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Original Article

PROTEIN-PROTEIN INTERACTION ANALYSIS TO IDENTIFY NUCLEAR FACTOR-ERYTHROID-2 FACTOR 2 (NRF2) INHIBITION BY EXTRACELLULAR ENZYMES FROM WATER KEFIR ORGANISMS

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ABSTRACT

Objective: The study aimed to investigate the interactions between twelve extracellular enzymes and nuclear factor erythroid 2–related factor 2 (Nrf2) in the active site.

Methods: The Zdock web server was accessed to perform molecular docking simulations for predicting interactions between the extracellular enzymes and the active site of Nrf2. The Z score analysis revealed enzymes with high scores, indicating strong and statistically significant interactions with Nrf2.

Results: DNase 1, α-amylase, and lecithinase C exhibited notably high Z scores, suggesting potential key players in modulating Nrf2-mediated signaling pathways. The examination of salt bridges showed enzymes with more ionic interactions, suggesting enhanced stability and potential for strong binding within the active site of Nrf2. This characteristic might be crucial for enzymatic inhibition of Nrf2's activity.

Conclusion: In conclusion, the findings highlight enzymes, including DNase 1, α-amylase, and lecithinase C, as promising candidates for further exploration as potential inhibitors of NRF2-mediated cellular responses.

Keywords: Protein-Protein Interaction, Nuclear factor-erythroid-2 factor 2 (Nrf2), Inhibition, Extracellular Enzymes, Water Kefir

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INTRODUCTION

Nrf2 is a transcription factor that plays a crucial role in regulating the expression of antioxidant and detoxification genes. Nrf2 is proven to stimulate the induction of enzymes that participate in metabolism. Nrf2 is activated by oxidative stress and electrophilic compounds, leading to its translocation to the nucleus and binding to antioxidant response elements (AREs) in the promoter regions of target genes [1]. Keap1 is a cytoplasmic protein that negatively regulates Nrf2 by promoting its ubiquitination and degradation [2]. Keap1 binds to Nrf2 through its Kelch domain, and this interaction is disrupted by electrophilic compounds, leading to Nrf2 activation. Inhibiting the Keap1-Nrf2 interaction has been proposed as a potential therapeutic strategy for various diseases, including cancer, neurodegenerative diseases, and diabetes [3].

Water kefir organisms, known for their probiotic and bioactive properties, have been gaining attention as a potential source of biologically active compounds with health benefits. It is a fermented beverage that contains a variety of microorganisms, including bacteria and yeasts. These microorganisms produce various extracellular enzymes that play a crucial role in the fermentation process and the health-promoting properties of water kefir. These enzymes include glucansucrases, polymerases, metabolic enzymes, dextransucrases, and acid-producing enzymes [4-8].

Glucansucrases are extracellular enzymes produced by lactic acid bacteria (LAB) that are responsible for the growth of water kefir grains. Their activity is optimal at a specific pH and calcium concentration [4]. Polymerases can occur cell wall-bound or extracellular and are responsible for polymerizing substrates such as sucrose or raffinose. Raffinose can only be used by certain microorganisms [5]. Microorganisms in water kefir also express various metabolic enzymes that are involved in carbohydrate metabolism and other metabolic pathways [6]. Dextransucrases are enzymes that produce dextran, which are exopolysaccharides found in water kefir grains. The dextran produced by water kefir grains are mostly O3-branched and contain an elevated portion of 1,3-linked glucose units compared to other dextran [7]. Microorganisms in water kefir also produce lactic and acetic acid through the activity of enzymes such as *Lactobacillus hilgardii* and *Acetobacter tropicalis* [8].

On the other side, water kefir has been shown to activate Nrf2 under hyperglycemic conditions in the kidneys of diabetic rats [9]. Glucansucrases produced by LAB, which are responsible for the water kefir grain growth, are extracellular enzymes [4]. Microorganisms immersed in kefir grain are responsible for the synthesis of extracellular components [10].

Protein-protein interactions (PPIs) play a crucial role in various cellular activities and pathways, making them promising targets for drug discovery. Research designs in the field of protein-protein interaction in drug discovery involve various approaches, such as identifying target proteins with well-defined binding sites, developing novel drug-like small molecules, utilizing virtual screening tools, using structural and biophysical methods, and applying machine learning and artificial intelligence techniques. These approaches aim to overcome the challenges presented by PPIs as drug targets and facilitate the development of new therapeutics for diseases [11].

There is limited research on the potential of extracellular enzymes from water kefir organisms to inhibit Nrf2, and further studies are needed to explore this potential therapeutic application. Therefore, the research aimed to identify extracellular enzymes from water kefir organisms that show significant interactions with the active site of Nrf2 based on Z scores obtained from protein-protein interaction analysis. By applying the computational tools to perform protein-protein interaction analysis between the identified extracellular enzymes and the active site of Nrf2, calculating Z scores for each interaction.

MATERIALS AND METHODS

Materials

For protein-protein interaction, the personal computer featured an Intel® CoreTM i7-7200U CPU running at 2.50 GHz (4 CPUs running at 2.7 GHz), 20 GB of RAM, and a Windows 10 Pro-64-bit operating system.

Preparation of extracellular enzymes and NRF2 structures

The 3D structures of extracellular enzymes from water kefir organisms, α-Amylase (PDB ID 1UA7), β-Amylase (PDB ID 1VEM), cellobiohydrolase (PDB ID 1I1Y), cellulase (PDB ID 1H11), DNase 2 (PDB ID 5I3E), DNase 1 (PDB ID 2DDR), extracellular serine protease (PDB ID 3HJR), gelatinase A (PDB ID 1GEN), gelatinase B (PDB ID 6ESM), glucosidase (PDB ID 1UOK), lecithinase C (PDB ID 1AH7), and Neutral protease (PDB ID 1NPC), and Nrf2 (PDB ID 4I7B) were obtained from the Protein Data nBank (PDB) (https://www.rcsb.org).

Protein-protein interaction analysis

The Zdock web server (https://zdock.umassmed.edu) was accessed to perform molecular docking simulations for predicting interactions between the extracellular enzymes and the active site of Nrf2. The extracellular enzymes were designated as ligands, while Nrf2 was set as the receptor during the docking simulations. The web server allowed for multiple docking runs to account for conformational flexibility and generate an ensemble of possible interactions.

Docking result analysis

The docking results obtained from the Zdock web server were downloaded and analyzed using the pdbsum1 application (https://www.ebi.ac.uk/thornton-srv/software/PDBsum1/). Z scores were calculated to assess the statistical significance of the predicted interactions, identifying extracellular enzymes with strong binding potentials to Nrf2. The application quantified the number of salt bridges, hydrogen bonds, and non-bonded contacts to understand the nature and stability of the enzyme-Nrf2 complexes. The results were analyzed and correlated with the Z scores, number of salt bridges, number of hydrogen bonds, and number of non-bonded contacts.

RESULTS AND DISCUSSION

Protein-protein interaction analysis

The protein-protein interaction analysis for the identification of nuclear factor-erythroid-2 related factor 2 (Nrf2) inhibitory extracellular enzymes from water kefir organisms was conducted using the web-based version of the Zdock application and the pdbsum1 application. The computations were performed on a high-performance server with sufficient processing power and memory to handle the computational demands of molecular docking and analysis. One important aspect of these simulations is the assessment of the binding interaction, which is often represented as a Z score. The Z score is a statistical measure used to evaluate the quality of the protein-protein interaction results by comparing the observed energy with a distribution of energies expected from random docking poses [11]. At the end, analyzed the Z scores for various extracellular enzymes obtained from protein-protein docking simulations.

Table 1: Docking score and bond interactions of the extracellular enzymes in the binding pocket of NRF2 (PDB ID 4I7B)

No.	Extracellular enzymes	PDB ID	Z score	Number of salt bridges	Number of hydrogen bonds	Number of non-bonded contacts
1	α-Amylase	1UA7	1624.022	4	21	435
2	β-Amylase	1VEM	1556.103	3	13	405
3	Cellobiohydrolase	1I1Y	1459.164	3	8	287
4	Cellulase	1H11	1557.836	3	7	319
5	DNase 2	5I3E	1449.446	0	8	303
6	DNase 1	2DDR	1935.147	0	6	373
7	Extracellular serine protease	3HJR	1338.119	1	8	327
8	Gelatinase A	1GEN	1266.218	1	6	349
9	Gelatinase B	6ESM	1581.293	2	4	499
10	Glucosidase	1UOK	1345.035	3	6	306
11	Lecithinase C	1AH7	1636.273	1	9	371
12	Neutral protease	1NPC	1554.477	2	14	524

Z score of Protein-Protein Docking Interactions

The results of the energy-related protein-protein interaction simulation with z-scores for extracellular enzymes in the active site of Nuclear factor-erythroid-2 related factor 2 (Nrf2) were discussed and compared. The z-score, a statistical measure indicating the standard deviation of an observation from the mean, provided valuable insights into the strength and significance of these interactions.

Upon analyzing the data, it was observed that some enzymes exhibited notably high z-scores, indicating strong interactions with the active site of Nrf2. DNase 1 had a z-score of 1935.147, making it one of the highest-scoring enzymes. α-amylase and lecithinase C also demonstrated substantial interactions, with z-scores of 1624.022 and 1636.273, respectively. These high z-scores suggested that these enzymes may have played critical roles in regulating Nrf2 activity or influencing downstream signaling pathways through energetic and stable interactions.

Other enzymes displayed moderate z-scores, indicating statistically significant interactions with the active site of Nrf2, but not as pronounced as the top-scoring enzymes. β-amylase, cellulase, and neutral protease showed z-scores of 1556.103, 1557.836, and 1554.477, respectively. These moderate z-scores suggested that these enzymes likely contributed to NRF2's function through energy-dependent binding and interaction mechanisms, though the magnitude of these interactions might have been relatively lower than the enzymes with higher z-scores.

On the other hand, some enzymes had lower z-scores, suggesting weaker interactions with the active site of NRF2. Cellobiohydrolase, DNase 2, glucosidase, extracellular serine protease, gelatinase A, and gelatinase B exhibited z-scores of 1459.164, 1449.446, 1345.035, 1338.119, 1266.218, and 1581.293, respectively. Although statistically significant, their energetic interactions with Nrf2 might have been less stable or have had lower binding affinities, indicating potentially lesser contributions to NRF2's overall function.

The energy-related protein-protein interaction simulation results with z-scores for extracellular enzymes in the active site of Nrf2 provided valuable insights into the energetic aspects of the interactions. Enzymes with high z-scores, such as DNase 1, α-amylase, and lecithinase C, demonstrated strong and stable energetic interactions with Nrf2, likely playing crucial roles in its activity. Enzymes with moderate z-scores, such as β-amylase, cellulase, and neutral protease, also exhibited significant energetic interactions, contributing to Nrf2 function, albeit to a lesser extent. Enzymes with lower Z-scores had weaker energetic interactions, suggesting potentially reduced contributions to Nrf2 activity.

It is essential to recognize that the z-score analysis provides a statistical representation of the energetic interactions and does not offer specific details about the precise nature or specific energy values of the interactions. Additional structural and biochemical studies, such as molecular dynamics simulations or binding energy calculations, would be necessary to gain a more comprehensive

understanding of the energetic aspects and dynamics of these protein-protein interactions with Nrf2.

In conclusion, the energy-related protein-protein interaction simulation results, together with z-scores for extracellular enzymes in the active site of Nrf2, have contributed valuable information about potential energetic contributions to Nrf2-mediated cellular responses. These findings further our understanding of the complex network of energy-driven protein interactions involved in Nrf2 signaling pathways and may have implications for therapeutic interventions targeting Nrf2 pathways in various diseases.

Analysis of protein-protein docking interactions

Protein-protein docking simulations are valuable tools to explore the interactions between proteins and gain insights into their binding modes. In this discussion, we will compare the number of salt bridges, hydrogen bonds, and non-bonded contacts for various extracellular enzymes obtained from protein-protein docking simulations (fig. 5). These interactions play essential roles in stabilizing protein complexes and are crucial for understanding their biological functions.

Salt bridges, hydrogen bonds, and non-bonded contacts are crucial factors influencing protein-protein interactions and stability (fig. 1). The number of salt bridges indicates the presence of ionic interactions between positively charged and negatively charged amino acid residues. Proteins with higher salt bridge counts, such as α-amylase, β-amylase, cellobiohydrolase, and cellulase, may have more stable interactions in the active site of Nrf2 due to these ionic bonds. In contrast, enzymes like DNase 2 and DNase 1, with no salt bridges, might have weaker electrostatic interactions with Nrf2. Overall, enzymes with more salt bridges were likely to have stronger interactions within the active site.

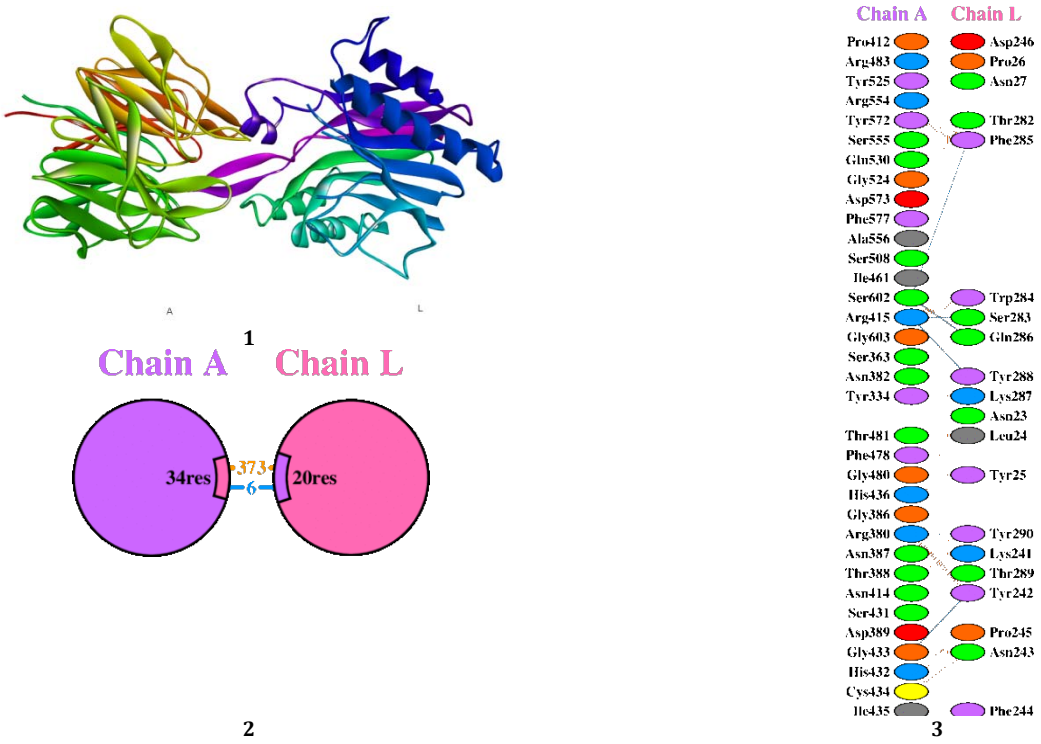

Fig. 1: Typical protein-protein interaction of extracellular enzyme. Chain A was NF-kB and chain L was DNase 1. Salt bridges (colored in red), hydrogen bonds (colored in blue), and non-bonded contacts (colored in orange)

Hydrogen bonds are essential for stabilizing protein structures and facilitating specific interactions between amino acid residues. Enzymes with a higher number of hydrogen bonds, like α-amylase and neutral protease, might form more stable complexes with Nrf2 in their active site. On the other hand, enzymes with lower hydrogen bond counts, such as gelatinase B and cellulase, may have relatively weaker interactions with Nrf2. The presence of a considerable number of hydrogen bonds indicates a stronger binding potential.

Non-bonded contacts represent van der Waals interactions between atoms in proteins. Enzymes with a higher number of non-bonded contacts, like neutral protease and gelatinase B, may have a larger interaction surface area with Nrf2. This could lead to increased binding strength and stability. Enzymes with lower non-bonded contact counts, such as cellobiohydrolase and glucosidase, might have less extensive interactions with Nrf2.

The Zdock web-based application allowed for easy access to the molecular docking algorithms without the need for local installation [12]. It provided a user-friendly interface for uploading protein structures and defining ligand-receptor interactions. The Zdock web server utilized parallel processing capabilities to expedite the docking simulations and generate results efficiently. The pdbsum1 application was utilized to analyze the docking results and extract essential information, including Z scores, number of salt bridges, number of hydrogen bonds, and number of non-bonded contacts [13]. The application featured a graphical user interface (GUI) for intuitive visualization and interpretation of the output data. It also facilitated the extraction of relevant interaction parameters from the docking results.

The Zdock protocol has been used in various studies, including the study of the intermolecular recognition mechanism between Keap1 and IKKβ [14]. In this study, the Zdock protocol was used to filter the docked poses, and the top 2000 poses were retained for evaluation using the ZRANK scoring function. Additionally, the Zdock protocol was used to achieve rigid docking in the study of microglia Sirt6 modulation of the transcriptional activity of NRF2 [15]. In this study, Sirt6 was found to deacetylate and stabilize NRF2 to increase its transcriptional activity. The Zdock protocol has also been used to design an *in silico* model of the DUX4-CDK1 complex in the study of NFE2L3 control of colon cancer cell growth [16]. Overall, the Zdock protocol is a useful tool in the field of protein-protein interaction in drug discovery, aiding in the prediction and analysis of protein-protein complexes.

NRF2 structural characteristics allowed the identification of reversible small-molecule inhibitors of the Keap1-Nrf2 interaction that can hopefully elucidate the therapeutic potential of Nrf2 activation [17]. The selective recognition mechanism of Keap1 with Nrf2 will be useful in the development of small molecule inhibitors of the Keap1-Nrf2 interaction [14]. Peptide inhibitors of the Keap1-Nrf2 protein-protein interaction have also been proposed as potential anti-inflammatory and cancer chemopreventive agents [18].

CONCLUSION

The identification of Nuclear factor-erythroid-2 related factor 2 (Nrf2) inhibitory extracellular enzymes from water kefir organisms through protein-protein interaction has been presented. The extracellular enzymes, such as DNase 1, α-amylase, and lecithinase C, hold promised as candidates for further investigation as potential inhibitors of Nrf2-mediated cellular responses. On the other side, the extracellular enzymes with higher counts of these interactions, such as α-amylase, neutral protease, and gelatinase B, were likely to form more stable complexes with Nrf2 and may play significant roles in its activity and regulation. However, it is essential to consider that these results are based solely on the provided data and further in-depth experimental and computational studies would be necessary to fully understand the intricacies of these protein-protein interactions with Nrf2.

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Nil

AUTHORS CONTRIBUTIONS

All the authors have equally contributed to the current study.

CONFLICT OF INTERESTS

All the authors declare no conflicts of interest.

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