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Note

Chain conformational analysis of beijeran by *n–h* map calculations

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Abstract

Possible regular helix models of beijeran, a new acidic heteropolysaccharide consisting of a trisaccharide as a repeating unit, were investigated. Conformational analyses of the three component disaccharides were first carried out by calculating their relaxed-residue energy maps with respect to the glycosidic bond rotations, Φ and Ψ. A search for possible beijeran chain conformations was carried out by calculating the two-dimensional map of the helix parameters; *n* (the number of asymmetric units per a fiber repeat) and *h* (the axial rise per the unit), on the basis of the Φ–Ψ conformations taken from the low energy regions of each of the three energy maps. The *n–h* values of the helix models with low steric energies were mostly found to be around the experimental values ($n = 2$ and $h = 1.20$–1.25 nm), which may support the present methodology. It was also suggested that the internal flexibility of beijeran chain allowed it to conform in diverse helical structures, each of which were reasonably in accord with the observed *n–h* values. The three representative helix models were finally proposed for the beijeran chain conformation in the crystal structure. © 1997 Elsevier Science Ltd. All rights reserved

Keywords: Beijeran; Chain conformational analysis; Relaxed-residue energy map; *n–h* map

1. Introduction

Beijeran is a new acidic polysaccharide extracted from *Azotobacter beijerinckii* YNM 1. It consists of linearly (1 → 3)-linked α-D-GalA, β-L-Rha, and α-D-Glc residues, while the glucose residue is *O*-acetylated at C6 position [1]. Its backbone structure therefore is described by the following sugar sequence:

→ 3)-α-D-GalA-(1 → 3)-β-L-Rha-(1 → 3)-
α-D-Glc-(1 →

It has been found that deacetylated beijeran exhibited a gel forming property in the presence of calcium ions, which raised the possibility of its application in the food and cosmetic industries [1]. We have recently reported the X-ray fiber diffraction data of calcium [2] and sodium [3] salts of deacetylated

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beijeran. In both crystalline forms, the extended, two-fold helical conformation has been suggested for the beijeran backbone chain where two trisaccharide units, each as an asymmetric unit, were involved in a fiber repeat. The helix parameters *n* (the number of asymmetric units per a fiber repeat) and *h* (the axial rise per the unit) of beijeran chain are 2 and about 1.20–1.25 nm, respectively. An attempt to determine the three-dimensional structure of the beijeran chain has been made based on these estimates. While, in the case of a simple homopolysaccharide, each set of possible *n*–*h* values corresponds to the respective set of the glycosidic bond rotations, Φ and Ψ, with a fixed residue geometry and glycosidic bond angle, the beijeran chain involves three sets of Φ–Ψ torsional angles and these values cannot be immediately defined from a given set of an *n*–*h* value. In addition, independent rotations of these six bonds generate an enormously large conformational space to be explored. Unfortunately, our first attempt to construct a helix model by adopting the minimum Φ–Ψ conformation at each glycosidic linkage failed to provide that having a reasonable *n*–*h* value. Therefore, an approach to perform a systematic search of the Φ–Ψ conformational space of beijeran chain must be developed. French reported the features and capabilities of the *n*–*h* maps calculated for some homopolysaccharides, where the stereochemically feasible helices were represented in the two-dimensional, *n*–*h* plot [4,5]. He also suggested the applicability of this technique to more complicated heteropolysaccharides. In the present study, the idea of the *n*–*h* map was extended to investigate the chain conformation of beijeran, complemented by the relaxed-residue, Φ–Ψ energy map calculations for each of the three disaccharide components.

2. Computational methods

The conformations of backbone chains are primarily described by the torsional angles of glycosidic bonds, defined as Φ = O5–C1–O1–C3′ and Ψ = C1–O1–C3′–C4′. Fig. 1 shows definitions of the Φ and Ψ torsional angles of the repeating unit of beijeran chain. Relaxed-residue energy maps were calculated by optimizing a disaccharide unit at each Φ and Ψ gird point. A whole Φ–Ψ range of each map was constructed on a 20° grid spacing and the low energy regions were further divided into a 10° grid spacing. The orientations of the secondary hydroxyl groups of each residue were restricted to either clockwise (*c*) or reverse-clockwise (*r*) direction [6,7]. The hydroxymethyl group of a D-Glc residue had either *gg* or *gt* orientation [8]. As for an orientation of the carboxyl group of a D-GalA residue, a carbonyl oxygen was initially set to be at *cis* with respect to an O5 ring oxygen. Combining these exocyclic conformers between two residues yielded the four starting exocyclic conformers for α-D-GalA-(1 → 3)-β-L-Rha-OMe and the eight, for α-D-Glc-(1 → 3)-α-D-GalA-OMe and β-L-Rha-(1 → 3)-α-D-Glc-OMe. All the exocyclic group conformers were used in the energy map calculations and the final map was produced by adopting the lowest energy value from an optimized disaccharide structure at each grid point. MM3(92) program [9–11] was used in optimization of disaccharide structures using the dielectric value 4.0 [12]. The Φ–Ψ grid point with steric energies less than 3 kcal/mol contour lines were selected from each of the three maps and the three sets of the Φ–Ψ values thus selected were combined to build a regular helix model of beijeran chain. In this procedure, the glycosidic bridge angle was fixed to be

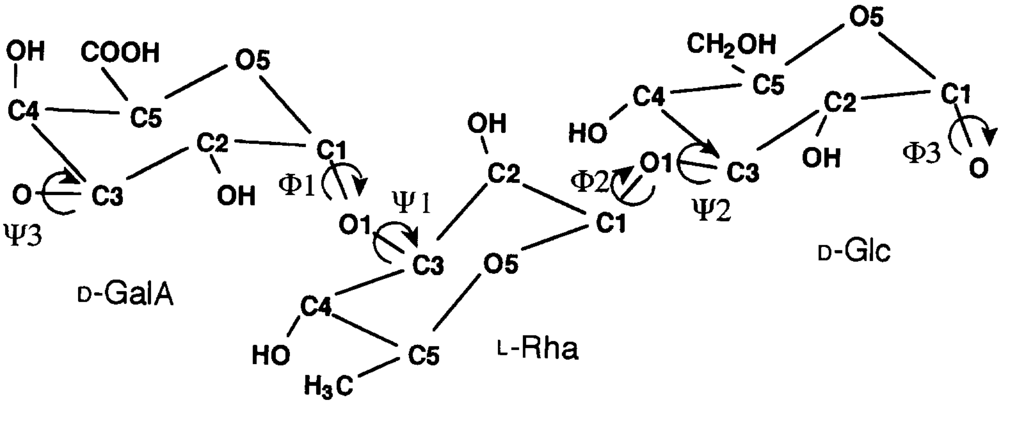

Fig. 1. Definition of the torsional angles Φ and Ψ in the chemical repeating unit of beijeran.

115.7°. All possible Φ–Ψ combinations in 10° spacing were tested and, for each resulting chain, a set of helix parameters, *n* and *h*, was calculated according to the method proposed by Sugeta and Miyazawa [13,14]. A distribution of *n*–*h* values of the low energy helix conformations was displayed in the two-dimensional map with respect to these values. While no further refinement of helix structures was carried out, a steric energy of a helix model was defined by a sum of three MM3 energies of the disaccharide Φ–Ψ conformers adopted in constructing the helix model.

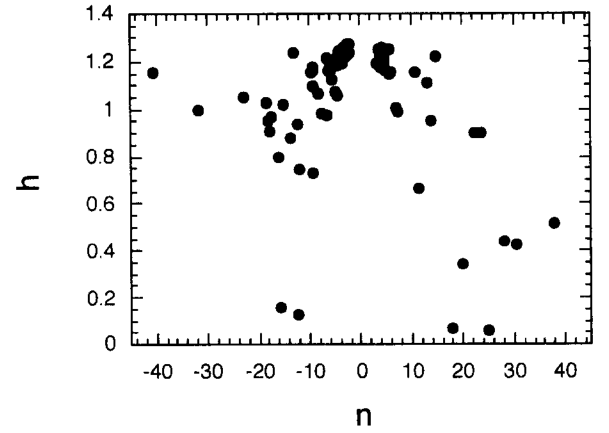

Fig. 3. The *n*–*h* map for beijeran chain with the steric energy less than 1.0 kcal/mol. Negative values of *n* indicate a left-handed helix.

3. Results and discussion

Fig. 2 shows the MM3-generated, relaxed-residue energy maps of the three disaccharide molecules, with iso-energy contour lines at 1 kcal/mol increments above the global minimum. While each map consists of two or three distinct minima, the area within the 3 kcal/mol contour lines comprises the lowest two minima in all the three maps. As denoted in the figures, the two respective potential wells that include the lowest and second lowest minima were given the notations A1 and B1 for the disaccharide α-D-GalA-(1 → 3)-β-L-Rha-OMe, A2 and B2 for β-L-Rha-(1 → 3)-α-D-Glc-OMe, and A3 and B3 for α-D-Glc-(1 → 3)-α-D-GalA-OMe. Combinations of all the 10° grid spacing, Φ–Ψ grids inside these potential wells generated 466, 990 helix models and, sub-

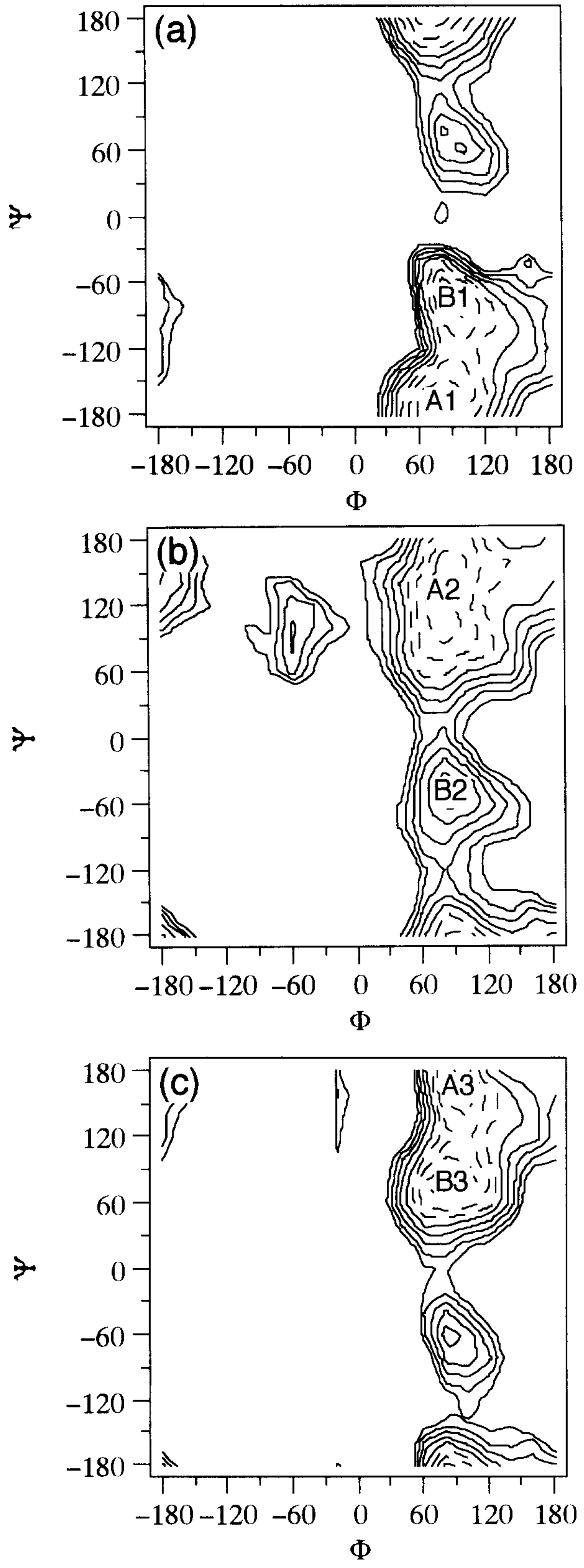

Fig. 2. MM3-generated relaxed-residue energy maps of α-D-GalA-(1 → 3)-β-L-Rha-*O*-Me (a), β-L-Rha-(1 → 3)-α-D-Glc-*O*-Me (b), and α-D-Glc-(1 → 3)-α-D-GalA-*O*-Me (c). The potential wells involving the global minimum (A) and the second global minimum (B) are indicated. Contour lines, given in solid or dashed lines, are drawn in 1 kcal/mol increments above the global minimum. The dashed lines are the 1, 2, and 3 kcal/mol contour lines.

Table 1
Number and average values of Φ–Ψ angles and steric energy of beijeran chain conformation models

Model	No.	Φ–Ψ angles (rms deviation)/deg.						Energy/kcal/mol
		$\Phi1$	$\Psi1$	$\Phi2$	$\Psi2$	$\Phi3$	$\Psi3$	
A1A2A3	2	80(0)	160(0)	65(5)	60(0)	80(0)	130(0)	7.6
A1A2B3	4901	74(15)	180(17)	77(18)	95(21)	75(18)	68(14)	4.9
A1B2A3	526	69(12)	−147(13)	85(5)	−44(5)	95(15)	165(17)	6.0
A1B2B3	3	97(5)	−120(0)	87(5)	−40(0)	110(0)	110(0)	8.0
B1A2A3	50	84(6)	−45(6)	98(14)	−178(8)	82(9)	−171(3)	7.3
B1A2B3	0	–	–	–	–	–	–	–
B1B2A3	38	85(9)	−99(6)	83(5)	−48(4)	94(8)	122(4)	6.4
B1B2B3	1012	90(14)	−75(16)	85(5)	−45(5)	91(19)	84(16)	5.5

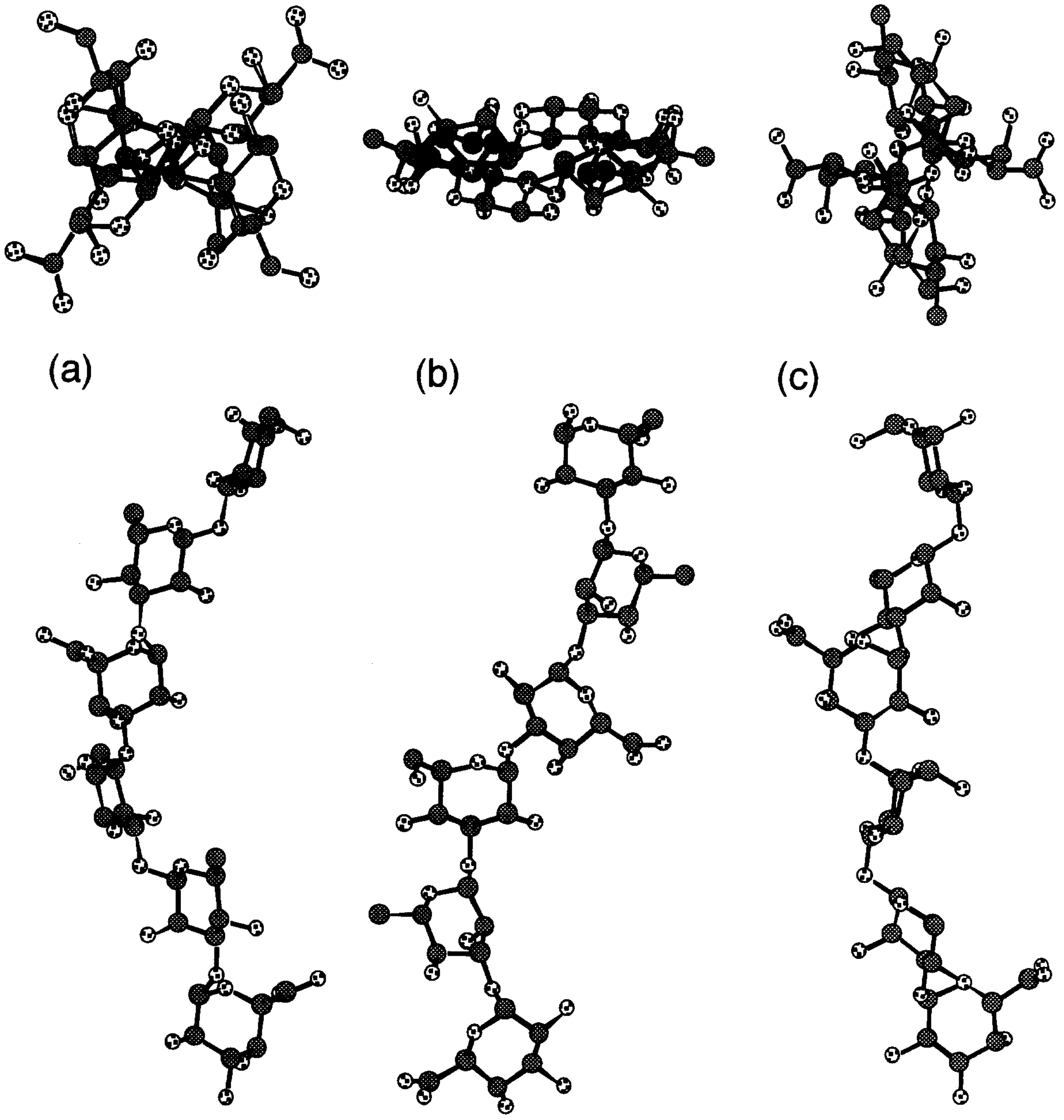

Fig. 4. The projections of the proposed helical conformations of beijeran. (a) A1A2B3, (b) B1B2B3 and (c) A1B2A3 models.

sequently, their total steric energies and helix parameters were calculated. Fig. 3 shows the n–h map of the chain models having the low steric energy (< 1.0 kcal/mol) where each n–h point has been based on the Φ–Ψ grids in 20°, instead of 10°, spacing for clarity of the map. As shown in Fig. 3, the n–h values are mainly distributed around those corresponding to the observed conformation. Some appearing at the small h range may suggest possible presences of large cyclic structures. In the next step, the chain models having the helix parameters $-2.1 < n < 2.1$ and $1.15 \text{ nm} < h < 1.25 \text{ nm}$ — about ±5% range of the observed values — were selected for the following examinations of the models. Table 1 lists the number of helix models of each Φ–Ψ conformation type along with the average Φ–Ψ and energy values. The notation given to each helix model represents the potential well of the relaxed energy map from which the Φ–Ψ conformation in the helix model has been adopted. The population of the A1A2B3 model is predominant over that of others and the model also shows the lowest value of the average energy. The moderate populations and energy values are observed for the B1B2B3 and A1B2A3 models. The number of the A1A2A3 models, obtained by combining the Φ–Ψ conformations in the lowest energy potential wells, is negligible. Furthermore, their average $\Phi 3$–$\Psi 3$ values considerably deviate from the exact position of the A3 minimum; rather they are near to the energy barrier toward the B3 potential well. The result given in Table 1 clearly suggests that, in the beijeran crystal structure, the backbone chain conformation is most likely to be the A1A2B3 type, and that the B1B2B3 and A1B2A3 type conformations may be present as a minor structure. The projections of the three representative helix models for beijeran chain are shown in Fig. 4.

In the present study, a D-GalA residue of a neutral form was employed throughout the energy map calculations instead of its metal salt form. We have assumed that a lack of the salt form in the residue would not significantly affect to predict the backbone conformation of beijeran salt chain. It should be also noted that the helix models has been constructed with the fixed residue geometry and glycosidic bridge angle. A complete structure refinement involving simultaneous changes in the whole residue geometry with the constraint of the helix symmetry would be necessary in order to evaluate more realistic values of the steric energy for each helix model. The programs specialized for the conformational analysis of polymers, such as LALS [15] and PS79 [16], may be capable of such stereochemical, full- or nearly full-optimization of a helical structure. The chain models proposed in the present study serve as a starting structure for the three-dimensional crystal structure analysis based on the X-ray diffraction intensity data.

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