterations in the conformation of the DNA-binding site (non-competitive mechanism).

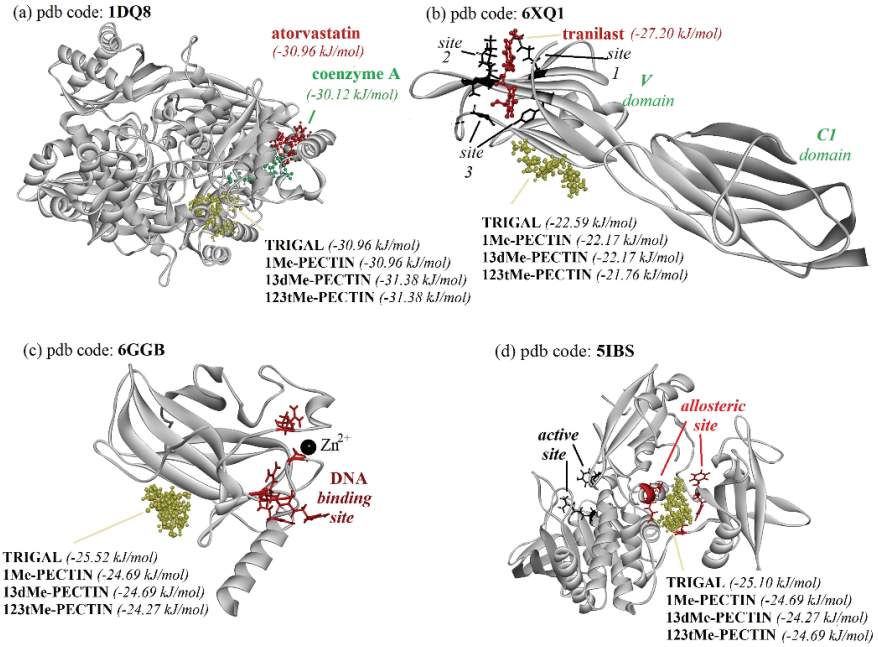


Fig. 1. Binding sites and binding energies of the investigated compounds for HMGR (a), the AGE receptors (b), p53 cancer mutant (c) and oncogenic phosphatase SHP2 (d).

Docking study on the oncogenic phosphatase SHP2

The Src homology 2-containing protein tyrosine phosphatase 2 (SHP2) is a non-receptor protein tyrosine phosphatase widely expressed, predominantly within the cytoplasm of various tissues. Src homology region 2-containing protein tyrosine phosphatase 2 (SHP2) is implicated in breast cancer, leukaemia, lung cancer, liver cancer, gastric cancer, laryngeal cancer, oral cancer and other malignancies.[28] Germline mutations in SHP2 lead to developmental disorders, while somatic mutations are observed in both childhood and adult cancers, contributing to leukaemia in murine models. SHP2 presents as a promising therapeutic target due to its involvement in numerous biological processes. For further details on the structure and function of the protein, refer to Supplementary

material. The docking results indicate non-competitive inhibition, as all four derivatives bind to an allosteric site on SHP2 (Fig. 1d). Binding energies are similar for all four tested compounds, ranging from –24.27 to –25.10 kJ/mol, suggesting that the degree of methylation does not play a significant role in recognition at the binding site on SHP2. The classical hydrogen bonds are responsible for the binding of derivatives to SHP2, although contributions are also made by carbon–hydrogen bonds and hydrophobic interactions. The formation of hydrogen bonds primarily includes Thr108, Glu110, His114, Thr218, Glu 232 and Arg229 (Fig. S-4 of the Supplementary material).

The radical mechanism is present in the protective mechanism of derivatized pectins from apples, citrus fruits and polygalacturonic acid, which reduce the growth of *Escherichia coli* and *Staphylococcus aureus*.[20] However, the antimicrobial action of pectins is not solely achieved through the radical mechanism but also by inhibiting relevant biomolecules. For this purpose, four targets were selected, which play a crucial role in antimicrobial activity.

Docking study on the Salmonella Typhi *TtsA*

S. Typhi TtsA is a protein crucial for secreting typhoid toxin, a virulence factor of *S. Typhi*. TtsA aids in transporting the toxin across the peptidoglycan layer of the bacterial cell wall, essential for its release into the *Salmonella*-containing vacuole.[29] A detailed description of the structure and function of the enzyme is given in the Supplementary material. To determine whether the investigated pectin derivatives possess inhibitory capabilities, a docking study was conducted, in which azithromycin (Supplementary material) was used as the test compound. Azithromycin and pectin derivatives are bound at the same site, between the amino-terminal catalytic domain (1–92) and carboxy-terminal substrate binding domain (93–180) of TtsA (Fig. 2a), forming hydrogen bonds mainly with the N-terminal histidine-epitope (Fig. S-5 of the Supplementary material). Pectin derivatives have higher binding energies (in the range of –30.12 to –30.96 kJ/mol) than azithromycin (–28.87 kJ/mol), which indicates a more pronounced inhibitory potential of derivatives. Stronger binding energies are due to a larger number of hydrogen bonds of pectin derivatives (about 4 bonds, Fig. S-5) than azithromycin (only 1 bond, Fig. S-5).

Docking study of Pseudomonas aeruginosa *Earp*

Protein glycosylation, the most widespread posttranslational modification in nature, significantly influences protein structure and function. Arginine glycosylation was reported as an L-rhamnosylation modification on a specific arginine residue within bacterial translation elongation factor P (EF-P). *P. aeruginosa* EarP may provide a platform for the development of new narrow-spectrum antibacterial agents to combat infections from *P. aeruginosa* and other EarP-con-

taining pathogenic bacteria. A detailed description of the structure and function of the Earp is given in the Supplementary material. Thymidine-5'-diphosphate (TDP) was used as a control compound (the structures of target and TDP were extracted from the crystal structure with the pdb code 6J7L).[30] The control compound is as expected docked on active site (Asp12, Asp16 and Glu272) with high binding energy –37.66 kJ/mol. The findings indicate that all four examined pectin derivatives demonstrate binding affinity to the same site (Fig. 2b), but with significantly lower binding energies (in the range of –28.45 to –28.87 kJ/mol). One can conclude that pectin derivatives possess inhibitory properties and methylation exerts minimal influence on the activity of these derivatives, by binding to this adjacent binding pocket, predominantly through hydrogen bonds with Asn13, Asp16, Thr296 and Lys300 (Fig. S-6 of the Supplementary material).

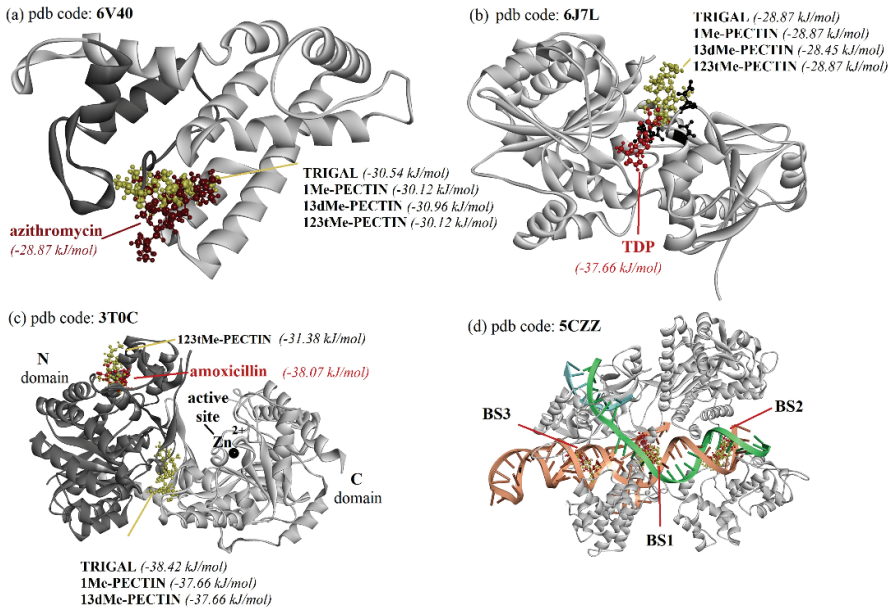


Fig. 2. The binding sites and binding energies of investigated compounds for *Salmonella typhi* TtsA (a), *Pseudomonas aeruginosa* Earp (b), *Streptococcus mutans* MetE (c) and *Staphylococcus aureus* Cas9 (d).

Docking results on Streptococcus mutans *MetE*

S. mutans MetE is an enzyme found in the bacterium *S. mutans*. Specifically, MetE is involved in the biosynthesis of methionine, an essential amino acid. *S. mutans* is primarily known for its role in dental caries (tooth decay) and dental plaque formation. However, it can also cause other oral health issues such as gingivitis and periodontitis when conditions favour its growth and colonization in the oral cavity. Additionally, *S. mutans* has been implicated in infective endo-

carditis, a serious infection of the heart valves, particularly in individuals with underlying heart conditions or compromised immune systems. A detailed description of the structure and function of the MetE is given in the Supplementary material.[31] Amoxicillin (AMX) is one of the most prescribed antibiotics globally, which for the purposes of this study is used as a control compound. Not one molecule from derivatives of neither pectin nor amoxicillin is docked on the active site where Zn is coordinated (Fig. 2c). Amoxicillin was docked for a site located in the N domain, with the binding energy of –38.07 kJ/mol), indicating its allosteric mechanism of inhibition. The 123tMe-PECTIN derivative was bound to the same site, forming hydrogen bonds with Arg43, Phe63, Leu65, Asp71, Il90, Asn131 and Asn132 (Fig. S-7 of the Supplementary material), with a significantly lower binding energy of –31.38 kJ/mol. It is obvious that the binding of these two compounds causes conformational changes in the N-domain, which are transmitted to the C-domain, interfering with the binding of the substrate to the active site. The remaining three pectin derivatives bind near the α-helix that connects two domains, with significantly higher binding energies (in range from –37.66 to –38.49 kJ/mol) compared to the 123tMe-pectin derivative. It is possible that the docked derivatives may act as an allosteric modulator inducing conformational changes that affect enzyme activity. The conventional hydrogen bonds with the Arg287, Asn288, His321 and Asn549 are mainly responsible for the binding of mentioned derivatives (Fig. S-7 of the Supplementary material).

Docking results on Staphylococcus aureus *Cas9*

S. aureus Cas9 (SaCas9) is an RNA-guided endonuclease derived from the bacterium *S. aureus*. The bacterial protein from *S. aureus* is associated with various human infections, including skin and soft tissue infections, pneumonia, bloodstream infections and others.[32] The RNA-guided DNA endonuclease Cas9 cleaves double-stranded DNA targets with a protospacer adjacent motif (PAM) and complementarity to the RNA guide. For the purposes of docking studies, the target structure of *S. aureus* Cas9 in a complex with sgRNA and its DNA targets was selected.[16] The cleavage site in the target DNA strand (the phosphodiester linkage between dC3 and dA4) is distant from the active site of the HNH domain (Asn580) and RuvC domain (Asp10), indicating that the present structure represents the inactive state. Triterpenoids, such as betulinic acid (BA), show promising antimicrobial properties and are a promising candidate for combating biofilm-associated infections.[33] The results of docking studies showed the existence of three binding sites (BS1, BS2 and BS3) of betulinic acid and pectin derivatives (Fig. 2d), with similar binding energies and number of conformations. However, all three places are not located on the enzyme, but in parts representing double helicoidal structures. The first binding site is located close to the cleavage site. All this leads to the conclusion that pectin derivatives and betulinic acid stabilize

the structure of the DNA double helix, thus preventing the DNA cleavage. The binding energy in the cleavage site (BS1) of betulinic acid (–47.70 kJ/mol) is higher than the binding energy of pectin derivatives (in range from –41.84 to –45.19 kJ/mol).

CONCLUSION

The present study investigated whether the esterification of pectin, particularly the methylation of carboxyl groups, affects the inhibitory potential of pectins, with a focus on their antimicrobial and anticancer properties. The results of docking analyses indicate that pectin derivatives exhibit binding affinities to target proteins in some extend especially in microbial infection. The results of calculations have revealed the minimal influence of methylation on chosen target set and mostly in allosteric mechanism of actions. It is also detected that pectin derivatives exhibit more affinity towards DNA compared to enzymes and that may unlock the potential for further investigation and therapeutically application.

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: https://www.shd-pub.org.rs/index.php/JSCS/article/view/12903, or from the corresponding author on request.

Acknowledgement. This work was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contracts No. 451-03-66/2024-03/ /200026 and 451-03-66/2024-03/ 200162).

ИЗВОД

ТЕОРИЈСКА ЕВАЛУАЦИЈА ТЕРАПИЈСКОГ ПОТЕНЦИЈАЛА ПЕКТИНА У ФУНКЦИЈИ СТЕПЕНА МЕТИЛОВАЊА

ЈЕЛЕНА Б. МАРТИНОВ НЕСТОРОВ[1], ГОРАН В. ЈАЊИЋ[2] и МАРИЈА М. ПЕТКОВИЋ БЕНАЗЗОУЗ[3]

[1]Универзитетски клинички центар Србије, Клиника за гастроентерологију и хепатологију, Медицински факултет Универзитета у Београду, Београд, [2]Универзитета у Београду – Институт за хемију, технологију и металургију, Институт од националног значаја за Републику Србију, Његошева 12, Београд и [3]Универзитет у Београду – Физички факултет, Студентски трг 12–16, Београд

Пектин је у фокусу научних интересовања како због својих физичко-хемијских и биохемијских особина, тако и због своје ниске токсичности. Метиловање пектина је природан процес који постоји као део одбрамбеног система ћелијског зида од напада различитих патогена. У овом раду урађена је докинг студија са циљем да се предвиди да ли метиловање и у ком степену утиче на антиканцерогене и антимикробне особине пектина. Коришћена су 4 деривата пектина са различитим степеном метиловања и два сета биомолекула. У првом сету су ензими одговорни за антиканцерогено дејство (HMG-CoA редуктаза, AGE рецептор, туморни протеин p53, онкогена фосфатаза SHP2) а у другом су ензими одговорни за антимикробно дејство (*Salmonella Typhi* TtsA, *Pseudomonas aeruginosa* Earp, *Streptococcus mutans* MetE и *Staphylococcus aureus* Cas9). Резултати докинга су указали да степен метиловања не игра одлучујућу улогу у поменутим активностима, јер се сва четири деривата везују на иста места са сличним енергијама везивања. Такође, показано је да деривати пектина имају већи афинитет везивања према ДНК него према

ензимима. Једино је потпуно метиловани дериват показао различито понашање, везујући се на другом везивном месту у случају *S. mutans* MetE.

(Примљено 22. априла, ревидирано 16. маја, прихваћено 2. јуна 2024)

REFERENCES

1. A. Dambuza, P. Rungqu, A. Omowunmi Oyedeji, G. Miya, A. Oluwabunmi Oriola, Y. Yiseyon Sunday Hosu, O. Oyehan Oyedeji, *Molecules* **29** (2024) 896 (https://doi.org/10.3390/molecules29040896)
2. N. Koropatkin, E. Cameron, E. Martens, *Nat. Rev. Microbiol.* **10** (2012) 323 (https://doi.org/10.1038/nrmicro2746)
3. K. Shinohara, Z. Ohashi, K. Kawasumi, A. Terada, T. Fujisawa, *Anaerobe* **16** (2010) 410 (https://doi.org/10.1016/j.anaerobe.2010.03.005)
4. R. Ciriminna, A. Fidalgo, F. Meneguzzo, A. Presentato, A. Scurria, D. Nuzzo, R. Alduina, L. M. Ilharco, M. Pagliaro, *ChemMedChem* **15** (2020) 2228 (https://doi.org/10.1002/cmdc.202000518)
5. M. C. Wu, H. C. Li, P. H. Wu, P. H. Huang, Y. T. Wang, *J. Food Sci*. **79** (2014) 1541 (https://doi.org/10.1111/1750-3841.12526)
6. E. Calce, E. Mignogna, V. Bugatti, M. Galdiero, V. Vittoria, S. De Luca, *Int. J. Biol. Macromol*. **68** (2014) 28 (https://doi.org/10.1016/j.ijbiomac.2014.04.011)
7. T. B. Emran, F. Islam, S. Mitra, S. Paul, N. Nat, Z. Khan, R. Das, D. Chandran, R. Sharma, C. M. Gonçalves Lima, A. A. Al Awadh, I. A. Almazni, A. H. Alhasaniah, R. P. F. Guinéet, *Molecules* **27** (2022) 7405 (https://doi.org/10.3390/molecules27217405)
8. V. V. Glinsky, *Carbohydr. Res*. **344** (2009) 1788 (https://doi.org/10.1016/j.carres.2008.08.038)
9. E. A. Almeida, S. P. Facchi, A. F. Martins, S. Nocchi, I. T. A. Schuquel, C. V. Nakamura, A. F. Rubira, E. C. Muniz, *Carbohydr. Polym*. **115** (2015) 139 (https://doi.org/10.1016/j.carbpol.2014.08.085)
10. E. S. Istvan, M. Palnitkar, S. K. Buchanan, J. Deisenhofer, *EMBO J.* **19** (2000) 819 (https://doi.org/10.1093/emboj/19.5.819)
11. N. Kozlyuk, B. A. Gilston, L. E. Salay, R. D. Gogliotti, P. P. Christov, K. Kim, M. Ovee, A. G. Waterson, W. J. Chazin, *Proteins* **89** (2021) 1399 (https://doi.org/10.1002/prot.26162)
12. J. R. LaRochelle, M. Fodor, X. Xu, I. Durzynska, L. Fan, T. Stams, H. M. Chan, M. J. LaMarche, R. Chopra, P. Wang, P. D. Fortin, M. G. Acker, S. C. Blacklow, *Biochemistry* **55** (2016) 2269 (https://doi.org/10.1021/acs.biochem.5b01287)
13. M. R. Bauer, R. N. Jones, R. K. Tareque, B. Springett, F. A. Dingler, L. Verduci, K. J. Patel, A. R. Fersht, A. C. Joerger, J. Spencer, *Future Med. Chem*. **11** (2019) 2491 (https://doi.org/10.4155/fmc-2019-0181)
14. He, N. Liu, F. Li, X. Jia, H. Peng, Y.Liu, Y.Xiao, *J. Bacteriol.* **201** (2019) 1 (https://doi.org/10.1128%2FJB.00128-19)
15. T. Geiger, M. Lara-Tejero, Y. Xiong, J. E. Galán, *eLife* **9** (2020) e53473 (https://doi.org/10.7554/eLife.53473)
16. H. Nishimasu, L. Cong, W. X. Yan, F. A. Ran, B. Zetsche, Y.Li, A.Kurabayashi, R. Ishitani, F. Zhang, O. Nureki, *Cell* **162** (2015) 1113 (https://doi.org/10.1016/j.cell.2015.08.007)
17. T.-M. Fu, J. Almqvist, Y.-H. Liang, L. Li, Y. Huang, X.-D- Su, *J. Mol. Biol.* **412** (2011) 688 (https://doi.org/10.1016/j.jmb.2011.08.005)

18. G. M. Morris, R. Huey, W. Lindstrom, M. F. Sanner, R. K. Belew, D. S. Goodsell, A. J. Olson, *J. Comput. Chem.* **30** (2009) 2785 (https://doi.org/10.1002/jcc.21256)
19. D. Biovia, H. Berman, J. Westbrook, Z. Feng, G. Gilliland, T. Bhat, T. J. T. J. o. C. P. Richmond, *Dassault Systèmes BIOVIA, Discovery Studio Visualizer, v. 17.2.0.16349*, Dassault Systèmes, San Diego, CA, 2016, 10 (2000) 0021-9991
20. J. Martinov, M. Krstić, S. Spasić, S. Miletić, J. Stefanović-Kojić, A. Nikolić-Kokić, D. Blagojević, I. Spasojević, M. B. Spasić, *Food Res. Int.* **100** (2017) 132 (https://doi.org/10.1016/j.foodres.2017.08.040)
21. H. S. Park, J. S. Choi, K. H. Kim, *Nutr. Res.* **20** (2000) 1783 (https://doi.org/10.1016/S0271-5317(00)00269-4)
22. A. Markowska, M. Antoszczak, J. Markowska, A. Huczyński, *Pharmaceuticals* **13** (2020) 422 (https://doi.org/10.3390/ph13120422)
23. S. Bongarzone, V. Savickas, F. Luzi, A. D. Gee, *J. Med. Chem.* **60** (2017) 7213 (https://doi.org/10.1021/acs.jmedchem.7b00058)
24. Z. Li, B. Zhou, T. Zheng, C. Zhao, Y. Gao, W. Wu, Y. Fan, X. Wang, M. Qiu, J. Fan, *Foods* **12** (2023) 423 (https://doi.org/10.3390/foods12020423)
25. H. Yan, X. Zhang, L. Yang, Y. Shen, L. Liu, *Food Chem.* **398** (2023) 133886 (https://doi.org/10.1016/j.foodchem.2022.133886)
26. T. B. Emran, F. Islam, S. Mitra, S. Paul, N. Nath, Z. Khan, R. Das, D. Chandran, R. Sharma, C. M. G. Lima, A. A. Al Awadh, I. A. Almazni, A. H. Alhasaniah, R. P. F. Guiné, *Molecules* **27** (2022) 7405 (https://doi.org/10.3390/molecules27217405)
27. L. Delphi, H. Sepehri, *Biomed. Pharmacother.* **84** (2016) 637 (https://doi.org/10.1016/j.biopha.2016.09.080)
28. J. Zhang, F. Zhang, R. Niua, *J. Cell. Mol. Med.* **19** (2015) 2075 (https://doi.org/10.1111/jcmm.12618)
29. J. P. Skittrall, D. Levy, C. Obichukwu, A.Gentle , M. A. Chattaway , D. Hayns , C. Etheridge, C. M. Parry, V. Wong, J. Whitehorn, *Clin. Infect. Pract.* **10** (2021) 100069 (https://doi.org/10.1016/j.clinpr.2021.100069)
30. C. He, N. Liu, F. Li, X. Jia, H. Peng, Y. Liu, Y. Xiao, *J. Bacteriol.* **201** (2019) (https://doi.org/10.1128/JB.00128-19)
31. T.-M. Fu, J. Almqvist, Y.-H. Liang, L. Li, Y. Huang, X. D. Su, *J. Mol. Biol.* **412** (2011) 688 (https://doi.org/10.1016/j.jmb.2011.08.005)
32. W. Chen, Y. Zhang, W.S. Yeo, T. Bae, Q. Ji, *J. Am. Chem. Soc.* **139** (2017) 3790 (https://doi.org/10.1021/jacs.6b13317)
33. G. Hamion, W. Aucher, A. Mercier, F. Tewes, M. Menard, J. Bertaux, M. Girardot, C. Imbert, *Int. J. Antimicrob. Agents* **63** (2024) 107166 **(**https://doi.org/10.1016/j.ijantimicag.2024.107166).