

# NMR, Molecular Modeling, and Crystallographic Studies of Lentil Lectin-Sucrose Interaction\*

(Received for publication, June 13, 1995, and in revised form, August 9, 1995)

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**The conformational features of sucrose in the combining site of lentil lectin have been characterized through elucidation of a crystalline complex at 1.9-Å resolution, transferred nuclear Overhauser effect experiments performed at 600 Mhz, and molecular modeling. In the crystal, the lentil lectin dimer binds one sucrose molecule per monomer. The locations of 229 water molecules have been identified. NMR experiments have provided 11 transferred NOEs. In parallel, the docking study and conformational analysis of sucrose in the combining site of lentil lectin indicate that three different conformations can be accommodated. Of these, the orientation with lowest energy is identical with the one observed in the crystalline complex and provides good agreement with the observed transferred NOEs. These structural investigations indicate that the bound sucrose has a unique conformation for the glycosidic linkage, close to the one observed in crystalline sucrose, whereas the fructofuranose ring remains relatively flexible and does not exhibit any strong interaction with the protein. Major differences in the hydrogen bonding network of sucrose are found. None of the two inter-residue hydrogen bonds in crystalline sucrose are conserved in the complex with the lectin. Instead, a water molecule bridges hydroxyl groups O2-g and O3-f of sucrose.**

Specific recognition of carbohydrates by protein receptors is of growing interest in biology. In addition to immunoglobulins, carriers, and toxins, carbohydrates can interact with lectins, a class of multivalent and ubiquitous carbohydrate binding pro-

teins (Sharon and Lis, 1989). Animal lectins have been shown to be implicated in the social life of the cells, mediating cell recognition and cell adhesion (Drickamer and Taylor, 1993). Plant lectins are also involved in recognition processes and play a role in the interaction with symbiots or pathogens (Diaz *et al.*, 1989; Chrispeels and Raikhel, 1991). They can be purified easily and their specificities make them useful as components of affinity columns for the separation of glycoconjugates or as markers of blood groups and tumor cell lines (Lis and Sharon, 1986).

Legume lectins are a large family of homologous proteins displaying a broad range of different carbohydrate specificities. Several x-ray structures of legume lectins complexed with carbohydrates have been determined recently: concanavalin A with mannose (Derewenda *et al.*, 1989), *Lathyrus ochrus* isolectins with mannose and glucose (Bourne *et al.*, 1990a), with a trisaccharide (Bourne *et al.*, 1990b), with a biantennary octasaccharide (Bourne *et al.*, 1992) and with a biantennary glycopeptide (Bourne *et al.*, 1994), *Erythrina corallodendron* lectin with lactose (Shaanan *et al.*, 1991), pea lectin with trimannose (Rini *et al.*, 1993), and *Griffonia simplicifolia* isolectin IV with the Lewis b determinant (Delbaere *et al.*, 1993).

To study recognition processes at the atomic level, different experimental and theoretical methods can be used. Many carbohydrate-protein complex structures have been elucidated recently by x-ray crystallography (Vyas, 1991; Bourne *et al.*, 1993) at high resolution. Detailed information derived from crystallographic data allows us to estimate the driving forces behind carbohydrate-protein interactions (Vyas, 1991; Imberty *et al.*, 1993). However, whereas x-ray crystallography provides us with a static picture, NMR experiments can provide information about the conformation as well as about the movement of the ligand in the binding site. In the special case of weakly bound ligands, with a high rate of exchange between the bound and the free states, transferred nuclear Overhauser effects (TRNOEs)<sup>1</sup> can be observed, giving information about the conformation of the carbohydrate in the binding site (Clore and Gronenborn, 1982; Ni, 1994). This method allowed the determination of the conformational changes undergone by oligosaccharides upon binding to ricin (Bevilacqua *et al.*, 1990, 1992), to antibodies (Glaudemans *et al.*, 1990, Bundle *et al.*, 1994, Arepalli *et al.*, 1995), and to a human lectin (Cooke *et al.*, 1994). Molecular modeling is a complementary tool for both x-ray and NMR methods. Several methods of docking monosaccharides to

\* This work was supported by Institut National de la Recherche Agronomique (INRA) and CNRS (to F. C., J.-R. B., C. T., S. P., A. I.) and V.L.A.B. Project of the Flemish government (to T. H., R. L., M.-H. D.-T., L. W., F. P.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The atomic coordinates and structure factors (code 1LES) have been deposited in the Protein Data Bank, Brookhaven National Laboratory, Upton, NY.

<sup>b</sup> These two authors are joint first authors.

<sup>c</sup> Supported by a grant from Ministère de l'Éducation Supérieure et de la Recherche.

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<sup>1</sup> The abbreviation used is: TRNOE, transferred nuclear Overhauser effect.

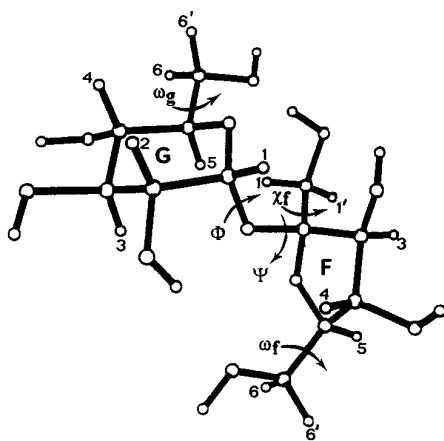


FIG. 1. **Perspective view of sucrose.** Hydrogen atoms of interest for the NMR study are labeled. Arrows indicate the torsion angles of interest.

protein receptors have been developed using molecular mechanics. Such methods allow for a systematic exploration of all possible positions and orientations of the ligand in the binding site (Rao *et al.*, 1989; Imberty *et al.*, 1991). In addition, it is possible, using computer simulations, to evaluate the conformational space accessible for flexible ligands such as oligosaccharides (Reddy and Rao, 1992; Imberty and Pérez, 1994).

The aim of this work is to study the conformation of sucrose in interaction with lentil lectin, using three different and complementary approaches: x-ray crystallography, NMR, and molecular modeling. Lentil lectin specifically recognizes mannose and glucose. The three-dimensional structure of this lectin was refined at 1.8-Å resolution in the uncomplexed form (Loris *et al.*, 1993, 1994a) and at 3.0-Å resolution for the complex with glucose (Loris *et al.*, 1994b). Sucrose is an abundant carbohydrate of interest to the food industry for its sweet taste. The molecular conformation of sucrose has been studied extensively, and many experimental and theoretical data are available (see review by Pérez (1994)). Sucrose has been shown to be

FIG. 2. **Stereoscopic representation of a dimer of the lentil lectin complexed with sucrose as determined by crystallography.**  $\beta$ -Sheets are shown as arrows. The two sucrose molecules, which are bound at opposite ends of the lectin dimer, are drawn as ball and stick models. This figure was prepared using MOLSCRIPT (Kraulis, 1991).



TABLE I  
Statistics of data collection and refinement

Space group	P2 <sub>1</sub>
Unit cell	$a = 50.03 \text{ \AA}$ $b = 124.0 \text{ \AA}$ $c = 50.02 \text{ \AA}$ $\beta = 111.80^\circ$ 1.9 Å
Resolution	1.9 Å
Number of observations	114,331
Number of unique reflections	43,108
Completeness (1.96 Å-1.90 Å, last shell)	69.9% (75.1%)
$R_{\text{merge}}^a$ (last shell, 1.96 Å-1.90 Å)	7.3% (29.5%)
$R$ value <sup>b</sup> after refinement (6.0 Å-1.9 Å)	18.8%
Number of protein atoms	3,838
Number of solvent atoms	228
Number of sugar atoms	46
Number of metal ions	2 Mn <sup>2+</sup> , 2 Ca <sup>2+</sup>
Root mean square on bond lengths	0.020 Å
Root mean square on bond angle restraints	2.3°
Root mean square on omega torsion angle planarity	5.3°
Distribution of non-Gly non-Pro $\Phi$ - $\Psi$ angles in the Ramachandran plot	89.3% in core regions 10.2% in additionally allowed regions 0.5% in generously allowed regions 0.0% in disallowed regions

<sup>a</sup>  $R_{\text{merge}} = \frac{\sum_i \sum_j (I_i - I_j) / \sum_j I_j}{\sum_i I_i}$

<sup>b</sup>  $R \text{ value} = \frac{\sum (F_o - F_c) / \sum F_o}{\sum F_o}$

flexible in dilute solution (Hervé du Penhoat *et al.*, 1991; Poppe and Van Halbeek, 1992), but its interactions with protein receptors have never been investigated. We report here the first x-ray structure of sucrose complexed with a protein. In addition, transferred NOEs have also been obtained for lentil lectin complexed with sucrose. Both sets of experimental data can be rationalized by comparison with the conformational behavior of sucrose in the binding site as predicted by molecular modeling.

## EXPERIMENTAL PROCEDURES

*Nomenclature*

The labeling of sucrose atoms and torsion angles is represented in Fig. 1. The glycosidic linkage can be described by two torsion angles  $\Phi$  and  $\Psi$ .  $\Phi = (\text{O5-g-C1-g-O1-g-C2-f})$ ;  $\Psi = (\text{C1-g-O1-g-C2-f-O5-f})$ .

The orientation of the three hydroxymethyl groups is given by the torsion angles  $\omega\text{g}$ ,  $\omega\text{f}$ , and  $\chi\text{f}$ .  $\omega\text{g} = (\text{O5-g-C5-g-C6-g-O6-g})$ ;  $\omega\text{f} = (\text{O5-f-C5-f-C6-f-O6-f})$ ;  $\chi\text{f} = (\text{O5-f-C2-f-C1-f-O1-f})$ .

The signs of the torsion angles are in agreement with the recommendations given by the IUPAC-IUB Commission of Biochemical Nomenclature (IUPAC-IUB, 1971). The three preferred orientations of the primary hydroxyl groups are referred to as either *gauche-gauche* (GG), *gauche-trans* (GT), or *trans-gauche* (TG), with respective values of  $-60^\circ$ ,  $60^\circ$ , and  $180^\circ$  (Marchessault and Pérez, 1979). The distortion of the fructofuranose ring is described by the Cremer-Pople puckering

parameters  $Q$  and  $\phi$  (Cremer and Pople, 1975).  $Q$  is the puckering amplitude which measures the deviation from the planarity, and  $\phi$  is the phase angle of puckering.

*Materials*

Lentil lectin was purified from the seeds of the common lentil (*Lens culinaris*) as described previously (Loris *et al.*, 1992). Commercial sucrose was used.

*Crystallization and Data Collection*

Crystallization conditions for the lentil lectin-sucrose complex were screened using the hanging drop method. Crystals of good quality were grown by equilibrating 10- $\mu\text{l}$  drops containing 6.25  $\text{mg} \cdot \text{ml}^{-1}$  protein in 50 mM cacodylate (pH 6.5), 25 mM sucrose, and 20% 2-methyl-2,4-pentanediol (v/v) against 1-ml reservoirs of 40% 2-methyl-2,4-pentanediol (v/v) in 100 mM cacodylate buffer, pH 6.5. X-ray data to a resolution of 1.9 Å were collected on a FAST area detector using two crystals. The crystals are of space group  $P2_1$  with unit cell parameters  $a = 50.03$  Å,  $b = 124.80$  Å,  $c = 50.02$  Å,  $\beta = 111.80^\circ$  and contain a complete lectin dimer in the asymmetric unit. A total of 108,320 observations were reduced to 41,579 unique reflections with an  $R_{\text{merge}}$  value of 0.073. Details about the data collection, space group, unit cell, and refinement are summarized in Table I. Molecular replacement calculations were performed with the AMORE package (Navaza, 1994) using the coordinates of orthorhombic lentil lectin (Loris *et al.*, 1994a) as a search model. Refinement was carried out with X-PLOR (Brünger,

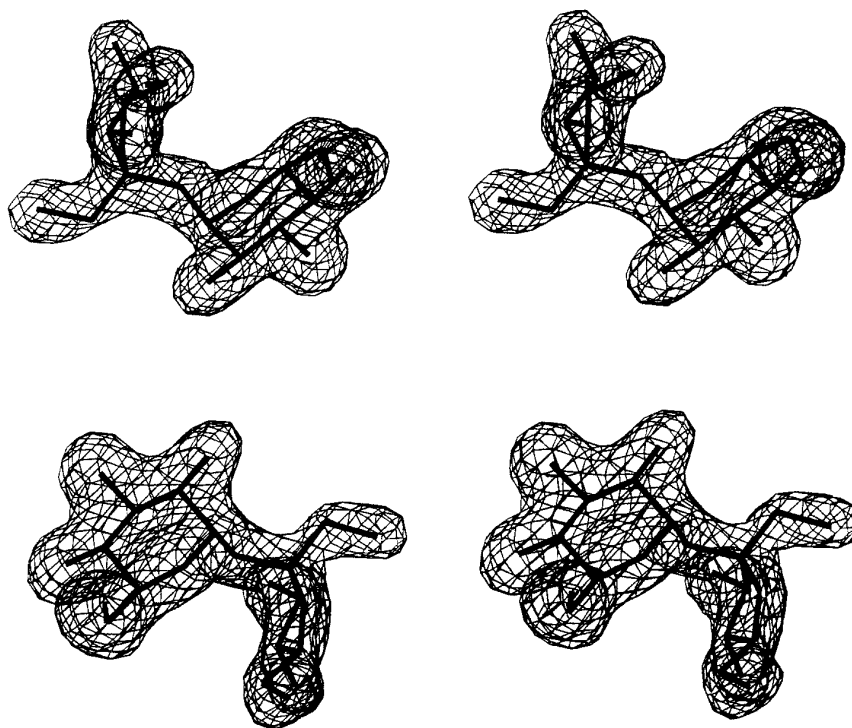


FIG. 3. Two different stereo views of the electron density of the sucrose molecule as determined by crystallography.

TABLE II  
Description of the hydrogen bonds between the lentil lectin binding site and sucrose

Hydrogen bonds <sup>a</sup>		Distance D...A	
Donor	Acceptor	Crystal structure (first monomer)	Lowest energy model
			Å
Ala-30.N	O5-g	3.04	3.11
Glu-31.N	O6-g	3.05	
O6-g	Asp-81.OD1	2.91	3.15
O4-g	Asp-81.OD2	2.64	2.59
Asn-125.N	O4-g	2.88	2.97
Gly-99.N	O3-g	2.82	2.95
Gly-97.N <sup>b</sup>	O1-f	2.77	

<sup>a</sup> Hydrogen bonds have been evaluated with the hydrogen atoms. Limits for hydrogen bonds are as follows:  $2.55 \text{ \AA} < \text{distance D...A} < 3.2 \text{ \AA}$  and angle  $\text{D-H...A} > 115^\circ$ .

<sup>b</sup> From the symmetry-related monomer.

FIG. 4. **Stereoscopic representation of the sucrose binding site in the crystal structure of lentil lectin-sucrose complex.** For the sake of clarity, only amino acids involved in intermolecular hydrogen bonding are shown. The sucrose molecule is displayed in *bold*. Hydrogen atoms have been generated on sucrose and on amino acids from the binding site in order to determine the hydrogen bond network (represented with *dotted lines*).

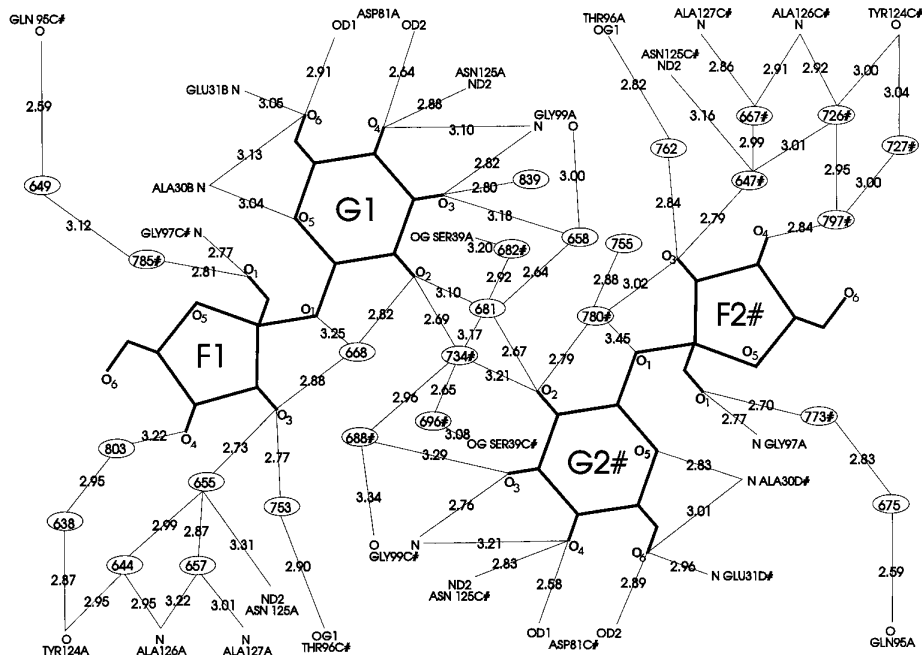
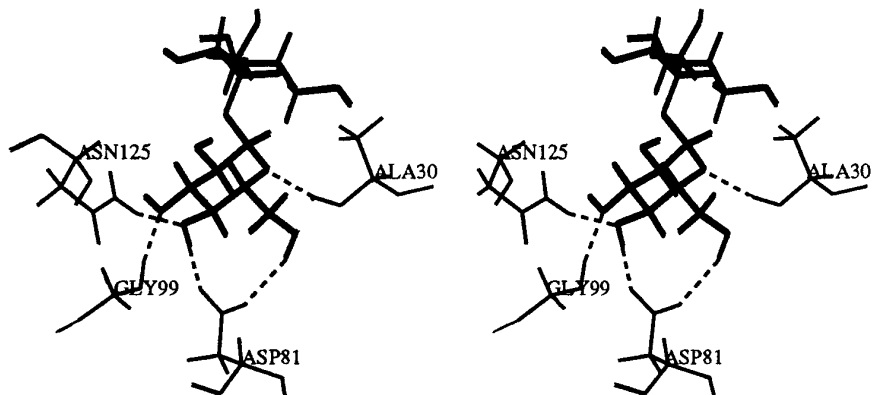
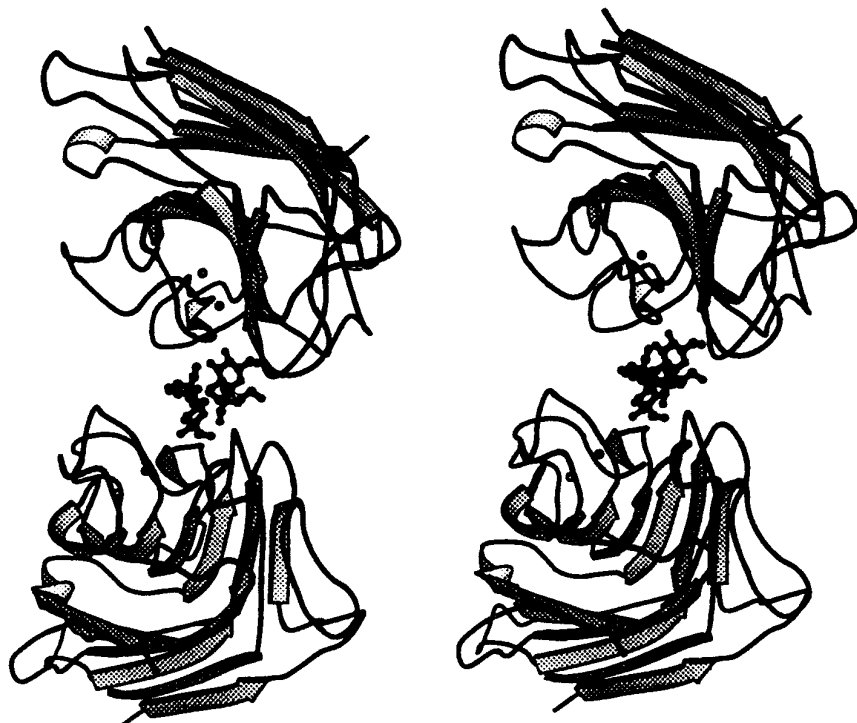


FIG. 5. **Hydrogen bond network of sucrose in the lentil lectin binding site with the water molecules.** The symmetry-related sucrose is also shown.

FIG. 6. **Stereoscopic representation of the sucrose-mediated crystal lattice interaction between both lectin monomers.** The two sucrose molecules in the center of this figure form a lattice contact via the water network that is drawn schematically in Fig. 5. The figure was prepared using MOLSCRIPT (Kraulis, 1991).



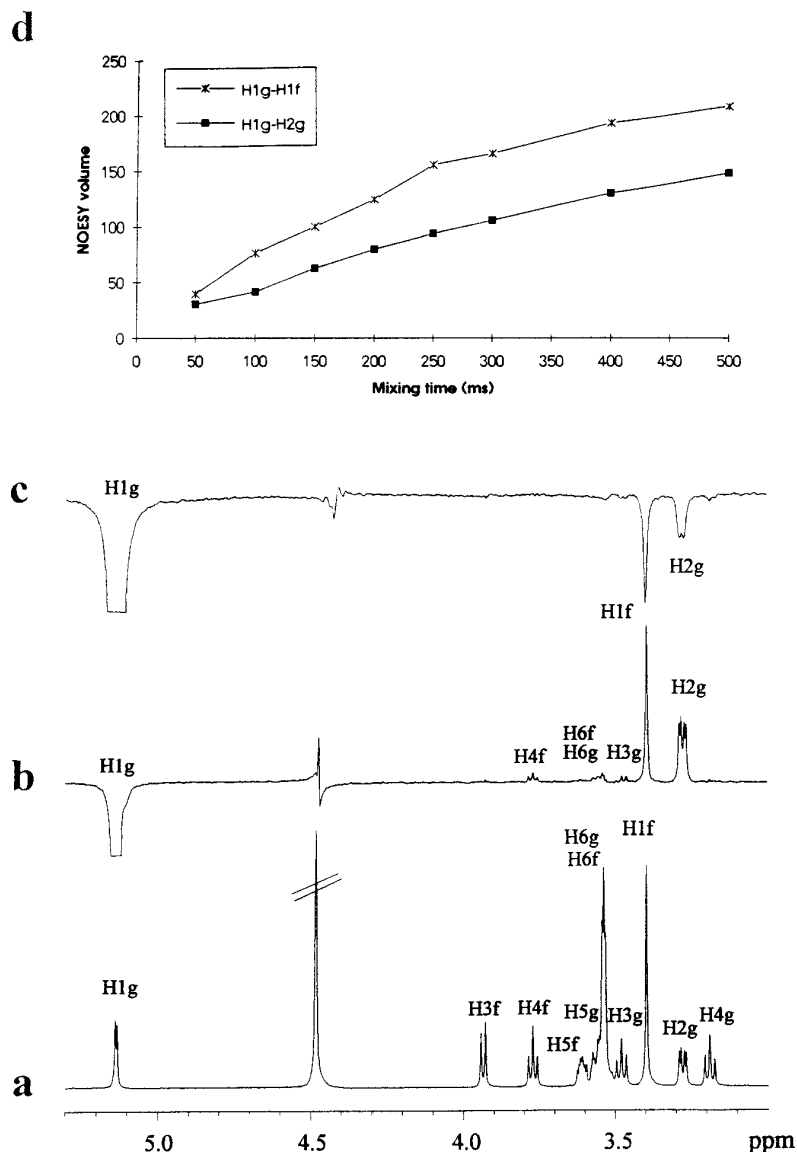


FIG. 7.  $^1\text{H}$  NMR spectra for the free and the bound ligand. *a*, one-dimensional spectrum of sucrose. *b*, one-dimensional NOESY of H1-g for the free ligand where positive NOEs are observed. *c*, one-dimensional TRNOE of H1-g for the complex of lentil lectin with sucrose, showing the negative NOEs. *d*, build-up curves of some observed transferred NOEs.

1990), and the final model was analyzed with PROCHECK (Laskowski *et al.*, 1993).

#### NMR Measurements

$^1\text{H}$  spectra were recorded at 30 °C on Bruker ARX 400 and AMX 600 NMR spectrometers. Experiments were performed without sample spinning and using standard software provided by Bruker. Transferred NOE experiments were recorded at 600 MHz. 2.75 mg of lentil lectin was dissolved in 0.470 ml of  $\text{D}_2\text{O}$  with 100 mM NaCl. Then, 0.03 ml of a 50 mM sucrose solution was added to obtain a ligand:protein ratio of 15:1. Phase-sensitive NOESY experiment using TPPI with a spin lock filter (Scherf and Anglister, 1993) and presaturation of the HDO signal were recorded for mixing times of 50, 100, 150, 200, 250, 300, 400, and 500 ms. 32 scans were collected per  $t_1$  increment with a pulse delay of 1 s. A  $\pi/2$ -shifted squared sine bell filter was applied to both dimensions prior to Fourier transformation. The baseline was corrected in  $f_2$  and  $f_1$  (third order polynomial) prior to integration of the cross-peak volumes. For the sucrose  $^1\text{H}$  spectra, 3 mM and 15 mM solutions were used after two lyophilizations in  $\text{D}_2\text{O}$  (99.9%). With the 15 mM solution, a phase-sensitive NOESY using TPPI and presaturation of water was also performed with a mixing time of 800 ms.

From the TRNOE experiments, build-up curves were drawn by plotting the NOE cross-peak volumes against the mixing times. Interproton distances and their standard deviations were calculated from the NOE build up curves by normalizing on a H2-g/H4-g theoretical distance of 2.47 Å and extrapolating to zero mixing time using a least square fit (Baleja *et al.*, 1990).

#### Molecular Modeling

**Energy Calculations**—The molecular modeling study of sucrose in the binding site of lentil lectin was performed using the SYBYL molecular modeling package (Sybyl V6.04, Tripos Associates, St. Louis, MO) running on a Silicon Graphics Elan 4000 Indigo. The Tripos force field (White and Guy, 1975) was used with appropriate energy parameters for carbohydrates (Imberty *et al.*, 1991; Pérez *et al.*, 1995). This force field takes into account the contribution of bond-stretching, angle-bending, torsional, Van der Waals, and electrostatic terms (Clark *et al.*, 1989), the hydrogen bonding contribution being included in the last energy term. Charges for the protein atoms were calculated using the Pullman method (Berthod and Pullman, 1965). Carbohydrate charges are the same as described by Imberty *et al.* (1991), and the  $\text{Mn}^{2+}$  and  $\text{Ca}^{2+}$  charges were fixed at 2. The dielectric constant was given a value of 4 to simulate the environment of the crystal.

**Geometry Optimization**—Geometry optimizations were performed using the MAXIMIN2 procedure of SYBYL (Press *et al.*, 1986). An energy minimizer consisting of a combination of SIMPLEX and conjugate gradient methods was used, with a gradient termination parameter fixed to 0.5 kcal/mol/Å<sup>2</sup>. Such energy minimizations can be performed on all the atoms of the molecule, or a subset of atoms can be kept in fixed positions and constraints can be imposed as well.

**Docking of Sucrose**—The coordinates of the glucose-lentil lectin complex determined by crystallography (Loris *et al.*, 1994b) were used as a starting point for the molecular modeling study of the sucrose-lentil lectin interaction. The glucose residue located in the lentil lectin binding site was extended to an  $\alpha\text{-D-GlcP}(1\rightarrow2)\beta\text{-D-Fruf}$  by adding a fructo-

TABLE III

Transferred NOESY volumes observed for sucrose complexed with lentil lectin for a mixing time of 500 ms and NOESY volumes for sucrose in solution for a mixing time of 800 ms. Standard deviations are given in *italic*

Transferred NOESY volumes were normalized to the H2-g/H4-g volume set at 100. Standard deviations were calculated for the two cross-peaks. Estimated error on NOESY experiment is of 10% for strong peaks and 20% for weak peaks and for TRNOESY.

Proton pair	Normalized NOESY volume	
	Lentil lectin-sucrose	Sucrose
H1-g/H2-g	-130 ( <i>12</i> )	150 ( <i>2</i> )
H1-g/H3-g		7 ( <i>4</i> )
H1-g/H5-g + H6-g + H6-f		24 ( <i>4</i> )
H2-g/H3-g	-87 ( <i>4</i> )	41 ( <i>2</i> )
H2-g/H4-g	-100 ( <i>11</i> )	100 ( <i>11</i> )
H3-g/H4-g	-49 ( <i>3</i> )	35 ( <i>2</i> )
H3-g/H5-g + H6-g + H6-f	— <sup>a</sup>	78 ( <i>2</i> )
H4-g/H5-g + H6-g + H6-f	-79 ( <i>2</i> )	78 ( <i>4</i> )
H1-f/H3-f	-14 ( <i>9</i> )	59 ( <i>4</i> )
H3-f/H4-f	-92 ( <i>7</i> )	24 ( <i>9</i> )
H3-f/H5-f	-38 ( <i>12</i> )	78 ( <i>2</i> )
H4-f/H5-f	-83 ( <i>15</i> )	— <sup>b</sup>
H4-f/H5-g + H6-g + H6-f	-137 ( <i>24</i> )	78 ( <i>4</i> )
H5-f/H5-g + H6-g + H6-f	— <sup>a</sup>	47 ( <i>11</i> )
H1-g/H1-f	-182 ( <i>11</i> )	120 ( <i>2</i> )
H1-g/H4-f		11 ( <i>2</i> )

<sup>a</sup> In the noise.

<sup>b</sup> Included with H4-f/H5-g + H6-g + H6-f.

furanosyl group. The starting geometry of sucrose was given a low energy conformation ( $\Phi = 110^\circ$  and  $\Psi = -60^\circ$ ) as described previously (Hervé du Penhoat *et al.*, 1991). In order to save computing time, only part of the protein was considered in the calculations. A protein region of 59 amino acids, corresponding to a sphere of 15 Å centered on the glucose residue, was extracted. Hydrogen atoms were added to the 59 amino acids and to the sucrose molecule, and their positions were optimized by several cycles of energy minimization. Then, 19 amino acids were defined as representing the glucose/sucrose binding site. In a second cycle of energy minimization, the positions of their side chains were fully optimized together with the position of the sucrose molecule.

**Conformational Analysis**—The conformational search of sucrose was performed varying the torsional angles  $\Phi$  and  $\Psi$  of sucrose by  $20^\circ$  increment steps. The origin of rotation was taken on the glucopyranose side, allowing therefore only the fructofuranose residue to take different positions with respect to the protein surface. Each primary hydroxyl group of fructofuranose was given its two preferred staggered orientations, *i.e.* GG and GT for  $\omega$ f and GG and TG for  $\chi$ f, as determined by screening all the sucrose-containing crystalline structures (Hervé du Penhoat *et al.*, 1991). The hydroxymethyl group of glucose always kept its GG orientation. This orientation establishes a strong hydrogen bond between the carbohydrate and the binding site, as observed in all glucose- or mannose-lectin crystalline complexes (Imberty *et al.*, 1993). For each combination of sucrose  $\Phi$  and  $\Psi$  torsion angles, the geometry of the 19 amino acid side chains in the binding site and of the sucrose molecule was optimized, with constraints on the values of these two torsion angles (20 kcal/mol/deg<sup>2</sup>). This procedure was very time-consuming since several orientations of pendant groups (side chains of the amino acids and hydroxyl of sucrose) were tested in order to determine the best geometry for each set of  $\Phi$  and  $\Psi$  torsion angles. The energy surfaces were then represented as a function of  $\Phi$  and  $\Psi$  and this for four different combinations of the sucrose hydroxymethyl groups. Several low energy conformers were extracted from these maps and were submitted to a final geometry optimization with no constraints on the glycosidic linkage conformation.

The same procedure was used to calculate the relaxed map of isolated sucrose with the TRIPOS force field.

## RESULTS

**Crystallography**—The refined lentil lectin-sucrose complex is a dimer which contains 3552 protein non-hydrogen atoms, 2 Mn<sup>2+</sup> and 2 Ca<sup>2+</sup> ions, 2 sucrose molecules, and 229 water molecules. The structure displays proper stereochemistry as analyzed with PROCHECK (Laskowski *et al.*, 1993). The root mean square deviations of bond lengths and bond angles from ideal values are 0.015 Å and 3.058°, respectively. No residues

are present in the disallowed regions of the Ramachandran plot. There are no large differences with the structures of the uncomplexed lentil lectin (Loris *et al.*, 1993, 1994a), or with other legume lectins, especially the pea or *L. ochrus* lectins. The three-dimensional structure of the dimer is represented in Fig. 2. Its main structural features are two large antiparallel  $\beta$ -sheets, one of them being continuous over the whole lectin dimer.

The final electron density of the sucrose moiety is represented in Fig. 3. The conformations of the two bound sucrose molecules are almost identical for the two subunits of the dimer and are characterized by torsion angles of  $\Phi = 107^\circ$  and  $105^\circ$  and  $\Psi = -58^\circ$  and  $-59^\circ$ , respectively. The torsion angles for the primary hydroxyl groups  $\omega$ g,  $\omega$ f, and  $\chi$ f have a GG, GT, and GG orientation, respectively. The puckering parameters of both fructose residues have values of  $Q = 0.47$  and  $\phi = 260^\circ$ , which corresponds to a shape between an E<sub>3</sub> envelope and a <sup>4</sup>T<sub>3</sub> twist, both which are observed in sucrose-containing crystalline compounds (Hervé du Penhoat *et al.*, 1991). Since both sucrose moieties are so similar, only the first one will be described in the following parts of the paper.

The hydrogen bond network between the sucrose molecule and the lentil lectin binding site has been investigated thoroughly (Table II). Starting from the crystal structure, hydrogen atoms were added, and their positions were optimized using the TRIPOS force field. The resulting hydrogen bond network is shown in Fig. 4. The glucose residue of sucrose displays the same hydrogen bonding scheme and the same stacking interaction between the hydrophobic face of glucose and the aromatic ring of Phe-123 $\beta$  as observed in the lentil lectin-glucose complex (Loris *et al.*, 1994b). The fructose residue displays some direct interactions or contacts with the amino acids from the binding site: hydrophobic contacts exist between the side chain of Phe-123 $\beta$  and the protons and H4-f and H6-f of the fructose moiety. One of the H6-f hydrogen atoms is located just between the aromatic ring of Phe-123 $\beta$  and the methyl group of Ala-30 $\alpha$ , creating a continuous hydrophobic cluster. In addition, oxygen O6-f is almost at hydrogen bonding distance from the acid group of Glu-31 $\alpha$ . In fact, many hydrogen bonds are mediated through one or two water molecules as shown in Fig. 5. Such water bridges are observed for O2-f and O3-f with Tyr-124 $\beta$ , Ala-126 $\beta$ , Ala-127 $\beta$ , and Asn-125 $\beta$ . No direct inter-residue hydrogen bonds are observed within the sucrose molecule, but one strong interaction is mediated by a water molecule between O3-f and O2-g.

As shown in Fig. 6, both carbohydrate binding sites interact with each other via crystal contacts. A large nearly symmetric water network connects the sucrose molecule bound to the first lectin monomer to a symmetry mate of the sucrose bound in the second monomer. A water bridge is present between the O2 atoms of both glucose residues, while the oxygen O1-f of each fructose residue is hydrogen bonded to Gly-97 $\beta$  of a symmetry-related lectin molecule. Other interactions of the sucrose molecules with symmetry-related lectin monomers are mediated via water bridges and involve Ser-39 $\alpha$ , Gln-95 $\beta$ , Thr-96 $\beta$ , Gly-99 $\beta$ .

**NMR**—The most important information about carbohydrate conformations is obtained from inter-residue NOEs volumes. As described previously (Hervé du Penhoat *et al.*, 1991; Poppe and van Halbeek, 1992), for sucrose in solution, inter-residue NOEs are observed between protons H1-g and H1-f, H1-g and H4-f, and H1-g and H6-f (Fig. 7b). The transferred NOEs obtained for sucrose interacting with lentil lectin are negative (Fig. 7c), in contrast with the NOEs of the free sucrose. Build-up curves have been calculated, and two of them, one for an intra-residue NOE (H1-g/H2-g) and one for an inter-residue

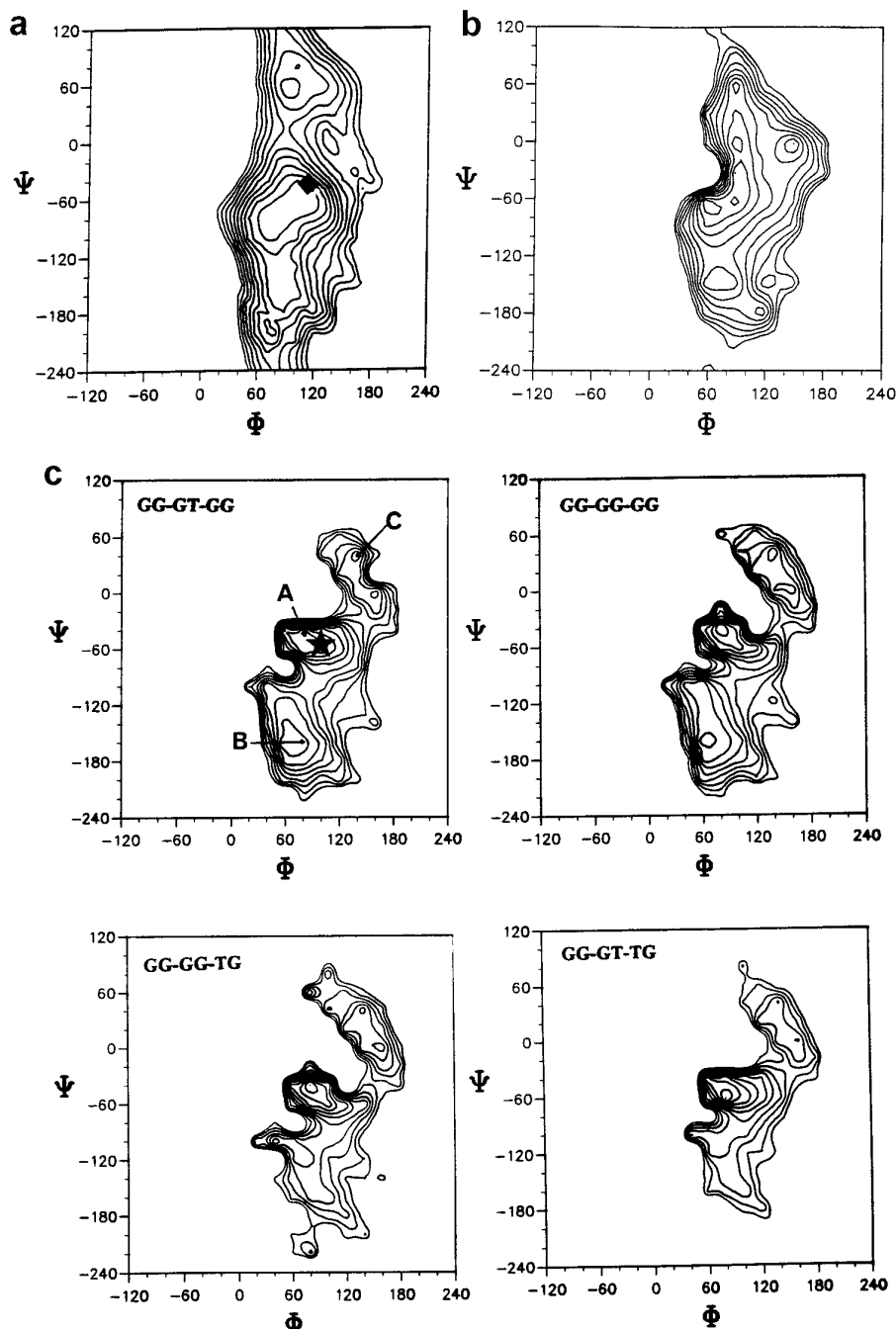


FIG. 8. *a*, adiabatic "relaxed" energy map of sucrose calculated with MM3. Energy was calculated as a function of  $\Phi$  and  $\Psi$  torsion angles with  $10^\circ$  increments (adapted from Casset *et al.*).<sup>2</sup> Isoenergy contours are drawn by interpolation of 1 kcal·mol<sup>-1</sup> above the absolute minimum in an energy window of 10 kcal·mol<sup>-1</sup>. Conformation of crystalline sucrose is indicated by a *diamond shape*. *b*, adiabatic "relaxed" energy map of sucrose calculated with TRIPOS. Energy was calculated as a function of  $\Phi$  and  $\Psi$  torsion angles with  $20^\circ$  increments. *c*, four "relaxed" potential energy surfaces of sucrose in lentil lectin binding site calculated with TRIPOS. Energy was calculated as a function of  $\Phi$  and  $\Psi$  torsion angles with  $20^\circ$  increments. Conformation of sucrose in the crystal structure of the complex is indicated by a *star* on the corresponding energy map, and the lowest energy conformations of the three main low energy regions have been indicated by A, B, and C.

NOE (H1-g/H1-f), are displayed in Fig. 7*d*. From the NOESY spectra, 11 transferred NOEs (Table III) can be observed. Comparison with the spectra of sucrose in solution allows the identification of a small inter-residue NOE present in the NOESY spectrum of sucrose, namely H1-g/H4-f, which is not observed in the complexed state. The absence of the latter NOE cannot be due to a lower sensitivity of the transferred NOE experiments compared to the classical one. Indeed, transferred NOEs are larger than direct NOEs for molecules of this size and should allow detection of the interaction between more distant protons. Therefore, the disappearance of this inter-residue NOE may be due to a change of the sucrose conformation upon binding, or, more likely, to a restriction of its conformational behavior (see "Discussion" below). However, possible spin diffusion involving protons from the protein might also be invoked to explain the differences between the spectra of free and bound sucrose.

**Molecular Modeling**—The four relaxed potential energy surfaces resulting from the systematic conformational search of the sucrose glycosidic linkage in the binding site are represented in Fig. 8*c*. They correspond to the four possible combinations of the fructose hydroxymethyl groups  $\omega$ f and  $\chi$ f: GG-TG, GG-GG, GT-GG, and GT-TG. These maps can be compared with those calculated for the free ligand using the same force field (Fig. 8*b*). Comparison has also been made with the adiabatic map (Pérez *et al.*, 1993) obtained with the molecular mechanics program MM3 (Allinger *et al.*, 1989). The energy maps of the sucrose molecule in the binding site do not show a drastic reduction of the allowed  $\Phi$ - $\Psi$  surface, indicating therefore that some flexibility could exist for the sucrose molecule in the bound state. However, when compared to the energy map of the isolated molecule, the lowest energy region is smaller and much deeper. The maps are restricted because the region located around  $\Phi = 80^\circ$  to  $100^\circ$  and  $\Psi = -60$  to  $80^\circ$  is not

FIG. 9. Stereoscopic representation of the main low energy conformers of sucrose in the binding site as found by molecular modeling. A, B, and C refer to the conformations of sucrose as located on the energy maps of Fig. 8b.

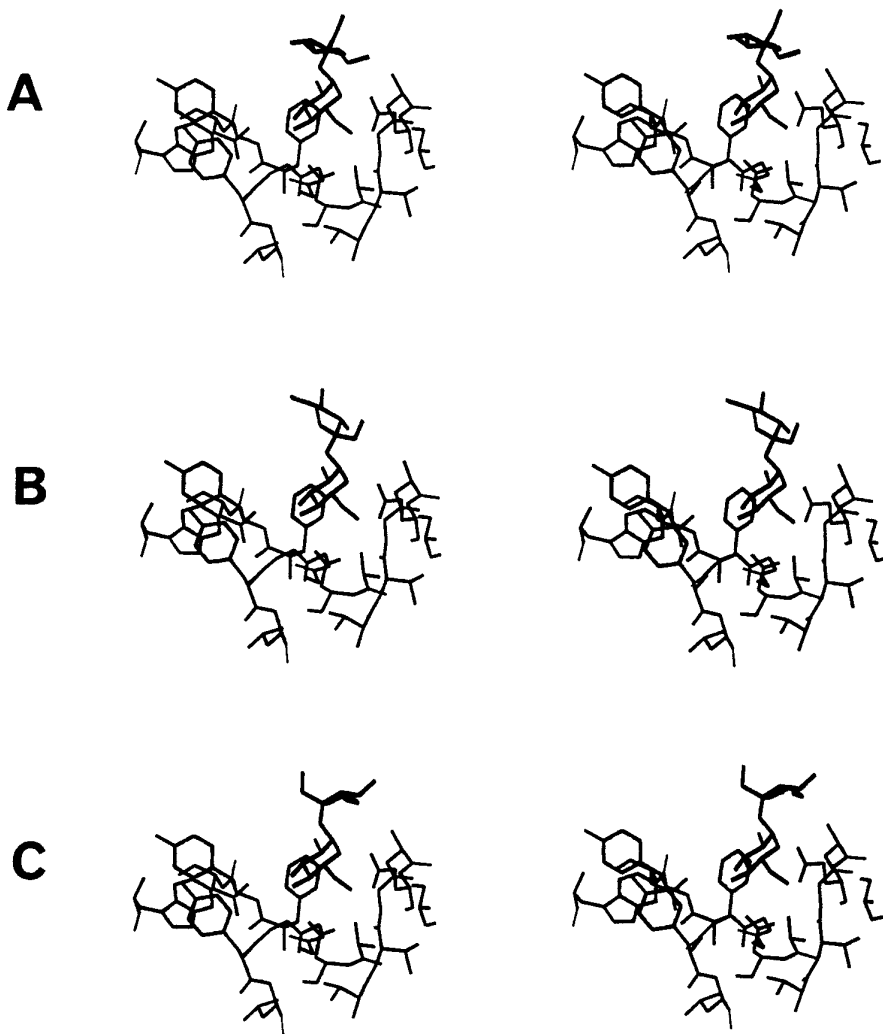


TABLE IV  
Structural and energetical characteristics of the three low energy models of the complex between the binding site of the lentil lectin and sucrose

	Models		
	A	B	C
$\Phi^a$	83.8	81.9	138.1
$\Psi$	-45.7	-161.2	37.5
$E_{\text{tot}}^a$	432.4	436.5	437.9
$E_{\text{prot}}^a$	449.5	449.0	448.7
$E_{\text{suc}}^a$	10.8	11.2	13.9
$E_{\text{inter}}^a$	-27.9	-23.7	-24.7

<sup>a</sup> Torsion angles are given in degrees, and energies are given in kcal·mol<sup>-1</sup>.

accessible due to steric conflicts between the fructose residue and Ala-30 $\alpha$ . Steric conflicts are also observed with Phe-123 $\beta$  for sucrose torsional angles around  $\Phi = 40^\circ$  and  $\Psi = -60^\circ$  to  $-20^\circ$ . The maps calculated for the GG-GT-GG and the GG-GG-GG orientations show a larger accessible space than the maps for the GG-GG-TG and GG-GT-TG orientations. Also, the minimum of each map, centered around  $\Phi = 80^\circ$   $\Psi = -60^\circ$ , is located in a quite deep low energy region, indicating favorable interactions in this given conformation. In the present calculations, the sucrose molecule exhibits an overall preference for this particular conformation.

Several low energy conformers from the three low energy domains of each map were selected and then fully energy minimized without constraints on the glycosidic torsion angles.

Releasing these constraints yields some minor adjustments in the  $\Phi$  and  $\Psi$  torsion angles ( $2^\circ$  to  $4^\circ$ ). The lowest energy conformer was obtained for the conformation GG-GT-GG with torsion angles  $\Phi = 84^\circ$  and  $\Psi = -46^\circ$  (conformer A). The hydrogen bond network of this global minimum is listed in Table II. The fructose residue is not involved in hydrogen bonding with the protein, but hydrophobic interactions exist between the fructose moiety (H4-f and H6-f) and the side chains of amino acids Phe-123 $\beta$  and Ala-30 $\alpha$ . In contrast, conformer B ( $\Phi = 82^\circ$  and  $\Psi = -161^\circ$ ) has hydrophobic contacts only between Ala-30 $\alpha$  and H6-f while conformer C ( $\Phi = 138^\circ$  and  $\Psi = 37^\circ$ ) has interactions only between Ala-30 $\alpha$  and H4-f. Fig. 9 shows these three different conformers, selected from the map GG-GT-GG, in the binding site of lentil lectin. For the three minima, details of the energy of interaction are listed in Table IV.

## DISCUSSION

### Comparison between Free and Bound Conformation of Sucrose

*In Solid State*—The sucrose molecule exhibits a slightly different conformation at the glycosidic linkage in the crystal structure of sucrose ( $\Phi = 108^\circ$ ,  $\Psi = -45^\circ$ ) (Brown and Levy, 1973; Hanson *et al.*, 1973) than in the crystal structure of the complex with lentil lectin ( $\Phi = 107^\circ$ ,  $\Psi = -58^\circ$ ). For the furanose ring, small differences are also observed: in crystalline sucrose, the furanose ring adopts a true twist conformation ( ${}^4T_3$  with  $\phi = 265^\circ$  and  $Q = 0.35$ ), whereas it tends to adopt an



FIG. 10. Stereoscopic representation of the sucrose molecule in the binding site of lentil lectin determined from the crystallographic study (black lines) and from the molecular modeling study (gray lines).

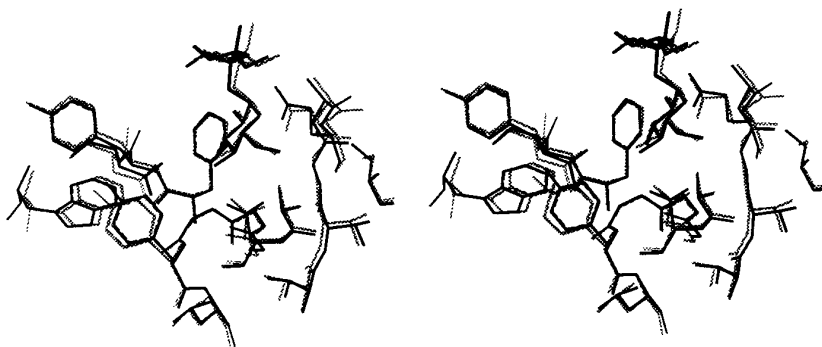


TABLE V  
Comparison of inter-proton distances for sucrose complexed in the binding site of lentil lectin as obtained from different studies.

Proton pair	Distances (Å) from		
	TRNOE build-up curves	Crystal structure of the complex	Molecular modeling (global minimum)
H1-g/H2-g	2.9	2.51	2.45
H2-g/H3-g	2.6	3.02	3.05
H2-g/H4-g	2.47 <sup>a</sup>	2.43	2.45
H3-g/H4-g	3.0	3.03	3.02
H3-f/H4-f	2.5	3.07	3.03
H4-f/H5-f	2.4	3.04	3.04
H3-f/H5-f	3.7	2.68	2.33
H1-g/H1-f	2.6	2.40	2.40

<sup>a</sup> Reference distance used for least square fit.

envelope shape in the lentil binding site with a more pronounced amplitude of deformation (between  ${}^4T_3$  and  $E_3$  with  $\phi = 260^\circ$  and  $Q = 0.47$ ). More differences are found in the orientation of the hydroxymethyl groups. Both the isolated and the complexed molecules display a GG orientation for the hydroxymethyl group of glucose. In contrast, for the fructose moiety,  $\omega$ f and  $\chi$ f have GG and GT orientations in crystalline sucrose but have GT and TG orientation in the lentil lectin complex.

Even if these differences in conformation are not very important, they result in major differences in the hydrogen bond network of sucrose. None of the two inter-residue hydrogen bonds in crystalline sucrose (O1-f  $\rightarrow$  O2-g and O6-f  $\rightarrow$  O5-g) are conserved in the complex with the lectin. A new feature in this complex is the presence of one water molecule located between the glucose and fructose residues. This water creates a strong water bridge between O2-g and O3-f. In a recent molecular dynamics simulation with explicit solvent, this given site is occupied by a water molecule during 25% of the trajectory (Engelsen *et al.*, 1995).

For sake of comparison, the conformation of both crystalline sucrose (Brown and Levy, 1973; Hanson *et al.*, 1973) and sucrose complexed with lentil lectin are indicated on the potential energy maps of Fig. 8. For the solid state sucrose, the crystal conformation belongs to the main energy well, but is about 2 kcal/mol higher in energy than the calculated global minimum (Pérez *et al.*, 1993). This small disagreement between solid state conformation and computation can be rationalized by either distortion due to packing effects or by the inadequacy of the force field for a molecule in which overlapping exoanomeric effects are present (French *et al.*, 1993). On the other hand, the conformation of sucrose complexed with lentil lectin corresponds well to the global energy minimum predicted by computer simulations.

*In Solution*—Flexibility of sucrose in solution has been shown by different studies, using NMR (Hervé du Penhoat *et al.*, 1991; Poppe and van Halbeek, 1992), chiro-optical (Stevens and Duda, 1991), and computational methods (Tran and Brady, 1990; Pérez *et al.*, 1993). Flexibility is present at different

levels: the glycosidic linkage and the three hydroxymethyl groups can adopt several conformations, and the fructose ring fluctuates between different shapes. These conclusions are confirmed by the most recent combined NMR and molecular mechanics studies<sup>2</sup> and by molecular dynamics in an explicit water environment (Engelsen *et al.*, 1995). The energy map of free sucrose in Fig. 8, calculated with MM3, is in agreement with the NMR data: the existence of different conformations has to be taken into account to explain all the observed inter-residue NOEs. In the global minimum ( $\Phi = 70^\circ$ ,  $\Psi = -83^\circ$ ), as calculated by MM3,<sup>2</sup> H1-g and H1-f are at a short distance of each other, whereas in the secondary minimum ( $\Phi = 96^\circ$ ,  $\Psi = 59^\circ$ ) H1-g and H4-f are close together. Therefore, the global minimum conformation is believed to be responsible for the observed H1-g/H1-f NOE and for the secondary minimum for the H1-g/H4-f NOE in sucrose solution. The situation is quite different for the transferred NOE experiment of the lentil lectin-sucrose complex where only one inter-residue NOE (*i.e.* H1-g/H1-f) is observed. This may indicate that the secondary minima are not populated in the complex, and therefore that the glycosidic linkage has a more restricted behavior in terms of the torsion angles  $\Phi$  and  $\Psi$ . However, the existence of conformer C, which possesses a short H1-g/H4-f distance, cannot be totally discarded, since in the complex, spin diffusion is possible through the methyl group of Ala-30 $\alpha$ , and this could explain the absence of the corresponding transferred cross NOE peak.

#### Comparison of Sucrose Conformation in the Lentil Binding Site as Inferred by Different Methods

The conformation of sucrose complexed with lentil lectin, as determined by x-ray crystallography, is indicated by a *star* on the potential energy maps of Fig. 8. This conformation corresponds precisely to the global minimum predicted by molecular modeling. This is true not only for the glycosidic linkage con-

<sup>2</sup> F. Casset, A. Imberty, C. Hervé du Penhoat, J. Koca, and S. Pérez, submitted for publication.

formation but also for the puckering of the fructose ring and for the orientations of the fructose hydroxymethyl groups (GG, GT, and GG for  $\omega_g$ ,  $\omega_f$ , and  $\chi_f$ , respectively). The experimental and theoretical conformations of sucrose, together with the binding site of lentil lectin have been superimposed in Fig. 10. At first glance, molecular modeling and crystallographic studies agree on defining a unique conformation of the sucrose molecule in the lectin binding site. This striking agreement between modeling and crystallography was not expected, as several factors are present that can produce artifacts in the results of both methods. 1) The involvement of the two sucrose molecules in crystal packing interactions may distort their conformations. 2) All modeling calculations are necessarily performed *in vacuum*, without explicit solvent interactions, while in the crystal structure, many water-mediated lectin-sucrose interactions are observed. Obviously, water molecules play an important role in the thermodynamic aspect of the interaction, but seem to have a less drastic influence on the establishment of the structural features of the complex since model predictions can be successful when they are omitted (Imberty *et al.*, 1991; Imberty and Pérez, 1994).

However, when looking at the data obtained from the transferred NOE study, the situation is quite different. Table V shows a comparison of inter-protons distance calculated using the three different approaches. The NMR distances are in good agreement with solid state and theoretical conformations for both the glucose ring and the interglycosidic distances. This good agreement confirms that the glycosidic linkage is immobilized in the lectin-sucrose complex. The situation is quite different for the fructose ring, where the agreement is not so good. This is related to the fact that, for a furanose ring, inter-proton distances vary widely for small distortions of the pucker (Olson and Sussman, 1982). Therefore, it seems quite likely that the fructose ring, which is not involved in a strong interaction with the protein, is still relatively mobile.

#### CONCLUSION

Both the crystallographic and NMR studies point toward a unique conformation for the glycosidic linkage in the bound sucrose molecule. Whereas the glycosidic linkage conformation is close to the one observed in crystalline sucrose, the orientations of the hydroxymethyl groups differ significantly. Therefore, the scheme of intramolecular hydrogen bonds within the sucrose molecule is completely different. Our NMR study provides some insight into the residual conformational flexibility of the sucrose in the protein binding site. This flexibility is mainly confined to fluctuations of the fructofuranose ring.

The global energy minimum predicted by the molecular modeling study has the same glycosidic linkage conformation as the conformation determined by experimental methods. The shape of the energy map also indicates that no large movements are allowed once the molecule is bound by the lentil lectin in this conformation. However, from the theoretical point of view, two other conformations of sucrose could also be bound by the lectin. The energies calculated for these complexes are only slightly higher than the global energy minimum (which is also the one observed in the crystal). It seems to us that the protein selects the most stable solution conformation of the ligand, as is also observed in most other lectin-carbohydrate and antibody-carbohydrate interactions.

*Acknowledgments*—We thank Maria Vanderveken for excellent technical assistance and E. Czerwiec for help with the purification of lentil lectin.

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