East European Journal of Physics. **2.** 463-469 (2024)
DOI:10.26565/2312-4334-2024-2-61 ISSN 2312-4334

INTERACTIONS OF AMYLOID FIBRILS WITH FUNCTIONAL PROTEINS: MODULATING EFFECT OF POLYPHENOLS

Valeriya Trusova*, Uliana Tarabara, Galyna Gorbenko
Department of Medical Physics and Biomedical Nanotechnologies, V.N. Karazin Kharkiv National University
4 Svobody Sq., Kharkiv, 61022, Ukraine
**Corresponding Author email: valerija.trusova@karazin.ua*
Received May 2, 2024, revised May 22, 2024; accepted May 26, 2024

The elucidation of interactions between functional proteins and amyloid fibrils is crucial for understanding the molecular basis of amyloid diseases, which are characterized by protein misfolding and aggregation. Polyphenols, due to their diverse biological properties, have garnered attention for their potential to modulate these protein-fibril interactions, thereby influencing disease progression and offering therapeutic possibilities. In this study, we investigated the effects of quercetin and its binary combinations with other polyphenols on the binding affinity between cytochrome *c*, in both its reduced and oxidized forms, and amyloid fibrils of insulin and apolipoprotein A-I. Our results demonstrate that quercetin complexation with cytochrome *c* decreases the binding affinity of insulin fibrils for both forms of the protein, while increasing the affinity for apolipoprotein A-I fibrils. This modulation was attributed to competitive or allosteric effects exerted by quercetin on cytochrome *c*. Additionally, while binary combinations of quercetin with other polyphenols did not reduce the affinity of insulin fibrils for oxidized cytochrome *c*, they did decrease the affinity in the case of reduced counterpart. These findings highlight the selective and significant impact of polyphenolic compounds on the interactions between amyloid fibrils and functional proteins, suggesting potential pathways for therapeutic intervention in amyloid-related disorders.
Keywords: *Amyloid fibrils; Functional proteins; Polyphenols; Molecular docking*
PACS: 87.14.C++c, 87.16.Dg

Amyloid fibrils, characterized by their insoluble, fibrous nature, are implicated in a myriad of pathological conditions, including neurodegenerative diseases [1]. These assemblies are formed through the misfolding and aggregation of peptides and proteins into highly ordered, β-sheet-rich structures [2,3]. Despite their notorious association with the diseases [4], recent investigations have illuminated the nuanced roles that amyloid fibrils may play in interacting with functional proteins [5], thereby potentially disrupting normal cellular processes or, conversely, participating in physiological mechanisms that are essential for maintaining cellular homeostasis. The modulation of these type of protein-protein interactions by bioactive compounds offers a promising avenue for therapeutic intervention. Among the wide range of different substances, polyphenols (PF) – a diverse group of naturally occurring compounds with potent biological activities – have garnered attention for their capacity to influence amyloid fibril formation and stability [6-8]. PF are known for their antioxidant properties and their ability to modulate protein aggregation pathways, which may mitigate the deleterious effects of amyloids on functional proteins [9]. The interaction between polyphenols and amyloid fibrils, therefore, represents a critical area of research that could lead to the development of novel therapeutic approaches for managing amyloid diseases. Motivated by these rationales, in the present paper we explore the modulative effects of polyphenolic compounds with quercetin serving as the primary PF under examination, on the interactions between the amyloid fibrils formed by insulin and apolipoprotein A-I with functional proteins, represented by reduced and oxidized forms of cytochrome *c*. Utilizing the molecular docking as our primary methodological approach, this study aims to provide a molecular-level understanding of how polyphenols can influence the binding interactions between fibrillar assemblies and endogenous proteins, offering insights into the mechanistic pathways through which PF exert their modulatory effects. To enhance the depth of our analysis, quercetin was not only studied in isolation but also in binary combinations with other polyphenolic compounds, each selected for their unique chemical properties and potential synergistic effects. These additional polyphenols include sesamin, curcumin, phenolic acid, gallic acid, and resveratrol. Through this detailed investigation, we seek to elucidate the potential of polyphenols as therapeutic agents in diseases associated with protein aggregation, highlighting their role in disrupting or stabilizing protein-protein interactions that are critical to disease progression.

METHODS

The blind docking of the examined complexes was performed using the web-based server HDOCK. This server implements an FFT-based hierarchical algorithm of rigid-body docking through mapping the receptor and ligand molecules onto grids and global sampling of the possible binding modes with an improved shape complementarity scoring method in which one molecule is fixed, while the second one adopts evenly distributed orientations in rotational Euler space and translational space within a grid. The resulting docking solutions are ranked according to their binding energy and clustered [10]. The three-dimensional X-ray crystal structures of oxidized and reduced cytochrome *c* were obtained

Cite as: V. Trusova, U. Tarabara, G. Gorbenko, East Eur. J. Phys. 2, 463 (2024), https://doi.org/10.26565/2312-4334-2024-2-61

from the Protein Data Bank (https://www.rcsb.org/) using the PDB IDs 2N9J and 2N9I, respectively. The 50-monomer fragment of insulin fibril model was provided by M. Sawaya (http://people.mbi.ucla.edu/sawaya/jmol/fibrilmodels/). The model amyloid fibrils of apoliporotein A-I were constructed using the CreateFibril tool based on the translational and rotational affine transformations providing several copies of a certain fragment of fibril core, whose subsequent stacking produces the elongated fibrillar aggregate [11]. The input structures for CreateFibril were generated from the monomers in the β-strand conformation with PatchDock. The selected docking poses were visualized with the UCSF Chimera software (version 1.14) and analyzed with BIOVIA Discovery Studio Visualizer, v21.1.0.20298, San Diego: Dassault Systemes; 2021.

RESULTS AND DISCUSSION

At the initial stage of the study, we meticulously explore the binding interactions of quercetin with cytochrome *c* (cyt *c*) in the reduced and oxidized form, and amyloid fibrils, represented by fibrillar insulin (InsF) and fibrillar apolipoprotein A-I (ApoAIF) as distinct control calculations. This foundational step is critical for establishing a baseline understanding of how polyphenols interact with each component individually before examining their tripartite interactions within a complex system. Fig. 1 represents the docking poses of the complexes between cyt *c* and quercetin (QR) corresponding to the best score of the docking. As seen from this figure, QR forms stable contacts with protein molecule both in the case of reduced and oxidized state of cyt *c*.

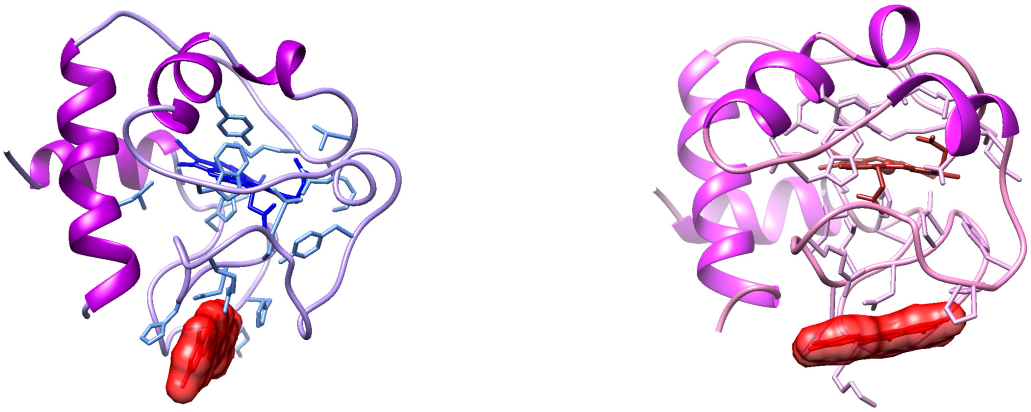

Figure 1. Representation of the optimal binding conformation of quercetin to the oxidized (left panel) and reduced (right panel) forms of cytochrome *c* as determined by the docking algorithm. Quercetin is shown in red

Cyt *c*, a small heme protein located within the mitochondrial intermembrane space, plays a pivotal role in the electron transport chain, facilitating the transfer of electrons between Complex III (cytochrome bc1 complex) and Complex IV (cytochrome c oxidase) [12]. This electron shuttling is crucial for the mitochondrial production of ATP, the energy currency of the cell, which underscores the fundamental role of cytochrome c in cellular energetics and metabolism. The functionality of cytochrome c is inherently linked to its redox state, with the protein undergoing reversible oxidation and reduction of its iron atom from the ferric (Fe^{3+}) to the ferrous (Fe^{2+}) state and *vice versa* [13]. From a physiological perspective, the redox state of cytochrome c not only dictates its electron-carrying capacity but also influences other critical cellular processes, including apoptosis. In its oxidized form (Fe^{3+}), cytochrome *c* is capable of inducing caspase activation via the apoptosome, a key component in the intrinsic pathway of apoptosis. This pro-apoptotic activity is attributed to the release of oxidized cytochrome *c*, cyt *c* oxy, into the cytosol following mitochondrial membrane permeabilization, which then interacts with apoptotic protease activating factor-1 to form the apoptosome, thereby initiating the caspase cascade leading to programmed cell death. Conversely, the reduced form of cytochrome *c*, cyt *c* red, (Fe^{2+}) exhibits a diminished capacity to initiate apoptosis, highlighting a regulatory mechanism by which the cellular redox state can influence apoptotic sensitivity. This redox-dependent modulation of apoptosis underscores the dual functionality of cytochrome c, serving both life-sustaining and cell death-promoting roles within the organism [14].

The binding scores obtained from the docking simulations were -123.51 for the oxidized form and -119.78 for the reduced form of the protein, indicating a slightly stronger affinity of quercetin for the oxidized state of the protein. These results suggest that the redox state of cyt *c* could influence its interaction with quercetin, potentially affecting the biological outcomes of this interaction. A detailed examination of the receptor-ligand interface residues reveals a largely conserved interaction pattern between the two redox states of cyt *c*, with only minor differences observed. Specifically, the interface residues involved in binding quercetin to the oxidized form of cyt *c* include THR_{19}, GLU_{21}, LYS_{22}, GLY_{23}, GLY_{24}, LYS_{25}, HIS_{26}, ASN_{31}, LEU_{32}, HIS_{33}, GLY_{34}, and ARG_{38}. Comparatively, in the reduced form, the interacting residues are GLU_{21}, LYS_{22}, GLY_{23}, GLY_{24}, LYS_{25}, HIS_{26}, ASN_{31}, HIS_{33}, GLY_{34}, ARG_{38}, ALA_{43}, and PRO_{44}. Notably, the core residues such as GLU_{21}, LYS_{22}, GLY_{23}, GLY_{24}, LYS_{25}, HIS_{26}, ASN_{31}, HIS_{33}, GLY_{34}, and ARG_{38} are conserved across both redox states, indicating a fundamental similarity in the binding mode of quercetin to cytochrome c irrespective of its oxidation state. The slight differences observed, specifically the additional involvement of ALA_{43} and PRO_{44} in the reduced form, suggest subtle conformational or electronic changes in cyt *c* upon reduction that might slightly alter the

binding landscape. These differences, while minor, could potentially influence the dynamics and stability of the quercetin-cytochrome *c* complex, possibly affecting the biological activity of cyt *c* in subtle ways.

Next, the binding of QR to amyloid fibrils of insulin and apolipoprotein A-I was explored. Fig. 2 represents the location of QR within the InsF and ApoAIF. The binding scores obtained were notably distinct, with QR exhibiting a binding score of -163.8 with InsF and -121.18 with ApoAIF. These results indicate a significantly stronger affinity of quercetin for InsF compared to those formed by ApoAIF. The analysis of the receptor-ligand interface residues provides further insights into the molecular basis of these interactions. For InsF, the interface residues involved in binding of QR include GLN_{115}, PHE_{201}, VAL_{202}, ASN_{203}, GLN_{204}, ILE_{2}, LEU_{13}, GLN_{15}, LEU_{113}, VAL_{202}, ASN_{203}, GLN_{204}, LEU_{206}, GLN_{5}. These residues are predominantly hydrophobic and polar in nature, which suggests that both hydrophobic interactions and hydrogen bonding might play crucial roles in stabilizing the quercetin-insulin fibril complex.

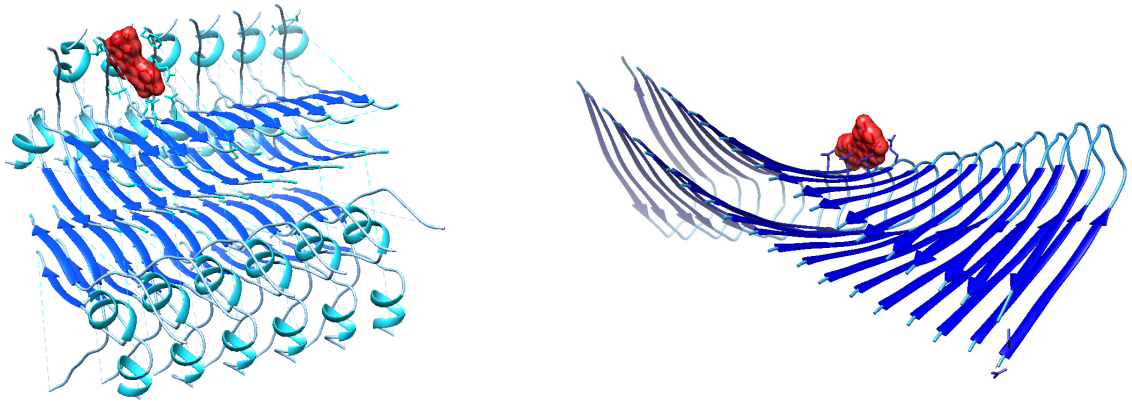

Figure 2. Location of QR within the amyloid insulin (left panel) and apolipoprotein A-I (right panel) in the best docking mode

The presence of multiple glutamine and asparagine residues could facilitate extensive hydrogen bonding, while hydrophobic residues like LEU, VAL, and PHE may contribute to the overall binding affinity through van der Waals interactions. In contrast, the interface residues for ApoAIF interacting with QR were identified as GLN_{271}, LEU_{272}, ASN_{273}, GLN_{298}, LEU_{299}, ASN_{300}, GLN_{325}, LEU_{326}, ASN_{327}, ASN_{354}. Similar to the insulin fibril interaction, this set also includes a mix of hydrophobic and polar residues, indicating a similar mode of interaction. However, the lower binding score observed with ApoAIF suggests that the specific arrangement and accessibility of these residues might not be as conducive to high-affinity binding as those in InsF. The differential binding affinities observed between QR and the two types of amyloid fibrils could be attributed to variations in the amyloid fibril structures, which in turn affect the availability and orientation of key residues at the binding interface. Amyloid fibrils, despite their general structural similarities, can exhibit significant variability in their surface characteristics depending on the specific protein from which they are formed. This structural variability can influence the docking and binding efficiency of small molecules like quercetin.

The final iteration of the control calculations was the molecular docking simulations of cyt *c* binding to the amyloid fibrils. Fig. 3 depicts the 3D structures of cyt *c* – amyloid complexes while Table 1 summarizes the interfacial amino acid residues on the fibril surface that are in contact with cyt *c*.

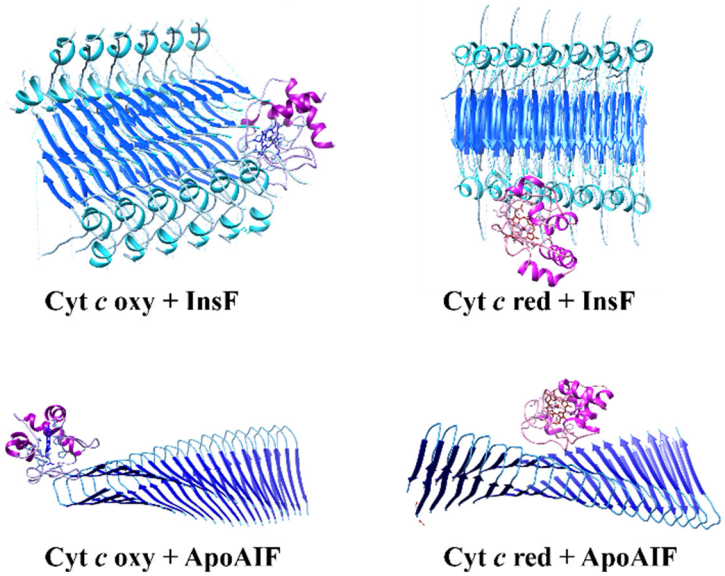

Figure 3. Docked poses corresponding to minimum energy for the complexes between reduced and oxidized form of cyt c and amyloid fibrils of insulin and apolipoprotein A-I. Cyt c is shown in pink

Analysis of the docking scores indicate a notably stronger binding affinity of the reduced form of cyt *c* to both InsF (-229.81) and ApoAIF (-174.63) compared to its oxidized counterpart, which exhibited scores of -218.95 and -164.58, respectively. This suggests that the electron state of cyt *c* significantly influences its interaction dynamics with amyloid fibrils. The reduced form, containing the ferrous state, may facilitate more favorable electrostatic or coordination interactions with the amyloid fibrils compared to the ferric state in the oxidized form. The interface residues identified on cytochrome c, which are in contact with the amyloid fibrils, further elucidate the molecular basis of these interactions. For the oxidized form of cyt *c*, residues such as SER_{15}, GLN_{16}, and LYS_{25} are implicated in binding to insulin fibrils, while residues including GLU_{21}, LYS_{22}, and GLY_{23} are involved in interactions with ApoAIF. Conversely, the reduced form of cyt *c* interacts with insulin fibrils through residues such as VAL_{20}, LYS_{22}, and ASN_{31}, and with ApoAIF through residues like LYS_{22}, GLY_{23}, and ASN_{31}. These residues, encompassing both polar and non-polar amino acids, suggest a complex interaction environment that might facilitate stabilization through various non-covalent interactions, including hydrogen bonds and hydrophobic contacts.

Table 1. Receptor-ligand interface residues in the complexes of cytochrome c with amyloid fibrils in the absence and presence of quercetin

Interface residues on cytochrome *c*	
Cyt *c* oxy	
InsF	
Without quercetin	SER_{15A}, GLN_{16A}, CYS_{17A}, LYS_{25A}, HIS_{26A}, LYS_{27A}, THR_{28A}, GLY_{45A}, TYR_{46A}, SER_{47A}, TYR_{48A}, THR_{49A}, ALA_{50A}, ALA_{51A}, LYS_{72A}, PRO_{76A}, GLY_{77A}, THR_{78A}, LYS_{79A}, MET_{80A}, ILE_{81A}, VAL_{83A}
With quercetin	GLN_{16A}, CYS_{17A}, LYS_{25A}, HIS_{26A}, LYS_{27A}, THR_{28A}, GLY_{29A}, GLY_{45A}, TYR_{46A}, SER_{47A}, TYR_{48A}, THR_{49A}, ALA_{50A}, GLY_{77A}, THR_{78A}, LYS_{79A}, ILE_{81A}
ApoAIF	
Without quercetin	GLU_{21A}, LYS_{22A}, GLY_{23A}, GLY_{24A}, LYS_{25A}, HIS_{26A}, ASN_{31A}, HIS_{33A}, GLY_{34A}, LEU_{35A}, PHE_{36A}, GLY_{37A}, ARG_{38A}, LYS_{39A}, GLN_{42A}, ALA_{43A}, PRO_{44A}, GLY_{45A}, LYS_{99A}, THR_{102A}, ASN_{103A}, GLU_{104A}
With quercetin	GLU_{21A}, LYS_{22A}, GLY_{23A}, GLY_{24A}, LYS_{25A}, HIS_{33A}, GLY_{34A}, LEU_{35A}, PHE_{36A}, GLY_{37A}, ARG_{38A}, LYS_{39A}, GLY_{41A}, GLN_{42A}, ALA_{43A}, PRO_{44A}, GLY_{45A}, LYS_{99A}, LYS_{100A}, THR_{102A}, ASN_{103A}, GLU_{104A}
Cyt *c* red	
InsF	
Without quercetin	VAL_{20A}, LYS_{22A}, GLY_{23A}, ASN_{31A}, HIS_{33A}, GLY_{34A}, PHE_{36A}, GLY_{37A}, ARG_{38A}, LYS_{39A}, THR_{40A}, GLY_{41A}, GLN_{42A}, ALA_{43A}, PRO_{44A}, GLY_{45A}, TYR_{46A}, SER_{47A}, TYR_{48A}, ASN_{52A}, LYS_{53A}, ASN_{54A}, LYS_{55A}, GLY_{56A}, ILE_{57A}, ILE_{58A}, LYS_{99A}, LYS_{100A}, ALA_{101A}, THR_{102A}, ASN_{103A}, GLU_{104A}
With quercetin	ALA_{51A}, ASN_{54A}, LYS_{55A}, GLY_{56A}, ILE_{57A}, ASP_{62A}, THR_{63A}, MET_{65A}, GLU_{66A}, TYR_{67A}, GLU_{69A}, ASN_{70A}, PRO_{71A}, LYS_{72A}, LYS_{73A}, TYR_{74A}, ILE_{75A}, PRO_{76A}, LYS_{79A}, MET_{80A}, ILE_{81A}, PHE_{82A}, VAL_{83A}, GLY_{84A}, ILE_{85A}, LYS_{86A}, LYS_{88A}, ARG_{91A}
ApoAIF	
Without quercetin	LYS_{22A}, GLY_{23A}, ASN_{31A}, HIS_{33A}, GLY_{34A}, LEU_{35A}, PHE_{36A}, GLY_{37A}, ARG_{38A}, LYS_{39A}, THR_{40A}, GLY_{41A}, GLN_{42A}, PRO_{44A}, GLY_{45A}, LYS_{53A}, ASN_{54A}, LYS_{55A}, GLY_{56A}, ILE_{57A}, ILE_{58A}, ASN_{103A}
With quercetin	GLU_{21A}, LYS_{22A}, GLY_{23A}, GLY_{24A}, LYS_{25A}, HIS_{33A}, GLY_{34A}, LEU_{35A}, PHE_{36A}, GLY_{37A}, ARG_{38A}, LYS_{39A}, GLY_{41A}, GLN_{42A}, ALA_{43A}, PRO_{44A}, GLY_{45A}, LYS_{99A}, LYS_{100A}, THR_{102A}, ASN_{103A}, GLU_{104A}

The implications of the interactions between cytochrome *c* in its reduced and oxidized forms with amyloid fibrils of insulin and apolipoprotein A-I extend beyond mere molecular docking scores and interface residues. These interactions provide a deeper understanding of the potential pathological and physiological roles of amyloid fibrils in relation to mitochondrial function, particularly in the context of neurodegenerative and systemic amyloid diseases. The differential binding affinities and interaction patterns of cyt *c* with amyloid fibrils, as observed in our study, suggest a nuanced influence of the protein's redox state on its association with amyloid structures. In diseases characterized by amyloidosis, such as Alzheimer's disease and type II diabetes, the deposition of amyloid fibrils is a hallmark [15]. These fibrils can sequester functional proteins like cytochrome *c*, potentially diverting them from their normal physiological roles. For instance, the stronger binding affinity of the reduced form of cyt *c* to amyloid fibrils might lead to a higher sequestration rate, thereby reducing its availability for electron transport activities. This could result in impaired mitochondrial function, decreased ATP production, and increased oxidative stress, all of which are critical factors in the progression of amyloid-related diseases. Moreover, the specific interaction sites and the nature of the binding (whether at the edge or side of the fibrils) could influence the structural integrity and toxicity of the amyloid fibrils themselves. By binding to specific sites, cytochrome c might stabilize certain conformations of the fibrils that are less toxic or, conversely, might promote configurations that are more detrimental to cellular health. On a physiological level, the interactions between cytochrome c and amyloid fibrils could also play a role in the natural regulation of apoptosis, a process in which cyt *c* is a key player.

Under normal conditions, cyt *c*, released into the cytosol from the mitochondria, initiates the apoptotic cascade. However, if cyt *c* is bound to amyloid fibrils, its release and subsequent initiation of apoptosis might be hindered, potentially affecting cell survival in stressed or damaged cells. This interaction could thus represent a double-edged sword, where it might either prevent unnecessary apoptosis in a protective manner or could hinder necessary cell death, leading to the persistence of dysfunctional cells.

After examination the binding patterns between different pairs of components, in the following we examined the interactions within the triad system QR + cyt *c* + amyloid fibrils. As seen from Table 2, the complexation of quercetin with cyt *c* leads to the decrease in the binding affinity of the insulin fibrils for both oxy and red forms of cyt *c*, and to the increase for the case of ApoA-I fibrils.

Table 2. The best scores of the complexes between cyt *c* and amyloid fibrils in the absence and presence of quercetin

	Amyloid fibrils	
	InsF	**ApoAIF**
Cyt *c* oxy	-218.95	-164.58
Cyt *c* red	-229.81	-174.63
Cyt *c* oxy + quercetin	-212.85	-174.40
Cyt *c* red +quercetin	-212.58	-186.39

The observed decrease in binding affinity may be attributed to the competitive or allosteric modulation effects exerted by QR on cyt *c*. Specifically, the presence of overlapping amino acid residues in the binding sites for quercetin and insulin on cytochrome c suggests a competitive interaction scenario. In the oxidized form of cyt *c*, residues such as LYS_{25} and HIS_{26}, and in the reduced form, residues including ASN_{31}, HIS_{33}, GLY_{34}, ARG_{38}, ALA_{43}, and PRO_{44}, are implicated in these interactions. These residues are crucial for the binding of cytochrome c to insulin fibrils, and their involvement in quercetin binding implies that QR may obstruct these critical sites, thereby hindering the effective interaction of cyt *c* with the amyloid fibrils. The ability of QR to modulate the interaction between cyt *c* and insulin amyloid fibrils has significant implications for understanding the pathological processes associated with amyloid diseases, particularly those related to mitochondrial dysfunction. The binding of cyt *c* to amyloid fibrils, as observed in amyloidogenic diseases, can disrupt its normal functions, leading to impaired mitochondrial electron transport and reduced apoptotic signaling. By reducing the affinity of cyt *c* for amyloid fibrils, QR could potentially restore or preserve the functional integrity of cyt *c*, thereby maintaining cellular energy production and promoting appropriate apoptotic responses. The interaction of QR with cyt *c* and its consequent effect on the protein affinity for amyloid fibrils could also have broader implications for cellular homeostasis and the progression of amyloid-related diseases. By potentially disfavoring the formation of non-functional complexes between cyt *c* and amyloid fibrils, quercetin may aid in mitigating the cytotoxic effects associated with amyloid accumulation. This protective mechanism could be particularly beneficial in the context of neurodegenerative diseases, where the deposition of amyloid fibrils is a hallmark feature.

In turn, the increased affinity of cyt *c* for ApoAIF could potentially enhance the undesirable impacts of amyloid fibrils on functional proteins. By binding more strongly to amyloid fibrils, cyt *c* may become increasingly sequestered in these complexes, thereby being unavailable for its normal physiological roles. This sequestration could contribute to mitochondrial dysfunction, a hallmark of many amyloid-related diseases, including Alzheimer's disease and systemic amyloidosis. Furthermore, the prevention of cyt *c* release into the cytosol could inhibit the initiation of the apoptotic cascade, potentially leading to the survival of damaged or dysfunctional cells that contribute to disease pathology.

Inspired by the revealed ability of quercetin to modulate the interactions between amyloid fibrils and cyt *c*, at the last step of the study we examined the synergetic potential of QR and other polyphenols to alter the binding profile of cyt *c* with InsF. As shown in Fig. 4, binary combinations of QR + sesamin / curcumin / phenolic acid / gallic acid / resveratrol was not effective in the attenuation of InsF affinity for cyt *c* oxy. The contrary effect was revealed for the case of cyt *c* red, where all sets of binary polyphenol complexes gave rise to the decrease to the protein-fibril binding affinity, with the maximum change of the best docking score being observed for gallic acid.

The observed lack of efficacy in attenuating the affinity of insulin amyloid fibrils for cyt *c* oxy in the presence of afore-mentioned binary polyphenolic complexes may be symptomatic of a specific conformational architecture of the oxidized form, which resists modulation by these polyphenols. This resistance may be attributed to the inherent stability of the interactions between oxidized cyt *c* and the amyloid fibrils which may not be sufficiently destabilized by the polyphenolic compounds tested. In addition, one should bear in mind, that when polyphenols reside at the interface between a protein and fibril, they may form additional contacts with amyloid fibrils, thereby increasing its binding affinity to the functional protein.

In stark contrast, the reduction in protein-fibril binding affinity for the reduced form of cyt *c* in the presence of these binary polyphenol complexes, especially that noted with gallic acid, underscores the potential allosteric or direct interaction effects of these compounds on the protein-fibril interface. This reduction in binding affinity suggests a destabilization of the amyloid fibril interaction with cyt *c*, thus potentially favoring the maintenance of the protein native functional state. The decrease in protein-fibril binding affinity, elicited by binary polyphenolic combinations, is particularly thought-provoking when considering the pathological ramifications of amyloid-protein interactions. The

propensity for amyloid fibrils to sequester functional proteins like cytochrome *c* can culminate in the impairment of critical cellular functions. Thus, the observed modulation of these interactions by polyphenolic compounds may hold therapeutic relevance, offering a biochemical scaffold for the development of interventions aimed at mitigating the deleterious consequences of amyloid-associated diseases.

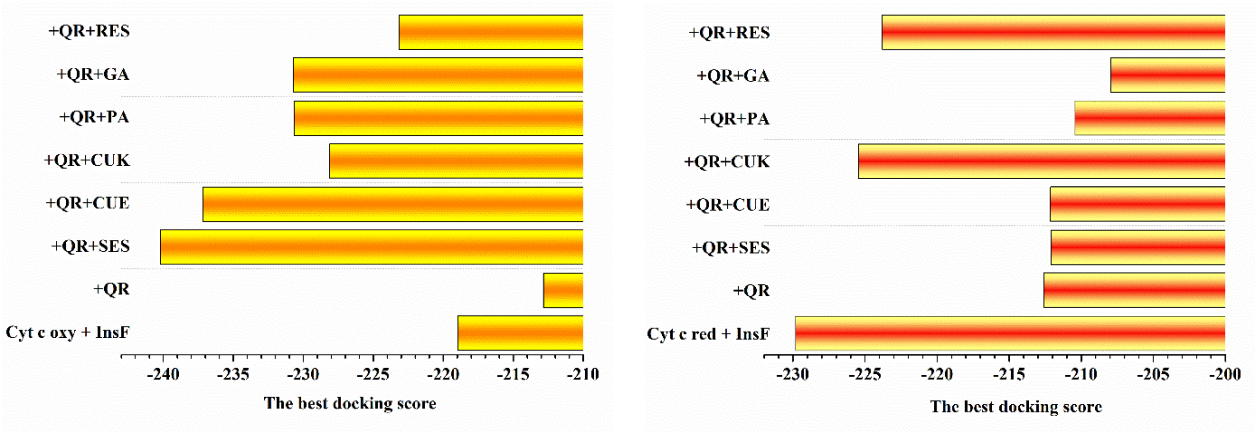

Figure 4. The changes in the best docking score calculated for the complexes cyt *c* oxy/red – quercetin – sesamin / curcumin / phenolic acid / gallic acid / resveratrol – InsF. The best docking score characterizes the affinity of InsF for cyt *c*. The abbreviations used are: quercetin (QR), sesamin (SES), curcumin enol form (CUE, curcumin keto form (CUK), phenolic acid (PA), gallic acid (GA), resveratrol (RES)

CONCLUSIONS

To summarize, the molecular docking studies delineating the interactions between cyt *c* and amyloid fibrils, alongside the modulatory presence of quercetin, provide an enlightening snapshot of the polyphenolic intervention in amyloidogenic landscapes. The complexation of QR with cyt *c* and the consequent modulation of its binding affinity to amyloid fibrils of insulin are suggestive of a polyphenol-mediated alteration in the protein-fibril interface, effectuating a decrease in affinity irrespective of the redox state of the cytochrome. This discovery highlights the potential of quercetin to impede non-specific interaction between functional proteins and amyloid fibrils, suggesting a protective mechanism that may preserve the physiological functions of cyt *c* against amyloid-induced perturbation. Such a phenomenon is of considerable interest, underscoring the broad utility of polyphenols as molecular agents capable of modifying the course of protein aggregation diseases by stabilizing functional proteins against the aberrant binding to amyloid fibrils.

Acknowledgements

This project has received funding through the EURIZON project, which is funded by the European Union under grant agreement No. 3049.

ORCID

Valeriya Trusova, https://orcid.org/0000-0002-7087-071X; **Uliana Tarabara,** https://orcid.org/0000-0002-7677-0779
Galyna Gorbenko, https://orcid.org/0000-0002-0954-5053

REFERENCES

[1] E. Chatani, K. Yuzu, Y. Ohhashi, and Y. Goto, Int. J. Mol. Sci. **22**, 4349 (2021). https://doi.org/10.3390/ijms22094349
[2] A. Buell, Chem. Sci. **12**, 10177 (2022). https://doi.org/10.1039/D1SC06782F
[3] D. Li, and C. Liu, Biochemistry 59, 639 (2020). https://doi.org/10.1021/acs.biochem.9b01069
[4] H. Koike, and M. Katsuno, Molecules **26**, 4611 (2021). https://doi.org/10.3390/molecules26154611
[5] H. Xie, and C. Guo, Front. Mol. Biosci. **7**, 629520 (2021). https://doi.org/10.3389/fmolb.2020.629520
[6] P. Bhosale, S. Ha, P. Vetrivel, H. Kim, S. Kim, and G. Kim, Transl. Cancer Res. **9**, 7619 (2020). https://doi.org/10.21037/tcr-20-2359
[7] A. Rana, M. Samtiya, T. Dhewa, V. Mishra, and R. Aluko, J. Food. Chem. **46**, e14264 (2022). https://doi.org/10.1111/jfbc.14264
[8] Y. Nian, Y. Zhang, C. Ruan, and B. Hu, Curr. Opin. Food Sci. **43**, 99 (2022). https://doi.org/10.1016/j.cofs.2021.11.005
[9] H. Kobayashi, M. Murata, S. Kawanishi, and S. Oikawa, Int. J. Mol. Sci. **21**, 3561 (2020). https://doi.org/10.3390/ijms21103561
[10] Y. Yan, H. Tao, J. He, and S-Y. Huang, Nat. Protoc. **15**, 1829–1852 (2020). https://doi.org/10.1038/s41596-020-0312-x
[11] D. D. Schneidman-Duhovny, Y. Inbar, R. Nussimov, and H. Wolfson, Nucl. Acid Res. **33**, W363 (2005). https://doi.org/10.1093/nar/gki481
[12] Z. Zhou, T. Arroum, X. Luo, R. Kang, Y. Lee, D. Tang, M. Hüttemann, and X. Song, Cell Death Differ. **31**, 387 (2024). https://doi.org/10.1038/s41418-024-01284-8
[13] G. Perez-Mejias, A. Diaz-Quintana, A. Guerra-Castellano, I. Diaz-Moreno, and M. De la Rosa, Coord. Chem. Rev. **450**, 214233 (2022). https://doi.org/10.1016/j.ccr.2021.214233
[14] S. Watson, G. McStay, Int. J. Mol. Sci. **21**, 7254 (2020). https://doi.org/10.3390/ijms21197254
[15] A. Bigi, R. Cascella, F. Chiti, C. Cecchi, Bioessays **44**, e2200086 (2022). https://doi.org/10.1002/bies.202200086

ВЗАЄМОДІЯ АМІЛОЇДНИХ ФІБРИЛ З ФУНКЦІОНАЛЬНИМИ БІЛКАМИ: МОДУЛЮЮЧИЙ ЕФЕКТ ПОЛІФЕНОЛІВ

Валерія Трусова, Уляна Тарабара, Галина Горбенко

Кафедра медичної фізики та біомедичних нанотехнологій, Харківський національний університет імені В.Н. Каразіна м. Свободи 4, Харків, 61022, Україна

Вивчення взаємодій між функціональними білками та амілоїдними фібрилами є ключовим для розуміння молекулярної основи амілоїдних захворювань, які характеризуються неправильним згортанням білків та їх агрегацією. Поліфеноли, завдяки своїм різноманітним біологічним властивостям, привертають увагу своєю потенційною здатністю модулювати ці взаємодії, тим самим впливаючи на прогресування захворювання та відкриваючи нові терапевтичні можливості. У даному дослідженні методом молекулярного докінгу було вивчено вплив кверцетину та його бінарних комбінацій з іншими поліфенолами на зв'язування між цитохромом *c* (відновлена та окиснена форми) та амілоїдними фібрилами інсуліну та аполіпопротеїну А-І. Отримані результаті свідчать на користь того, що комплексування кверцетину з цитохромом *c* знижує силу зв'язування фібрил інсуліну для обох форм білка. Протилежний ефект було виявлено для фібрил аполіпопротеїну А-І. Таке модулювання було інтерпретовано у рамках конкурентних або алостеричних ефектів. Крім того, хоча бінарні комбінації кверцетину з іншими поліфенолами не знижували силу зв'язування фібрил інсуліну для окисленого цитохрому *c*, вони знижували силу зв'язування у випадку відновленої форми. Ці дані підкреслюють вибірковий та значний вплив поліфенольних сполук на взаємодії між амілоїдними фібрилами та функціональними білками, пропонуючи потенційні шляхи для терапевтичних анти-амілоїдних стратегій.

Ключові слова: *амілоїдні фібрили; функціональні білки; поліфеноли; молекулярний докінг*