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Molecular Requirements for Recognition at a Glucoreceptor for Insulin Release

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SUMMARY

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Alloxan and ninhydrin affect insulin release by first stimulation and then inhibition of subsequent glucose-induced insulin release from the β-cells in the islets of Langerhans. The structures of D-glucose and D-mannose, the two hexoses that protect against them and initiate insulin release, were analyzed and found to share with alloxan and ninhydrin common molecular properties. These common molecular properties were: An oxygen at C(1); a hydroxyl at C(2), either axial or equatorial; an equatorial oxygen at C(3); and at position 5 an electron density. Further structural analysis revealed that alloxan and ninhydrin would require little, if any, additional volume than that required for recognition of the active hexoses, D-glucose and D-mannose at a common site. Other hexoses (1-deoxy-D-glucose, 2-deoxy-D-glucose, D-allose, D-galactose, lyxose, xylose, 6-deoxy-D-glucose, and L-glucose) which either did not protect against alloxan and ninhydrin or did not initiate insulin release were found to vary in their structure from the active hexoses. Based on the presently available evidence, a hypothesis was advanced that alloxan, ninhydrin, and the active hexoses interact at a common receptor to initiate the first phase of insulin release.

INTRODUCTION

D-glucose is the major physiological stimulus for release of insulin from the β-cells in the islets of Langerhans. The molecular mechanism of glucose-induced insulin release remains, however, unclear (1). Recent studies with alloxan have provided evidence that D-glucose itself may be the molecule recognized by the β-cell to release insulin from the islets of Langerhans. Alloxan produces first a monophasic burst of insulin release, then irreversible inhibition of subsequent glucose-induced insulin release (2, 3). Moreover, D-glucose, when present during the alloxan exposure, can competitively prevent the inhibition of insulin release (3). This interaction between the two compounds has led to suggestions that alloxan and D-glucose compete for a glucoreceptor to initiate insulin release (2–4).

In support of this hypothesis, a preliminary chemical comparison demonstrated an analogy between the structures of alloxan and D-glucose (3). The purpose of this study is to define the characteristics of molecular recognition that lead to glucose-induced insulin release. We employ a receptor

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mapping approach in which the structures of D-glucose, alloxan and their analogues are analyzed by a computer for molecular similarity. This study, which is the first systematic analysis of these agents, demonstrates structural similarities among the active molecules, computes the volume required to accommodate them at a common site, and proposes a model for their molecular recognition at a glucoreceptor. This receptor hypothesis leads to testable predictions for molecular recognition and insulin release.

MATERIALS AND METHODS

Steric analysis. Crystallography results were used to determine the conformation of a compound. The structures of β-D-glucose (5), α-D-glucose (6), α-D-galactose (7), and alloxan monohydrate (8) were based on single crystal analyses. β-D-galactose was extracted from the average atomic coordinates of the molecule as found in α-lactose monohydrate (9) and 4-0-β-galactopyranosyl-L-rhamnitol (10). α-D-mannose was based on coordinates averaged from methyl α-D-mannopyranoside (11) and α-rhamnose monohydrate (12). Built after a single rotation about a carbon were, β-D-mannose from α-D-mannose, and D-allose from β-D-glucose. Ninhydrin was built from anhydrous ninhydrin (13) and the *gem*-dihydroxy group of alloxan with the aid of ring-closure program that predicted on the basis of bond angles and lengths the pucker in the ninhydrin five-member ring.

The comparison of molecules was made with the aid of a molecular modeling system (MMS-X)[1] developed at Washington University that allowed for the translation, rotation and display of each molecule. Molecules were aligned with one another by the aid of a program that computed the minimal least-squares variance of individual atoms. The electron density of the molecules was generated from the Gaussian atom density distribution and contoured to provide a surface representative of the van der Waals radii. Figures 3 and 4 were composed of computer-generated molecules photographed directly from the cathode-ray tube.

[1] The abbreviation used is: MMS-X, molecular modeling system.

Medium and chemicals. All incubation
were accomplished with a modified Krebs
Ringer bicarbonate medium (14), and a
required, D-glucose (dextrose, National Bu
reau of Standards, Washington, D. C.), 1
deoxy-D-glucose, (Sigma Chemical Co., St
Louis, MO), D-lyxose, D-xylose (Pfanstieh
Labs, Waukegan, IL), and 6-deoxy-D-glu
cose (KRB, Elk Grove, IL). The mediun
was equilibrated to 37° and pH 7.4 with
humidified mixture of O_2/CO_2 (95%/5%).

Static incubation of islets. Insulin releas
was determined in a static incubation sys
tem described previously (14). Islets were
isolated by the collagenase technique (15)
and placed in a glass vial containing 200 μ
of medium. The glass vial was inserted into
a scintillation vial equipped with rubber
stopper, gassed with O_2/CO_2 (95%/5%) and
shaken in a Dubnoff metabolic shaker (70-
100 cycles/min). The islets were preincu
bated for 20–25 min in 200 μl of 5.5 mM D-
glucose medium and then incubated with
200 μl of medium with either the test agent
plus 5.5 mM D-glucose, 27.5 mM D-glucose
(control), or 5.5 mM D-glucose (basal) for
30 min at 37°. At the end of the stimulation
period, the media were removed and frozen
for subsequent insulin assay by the method
of Wright *et al.* (16). The results were an-
alyzed with the *t*-test for independent sam-
ples.

RESULTS

*Structure-activity relationships of al-
loxan.* Alloxan is a heterocyclic six-member
ring which has been depicted at carbon five
with either a ketone group (anhydrous al-
loxan) or with a *gem*-dihydroxy group (al-
loxan monohydrate). Anhydrous alloxan is
an unstable compound that rapidly absorbs
water from the air to form the monohydrate
(17). Crystallographic studies have shown
that alloxan when recrystallized from water
exists as the monohydrate (8). The mono-
hydrate structure is also supported by nu-
clear magnetic resonance (18) and infrared
spectrum studies (19). Based on these find-
ings, the most likely configuration for al-
loxan in solution is with a *gem*-dihydroxy
group at C(5) (Fig. 1).

The X-ray studies by Singh (8) demon-
strated that the alloxan molecule is puck-

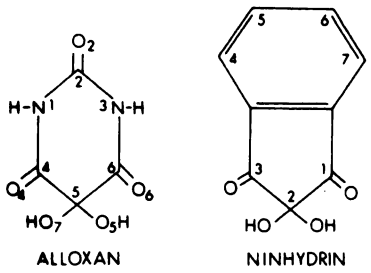

FIG. 1. *Chemical formulas of alloxan monohydrate and ninhydrin*

ered, with the C(5) group projecting out of the principle plane of the rest of the molecule. Comparison of the bond lengths shows that the C—OH, C—C, N—H and O—H distances are consistent with single-bond lengths and the C=O distance is consistent with a double-bond length. The C—N value of 1.37 Å differed significantly from the single or double-bond values and corresponded to 23% double-bond character using Pauling's equation (20). These findings indicate that the correct description of alloxan is 2,4,6-triketo-5,5-dihydroxypyrimidine with resonance occurring across the C—N bonds. The existence of resonance here is consistent with the presence of C(4), N(3), C(2), N(1) and C(6) in a common plane. The group that extends out of this plane is the *gem*-dihydroxy group at C(5) with one of its hydroxyls in essentially the axial position [O(7)], and the other in the equatorial position [O(5)].

To define that part of the alloxan molecule that is recognized by the β-cell, analogues of alloxan have been studied. Ninhydrin, which shares with alloxan the *gem*-dihydroxy group and the adjacent two carbonyls (Fig. 1), has been shown to both stimulate insulin release (F. M. Matschinsky, personal communication) and to inhibit subsequent glucose-induced insulin release (21). The inhibition of insulin release by ninhydrin could be prevented by D-glucose in a manner similar to alloxan inhibition. Analysis of the molecular structure of ninhydrin showed a close similarity to alloxan in the conformation of the *gem*-dihydroxy group and the two adjacent carbonyls. The similarity in conformation of this moiety is consistent with studies showing similarities in the chemical reactions of alloxan and ninhydrin (22). It seems likely that their common effects on insulin release involve this reactive moiety shared by alloxan and ninhydrin. In addition to the molecular similarity in the region of *gem*-dihydroxy group, alloxan and ninhydrin show a common feature at the opposite ends of the molecules–an electron dense group. Alloxan at C(2) has an attached oxygen that aligns near the aromatic ring of ninhydrin. Thus, there are two regions of similarity: the common *gem*-dihydroxy group, its adjacent carbonyls, and an electron dense region.

Structure-activity relationships of D-glucose. D-glucose is a hexose which, both in solution (23) and in the crystalline form (5), exists primarily as a six-member ring in the chair conformation with hydroxyls at C(2), C(3) and C(4) in the equatorial position (Fig. 2). The position of the hydroxyl group at C(1) defines the anomer of D-glucose. With the hydroxyl in the equatorial position at C(1), the molecule is β-D-glucose, and with the hydroxyl in the axial position the molecule is α-D-glucose. The α anomer stimulates insulin release and protects against alloxan and ninhydrin better than the β anomer at low concentra-

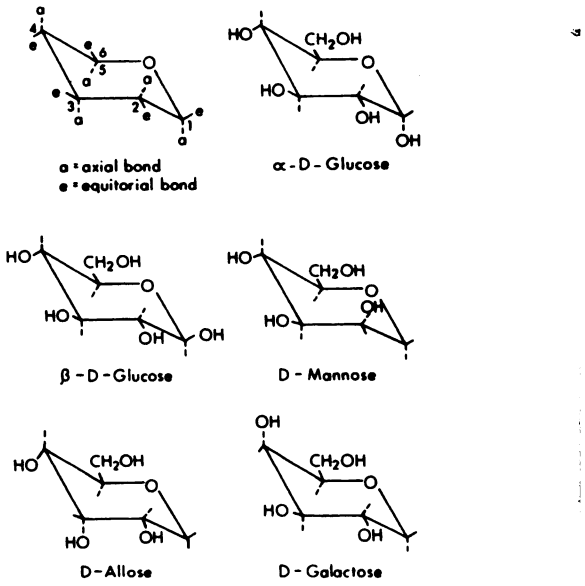

FIG. 2. *D-glucose and its anomers and epimers*
The C(1) hydroxyl of the epimers has been deleted for clarity.

TABLE 1

Effect of glucose analogues on insulin release

Islets were pre-incubated for 20 min in 5.5 mM D-glucose, then exposed for 30 min to either the test agent (27.5 mM) plus 5.5 mM D-glucose, 5.5 mM D-glucose alone (basal release), or 27.5 mM D-glucose (stimulatory control). Mean ± SEM; n = 10 in all cases.

Agent (27.5 mM)	Insulin Release (μU/islet/min)		
	Agent + 5.5 mM glucose	Basal	Control
1-deoxy-D-glucose	0.25 ± 0.03	0.39 ± 0.04	2.93 ± 0.45
6-deoxy-D-glucose	0.21 ± 0.03	0.45 ± 0.06	3.34 ± 0.10
Xylose	0.19 ± 0.04	0.35 ± 0.11	2.28 ± 0.11
Lyxose	0.62 ± 0.14	0.47 ± 0.10	5.03 ± 0.23

tions, but both anomers are equally effective at higher concentrations (21, 24, 25). These findings suggest that, though the α anomer is recognized better than the β anomer, the recognition is not absolute for the position of the hydroxyl at C(1). If the hydroxyl is removed at C(1), as in 1-deoxy-D-glucose, the compound is no longer able to initiate insulin release (Table 1). The results of the analysis indicate that, although the position of the C(1) hydroxyl can vary, its presence seems necessary for recognition by the insulin release mechanism.

D-Mannose (Fig. 2) gives further insight into the steric requirements at C(2) for recognition of a hexose. D-mannose, which has an axial instead of an equatorial hydroxyl at C(2), stimulates insulin release (26, 27) and protects against alloxan and ninhydrin although not as well as D-glucose. If the hydroxyl is removed at C(2), as in 2-deoxy-D-glucose, the sugar neither stimulates insulin release nor provides much protection against alloxan (2, 28). These findings indicate that at C(2), as at C(1), the hydroxyl can be either axial or equatorial, but its presence appears essential for recognition of a hexose.

D-Allose and D-galactose, the C(3) and C(4) epimers of D-glucose, have axial hydroxyls at C(3) and C(4), respectively. Although D-allose and D-galactose differ from D-glucose merely in the position of one hydroxyl (Fig. 2), neither hexose stimulates insulin release (28, 29) nor protects against alloxan or ninhydrin (2, 21, 29). It appears, then, that a hexose with an axial hydroxyl at either C(3) or C(4), is not recognized by the insulin release mechanism.

Table 1 shows the results of the analysis of hexoses which differ from the active hexoses (D-glucose and D-mannose) at the C(6) position. When the terminal CH_2OH group is removed from either D-glucose, as in D-xylose, or D-mannose, as in D-lyxose, the sugar is incapable of initiating insulin release. Moreover, if the hydroxyl is removed at C(6), as in 6-deoxy-D-glucose, that sugar also fails to initiate release of insulin. These findings emphasize the importance of an electron dense group at the C(6) position. The results of the analysis of D-glucose and its analogues suggest that recognition of a hexose demands an electron density near C(6), equatorial hydroxyls at C(4) and C(3), and prefers an equatorial hydroxyl at C(2) and an axial hydroxyl at C(1).

Structure analysis of alloxan and active hexoses. D-glucose, D-mannose and their anomers are the only known hexoses to both initiate insulin release and protect against alloxan, and will for this discussion be referred to as the active hexoses.

Alloxan and D-glucose are both heterocyclic six-member rings with protruding oxygens. A structural comparison (Fig. 3) reveals that the oxygens of alloxan suggested earlier to be involved in recognition [O(4), O(5) and O(6)] align with O(1), O(2) and O(3) of β-D-glucose. The slight puckering of the alloxan ring at C(5) allows for alloxan to more closely approximate the D-glucose molecule. The net result of this alignment is that the carbon atoms of alloxan at C(4), C(5), and C(6) match closely with C(1), C(2), and C(3), respectively, of D-glucose. In addition, the matching of the C(5) and O(5) of alloxan at the central C(2) and O(2) of D-glucose allows for the hydrogens of these two hydroxyls to occupy approximately the same space. This results in a total of seven atoms of alloxan [C(4), O(4), C(5), O(5), H(5), C(6), O(6)] in close alignment with seven atoms of β-D-glucose [C(1), O(1), C(2), O(2), H(2), C(3), O(3)].

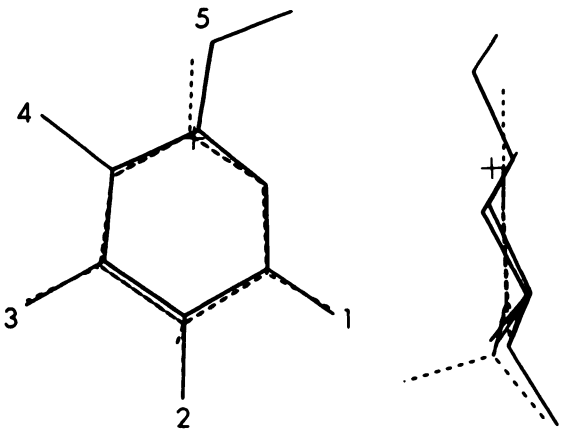

FIG. 3. *Comparison of the molecular structures of alloxan* (- - -) *and D-glucose* (——) *in flat (left) or orthogonal (right) projection*

The numbers correspond to the ring atoms of D-glucose.

Moreover, aligned in this way with D-glucose, alloxan has the electron-dense carbonyl at C(2) near the terminal CH_2OH group of D-glucose. The result of this alignment is that except for its axial hydroxyl, O(7), alloxan will fit nearly within the space needed by β-D-glucose. Further analysis reveals, however, that this axial hydroxyl of alloxan [O(7)] aligns with the axial hydroxyl at C(2) of the other active hexose, D-mannose. Thus, the axial and equatorial hydroxyls of alloxan's *gem*-dihydroxy group align at C(2) with both the axial hydroxyl of D-mannose and the equatorial hydroxyl of D-glucose. This steric analysis indicates that the chemically reactive moiety of alloxan aligns with hydroxyls at C(1), C(2) and C(3) of D-glucose and D-mannose.

Previously (3), we had employed another alignment of alloxan and D-glucose in which the C(5) of alloxan was aligned with the C(3) of D-glucose. Subsquent analysis has revealed, however, that this alignment would require additional space at the recognition site for the C(2) carbonyl and the axial [O(7)] hydroxyl of alloxan and could not account for the inactivity of D-allose. Based on the presently available evidence, we prefer the alignment of the C(5) of alloxan with the C(2) of D-glucose and D-mannose. In this alignment, the whole alloxan molecule fits entirely within the space needed by the active hexoses (D-glucose and D-mannose) to initiate insulin release.

The active hexoses, D-glucose, D-mannose, and their anomers, differ in their potency both for stimulation of insulin release and protection against alloxan. At low concentrations the α anomer of D-glucose is more potent than the β anomer, which suggests that recognition of a hexose prefers at C(1) an axial hydroxyl. At C(2), recognition for insulin release appears to prefer an equatorial hydroxyl, on the basis of the finding that D-glucose is more potent than D-mannose at low concentrations. This variance in potency for the active hexoses can be explained by proposing that the recognition involves some preference for an axial hydroxyl at C(1) and an equatorial hydroxyl at C(2).

Analysis of D-allose and D-galactose. The inactive hexoses, D-allose and D-galactose, have axial hydroxyls at C(3) and C(4) respectively, that align neither with D-glucose or alloxan. Steric analysis reveals that these axial hydroxyls of D-allose and D-galactose occupied space not used by either alloxan or D-glucose. Thus, these two hexoses not only lack an equatorial hydroxyl at C(3) and C(4) but have additional space requirements in the axial position for their hydroxyl. These findings indicate that the inactive epimers of D-glucose vary in the structure from any of the agents tested that initiate insulin release; alloxan, ninhydrin, D-glucose, D-mannose, or their anomers.

Lack of recognition of L-glucose. L-glu-

cose, which does not stimulate insulin release or protect against alloxan and ninhydrin (2, 21, 28), gives further insight into the steric requirements for recognition. There is symmetry in the six-member ring of glucose that allows any one of the ring atoms of L-glucose to be aligned with any of the six ring atoms of D-glucose. In each of these six possible alignments, though, L-glucose would have groups (OH or CH_2OH) that protrude beyond the space occupied by D-glucose. Although it is possible for L-glucose to fulfill the recognition requirements at appropriately positioned hydroxyls at C(1), C(2), C(3) and C(6), new volume in the region of the ring oxygen of D-glucose would be required. The inactivity of L-glucose suggests that such an additional volume at the ring atom of D-glucose is not tolerated. A single comparison of D-glucose and L-glucose has been illustrated previously (29).

Recognition of ninhydrin. The chemically reactive moiety of ninhydrin shares with alloxan the ability to align with hydroxyls of D-glucose and D-mannose at C(1), C(2) and C(3). When aligned with these hexose hydroxyls, the electron-dense aromatic ring of ninhydrin lies near the freely rotatable CH_2OH group of the hexoses. Although the exact position of the CH_2OH group is not known, it can be positioned to include most of the volume in ninhydrin's aromatic region. Indeed, only in the region slightly outside the hexose ring at O(5) does ninhydrin require some additional space. Thus, like alloxan, ninhydrin fits almost entirely within the volume needed by the active hexoses that protect against them and initiate insulin release.

DISCUSSION

The present study demonstrates a similarity in the molecular structure of alloxan, ninhydrin and the two hexoses that both provide protection against them and initiate insulin release—D-glucose and D-mannose. Both alloxan and ninhydrin have a slight pucker in the ring that allows their reactive moiety (*gem*-dihydroxy group and two adjacent carbonyls) to align with hydroxyls of D-glucose and D-mannose at C(1), C(2) and C(3). The *gem*-dihydroxy group of both alloxan and ninhydrin has an equatorial and an axial hydroxyl that align at the hexose C(2) with both the equatorial hydroxyl of D-glucose and the axial hydroxyl of D-mannose. Furthermore, the mean plane of alloxan and ninhydrin lies near the mean plane of the hexoses with their terminal electron-rich region, a carbonyl or aromatic ring, near the terminal CH_2OH group of the hexoses. The net result of this alignment is that alloxan and ninhydrin fit almost entirely with the volume demanded by the active hexoses to initiate insulin release.

Based on their similar biological actions and molecular structures, we propose a hypothesis that alloxan, ninhydrin and the active hexoses interact at a common receptor to initiate the first phase of insulin release. This receptor must have volume to accommodate the recognized agents and sense a particular molecular architecture to initiate insulin release. The volume to accommodate all of the recognized agents (alloxan, ninhydrin, D-glucose, D-mannose and their anomers) is illustrated in Figure 4, which is a union of the volume of these active agents. This union represents the receptor-excluded volume—the volume the receptor cannot occupy. To analyze the molecular architecture that may be essential to initiate insulin release, we determined the common features among the recognized agents. Figure 5 shows that the molecular architecture in common among all of the recognized molecules is: an oxygen at position one, either axial or equatorial; a hydroxyl at position two, either axial or equatorial; an equatorial oxygen at position three; and at position five an electron-rich region. This molecular architecture represents the particular chemical and structural properties sensed by the glucoreceptor—the pharmacophore. These results suggest that a new compound that has this pharmacophore could be used to test the receptor hypothesis of hexose-induced insulin release. We wish to emphasize that this hypothesis refers only to the events involved in initiating the first phase of insulin release. The rapid metabolism of D-glucose may maintain release of insulin during the second phase.

Several hexoses that neither protected

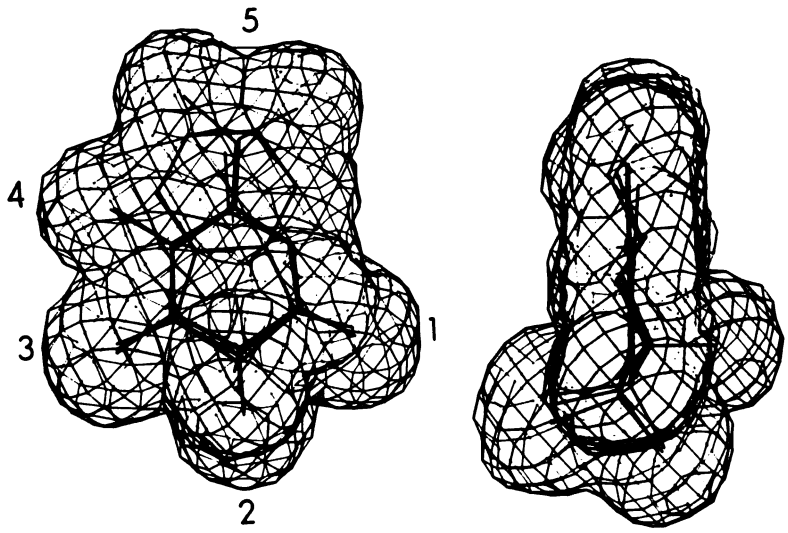

FIG. 4. *The receptor-excluded volume in flat (left) or orthogonal projection (right)*
This volume represents the union of the volume required for alloxan, ninhydrin, D-glucose, D-mannose and their anomers. The darker internal lines represent their interatomic bonds. The outer lines represent the molecular volume that was generated from the Gaussian atom density distribution and contoured to provide a surface representative of the van der Waals radii.

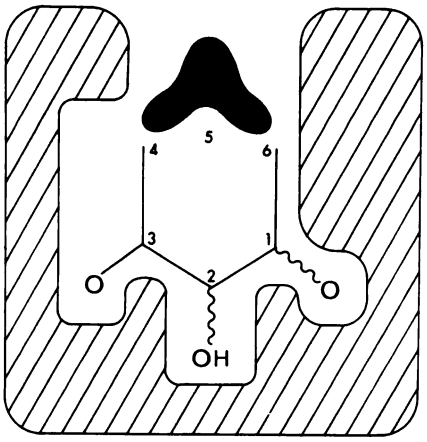

FIG. 5. *The pharmacophore for recognition at a glucoreceptor*
The common properties for insulin release are: An oxygen at position 1, either axial or equatorial; a hydroxyl at position 2, either axial or equatorial; an equatorial oxygen at position 3; and at position 5 an electron-rich region. Structural analysis of inactive hexoses suggests that the receptor has essential volume near position 6 and near axial substituents at positions 3 and 4.

against alloxan nor initiated insulin release were analyzed and found to vary in their structure from the recognized agents. D-Allose and D-galactose, epimers of D-glucose, have axial hydroxyls at C(3) and C(4), respectively, that would demand additional space at the glucoreceptor. Analysis of the structure of L-glucose provided suggestive evidence for intolerance for a protruding group at the ring oxygen of D-glucose. Although the inactivity of these isomers may be unrelated to their inability to interact with the recognition site, the results are suggestive of intolerance for a protruding group at position six or axial hydroxyls at positions three and four of the receptor. This positional intolerance can be explained by proposing that the receptor has essential volume at positions three, four and six that prevents the accommodation of the inactive hexoses.

In conclusion, biological and structural studies suggest that there is a glucoreceptor for insulin release with specific steric requirements. D-glucose, D-mannose, alloxan and ninhydrin share a common molecular architecture that could account for their recognition by the glucoreceptor to initiate the first phase of insulin release.

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