ANALYSIS OF GLUCOSE-BIDING SITES

OF PROTEINS WITH GLUCOSE SPECIFICITY

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ABSTRACT

There is a need for synthesizing glucose-sensitive molecules which can be used in glucose sensors and self-regulating insulin delivery devices. Currently, glucose sensitive proteins, such as glucose oxidase or concanavalin A (Con-A), are used for detecting glucose molecules. For long-term *in vivo* applications, it is necessary to synthesize non-proteineous glucose-sensitive molecules which are biocompatible, nontoxic, cost-effective, and independent on the environmental factors such as the pH, ionic strength, or the presence of divalent cations.

As a first step toward synthesizing glucose sensitive molecules, we have analyzed glucose-binding sites of 4 different types of glucose-binding proteins. They are human β-Cell glucokinase, D-xylose isomerase, lectins (*Lathyrus ochrus* isolectin I and Con-A), and glucose/galactose binding protein. Analysis of the glucose-binding sites of their 3-dimensional crystal structures showed that the hydrogen bonds between the hydroxyl groups of glucose and a few types of amino acid residues of proteins provide the main attraction. Hydrophobic interactions between the pyranose ring of glucose and aromatic rings of hydrophobic amino acid residues also play an important role in the glucose specificity. This information on the spatial arrangement of certain amino acid residues around the glucose molecule may provide the starting point in the synthesis of glucose-binding molecules.

Keywords: glucose, glucose binding protein, glucose sensor, molecular modeling, hydrogen bond, hydrophobic interaction

INTRODUCTION

For those with insulin-dependent diabetes, the tight control of blood glucose levels is critically important in preventing devastating complications. The key to the tight control of the blood glucose levels is frequent monitoring of blood glucose levels. While it is most desirable to continuously monitor glucose levels by non-invasive methods such as infrared spectroscopy (1) and transdermal extraction (2,3), such technologies are still in their infant stages. So far, invasive approaches are most commonly used for the measurement of blood glucose levels. Partial or total implantable glucose sensors, such as needle-type glucose sensors (4), microdialysis fiber systems (5), and total implantable glucose sensors (6), have also been studied with limited successes. Pricking fingers has been and will be the method of choice for the measurement of blood glucose levels for a majority of patients, at least for a while. For implantable glucose sensors and glucose measurement by blood drops, quantitation of the blood glucose levels is based on the glucose-sensitive molecules, such as glucose oxidase and concanavalin A (Con-A).

While glucose oxidase and Con-A have been used successfully in the development of glucose sensors, their long-term application has been limited due to the proteineous nature. The use of these proteins is particularly problematic for implantable glucose sensors. They may lose their bioactivity in time. In addition, Con-A is known to be immunotoxic and require the presence of divalent cations such as Mn^{2+} and Ca^{2+} and twice higher ionic strength than the physiological (7,8). The same concerns exist for the *in vivo* application of self-regulating insulin delivery devices which require a glucose sensing part. To avoid possible problems associated with protein-based glucose sensing, it would be highly desirable, if we could synthesize glucose-specific molecules which are non-toxic and stable for long-term *in vivo* applications.

As a first step towards synthesizing glucose-sensitive molecules, we have analyzed the glucose-binding sites of several proteins with glucose specificity. The analysis of such glucose-binding sites is expected to provide the basic information on the design of artificial glucose-binding molecules.

PROTEINS WITH GLUCOSE SPECIFICITY

In this study, we have analyzed specific interactions between glucose and five glucose-binding proteins, such as human β-Cell glucokinase, D-xylose isomerase, lectins (*Lathyrus ochrus* isolectin I and Con-A), and glucose/galactose binding protein. Glucokinase, which phosphorylates glucose (9), is known to participate in regulating glucose metabolism in liver and pancreatic β-cells (10-12). D-xylose isomerase converts D-glucose to D-fructose (13-15). Its catalytic activity depends on the presence of divalent metal cations, such as Mn^{2+}, Mg^{2+}, and Co^{2+}. Isolectin I and Con-A are lectins which are sugar-specific, cell-agglutinating proteins found in plants, animals, and microorganisms (16-19). The glucose binding proteins of gram-negative bacteria act as receptors for various sugars, oxyanions, amino acids, oligopeptides, and other nutrients (20-22).

Ribbon models of 3-dimensional (3-D) structures of the proteins with bound glucose molecules are shown in Fig. 1. The graphics in Fig. 1 were prepared by RasMol 2.6 (23). The 3-D structures of the proteins used in this study were obtained from Brookhaven Protein Data Bank (24,25). Since the data from the Protein Data Bank did not include hydrogen atoms except polar hydrogen atoms, the remaining hydrogen atoms were added using a molecular modeling program, Quanta (Molecular Simulation Inc.). This was done for all proteins used in our study.

The data of 3-D structure of human β-cell glucokinase were found under the code name of 1GLK. The structure has 465 amino acid residues and one bound β-D-glucose molecule. No water molecules and no metal ions are involved. The crystal structure of hexokinases is not yet available for any of the mammalian hexokinases. For this reason, the 3-D structure of human β-cell glucokinase was modeled (12) from structures of yeast hexokinase which was already studied by X-ray crystallography (26). The modeling was done by finding amino acid sequences analogous to the known yeast hexokinase B and aligning the amino acid sequences of two structures. The human β-cell glucokinase has 465 amino acid residues while yeast hexokinase has 486 residues. The sequence alignment shows 31.1% identity, mainly in the protein core and around the glucose-binding site (12).

The data of 3-D structure of D-xylose isomerase were obtained under the code name of 1XIF (27-29). The 3-D structure of D-xylose isomerase shows 388 amino acid residues, 380 water molecules, 2 Mn^{2+}, and 1 α-D-glucose molecule bound to the binding site.

The crystallographic structures of isolectin I and Con-A (complexed with methyl-α-D-mannopyranoside) were obtained under the code names of 1LOA and 5CNA, respectively. The 3-D structure of isolectin from *Lathyrus ochrus* shows two dimers with two monomers each (30). Each monomer consists of the 181-residue α chain and the 52-residue β chain. One methyl-α-D-glucopyranoside is bound to each monomer. Con-A was the first lectin of which amino acid sequence was determined (31). The crystalline structure of Con-A (32-35) is quite similar to the structure of isolectin. Con-A also has two dimers which have two monomers each. Each monomer consists of 237 amino acid residues and has one saccharide-binding site.

The data of glucose/galactose binding protein were obtained under the code name of 2GBP. The structure data of this protein consist of 309 amino acid residues, one calcium ion, one bound β-D-glucose, and 214 water molecules. The 3-dimensional (3-D) structure of D-glucose/D-galactose binding protein used in our study was from *Escherichia coli* (36).

All the structures of proteins and glucose in Figs. 2-7 were prepared by Raster3D (37,38). The analysis of glucose binding to proteins and modeling were done using Quanta.

ANALYSIS OF GLUCOSE-BINDING SITES

I. Human b-Cell Glucokinase (molecular model)

As shown in Fig. 1-A, the glucose binding site of human β-cell glucokinase is rather wide open. Fig. 2-A shows a glucose molecule surrounded by amino acid residues which provide hydrogen bonding with the glucose molecule. The glucose molecule is shown in black while others are in gray. In the model shown in Fig. 2-B, glucose molecule is bound to the receptor site through 9 hydrogen bonds with the surrounding amino acid

residues. These hydrogen bonds are formed either through hydrogen atoms of glucose hydroxyl groups and oxygen atoms of Glu and Asp, or through oxygen atoms of glucose hydroxyl groups and hydrogen atoms of Asn and Gly. It is noted that each of the five hydroxyl groups of glucose has at least one hydrogen bond with glucokinase. The ring oxygen (O5 in Fig. 2-B) of glucose has no hydrogen bonding with any of the surrounding amino acid residues. The hydrogen and oxygen atoms involved in the 9 hydrogen bonds are listed in Table 1. Table 1 also summarizes the distances and angles of these hydrogen bonds. Strong hydrogen bonds with bond length of 2 Å or less exist between glucose O1 and Glu290, O3 and Glu256, O4 and Asp205, O4 and Asn204, and O4 and Asn231. Glu and Asp are good hydrogen acceptors due to their negative charges on terminal oxygens. Asn204 and Asn231 not only interacted with oxygen atoms of glucose but also form hydrogen bonds with Asp205 and Glu256 (not shown in Fig. 2-B). This network of hydrogen bonds appears to present the glucose specific binding site.

This glucokinase model suggests both α- and β-anomer have the same binding affinity since O1 hydroxyl group of either anomer can form hydrogen bond with one of two oxygen of Glu290. This was also proven by experiments with glucokinase obtained from rabbit liver (9). It was also shown that glucose had stronger affinity than mannose to glucokinase. Michaelis constants were 12 mM and 33 mM for glucose and mannose, respectively, in the presence of 2 mM MgATP (9). The hydrogen bond between hydrogen of O2 hydroxyl group of glucose and Glu256, which is further stabilized by the salt bridge with Lys56 (as shown in Fig 2-B), could attribute to the higher specificity toward glucose.

II. D-Xylose Isomerase

Fig. 3-A shows an α-D-glucose molecule (shown in black) bound to the binding site of D-xylose isomerase. As shown in Fig. 3-B, glucose molecule has 13 hydrogen bonds with surrounding amino acid residues and water molecules. The glucose molecule binds to the binding site with C1-C2 bond facing outside. The glucose binding site involves two manganese ions (Mn1 and Mn2 in Fig. 3-A). The two manganese ions with octahedral coordination appear to stabilize the binding pocket by interacting not only with glucose but also with amino acids and water molecules. Mn1 directly coordinates O3 and O4 of glucose and amino acid residues (Glu181, Asp245, Asp287, and Glu217). Another manganese ion (Mn2) bonds with a water molecule which forms three hydrogen

bonds with hydrogen atoms of O2 and O3 of glucose. Mn2 also coordinates His220, and two oxygen atoms from each of Asp255, Asp257 and Glu217. Mn1 and Mn2 are linked together by Glu217. The interaction of the O2~O3~O4 edge of the glucose molecule with the protein is much stronger than the other edge (O1~O5~C5) of the glucose molecule, where less stronger hydrogen bonds are formed with 3 water molecules, one histidine and one threonine. The specifics of the hydrogen bonds formed among glucose, amino acid residues, and water molecules are summarized in Table 2. O2 and O3 of glucose have the two strongest hydrogen bonds with water molecules. It is interesting to observe that water molecules play important roles in providing hydrogen bonds with glucose as well as amino acid residues and thus in stabilizing the glucose binding site.

In addition to hydrogen bonds, the glucose binding appears to be further stabilized by the presence of indolyl rings of Trp16 and Trp137 on both faces of the bound glucose molecule (shown in dark gray in Fig. 3-A). The hydrophobic interaction between indolyl rings and hydrophobic faces of glucose pyranose ring may provide additional specificity, both geometrically and energetically.

Unlike α-D-glucose, β-D-glucose molecule can not bind to the isomerase. If we changed the α-anomer to the β-anomer in the binding structure shown in Fig. 3, O1 hydroxyl group of glucose could sterically interfere with Trp137 and Phe94 (not shown). This interference could be energy-unstable due to the interaction between hydrophilic hydroxyl and hydrophobic indolyl ring of Trp137 and phenyl ring of Phe94. This argument is supported by experimental studies by others (29,39). In addition, D-mannose is not a substrate for D-xylose isomerase. Experiments showed that D-mannose gave no isomeration compared to 43.9% conversion of D-glucose after incubation with D-xylose isomerase for 24 hours (39). Based on the 3-D structure of the binding site of D-xylose isomerase, one can observe that O2 hydroxyl of D-mannose could cause steric hindrance with Trp137. It appears that this is the reason that D-mannose has difficulty to bind to D-xylose isomerase.

III. *Lathyrus ochrus* Isolectin I and Concanavalin A

A. *Lathyrus ochrus* Isolectin I

One of the glucose binding sites of *Lathyrus ochrus* Isolectin I in Fig. 1-C is shown in more detail in Fig. 4. Both Figs. 1-C and 4-A clearly show that methyl-α-D-glucopyranoside molecule is not really embedded deeply into the binding pocket. Only O3, O4, O5 and O6 of glucose form hydrogen bonds with the surrounding amino acid residues, while O1 and O2 extending outside form no hydrogen bonds at all (Fig. 4-B). There are a total of 9 hydrogen bonds between glucoside and the protein and water molecules as listed in Table 3. O3 and O4 of glucose form four hydrogen bonds with residues interacting with metal ions, and O6 and O5 form five hydrogen bonds with residues relatively further away from metal ions. One Ca^{2+} and one Mn^{2+} provide the stability of the amino acid residues involved in the binding site. The two ions are linked by Asp129 and Asp121. Both metal ions have octahedral coordination with two water molecules and four amino acid residues (Asp129, Asn125, Phe123 and Asp121 for Ca^{2+}, and Glu119, His136, Asp129 and Asp121 for Mn^{2+} as shown in Fig. 4-A). The aromatic ring of Phe123, which is coordinated with Ca^{2+}, forms hydrophobic interaction with C5 and C6 of the glucoside.

The binding experiments between isolectin and sugars were done by Rouge et al. (40). The affinity of a sugar was characterized by its minimum concentration to completely inhibit the hemagglutination of isolectin. The concentrations of D-mannose and D-glucose required for the total blocking of the hemagglutination of 37.5 μg/ml of isolectin were 6.25 mM and 12.5 mM, respectively. The concentrations D-mannose and D-glucose in case of Con-A were 3.12 mM and 12.5 mM, respectively. Comparison of the binding sites of mannoside and glucoside with isolectin indicated that O2 atom of neither mannoside or glucoside was involved in forming any hydrogen bonds with the protein, but O2 of mannoside did form more hydrophobic interactions with the protein than O2 of glucoside did (30). This may explain why mannose has higher affinity than glucose.

B. Concanavalin A

The glucose binding site of Con-A (Fig. 5-A) is very similar to that of isolectin. O3, O4, O5 and O6 of methyl-α-D-mannopyranoside form hydrogen bonds with Con-A, while O1 extends away from the protein without forming any hydrogen bond (Fig. 5-B). O2 does not form any hydrogen bond with the protein either, but does with two water molecules. As in isolectin, the presence of metal ions is critical in maintaining the stability of the binding site. The total of 11 hydrogen bonds formed in the binding site are listed in Table 4. The number and

composition of hydrogen bonds in Table 4 are very similar to those in Table 3. Arg228, Asn14, Asp208, Tyr100, and Leu99, which are amino acid residues of Con-A involved in hydrogen bonding with saccharide, are equivalent to Gly99, Asn125, Asp81, Glu31, and Ala30 in isolectin. O3, O5, and O6 have strong hydrogen bonds with the protein due to nearly liner bond angles and short bond lengths. Moreover, the aromatic ring of Tyr12 provides hydrophobic interaction with C5 and C6 (Fig. 5-A), as Phe123 does in isolectin (Fig. 4-A). The hydrophobic interaction is certainly expected to increase the binding ability and stabilize the binding site.

Both mannoside and glucoside bind to Con-A, but their binding affinities are quite different. Con-A has higher binding affinity for mannose/mannoside than for glucose/glucoside (40). The reason for this may be similar to the one described above for isolectin. Mannose could form an extensive van der Waals interaction with amino acids residues around the O2 atom. For methyl-α-D-mannopyranoside in Con-A, there are 18 atoms within the distance of 4Å to the O2 hydroxyl group. These atoms are from 3 water molecules, Gly98, Gly227, Leu99, and Arg228. On the other hand, for methyl-α-D-glucopyranoside in isolectin, there are only 5 atoms (from 3 water molecules and Gly98) within the distance of 4Å to the O2 hydroxyl group. β-anomers of either mannoside or glucoside has lower binding affinity to Con-A than α-anomers (41). It appears that the methyl group of β-anomer could cause steric hindrance with Gly98 and Leu99.

IV. Glucose Binding Protein

The bound glucose molecule is buried deeply inside the pocket of glucose binding protein (Fig. 1-D and Fig. 6-A). There are a total of 13 hydrogen bonds, as summarized in Table 5, between glucose and the protein. It is the network of these hydrogen bonds that provides the binding specificity and stabilizes the binding site. O1 and O2 of glucose form 8 hydrogen bonds out of the total 13. O1 and O2 form hydrogen bonds with Asn256 and Arg158 simultaneously through different hydrogen atoms of the same carboxylate ion. The hydrogen atoms of O1 and O2 hydroxyl groups form three hydrogen bonds with Asp154, Asp236, and a water molecule. Both O3 and O4 form hydrogen bonds with the same hydrogen atom of $-NH_2$ of Asn211. Hydrogen atoms of the O3 and O4 hydroxyl groups form hydrogen bonds with a water molecule and Asp14, respectively. O5 forms one hydrogen bond with Asn91. No hydrogen bond is formed with O6.

The interaction between glucose and glucose binding protein is further strengthened by hydrophobic interactions between the pyranose ring and aromatic rings. As shown in Fig. 6-A, the lower indolyl of Trp183 and upper phenyl of Phe16 sandwich the pyranose ring of the bound glucose. C1, C3 and C5 interact closely with the indolyl ring and C2 with the phenyl ring.

MAIN INTERACTIONS BETWEEN GLUCOSE AND ITS BINDING SITES

The above analysis suggests that the main interaction providing glucose specificity is hydrogen bonding. Almost every hydroxyl group of the glucose molecule has at least one hydrogen bond with amino acid residues or water molecules. It is very common that each hydroxyl group forms multiple hydrogen bonds with many amino acid residues. Certain amino acid residues could form multiple hydrogen bonds with different hydroxyl groups of the glucose molecule. This network of hydrogen bonds in the right spatial arrangement is expected to provide glucose specificity. The most common amino acid residues involved in the hydrogen bonds are Asp, Glu, and Asn. Either Asp or Glu utilizes one or both oxygen atoms of its $-COO^-$ functional group to form hydrogen bonds with hydrogen atoms of hydroxyl groups of glucose. Asn uses one or both hydrogen atoms of its $-NH_2$ group to form hydrogen bonds with oxygen atoms of hydroxyl groups of glucose. In the human β-cell glucokinase and glucose binding protein, one can see the fork-shape double hydrogen bonds formed between one functional group of Asp, Glu, or Asn and two hydroxyl groups of the bound glucose molecule. This fork-shape interaction helps increase the binding specificity and binding strength. It is important to notice that these functional groups can bind two anomers or two epimers of glucose with the comparable strengths since either one of two interacting atoms of $-COO^-$ or $-NH_2$ group can form a hydrogen bond with either axial or equatorial hydroxyl group.

Hydrophobic interaction is also an important contributor for glucose binding as shown in the cases of D-xylose isomerase and glucose binding protein. Since the pyranose ring of the glucose molecule is hydrophobic on both faces, they can interact with aromatic rings, such as indolyl and phenyl rings. For β-D-glucose, the C1, C3 and C5 atoms along one face, and, the C2 and C4 atoms along the other face can interact with aromatic rings of

amino acid residues, such as Phe and Trp. The two aromatic rings sandwiching the pyranose ring could further enhance the binding specificity. The ionic coordination between oxygen atoms of the hydroxyl groups and metal ions increases the binding strength and stabilize the binding site in isolectin I, Con-A, and D-xylose isomerase.

DESIGN OF GLUCOSE RECEPTORS

The three types of interactions found in glucose binding are hydrogen bonding, hydrophobic interaction, and ionic coordination. In the design of synthetic glucose binding molecules, both hydrogen bonding and hydrophobic interaction can be used. The ionic coordination may not be desirable in the design of glucose binding molecules, since it means that the glucose binding is influenced by the concentration of metallic ions in the environment. As shown in Figs. 2-6, the glucose specificity is provided by the certain spatial arrangement of hydrogen bonds and hydrophobic interactions. All the hydroxyl groups and the C6 atom of glucose are at equitorial positions, therefore, the functional groups forming hydrogen bonds with hydroxyl groups, such as Asp, Asn, and Glu, can be placed around the edge of the pyranose ring. In addition, two faces of the pyranose ring can be involved in hydrophobic interactions with aromatic rings such as phenyl of Phe and indolyl of Trp. The sandwich-type coupling makes glucose binding even stronger.

Fig. 7 shows a hypothetical binding site consisting of amino acid residues in a certain spatial arrangement to provide hydrogen bonds around the pyranose ring and hydrophobic interactions on both faces of the pyranose ring. Asp and Asn can be aligned to provide hydrogen bonds with oxygen or hydrogen atoms of glucose, while Phe can provide hydrophobic interactions. For the synthesis of glucose binding molecules, one can simply connect the amino acid residues shown in Fig. 7. This may not be easy, but it, at least, provides the starting point for the design of customized glucose-binding molecules.

The possibility of synthesizing glucose binding molecules is important not only for the glucose sensing but also for the development of self-regulating insulin delivery devices. Recently, we have shown that glucose-sensitive hydrogel systems can be used to prepare self-regulating insulin delivery systems (42-46). In those

studies, Con-A was used as a glucose-sensitive molecule. The use of synthetic glucose-binding molecules will eliminate all the limitations and disadvantages of using Con-A, such as toxicity, poor stability, and high cost.

The premise of custom-design of receptors has barely begun to be realized and *de novo* design of glucose binding sites may be within reach. Supramolecular chemistry has been applied to the design of receptors for detection of clinically important molecules such as creatinine (47). Various interactions, such as hydrogen bonding, ion-pairing, metal-ion-to-ligand attractions, π-acid-to-π-base attractions, van der Waals attractions, and the entropic component of desolvation, can be used to create the binding site (48). Of these interactions, hydrogen bonding is quite often used for molecular recognition (47). In our study, we also noted that hydrogen bonding, along with hydrophobic interaction, may be enough to provide a glucose binding site by prearranging amino acids in the right spatial arrangement. Many reports have already shown that molecular imprinting can be used to synthesize molecules with specific binding properties (49-51). In addition, a cage can be crafted for the molecule to slide in and out (52). To create a molecule that binds to glucose, one can pick amino acids that interact with glucose molecules as shown in Fig. 7, put them together in many ways using combinatorial methods, and come up with compounds that fit just right. The use of combinatorial synthesis may examine all possible amino acids for the synthesis of the glucose-binding molecules with the highest affinity. We are currently synthesizing glucose-binding molecules based on the arrangement of amino acids as shown in Fig. 7.

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Table 1. Summary of hydrogen bonds between glucose and human β-cell glucokinase.

H acceptor	H donor	H bond length (Å)	H bond angle (°)
Glu290 OE1	O1	2.0	174.5
Glu256 OE1	O2	3.0	108.4
Glu256 OE2	O3	1.9	171.1
O3	Asn204 ND2	2.8	92.0
O3	Asn204 ND2	2.5	109.1
Asp205 OD2	O4	2.0	148.4
O4	Asn204 ND2	2.1	175.2
O4	Asn231 ND2	1.9	160.9
O6	Gly229 N	2.8	124.3

Table 2. Summary of hydrogen bonds between glucose and D-xylose isomerase including water molecules.

H acceptor	H donor	H bond length (Å)	H bond angle (°)
O1	2.1	102.5	
His54 NE2	O1	2.7	129.2
O1	H_2O	2.6	106.4
H_2O	O2	1.7	145.0
H_2O	O2	2.8	115.5
O2	H_2O	2.8	117.6
Glu181 OE2	O3	2.8	93.5
O3	H_2O	1.8	157.1
H_2O#	O4	2.3	112.4
O4	H_2O#	2.4	104.7
O5	H_2O*	2.6	141.0
H_2O*	O6	2.7	90.7
Thr90 OG1	O6	2.6	147.4

The water molecules marked with superscripts, # or *, are the same water molecules.

Table 3. Summary of hydrogen bonds between methyl-α-D-glucopyranoside and *Lathyrus ochrus* Isolectin I.

H acceptor	H donor	H bond length (Å)	H bond angle (°)
O3			
Gly99 N	1.8	154.0	
O4	Gly99 N	2.2	117.5
O4	Asn125 ND2	2.0	148.7
Asp81 OD1	O4	2.0	139.6
O5	Ala30 N	2.1	161.7
O6	Ala30 N	2.3	117.7
O6	Glu31 N	2.1	167.7
O6	Gly29 N	2.9	97.9
Glu31 O	O6	2.6	130.5

Table 4. Summary of hydrogen bonds between methyl-α-D-glucopyranoside and concanavalin A.

H acceptor	H donor	H bond length (Å)	H bond angle (°)
H_2O	2.3	121.3	O2
O2	H_2O^*	2.1	113.0
H_2O^*	O2	1.8	135.4
O3	Arg228 N	1.8	172.8
O4	Asn14 ND2	2.0	144.6
O4	Arg228 N	2.6	114.0
Asp208 OD2	O4	1.8	126.3
O5	Leu99 N	2.0	166.7
O6	Leu99 N	2.5	113.2
O6	Tyr100 N	1.9	160.0
Tyr100 O	O6	2.8	121.2

The water molecules marked with superscript * are the same molecule.

Table 5. Summary of hydrogen bonds between glucose and the glucose binding protein.

H acceptor	H donor	H bond length (Å)	H bond angle (°)
Arg158 NH2	2.4	137.9	
O1	Asn256 ND2	2.5	111.4
Asp154 OD1	O1	1.6	150.0
H_2O	O1	2.9	134.1
O2	Arg158 NH2	2.4	142.2
O2	Arg158 NH1	1.7	165.7
O2	Asn256 ND2	2.4	142.9
Asp236 OD2	O2	1.8	128.0
O3	Asn211 ND2	2.0	153.0
H_2O	O3	2.2	121.5
O4	Asn211 ND2	2.8	133.1
Asp14 OD1	O4	1.8	136.1
O5	Asn91 ND2	2.0	162.8

O1

LEGENDS

Fig. 1. The ribbon models of 3-dimensional structures of human β-Cell glucokinase (A), D-xylose isomerase (B), *Lathyrus ochrus* isolectin I (C), and glucose binding protein (D). Glucose molecules bound to the receptor sites are shown as space-filling models.

Fig. 2. Side (A) and top (B) views of the glucose molecule in contact with amino acid residues in the immediate vicinity of the binding site of human β-cell glucokinase. The glucose molecule is shown in black. In panel B, only those amino acid residues participating in hydrogen bonding with glucose are shown. Hydrogen bonds are shown as thin lines.

Fig. 3. Side (A) and top (B) views of the glucose molecule in contact with amino acid residues in the immediate vicinity of the binding site of D-xylose isomerase. The glucose molecule is shown in black. In panel B, only those amino acid residues participating in hydrogen bonding with glucose are shown. Hydrogen bonds are shown as thin lines. Ionic coordinations by two manganese ions (Mn1 and Mn2) are also shown by thin lines.

Fig. 4. Side (A) and top (B) views of the glucose molecule in contact with amino acid residues in the immediate vicinity of the binding site of isolectin I. The glucose molecule is shown in black. In panel B, only those amino acid residues participating in hydrogen bonding with glucose are shown. Hydrogen bonds are shown as thin lines. Ionic coordinations by manganese ion and calcium ion are also shown by thin lines.

Fig. 5. Side (A) and top (B) views of the glucose molecule in contact with amino acid residues in the immediate vicinity of the binding site of concanavalin A. The glucose molecule is shown in black. In panel B, only those amino acid residues participating in hydrogen bonding with glucose are shown. Hydrogen bonds

are shown as thin lines. Some of octahedral coordinations by manganese ion and calcium ion related to the glucose binding sites are also shown by thin lines.

Fig. 6. Side (A) and top (B) views of the glucose molecule in contact with amino acid residues in the immediate vicinity of the binding site of glucose binding protein. The glucose molecule is shown in black. In panel B, only those amino acid residues participating in hydrogen bonding with glucose are shown. Hydrogen bonds are shown as thin lines.

Fig. 7. Side (A) and top (B) views of a possible binding site for glucose. Asp and Asn can be placed around glucose to provide hydrogen bonds and Phe for hydrophobic interaction.

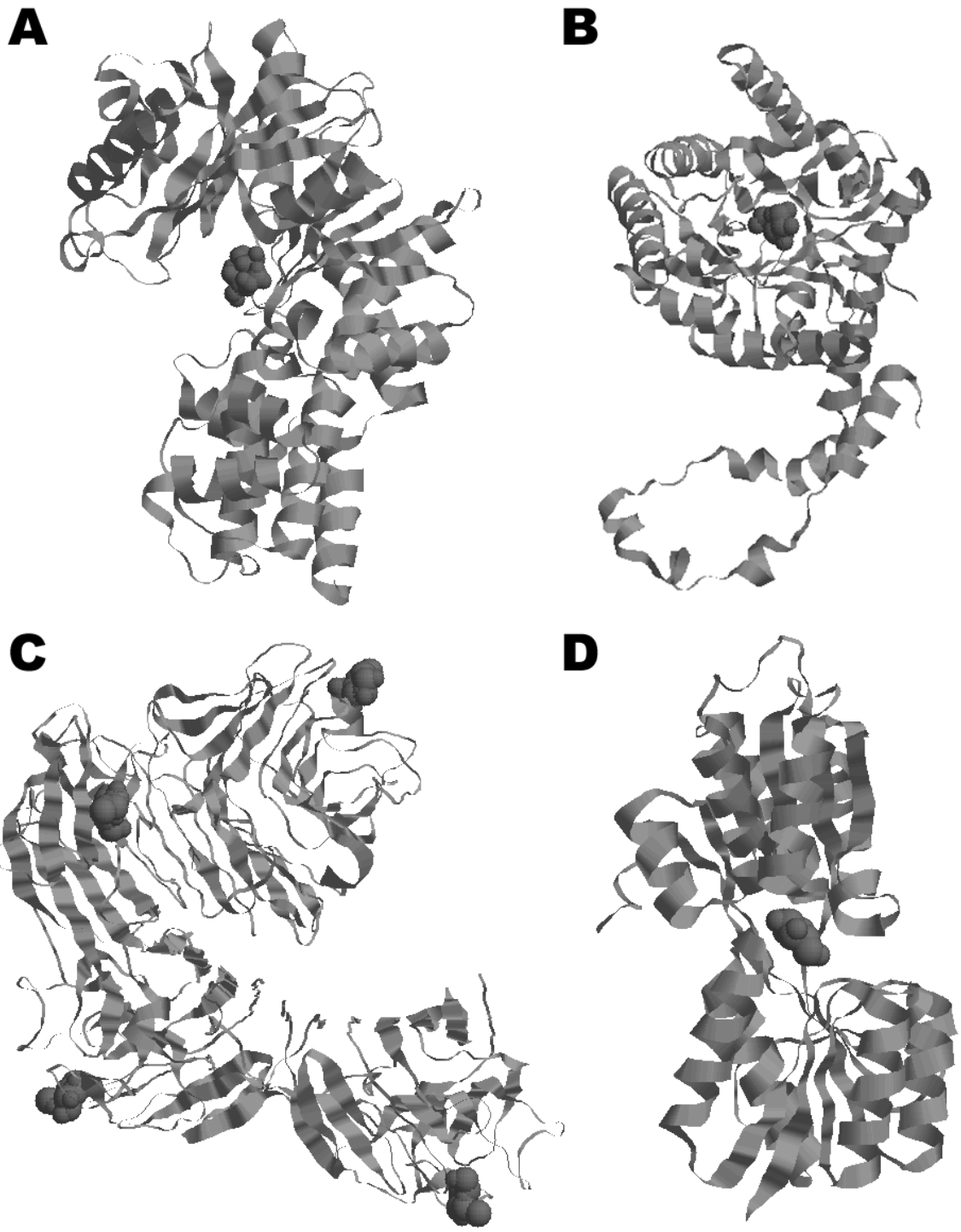
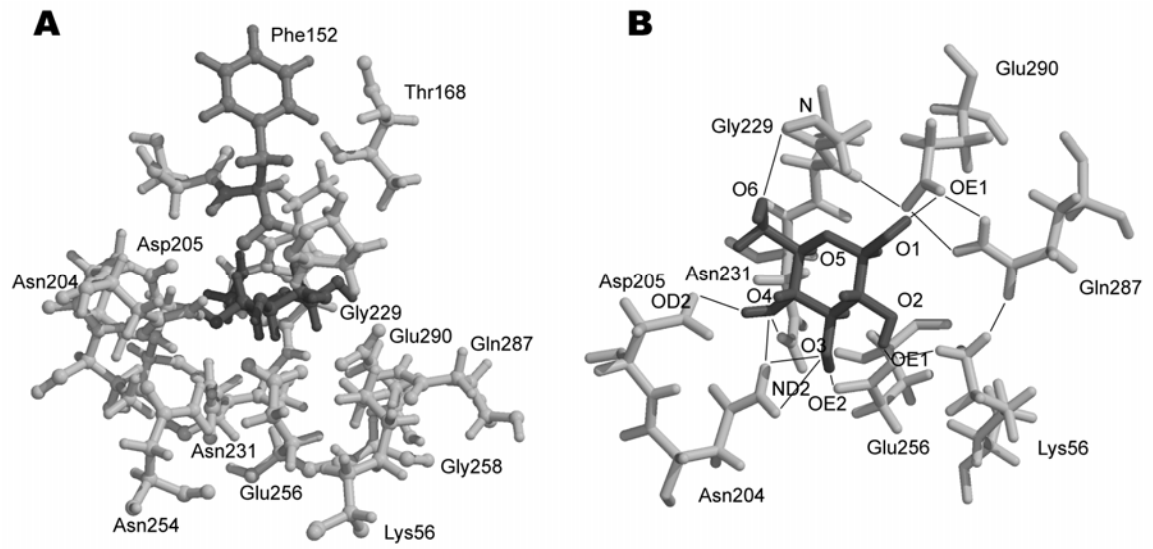


Figure 1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O5
O1
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Figure 2

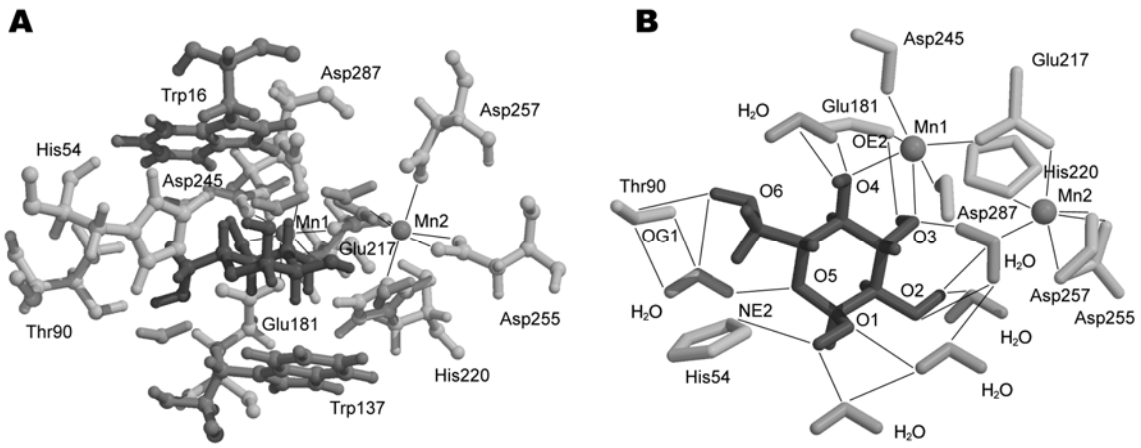
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Figure 3

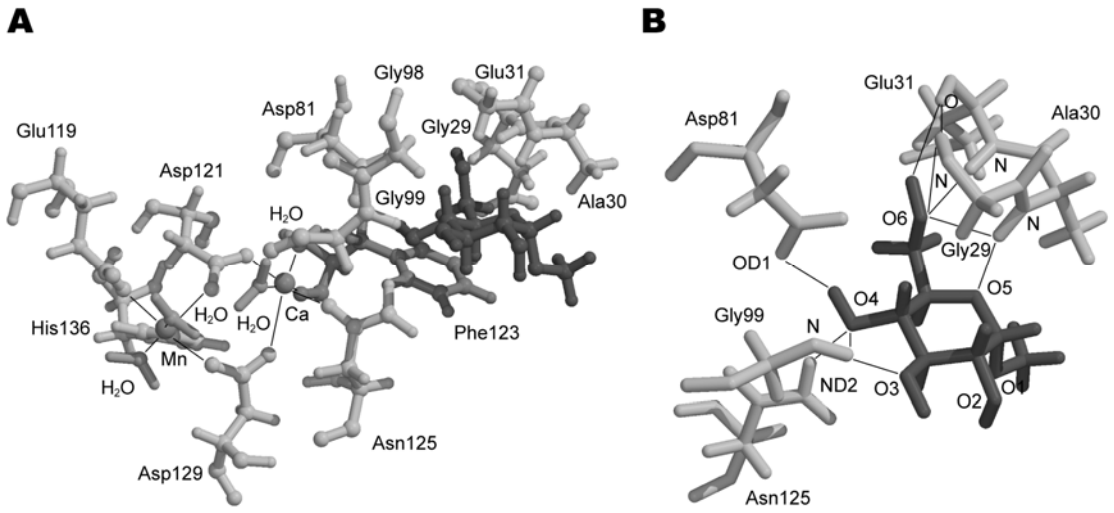
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Figure 4

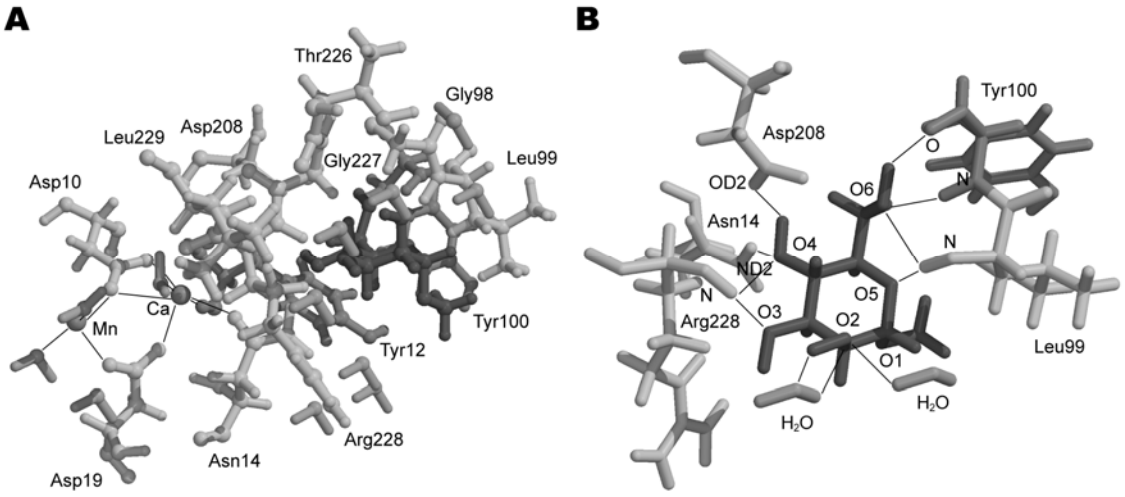
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Figure 5

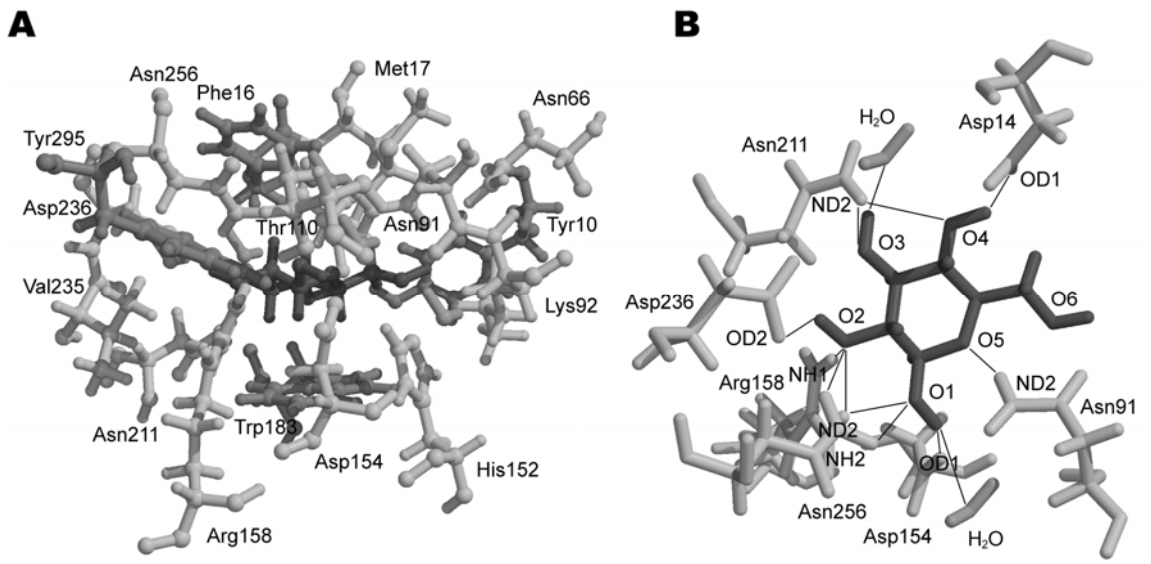
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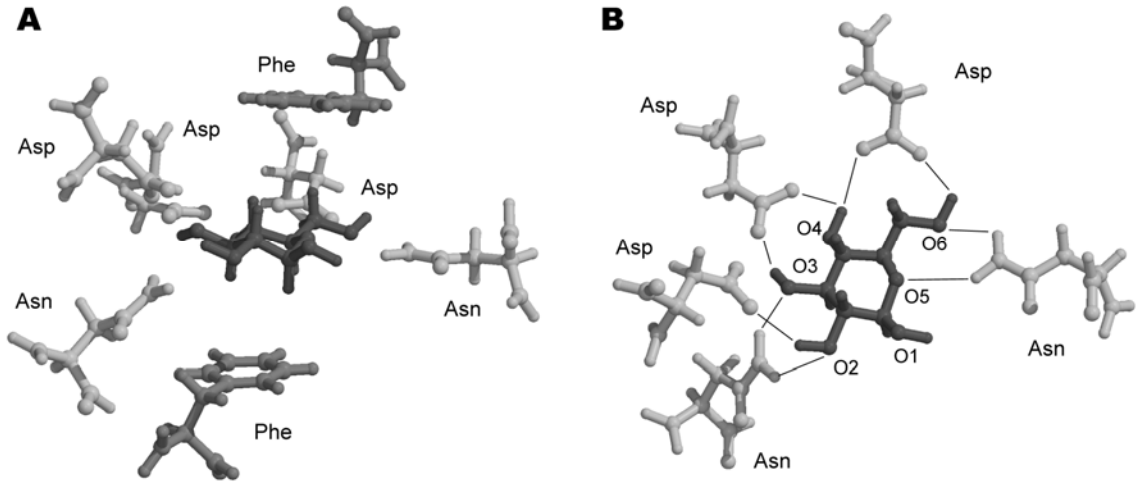
Figure 6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Asn

Figure 7