Replica exchange molecular dynamics simulations reveal the structural and molecular properties of levan-type fructo-oligosaccharides of various chain lengths

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Abstract

**Background:** Levan and levan-type fructo-oligosaccharides (LFOs) have various potential applications in pharmaceutical and food industries due to their beneficial properties such as their low intrinsic viscosity and high water solubility. Previous studies showed that they exhibited prebiotic effects, anti-inflammatory and anti-tumor activities against Sarcoma-180 tumor cells of human. Despite their various potential applications, the structural and molecular properties of LFOs of various chain lengths are not well understood.

**Results:** We employed the replica-exchange molecular dynamics simulations method (REMD) in AMBER14 to elucidate structural and molecular properties of LFOs with chain lengths of 5 (LFO⁵), 10 (LFO¹⁰) and 15 (LFO¹⁵) residues in two models of generalized Born implicit solvent (GBΗCΤ and GBΟBC1). For LFO¹⁰ and LFO¹⁵, four distinct conformations (helix-like, partial helix, zig-zag and random structures) were characterized by their upper-middle and lower-middle torsions. For LFO⁵, two distinct conformations (partial helix and random structures) were characterized by their middle torsion and molecular angle of residues 1, 3 and 5. To determine hydrogen bonds important for the formation of helix-like structures of LFO¹⁰ and LFO¹⁵, occurrence frequencies of hydrogen bonds were analyzed, and the O₆(i)–H₃O(i+1) hydrogen bond was found with the highest frequency, suggesting its importance in helix formation. Among three dihedral angles between two fructosyl units [ϕ (O⁵’-C₂’-O₆-C₆), ψ (C₂’-O₆-C₆-C₅) and ω (O₆-C₆-C₅-C₄)], dihedral angle distributions showed that ω was the most flexible dihedral angle and probably responsible for conformational differences of LFOs.

**Conclusions:** Our study provides important insights into the structural and molecular properties of LFOs, which tend to form helical structures as the chain length increases from 5 to 15 residues. This information could be beneficial for the selection of LFOs with appropriate lengths and properties for pharmaceutical and biological applications.

**Keywords:** Levan, Helix, Replica exchange molecular dynamics simulation, Oligosaccharide, Generalized Born implicit solvent

**Abbreviations:** GB, Generalized Born implicit solvent; LFOs, Levan-type fructo-oligosaccharides; REMD, Replica-exchange molecular dynamics simulations.

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**Background**

Levan-type fructo-oligosaccharides (LFOs) are short chain fructans that contain D-fructofuranosyl residues and are predominantly linked by $\beta$-(2, 6) linkages in a main chain with some $\beta$-(2, 1) linked branching points (Fig. 1a). Produced by levansucrase, levan and LFOs are found in various microorganisms such as *Bacillus subtilis* [1], *Zymomonas mobilis* [2], and *Leuconostoc mesenteroides* [3], and play important roles as sources for energy utilization and biofilm formation [4, 5]. The properties of levan and LFOs depend on their chain lengths and branching degrees [6], and they have various desirable properties such as their unusually low intrinsic viscosity [7] and high water solubility [8]. These properties are very beneficial for various industrial applications, especially in food and pharmaceutical industries. For example, levan and LFOs showed prebiotic effects, stimulating the growth of beneficial intestinal bacteria, and also could potentially act as cholesterol lowering agents (MW 2000 kDa) [9, 10]. Furthermore, they could also be served as carbon sources for probiotics such as four strains of *Bifidobacterium sp.* that produce short chain fatty acids, lactate and acetate (MW < 3600 Da) [11]. Moreover, they exhibited anti-inflammatory and anti-tumor activities against Sarcoma-180 tumor cells of human (MW 380–710 kDa) [12, 13]. Despite their various potential applications, the knowledge on the structural and molecular properties of levan and LFOs of various chain lengths is still limited.

Replica exchange molecular dynamics (REMD) method provides an extensive conformational sampling at various temperatures by allowing each replica to exchange their configurations through parallel tempering [14]. Raising the temperature can increase the probability of the system to overcome the energy barrier, consequently enhancing the probability of attaining the global minimum and allowing the sampling of large volumes of phase space. Therefore, the incorporation of higher temperature systems allow the lower temperature systems to access a representative set of the low free energy minima that are accessible by the higher temperature systems [15]. This technique has been used to investigate the properties of oligosaccharides in solution. For example, Re et al. employed REMD to elucidate the structural diversity and the changes in conformational equilibria of biantennary

![Fig. 1](image-url)
complex-type N-glycans [16]. Moreover, Nishima et al. used this technique to investigate the effects of bisecting GlcNAc and core fucosylation on conformational properties of biantennary complex-type N-glycans [17]. Recently, Jo et al. employed this technique to examine the conformational freedom of the N-glycan core pentasaccharide moiety in solution and found that the conformational variability of the pentasaccharide in solution was more restricted than the N-glycan on the protein surface [18]. This method was also employed to investigate conformational flexibility of cellulose oligomers as well as their chain length and temperature dependence [19]. However, to our knowledge, REMD method has not been used in the elucidation of the properties of LFOs of various chain lengths.

In this study, we performed REMD on the models of LFOs with the chain lengths of 5, 10 and 15 residues in two models of generalized Born implicit solvent (GB_{HCT} and GB_{OBC1}) to elucidate their structural and molecular properties as well as the relationship between these properties and the chain length. Such information would be beneficial for the selection of LFOs with appropriate lengths and properties for pharmaceutical and biological applications.

**Methods**

**Structure preparation and minimization**

The structures of LFO_5, LFO_10 and LFO_15 were constructed using the LEaP module in AMBER14 [20], and their atom types and force field parameters were assigned based on GLYCAM06j-1 [21]. Two implicit solvent models (GB_{HCT} and GB_{OBC1}) were used in the minimization and simulations of each system. All systems were minimized with 2500 steepest-descent minimization cycles and 2500 conjugate-gradient minimization cycles [22, 23].

**Replica exchange molecular dynamics simulations**

Sixteen replicas of each system were initially equilibrated for 500 ps to reach the desired temperature range from 262 to 802 K. REMD of all systems were performed using the SANDER module in AMBER14. Langevin dynamics with a collision frequency of 1 ps^{-1} were used to control the temperatures in all systems. Initial velocity of each system was reseeded by the random number generator [24]. A cut off of 999 Å was used to truncate nonbonded pairs, and the maximum distance of 999 Å between atom pairs was employed to compute the pairwise summation involved in the effective Born radii calculation. All bond-stretching freedoms associated with hydrogen were eliminated by SHAKE algorithm, allowing a time step of 0.002 ps [25]. Each replica was simulated for 100 ns and exchanged every 2 ps. The replicas at 298 K were employed for the analyses of the structural and molecular properties of LFOs with different chain lengths.

To quantify the sizes of all systems, their average radii of gyration (ROG) were determined. To determine possible representative structures of LFOs of each chain length, K-means clustering algorithm, as implemented in MMTSB tool sets [26], was employed to cluster the structures from their 100 ns trajectories based on their structural similarities, calculated from their heavy-atom root-mean-square-deviation. To determine a reasonable representative of each cluster, a structure that is most similar to the average structure of all members of each cluster was chosen to be a “centroid”; i.e., a “centroid” is a structure with the lowest heavy-atom root-mean-square-deviation to the average structure. Based on their shapes, these “centroids” were further classified into helix-like, partial helix, zig-zag or random structures as major representative conformers. Helix-like structures were defined as conformations that had more than 1 helical turn, while partial helix structures were defined as conformations that had 1 helical turn. Zig-zag structures were defined as conformations that had zigzag shapes. Random structures were defined as structures that were not classified as helix-like, partial helix or zig-zag structures.

To plot the free energy maps, various parameters were employed to characterize the structures of LFOs. Since helix-like conformations were observed with high frequencies in LFO_10 and LFO_15 and they tended to have similar values of upper-middle and lower-middle torsions, their upper-middle and lower-middle torsions were used to characterize the structures of LFO_10 and LFO_15. Their upper-middle torsions were computed by measuring the torsion angles of the centers of masses (CM) of residues 5, 6, 7 and 8 (defined as χ_{5-7} = CM_5-CM_6-CM_7-CM_8) and residues 3, 4, 5 and 6 (defined as χ_{3-5} = CM_3-CM_4-CM_5-CM_6) for LFO_15 and LFO_10, respectively. Their lower-middle torsions were computed by measuring the torsion angles of CMs of residues 8, 9, 10 and 11 (defined as χ_{8-10} = CM_8-CM_9-CM_10-CM_11) and residues 6, 7, 8 and 9 (defined as χ_{6-8} = CM_6-CM_7-CM_8-CM_9) for LFO_15 and LFO_10, respectively (Fig. 1b, c). For LFO_5, the molecular angles and middle torsions were computed by measuring the angles and torsion angles of CMs of residues 1, 3 and 5 (defined as θ_a = CM_1-CM_3-CM_5) and residues 2, 3, 4 and 5 (defined as χ_{3-4} = CM_3-CM_4-CM_5) respectively (Fig. 1d).

To measure conformational flexibilities, the occurrence frequencies of three dihedral angles between every two fructosyl residues, ω (C4-C5-C6-C0), ψ (C5-C6-C0-C2) and φ (C6-C0-C2-C05) (Fig. 1e) were computed. To identify hydrogen bonds important for the formation of helix-like structures of LFO_10 and LFO_15, the occurrence frequencies of hydrogen bonds were measured. Only the hydrogen bonds with the occurrence frequency of at least 1% were used for further analysis.
Results and discussion

Reliability of REMD simulations

To determine whether the temperatures were optimally distributed and the number of replicas was sufficient, the acceptance ratios of replica exchange were calculated. The acceptance ratios of the simulations of LFO₁₅ in the GB_HCT model were almost constant around 28%, implying a free random walk in the replica (temperature) space (Additional file 1: Figure S1a). Moreover, a free random walk both in the replica space (Additional file 1: Figure S1b) and the temperature space (Additional file 1: Figure S1c) were also confirmed. Furthermore, the canonical probability distribution of the total potential energy at each temperature had sufficient overlap with those of neighbors (Additional file 1: Figure S1d). The results of the REMD simulations of LFO₁₀ and LFO₅ in the GB_HCT model were also similar, and their average acceptance ratios were almost constant around 37 and 50% for LFO₁₀ and LFO₅, respectively. For the systems simulated in the GB_OBC₁ model, the results of REMD simulations were also similar to those simulated in the GB_HCT model, and their average acceptance ratios were almost constant around 28, 36 and 50% for LFO₁₅, LFO₁₀ and LFO₅, respectively. These results indicate good reliability of the REMD simulations of all systems.

Sizes of LFOs

The sizes of LFOs were determined by measuring their radii of gyration. Figure 2 shows that the trends of the radii of gyration of LFOs simulated in the GB_HCT model and those simulated in the GB_OBC₁ model are similar. The radii of gyration of LFOs tended to increase as their chain lengths increased from 5 to 15 residues. These results suggest the extension of the structures of LFOs as their chain lengths increase.

Conformations of LFO₁₅, LFO₁₀ and LFO₅

Figure 3 shows the free-energy maps of LFO₁₅, LFO₁₀ and LFO₅ as simulated in GB_HCT and GB_OBC₁ models as well as their major representative conformers and their population sizes from clustering analysis and centroid classification. For LFO₁₅, four major conformations such as helix-like (a), partial helix (b), zig-zag (c) and random (d) structures were observed after clustering analysis and centroid classification (Fig. 3a, b and Additional file 1: Figure S2 and Figure S5), and they were characterized by their upper-middle and lower-middle torsions (χ_6-7 and χ_9-10). Helix-like structures were found with the highest population of 54.1 and 63.2% for those simulated in GB_HCT and GB_OBC₁ models, respectively. Helix-like structures took up conformations of left-handed 3-fold helices and tended to have their upper-middle and lower-middle torsions in the similar range of around 240–315°. The conformations with the second highest population were partial helix structures, and their population sizes were 33.9 and 22.3% for systems simulated in GB_HCT and GB_OBC₁ models, respectively. The other two conformations were zig-zag and random structures. Zig-zag structures were found with the population sizes of 2.8 and 6.7% for systems simulated in GB_HCT and GB_OBC₁ models, respectively. The population sizes of random structures simulated in GB_HCT and GB_OBC₁ models were 9.2 and 7.8%, respectively.

For LFO₁₀, two major conformations such as partial helix (b) and random (d) structures were observed after clustering analysis and centroid classification, probably due to its shorter chain length as compared to those of...
LFO₁₀ and LFO₁₅ (Fig. 3e, f and Additional file 1: Figure S4 and Figure S7). These conformations were characterized by their molecular angles (θ) and middle torsions (χ₃-₄). Partial helix structures were observed with the population sizes of 92.8 and 92.5 % for those simulated in GB_HCT and GB_OBC₁ models, respectively. Random structures were also found with the population sizes of 7.2 and 7.5 % for those simulated in GB_HCT and GB_OBC₁ models, respectively.

Table 1 shows the populations of major representative conformers of LFO₁₅, LFO₁₀ and LFO₅ simulated in GB_HCT and GB_OBC₁ models as determined from clustering analysis and centroid classification. As the chain length increased, the population of the helix-like structures tended to increase. These results may suggest that LFOs have tendencies to form helices as their chain lengths are extended.

**Hydrogen bonds important for the formation of helix-like structures**

To elucidate the hydrogen bonds important for the formation of helix-like structures, the occurrence...
The populations of major representative conformers of LFO\textsubscript{15}, LFO\textsubscript{10} and LFO\textsubscript{5} simulated in GB\textsubscript{HCT} and GB\textsubscript{OBC1} models as determined from clustering analysis and centroid classification are shown in Table 1. The occurrence frequencies of hydrogen bonds in helix-like structures of LFO\textsubscript{15} and LFO\textsubscript{10} with the occurrence frequencies of at least 1 % were analyzed. For the systems simulated in the GB\textsubscript{HCT} model, the O6(0)–H3O(i+1) hydrogen bonds (between residue i and i + 1) were found with the highest frequency, and their glycosidic oxygens acted as important hydrogen bond acceptors that interacted with the hydroxyl groups of C3 atoms of the furanose rings and probably helped stabilize the helix-like structures (Table 2 and Fig. 4). The hydrogen bonds with the second and third highest occurrence frequencies for both LFO\textsubscript{15} and LFO\textsubscript{10} were the O1(0)–H3O(i) and O5(0)–H1O(i) hydrogen bonds, which were the hydrogen bonds within the same residue (Table 2 and Fig. 4). The trends of the occurrence frequencies of the hydrogen bonds of LFO\textsubscript{15} and LFO\textsubscript{10} in the GB\textsubscript{OBC1} model were also similar to those in the GB\textsubscript{HCT} model (Table 2). These three hydrogen bonds (O6(0)–H3O(i+1), O1(0)–H3O(i) and O5(0)–H1O(i) hydrogen bonds), especially the O6(0)–H3O(i+1) hydrogen bond that was found with the highest frequency, are probably important for the formation of helix-like structures of LFO\textsubscript{15} and LFO\textsubscript{10} as their occurrence frequencies are higher than other hydrogen bonds.

### Table 1

| Solvent model | Major representative conformer | Population (%) | LFO\textsubscript{15} | LFO\textsubscript{10} | LFO\textsubscript{5} |
|---------------|--------------------------------|----------------|------------------------|------------------------|------------------------|
| GB\textsubscript{HCT} | Helix-like structure | 51.4 | 50.5 | - |
| | Partial helix structure | 33.9 | 34.5 | 92.8 |
| | Zig-zag structure | 2.8 | 6.8 | - |
| | Random structure | 9.2 | 8.2 | 7.2 |
| GB\textsubscript{OBC1} | Helix-like structure | 63.2 | 57.5 | - |
| | Partial helix structure | 22.3 | 25.4 | 92.5 |
| | Zig-zag structure | 6.7 | 8.7 | - |
| | Random structure | 7.8 | 8.4 | 7.5 |

### Conformational flexibilities

To investigate the conformational flexibilities of LFO\textsubscript{15}, LFO\textsubscript{10} and LFO\textsubscript{5}, the occurrence frequencies of ω, ψ and φ of all glycosidic bonds were measured. For the systems simulated in the GB\textsubscript{HCT} model, ψ and φ of all glycosidic linkages of all LFOs exhibited single major peaks around 173° and -63°, respectively (Fig. 5). However, ω was more flexible than ψ and φ as it exhibited one major peak and two minor peaks (Fig. 5). The results from the systems simulated in the GB\textsubscript{OBC1} model were similar (Additional file 1: Figure S8); ω exhibited more peaks and was more flexible than ψ and φ. These results suggest that the flexibility of ω may be responsible for the conformational diversity of LFOs since this dihedral angle has more possibilities in rotating and changing the conformations of LFOs.

### Conclusions

To elucidate the structural and molecular properties of LFOs as well as the relationship between these properties and their chain lengths, REMD were performed on systems of LFO\textsubscript{5}, LFO\textsubscript{10} and LFO\textsubscript{15} in GB\textsubscript{HCT} and GB\textsubscript{OBC1} solvent models. We found that as the chain length increased, the radii of gyration tended to increase, suggesting the extension of the conformations as the chain length increases. After clustering analysis and centroid classifications, four major representative conformations (helix-like, partial helix, zig-zag and random structures) were found for LFO\textsubscript{15} and LFO\textsubscript{10}, while two conformations (partial helix and random structures) were identified for LFO\textsubscript{5}. The free energy maps show that the four conformations of LFO\textsubscript{15} and LFO\textsubscript{10} were characterized by their upper-middle and lower-middle torsions, whereas the two conformations of LFO\textsubscript{5} were characterized by their molecular angles and middle torsions. As the chain length increased from 5 to 15 residues, the conformation populations of the helix-like structures tended to increase, suggesting the possible tendency of LFOs to form helices as their chain lengths are extended. Moreover, the O6(0)–H3O(i+1) hydrogen bond was found with the highest frequency, suggesting its importance in helix formation of LFO\textsubscript{15} and LFO\textsubscript{10}. Furthermore, ω was found to be more flexible than ψ and φ and probably responsible for the conformational diversity of LFOs. This study gives important insights into the structural and molecular properties of LFOs; they tend to form helical structures as the chain length increases from 5 to 15 residues. Our findings may be useful in the selection of LFOs with appropriate chain lengths and structural properties for pharmaceutical and biological applications.

### Table 2

| Solvent model | Residue that form a hydrogen bond | Type | Occurrence frequency (\%) | LFO\textsubscript{15} | LFO\textsubscript{10} |
|---------------|---------------------------------|------|--------------------------|------------------------|------------------------|
| GB\textsubscript{HCT} | i, i | O1(0)–H3O(i) | 15.4 | 15.3 |
| | | O5(0)–H1O(i) | 11.5 | 12.5 |
| | i, (i + 1) | O6(0)–H3O(i+1) | 65.0 | 60.2 |
| GB\textsubscript{OBC1} | i, i | O1(0)–H3O(i) | 10.0 | 10.0 |
| | | O5(0)–H1O(i) | 8.3 | 7.8 |
| | i, (i + 1) | O6(0)–H3O(i+1) | 37.5 | 34.8 |

*Only hydrogen bonds with the occurrence frequency of at least 7 % are shown.*
Fig. 4 Hydrogen bonds important for the formation of helix-like structures (LFO$_{15}$ simulated in the GB$_H$CT model is shown as an example). Middle; the O6$_{(i)}$ – H3O$_{(i+1)}$ hydrogen bond (occurrence frequency = 65.0 %). Right; the O1$_{(i)}$ – H3O$_{(i)}$ hydrogen bond. (occurrence frequency = 15.4 %). Left; the O5$_{(i)}$ – H1O$_{(i)}$ hydrogen bond (occurrence frequency = 11.5 %). Hydrogen bonds are represented as dash lines. The LFO chain and fructosyl units are represented as ribbon and filled yellow color representations, respectively.

Fig. 5 The frequencies of the three dihedral angles of all glycosidic linkage of LFO$_{15}$, LFO$_{10}$ and LFO$_{5}$ in the GB$_H$CT model. Each dihedral angle is shown in different color.
