chemistry proceedings

MDPI

Proceeding Paper

Insights on the Interaction between Kefiran and Whey Proteins Using Computational Analyses †

Carlos Jiménez-Pérez [1], Laura Roldán-Hernández [2], Alma Cruz-Guerrero [1], John F. Trant [3] and Sergio Alatorre-Santamaría [1,*]

[1] Departamento de Biotecnología, División de Ciencias Biológicas y de la Salud, Universidad Autónoma Metropolitana, Unidad Iztapalapa, Avenida San Rafael Atlixco 186, Ciudad de México 09340, Mexico; ibi.cjimenez@xanum.uam.mx (C.J.-P.); aec@xanum.uam.mx (A.C.-G.)

[2] Facultad de Química, Universidad Nacional Autónoma de México, Circuito Escolar S/N, Coyoacán, Cd. Universitaria, Ciudad de México 04510, Mexico; 110004060@quimica.unam.mx

[3] Department of Chemistry and Biochemistry, University of Windsor, 401 Sunset Avenue, Windsor, ON N9B 3P4, Canada; j.trant@uwindsor.ca

* Correspondence: salatorre@xanum.uam.mx

† Presented at the 27th International Electronic Conference on Synthetic Organic Chemistry (ECSOC-27), 15–30 November 2023; Available online: https://ecsoc-27.sciforum.net/.

Abstract: Kefiran is an exopolysaccharide produced by milk fermentation that is widely used in the food industry, mainly to improve the rheological properties of foods, as well as for the formation of biofilms. Most of these properties are related to its interaction with milk proteins. It has been reported that kefiran has a molecular weight of up to 10^7 Da and that it is formed by glucose and galactose molecules in equimolar quantities; however, its folding form is not completely understood. In this work it was elucidated that a model of the 20 kDa kefiran folds in a helical shape. In addition, it was determined that whey proteins can form complexes with kefiran.

Keywords: kefiran; molecular docking; whey proteins; kefir granules

Citation: Jiménez-Pérez, C.; Roldán-Hernández, L.; Cruz-Guerrero, A.; Trant, J.F.; Alatorre-Santamaría, S. Insights on the Interaction between Kefiran and Whey Proteins Using Computational Analyses. *Chem. Proc.* **2023**, *14*, 47. https://doi.org/10.3390/ecsoc-27-16128

Academic Editor: Julio A. Seijas

Published: 15 November 2023

1. Introduction

Kefiran is an exopolysaccharide (EPS) produced during the lactic-alcoholic fermentation of milk with kefir granules, and is a complex matrix that behaves like a symbiont, formed by bacteria (lactic and acetic acid) and yeast [1]. It is a heteropolysaccharide consisting of subunits of D-glucopyranose and D-galactopyranose in equimolar amounts [2,3] with a molecular weight (MW) ranging between 55 and 10,000 kDa, depending on extraction and purification conditions [4,5]. Figure 1 shows the chemical structure of a subunit of kefiran proposed by Micheli et al. [4], which has an approximate MW of 1 kDa. Due to the β-glycosides, like cellulose, it has been reported that it cannot be hydrolyzed by the enzymes of the digestive tract, giving it a prebiotic character [5–7].

The EPS is produced mainly by Lactobacillus lactic acid bacteria (LAB), including Lactobacillus kefiranofaciens, Lactobacillus kefirgranum, Lactobacillus parakefir, Lactobacillus kefir and Lactobacillus delbrueckii subsp. bulgaricus [7], although it has been noted that producing this EPS with an isolated strain, rather than the complete microbial consortium, does not generate a polymer with the same characteristics as those present in kefir [8]. This suggests a complex interplay between the various bacteria, and potentially a co-operative biosynthesis. Kefiran interactions with milk proteins have been reported to improve the rheological properties of milk derivative products [7]. Some of the best known applications of this polysaccharide are as an emulsifying agent [9], stabilizer thickener or gelling agent [10]. It has also been reported to have antioxidant [11], antimicrobial, [12] and even hypotensive activity [13]. Additionally, due to their resistance to digestive hydrolysis, kefirans can be used as vehicles for the protected delivery of probiotic bacteria and even drugs [5].

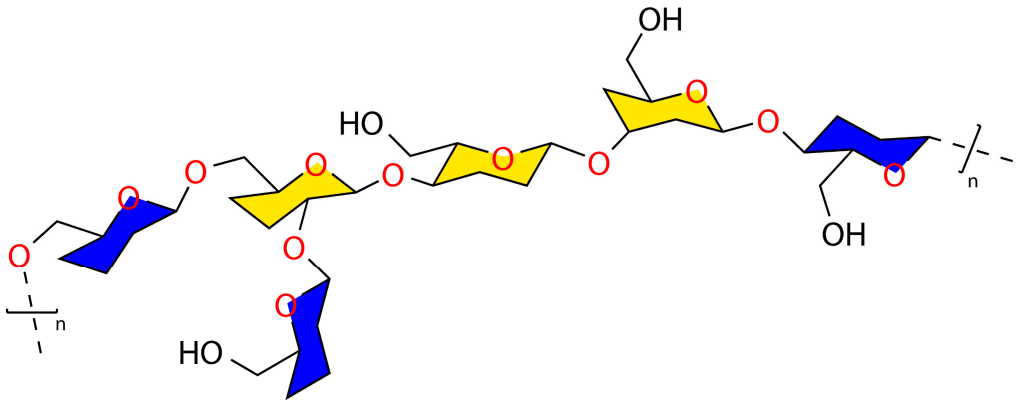

Figure 1. Chemical structure of the kefiran repeating subunit [4]. D-glucose (blue) and D-galactose (yellow). N = [55–10,000] (Backbone [→6)βDGlc*p*(1→2,6)βDGal*p*(1→4)αDGal*p*(1→3)βDGal*p*(1→4)βDGlc*p*(1→], with a branched [βDGlc*p*(1→2)] to the first βDGal*p* residue).

As part of our work to characterize the interaction of EPS with mycotoxins in milk, we want to present preliminary results where we have modelled a sub-portion of the kefiran structure and evaluated the interaction of this saccharide with two of the main lactic proteins: α-lactalbumin (ALA) and β-lactoglobulin (BLG). This will allow us to access a broader perspective of the possible interference these proteins can cause in the interactions between kefiran and the mycotoxins.

2. Materials and Methods

2.1. Design and Geometric Optimization of Kefiran

Starting from 20 subunits of kefiran (≈20 kDa) [6], the 3D model was built using the programs ChemDraw and Chem3D (22.2 version); this was optimized by energy minimizations using the MM2 forcefield with a maximum of 10,000 iterations.

2.2. Molecular Docking

Prior to the evaluation of the molecular docking (MDock), the structural models of the whey proteins obtained from the Protein Data Bank, ALA (1HFZ) and BLG (1BEB), were corrected by the addition of missing hydrogens according to the pKa of the titratable amino acids at pH 6.8 (pH of milk for EPS interaction assays with mycotoxins), using the ProteinPrepare tool on the PlayMolecule server [14].

MDock evaluations were performed on the SwissDock server [15] using the the "Accurate with certain limitations" docking protocol, as this program performs dockings that are based on the interactions with small molecules (maximum 250 atoms). Through the program BIOVIA Discovery Studio Visualizer v20.1.0.19295, the optimized model (Section 2.2) was cut into two subunits (226 atoms each) to perform the evaluation of their interactions with the previously protonated whey proteins.

3. Results and Discussion

3.1. Design and Geometric Optimization of the Kefiran Model

According to the optimization of the 20 subunits of the kefiran structure, it was observed that it tends to fold into an α-helix-like conformation (Figure 2). This result is in agreement with Exarhopoulos et al. [16], as they reported that kefiran solubilized in an aqueous solution adopts a helix.

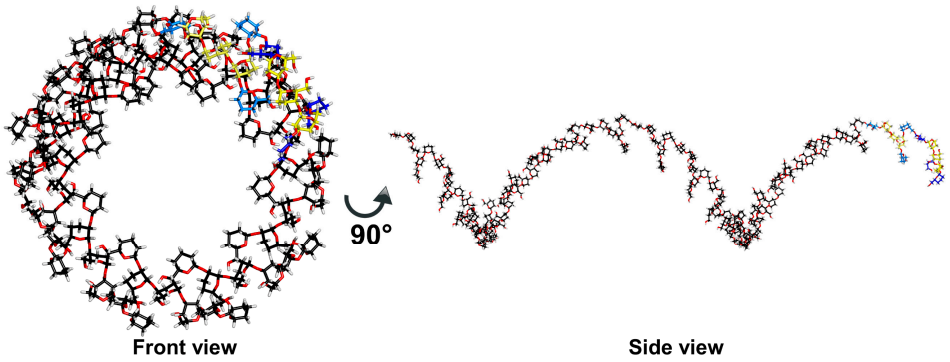

Figure 2. Front and side views of the 3D model of 20-subunit kefiran. D-glucose (blue) and D-galactose (yellow).

3.2. Molecular Docking

According to the free binding energies (ΔG_b) obtained from MDock evaluations (Table 1), kefiran can form complexes with both proteins. These data showed that they have a similar affinity for EPS. In addition, it was observed that interactions occur in the hydrophobic regions of the protein (Figure 3). Something similar happened when the interaction between an EPS produced by *Streptococcus thermophilus* LY03 and BLG was evaluated, where MDock showed that the formation of the complexes depends not only on hydrogen bonds, but also on hydrophobic interactions [17]. With these results, and taking as a reference the model in Figure 2 (Section 3.1), it is considered that the way in which the two proteins participate in the interaction with EPS is like that of the ALA binding the region where the chain bend is formed (Figure 3A), while the BLG interacts in the linear regions (Figure 3B). Therefore, if a greater number of protein units were considered, it is likely that they would surround the EPS, creating the conditions for enhanced interactions between proteins and the polysaccharide to allow the formation of more extensive networks. This phenomenon would result in an increase in rheological properties, such as viscosity. However, the fact that more EPSs and proteins bind should create interference in the interactions between mycotoxins and kefiran.

Table 1. Free binding energy of complexes formed between the two subunits of kefiran with whey proteins.

Complex	ΔG_b (kcal/mol)
ALA-kefiran	−10.52
BLG-kefiran	−11.01

ALA: α-lactalbumin; BLG: β-lactoglobulin; ΔG_b: binding free energy.

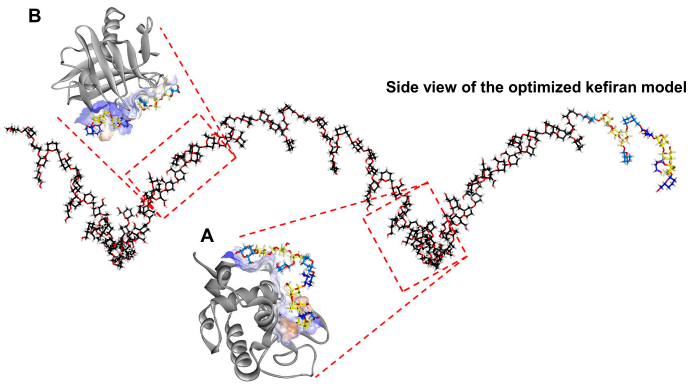

Figure 3. Side view of the 3D model of 20-subunit kefiran. (**A**) Complex formed between 2 subunits of kefiran with ALA. (**B**) Complex formed between 2 subunits of kefiran with BLG. D-glucose (blue) and D-galactose (yellow).

The proposed regions in the kefiran structure where ALA and BLG bind are based on the work undertaken by Ayala-Hernandez et al. [18], who evaluated the interaction of milk proteins with an EPS produced by *Lactococcus lactis* ssp. *cremoris* using scanning electron microscopy. They found that the proteins interact by wrapping the filament-shaped EPS, whose structure they attribute to the dehydration process suffered by the samples. In addition, they observed that proteins began to aggregate around the EPS, which allowed the formation of a biofilm.

4. Conclusions

According to the results obtained, it was possible to identify that kefiran folds helically, which allows it to have a larger surface to interact with whey proteins. Likewise, it was determined that the complexes formed between ALA and BLG with EPS are mainly due to hydrophobic interactions.

Author Contributions: Conceptualization, C.J.-P. and S.A.-S.; methodology, C.J.-P., L.R.-H. and S.A.-S.; software, C.J.-P.; validation, A.C.-G., J.F.T. and S.A.-S.; formal analysis, A.C.-G., J.F.T. and S.A.-S.; investigation, C.J.-P. and L.R.-H.; resources, S.A.-S.; data curation, C.J.-P., J.F.T. and S.A.-S.; writing—original draft preparation, C.J.-P. and L.R.-H.; writing—review and editing, C.J.-P., J.F.T. and S.A.-S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by CONAHCYT (Mexico), through Frontiers of Science, grant number CF-2023-I-1168, and by NSERC (Canada) through the International Alliance Collaboration Program, grant number ALLRP 585962–23.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available because the repository is not yet created.

Acknowledgments: C.J.-P. would like to acknowledge CONAHCYT for the postdoctoral fellowship.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Taniguchi, M.; Nomura, M.; Itaya, T.; Tanaka, T. Kefiran Production by Lactobacillus Kefiranofaciens under the Culture Conditions Established by Mimicking the Existence and Activities of Yeast in Kefir Grains. *Food Sci. Technol. Res.* **2001**, *7*, 333–337. [CrossRef]
2. la Riviére, J.; Kooiman, P.; Schmidt, K. Kefiran, a Novel Polysaccharide Produced in the Kefir Grain by Lactobacillus Brevis. *Arch. Mikrobiol.* **1967**, *59*, 269–278. [CrossRef] [PubMed]
3. Marshall, V.; Cole, W. Methods for Making Kefir and Fermented Milks Based on Kefir. *J. Dairy Res.* **1985**, *52*, 451–456. [CrossRef]
4. Micheli, L.; Uccelletti, D.; Palleschi, C.; Crescenzi, V. Isolation and Characterisation of a Ropy Lactobacillus Strain Producing the Exopolysaccharide Kefiran. *Appl. Microbiol. Biotechnol.* **1999**, *53*, 69–74. [CrossRef] [PubMed]
5. Gagliarini, N.; Diosma, G.; Garrote, G.; Abraham, A.; Piermaria, J. Whey Protein-Kefiran Films as Driver of Probiotics to the Gut. *Lwt* **2019**, *105*, 321–328. [CrossRef]
6. Moradi, Z.; Kalanpour, N. Kefiran, a Branched Polysaccharide: Preparation, Properties and Applications: A Review. *Carbohydr. Polym.* **2019**, *223*, 115100. [CrossRef]
7. Gentry, B.; Cazón, P.; O'Brien, K. A Comprehensive Review of the Production, Beneficial Properties, and Applications of Kefiran, the Kefir Grain Exopolysaccharide. *Int. Dairy J.* **2023**, *144*, 105691. [CrossRef]
8. Nielsen, B.; Gürakan, G.; Ünlü, G. Kefir: A Multifaceted Fermented Dairy Product. *Probiotics Antimicrob. Proteins* **2014**, *6*, 123–135. [CrossRef] [PubMed]
9. Wang, Y.; Ahmed, Z.; Feng, W.; Li, C.; Song, S. Physicochemical Properties of Exopolysaccharide Produced by Lactobacillus Kefiranofaciens ZW3 Isolated from Tibet Kefir. *Int. J. Biol. Macromol.* **2008**, *43*, 283–288. [CrossRef] [PubMed]
10. Rimada, P.; Abraham, A. Kefiran Improves Rheological Properties of Glucono-δ-Lactone Induced Skim Milk Gels. *Int. Dairy J.* **2006**, *16*, 33–39. [CrossRef]
11. Chen, Z.; Shi, J.; Yang, X.; Nan, B.; Liu, Y.; Wang, Z. Chemical and Physical Characteristics and Antioxidant Activities of the Exopolysaccharide Produced by Tibetan Kefir Grains during Milk Fermentation. *Int. Dairy J.* **2015**, *43*, 15–21. [CrossRef]
12. Rodrigues, K.; Gaudino Caputo, L.; Tavares Carvalho, J.; Evangelista, J.; Schneedorf, J. Antimicrobial and Healing Activity of Kefir and Kefiran Extract. *Int. J. Antimicrob. Agents* **2005**, *25*, 404–408. [CrossRef] [PubMed]
13. Maeda, H.; Zhu, X.; Omura, K.; Suzuki, S.; Kitamura, S. Effects of an Exopolysaccharide (Kefiran) on Lipids, Blood Pressure, Blood Glucose, and Constipation. *BioFactors* **2004**, *22*, 197–200. [CrossRef] [PubMed]

14. Martínez-Rosell, G.; Giorgino, T.; De Fabritiis, G. PlayMolecule ProteinPrepare: A Web Application for Protein Preparation for Molecular Dynamics Simulations. *J. Chem. Inf. Model.* **2017**, *57*, 1511–1516. [CrossRef] [PubMed]
15. Bitencourt-Ferreira, G.; Azevedo, W. Docking with SwissDock. In *Docking Screens for Drug Discovery*; Filgueira de Azevedo, W., Ed.; Human Press: New York, NY, USA, 2019; pp. 189–202.
16. Exarhopoulos, S.; Raphaelides, S.; Kontominas, M. Conformational Studies and Molecular Characterization of the Polysaccharide Kefiran. *Food Hydrocoll.* **2018**, *77*, 347–356. [CrossRef]
17. Birch, J.; Khan, S.; Madsen, M.; Kjeldsen, C.; Møller, M.; Stender, E.; Peters, G.; Duus, J.; Kragelund, B.; Svensson, B. Binding Sites for Oligosaccharide Repeats from Lactic Acid Bacteria Exopolysaccharides on Bovine β-Lactoglobulin Identified by NMR Spectroscopy. *ACS Omega* **2021**, *6*, 9039–9052. [CrossRef] [PubMed]
18. Ayala-Hernandez, I.; Goff, H.; Corredig, M. Interactions between Milk Proteins and Exopolysaccharides Produced by Lactococcus Lactis Observed by Scanning Electron Microscopy. *J. Dairy Sci.* **2008**, *91*, 2583–2590. [CrossRef] [PubMed]