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# Conformational analysis of biantennary glycans and molecular modeling of their complexes with lentil lectin

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*Some mannose-binding legume lectins show higher affinity for fucosylated glycans than for glycans without fucose. These lectins possess a secondary binding site. Owing to the possibility of additional fucose binding, oligosaccharides adopt different conformations depending on whether they contain fucose or not. To study these conformational differences, complexes of fucosylated and unfucosylated glycans with* Lens culinaris *lectin have been modeled. Starting points were X-ray structures of lentil lectin and complexes of the homologous* Lathyrus ochrus *lectin. The SYBYL molecular modeling package with the TRIPOS force field was used. Two different models were built, displaying in both a network of hydrogen bonds between the saccharide and the binding site. Furthermore, to compare the free and bound ligand, conformational analysis in the free state has been performed. A complete analysis of all possible disaccharide fragments has been performed using the MM3 force field. A CICADA analysis employing the same force field was carried out to study the complete oligosaccharide. Low-energy conformers found by CICADA were clustered in conformational families and analyzed in terms of flexibility and rotational barriers. All values of glycosidic torsion angles are in the range as calculated by MM3 for the disaccharides. © 1997 by Elsevier Science Inc.*

*Keywords*: lentil lectin, Lens culinaris, *oligosaccharide, molecular modeling*

## INTRODUCTION

Lectins of the tribe Viciae, such as *Lathyrus ochrus, Lens culinaris,* and *Pisum sativum,* are specific for mannose and glucose but show higher affinity for fucosylated oligosaccharides than for glycans without fucose [1]. Other lectins, such as concanavalin A (ConA), do not discriminate between these structures. The reason for this is a secondary binding site for fucose. Owing to the possibility of additional fucose binding, glycans adopt different conformations when binding to the lectin, depending on whether a fucose residue (1,6)-linked to the first *N*-acetylglucosamine is present. This has been shown by Bourne et al. for crystal structures of *L. ochrus* lectin complexed with different glycans [2,3].

In comparing complexes with fucosylated and unfucosylated biantennary glycans, it is seen that in either type (1,3)-Man is localized in the major binding site close to a $Ca^{2+}$ ion. If fucose (1,6)-linked to the chitobiose core is present, it is bound to a secondary binding site while the 1,6-branch is flexible in solvent. But if the chitobiose is cleaved, the remaining GlcNAc is exposed to solvent while the 1,6-arm is bound in the secondary binding site. This conformational change is due to a 180° difference of $\Psi$ in the Man-(1,3)–Man glycosidic linkage [2,3].

To study these conformational differences, complexes of fucosylated and unfucosylated glycans with *L. culinaris* lectin (LCL) have been modeled. Starting points included X-ray crystal structures of lentil lectin [4–6] and of complexes of the homologous *L. ochrus* lectin (LOL) [2,3]. The SYBYL molecular modeling package with the TRIPOS force field [7] was used with the addition of energy parameters appropriate for protein–carbohydrates interactions [8]. Two different models were built, both showing a network of hydrogen bonds between the saccharides and the binding sites.

Furthermore, to compare the free and bound ligands, conformational analyses of the two oligosaccharides in the free state have been carried out. A complete conformational analysis of all possible disaccharide fragments was done using the MM3 force field [9,10]. A CICADA analysis employing the same

Color Plates for this article are on page 54.

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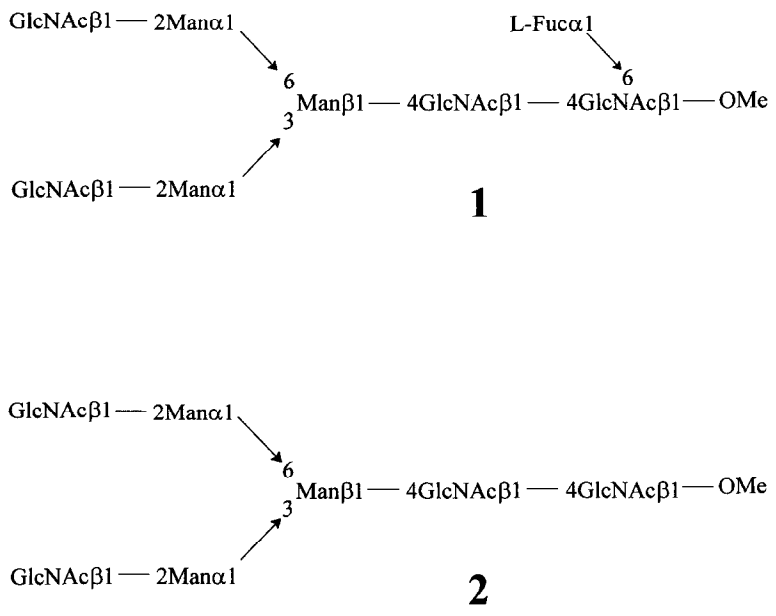


*Figure 1. Schematic representations of the glycans (oligosaccharides **1** and **2**) used in the present study.*

force field was carried out to study the complete oligosaccharides [11,12]. Low-energy conformations found by CICADA were combined into conformational families and analyzed in terms of flexibility and rotational energy barriers. All values of the glycosidic angles are in the range calculated by MM3 for the disaccharides.

## MODELING OF LENTIL LECTIN–OLIGOSACCHARIDE COMPLEXES

In this study complexes of lentil lectin (LCL) with biantennary oligosaccharides were modeled. Lentil lectin is highly homologous to the isolectins of *L. ochrus* (LOL). The crystal structure and the monosaccharide-binding site of lentil lectin are known [6,13]. Models of complexes with the two oligosaccharides (**1** and **2**) displayed in Figure 1 in different conformations have been built. Conformations of the oligosaccharides present in different crystal structures of *L. ochrus* complexes [2,3] served as starting points for the modeling. The models were built and energetically optimized using the SYBYL molecular modeling package with the TRIPOS force field [7] specially parametrized for carbohydrates [8].

Starting with the X-ray crystal structure of lentil lectin, hydrogen atoms were added on carbon, nitrogen, and oxygen atoms, successively. After each step, the energy was minimized. Water was neglected except for six molecules preserved in all legume lectins [14]. In the next step the oligosaccharide was complexed with the lectin. Using the crystal structure of lentil lectin–sucrose complex [6], the (1,3)-mannose was fitted onto the glucose residue of sucrose and then merged into the binding site. Subsequently, several cycles for establishing hydrogen bonds and optimizing the energy were carried out. Hydrogen atoms, then side chains, and finally the complete oligosaccharide and all amino acids in the binding site were considered for the optimization.

While the complex with oligosaccharide **1** was modeled using a single conformation of the glycan, two conformations were considered for oligosaccharide **2**: one conformation with the chitobiose part bound to the protein and one with the 1,6-branch in this position. The starting conformations were optimized previous to modeling the complex.

The optimized complex between LCL and octasaccharide **1** is displayed in Figures 2 and 3. The predicted hydrogen-bonding network between the oligosaccharide and the protein (Table 1) is similar to the one found in the crystal structure of the *L. ochrus* isolectin II [3].

Two complexes were modeled for the heptasaccharide **2**. In complex a chitobiose is located in the secondary binding site (Color Plate 1a) while in complex b the terminal GlcNAc of the

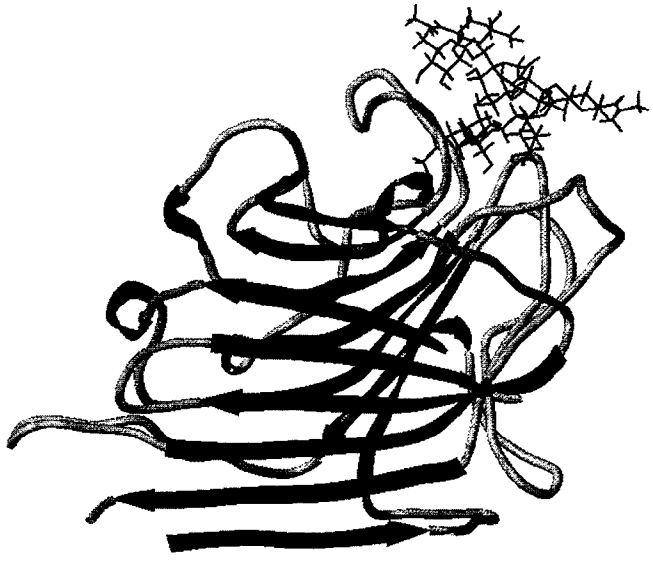

*Figure 2. Backbone of lentil lectin complexed with octasaccharide **1**.*

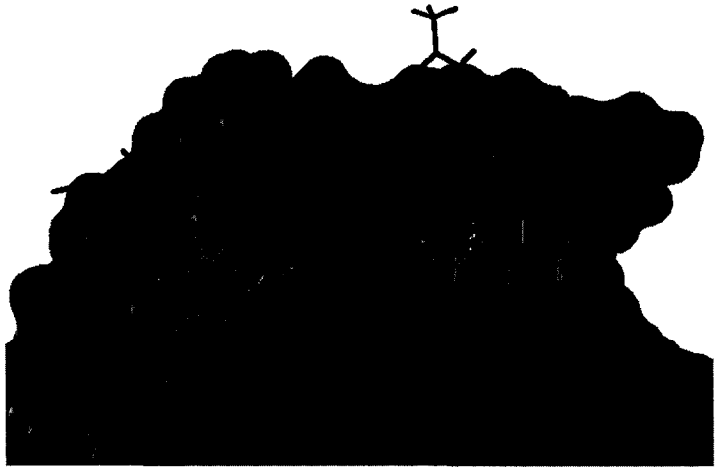

*Figure 3. Octasaccharide **1** in the binding site of lentil lectin. (1,3)-mannose and fucose are displayed with light bonds on the left side and the right side of the picture, respectively.*

1,6-arm is in the secondary binding site and the chitobiose part is exposed to the solvent (Color Plate 1b). Both structures show a number of ligand–protein hydrogen bonds (Table 2). Although in complex a three more polar contacts could be established, a clear preference for this structure cannot be made.

## CONFORMATIONAL ANALYSIS OF FREE OLIGOSACCHARIDES

To learn about possible conformations and flexibility of the oligosaccharides, both octasaccharide **1** and heptasaccharide **2**

**Table 1. Comparison of protein–ligand hydrogen bondings in the model of lentil lectin complexed with oligosaccharide 1 and X-ray crystal structure of *Lathyrus ochrus* isolectin II**

| Sugar ring | Sugar atom of oligosaccharide **1** | Protein atom of LCL | Sugar atom of glycopeptide[a] | Protein atom of LOLII |
|---|---|---|---|---|
| Fuc 1′ | HO2 | OE1 (Glu-212) | O2 | OE1 (Glu-212) |
| | HO2 | OE2 (Glu-212) | O2 | OE2 (Glu-212) |
| | HO3 | OE1 (Glu-212) | O3 | E2 (Glu-212) |
| | O3 | NH2 (Arg-135) | — | — |
| GlcNAc 1 | O7 | ND2 (Asn-78) | O7 | OD1 (Asn-78) |
| | — | — | O5 | ND2 (Asn-78) |
| | — | — | O6 | ND2 (Asn-78) |
| GlcNAc 2 | HO6 | OE2 (Glu-212) | O5 | OE2 (Glu-212) |
| Man 3 | — | — | — | — |
| Man 4′ (1,6-arm) | HO3 | O (Tyr-124) | — | — |
| GlcNAc 5′ (1,6-arm) | O7 | HNZ (Lys-133) | — | — |
| Man 4 (1,3-arm) | O3 | HN (Gly-99) | O3 | HN (Gly-99) |
| | O4 | HN (Gly-99) | O4 | HN (Gly-99) |
| | O4 | HND2 (Asn-125) | O4 | HND2 (Asn-125) |
| | HO4 | OD1 (Asp-81) | O4 | OD2 (Asp-81) |
| | O5 | HN (Ala-211) | O5 | HN (Ala-209) |
| | O6 | HN (Glu-212) | O6 | HN (Glu-210) |
| | O6 | HN (Ala-211) | O6 | HN (Ala-209) |
| | HO6 | OD1 (Asp-81) | O6 | OD1 (Asp-81) |
| | HO6 | OD2 (Asp-81) | O6 | NH (Gly-208) |
| GlcNAc 5 (1,3-arm) | — | — | O5 | OD1 (Asn-39) |
| | — | — | O6 | HN (Ala-209) |

[a] From Ref. 3. Only atoms of comparable sugar residues are listed.
Abbreviations: LCL, *Lens culinaris* lectin; LOL, *Lathyrus ochrus* lectin.

**Table 2. Hydrogen bondings in the two models of heptasaccharide–*Lens culinaris* lectin complex**

| | Complex a | | Complex b | |
|---|---|---|---|---|
| Sugar ring | Sugar atom | Protein atom | Sugar atom | Protein atom |
| GlcNAc 1 | HO6 | HO (Tyr-77) | — | — |
| | O7 | O (Tyr-77) | — | — |
| GlcNAc 2 | — | — | — | — |
| Man 3 | — | — | — | — |
| Man 4′ (6-arm) | O3 | HO (Tyr-77) | — | — |
| | HO3 | O (Tyr-77) | — | — |
| GlcNAc 5′ (6-arm) | — | — | HO6 | O (Tyr-124) |
| | — | — | O7 | OE2 (Glu-212) |
| Man 4 (3-arm) | O3 | HN (Gly-99) | O3 | HN (Gly-99) |
| | O4 | HND2 (Asp-125) | O4 | |
| | O4 | HN (Gly-99) | — | — |
| | HO4 | OD1 (Asp-81) | HO4 | OD1 (Asp-81) |
| | O5 | HN (Ala-211) | O5 | HN (Ala-211) |
| | O6 | HN (Glu-212) | O6 | HN (Glu-212) |
| | O6 | HN (Ala-211) | O6 | HN (Ala-211) |
| | HO6 | OD1 (Asp-81) | — | — |
| | HO6 | OD2 (Asp-81) | HO6 | OD2 (Asp-81) |
| GlcNAc 5 (3-arm) | — | — | O5 | HN (Ala-211) |

were studied in the free state. First, all constituting disaccharide fragments were completely analyzed using MM3 [8,9]. All calculations were carried out using a dielectric constant of 78.5. Relaxed energy maps for Φ and Ψ angles were drawn in order to determine all energetical minima for each glycosidic linkage. For 1,6-glycosidic linkages [Man-(1,6)–Man and Fuc-(1,6)–GlcNAc], the ω angles were also driven. In this case either three-dimensional relaxation maps representing the conformational space or two-dimensional projections on the Φ, Ψ-plane for all ω angles can be used to determine the minima. The crystallographic definition of the torsional angles was used in the study (see Figure 4).

Several conformations of oligosaccharides **1** and **2** were built and energetically optimized. These models served as starting conformations for the CICADA calculation [10,11]. This program uses the MM3 force field and can be used to explore the conformational space of rather large molecules when a grid search is impracticable. With CICADA, minima and also transition states of molecules can be calculated, i.e., pathways on the potential surface can be explored. For the calculations all torsional angles of the glycosidic linkages were driven. In addition, the torsional angles of NAc and hydroxymethyl groups were monitored. Altogether, 25 torsion angles were taken into account for octasaccharide **1**, and 22 torsion angles for heptasaccharide **2**.

To see whether the low-energy conformations of the oligosaccharide correspond to the energy minima of its constituting disaccharides, a comparison is made between the disaccharide grid search and the oligosaccharide CICADA calculations. Every oligosaccharide conformation with a relative energy less than 8 kcal/mol is superimposed on the energy map of the constituting disaccharide (Figure 5).

As shown in the graphics, all torsional angles of low-energy conformations of the complete oligosaccharide belong to the lowest energy region of the disaccharide fragments. It is interesting to notice that the terminal GlcNAc-(1,2)–Man linkage of the 1,6-arm is less flexible than the linkage of the 1,3-arm. In the latter, the *trans* conformation, i.e., the one with Φ about 60°, is populated, whereas the GlcNAc-(1,2)–Man linkage of the 1,6-arm is almost fixed in the global minimum. A conformation corresponding to this precise secondary minimum has been observed in one of the *L. ochrus* complexes [3].

The biantennary oligosaccharides studied here are very flexible (Figure 6). The low-energy conformations were analyzed by a home-made clustering method. Two conformations belong to the same family if none of the torsion angles of the core differs by more than 30° from at least one conformation of the same family. When considering an energy cutoff of 10 kcal/mol for oligosaccharide **1** (918 of the 2042 calculated minima), 17 families can be defined, which comprise more than 96% of the conformers.

Since solvent molecules were not explicitly taken into account in the present study, it must be stated that too much emphasis is probably given to the conformations with strong

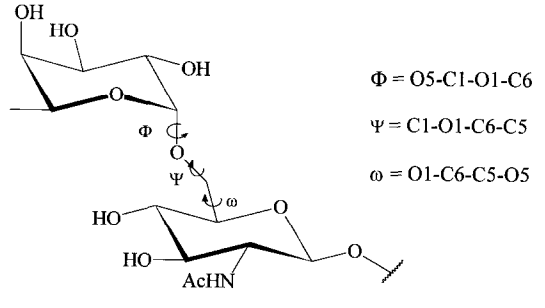


*Figure 4. Definition of Φ, Ψ, and ω torsion angle in the L-Fuc(1,6)-GlcNAc disaccharide.*

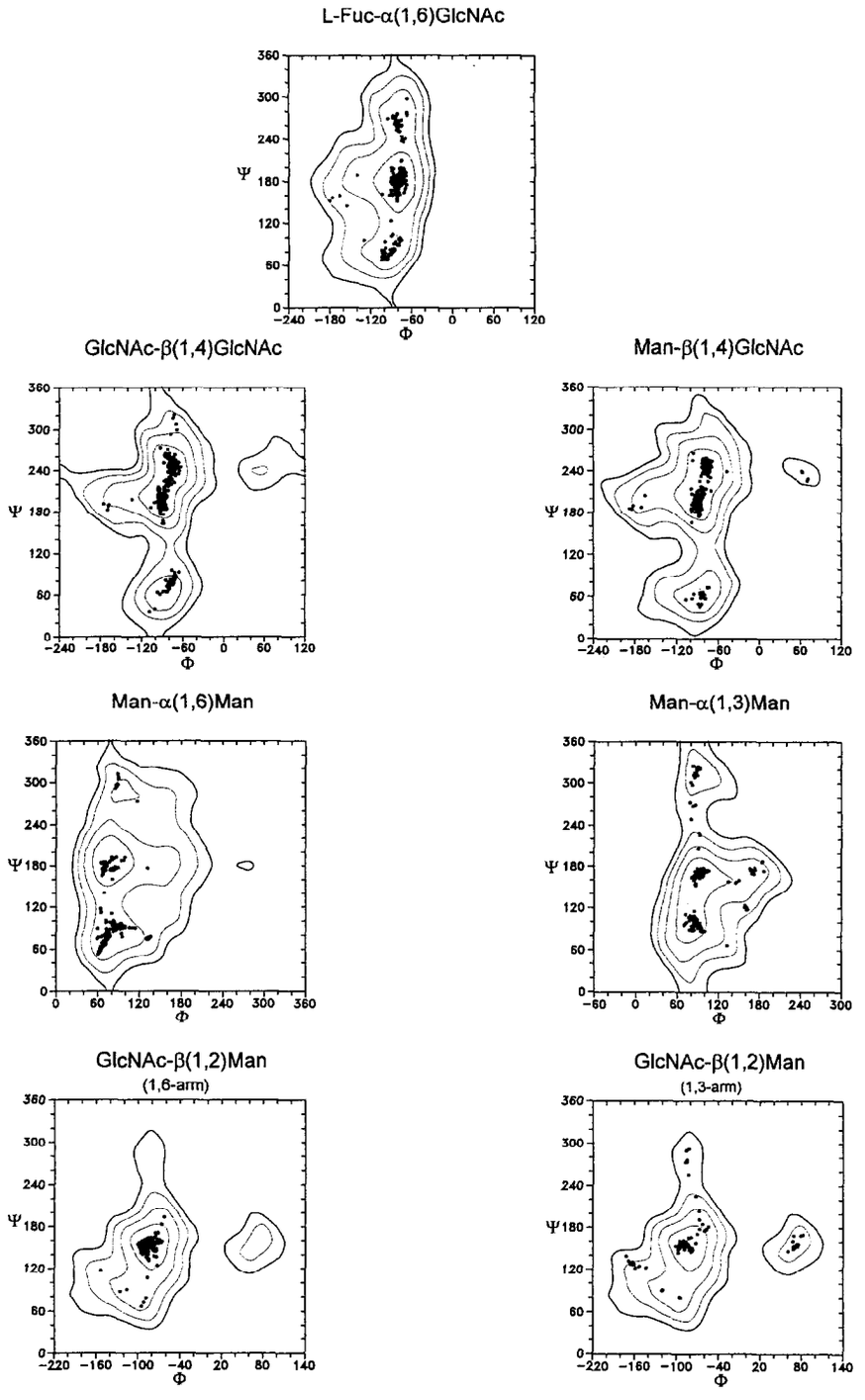


*Figure 5. Relaxed energy maps of disaccharides constituting octasaccharide* ***1****. Isoenergy contours are displayed at values of 2, 4, 6, and 8 kcal/mol. Each dot corresponds to a conformation of the octasaccharide with an energy <8 kcal/mol above the global minimum. Last row: linkage GlcNAc-(1,2)–Man, (1,6)-arm on left, (1,3)-arm on right.*

intraresidue contacts. Nevertheless, this conformational analysis shows the high flexibility of biantennary oligosaccharides, fucosylated or not, and gives results that are in agreement with experimental data [3], such as the conformation of the terminal GlcNAc-(1,2)–Man linkage.

## CONCLUSION

The present study establishes the conformational space of fucosylated and nonfucosylated biantennary oligosaccharides. When modeling the conformers complexed by lentil lectin all

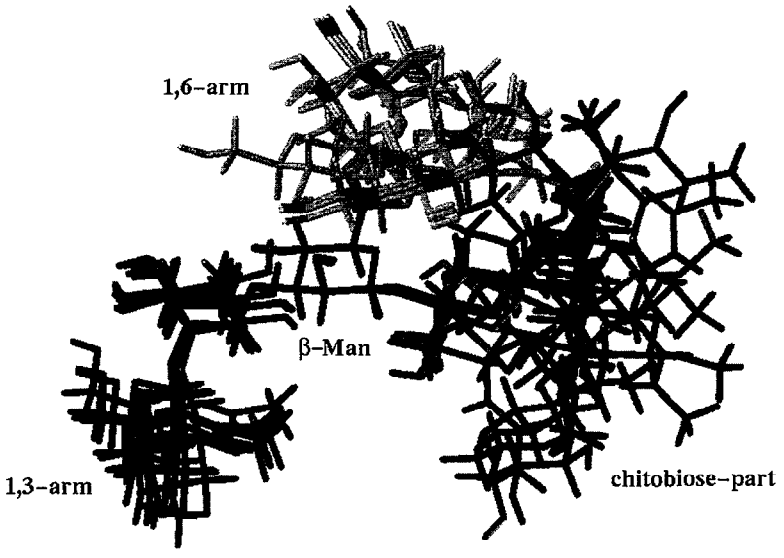


*Figure 6. Five low-energy conformations of octasaccharide* ***1*** *fitted on the β-Man residue and superimposed.*

Φ and Ψ angles correspond to energy minima of the constituting disaccharides. This shows that, by binding to the lectin, there is no creation of a bioactive conformation but a selection of one conformer already present in solution. For the unfucosylated glycan two different complexes were modeled. These different structures are quite similar in terms of energy, so that a clear statement for either of them cannot be made. This can be achieved only in comparison with experimental data.

This study is the theoretical part of a project concerning interaction of N-type glycans with lectins. The models will serve as the starting hypothesis for the experimental work that will investigate the described complexes by nuclear magnetic resonance spectroscopy.

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