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Effect of Alloxan on the Incorporation of Uridine Diphospho D-Galactose into Langerhans' Islets of the Rat

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The effect of alloxan on pancreatic islets of the rat was studied by following the change in the incorporation of uridine diphospho (UDP) D-[U-^{14}C]galactose into glycoproteins (chloroform/methanol-insoluble fraction) and glycolipids (chloroform/methanol-soluble fraction). Islets exposed to either alloxan or alloxan plus D-glucose anomers (16.7 mM) at 37° for 5 min, were incubated in a medium containing UDP-[U-^{14}C]galactose at 37° for 120 min.

The incorporation of UDP-[U-^{14}C]galactose into glycoproteins and glycolipids was unaffected by 1.25 mM alloxan, but was slightly reduced by 6.25 mM alloxan. Alloxan at 31.2 mM severely inhibited the incorporation. The combination of alloxan (31.2 mM) plus the α anomer abolished the inhibitory effect of alloxan to a considerable extent, while the β anomer provided no protection against the action of alloxan.

The label distribution on polyacrylamide gel electrophoresis of islets incubated with UDP-galactose was similar to that obtained after labeling with galactose oxidase and NaB^3H_4.

In conclusion, the present data indicate that pancreatic islets of the rat can transfer galactose from UDP-galactose on the plasma membrane and that the α anomer of glucose is more effective than the β anomer in overcoming the inhibitory effect of alloxan.

Keywords——galactosyltransferase; glycoprotein; glycolipid; alloxan; α-D-glucose; β-D-glucose

Introduction

In earlier studies, it has been demonstrated that both the biosynthesis and the release of insulin are markedly inhibited by exposure of pancreatic islets of the rat to alloxan and that the α anomer of D-glucose provides a significantly greater protection against the inhibitory effect of alloxan than the β anomer.[2,3]

Since then, several lines of evidence supporting the existence of the glucoreceptor controlling insulin release and biosynthesis have been accumulated. However, the precise mechanisms by which glucose stimulates insulin release and biosynthesis, and alloxan inhibits them, remain still unclear. Maier and Pfeiffer[4] (1971) suggested that sialic acid, possibly attached to membrane proteins of islets, might play an important role in glucose-induced insulin release. Glycosyltransferases have been found in the cell surface and the Golgi apparatus.[5] In addition, it was recently observed that alloxan inhibits the incorporations of D-glucose and UDP-glucose into cultured pancreatic cells of the rat.[6]

In the present work, we found that pancreatic islets of the rat possess galactosyltransferases on the plasma membrane and that the α anomer of D-glucose was more effective than the β anomer in providing protection against the inhibitory effect of alloxan on the activity of galactosyltransferases.

Materials and Methods

Reagents——All reagents used were of special grade. Collagenase, α-D-glucose, β-D-glucose, galactose oxidase (100 units/2 mg) and tris(hydroxymethyl)aminomethane were purchased from Sigma Chemical Co., U.S.A. Bovine serum albumin (Fraction V), was from Armour Laboratories, U.S.A. PCS and NCS

solubilizers were from Amersham Co., U.S.A. Uridine diphospho D-[U-^{14}C]galactose (347 mCi/mmol), D-[5-^{3}H]glucose (12 Ci/mmol) and NaB^3H_4 (309 mCi/mmol) were from the Radiochemical Centre, Amersham, England. The kit of protein standards for SDS-polyacrylamide gel electrophoresis was from Boehringer Mannheim GmbH, W. Germany. Coomassie brilliant blue R-250 was from Nakarai Chemical Co., Japan. D-Glucose, $MnCl_2$, alloxan and other reagents were products of Wako Pure Chemical Industries, Japan.

Media for the Isolation of Islets and the Incorporation of UDP-[U-^{14}C]galactose——The basal medium used for the isolation of rat islets and alloxan treatment was a Krebs-Henseleit bicarbonate buffer (pH 7.35—7.40, KHB buffer) containing 0.5% bovine serum albumin. In experiments with UDP-[U-^{14}C]galactose, Tris buffer containing 5.0 mM Tris, 139 mM Na^+, 4.7 mM K^+, 2.6 mM Ca^{2+}, 1.2 mM Mg^{2+}, 1 mM Mn^{2+} and 153.3 mM Cl^- was used (pH 6.8).

Isolation of Islets of the Rat——Islets of the rat were isolated from male Sprague-Dawley rats weighing 200—300 g, which had been fasted, according to a modification of the method of Lacy and Kostianovsky[7] (1967) as described in our previous report.[8]

Incorporation of Galactose from UDP-[U-^{14}C]galactose into Glycoproteins and Glycolipids——Each batch of 100 islets was equilibrated in a medium containing 2.8 mM D-glucose and 0.5% bovine serum albumin at 37° for 30 min. Islets were preincubated in 400 μl of media containing alloxan or alloxan plus D-glucose anomers at 16.7 mM at 37°. After 5 min, the islets were washed with KHB buffer and then incubated in 200 μl of Tris buffer containing 1 mM $MnCl_2$ and UDP-[U-^{14}C]galactose (0.76 μCi/100 μl) at 37° for 120 min. The labeled islets were then disrupted by sonication. Aliquots (100 μl) of the sonicated sample were transferred to 500 μl of 5% TCA and centrifuged at 1×10^4 g for 5 min. After the addition of 40 μl of 1 N NaOH and subsequently 1 ml of cold 5% TCA to the pellet, the suspension was re-centrifuged at 1×10^4 g. Precipitated materials was extracted twice with 500 μl of chloroform/methanol (2: 1); the extract soluble in chloroform/methanol contained glycolipids and the residue contained glycoproteins. Chloroform and methanol were removed from glycolipids by heating at 100° for 5 min in a scintillation vial. Glycoproteins and glycolipids were each dissolved with 10 ml of PCS-xylene (2: 1) and their radioactivities were determined in a liquid scintillation counter (Packard 3385, Packard Instrument Company Inc., U.S.A.). The blank value of the reaction, which was obtained by incubating islets boiled for 3 min prior to alloxan exposure with labeled UDP-galactose, was subtracted from each measurement.

Labeling of the Plasma Membrane of Islets by Galactose Oxidase and NaB^3H_4——Three hundred islets were labeled by treatment with galactose oxidase (25 units/ml) and NaB^3H_4 (0.5 μCi/300 μl) at 20° for 60 min, according to a modification of the method of Gahmberg and Hakomori[9] (1973).

SDS-Polyacrylamide Gel Electrophoresis——SDS-Polyacrylamide gel electrophoresis was carried out by the method of Fairbanks *et al.*[10] (1971), as described in our previous report.[6]

The Utilization of Glucose in Rat Islets treated with Alloxan——In order to clarify the viability of alloxan-treated islets, the utilization of glucose in the islets was studied by using 2 mM D-[5-^{3}H]glucose as described in our previous report.[11]

Calculation——Student's "t" test was used to determine statistical significance.

Results

The incorporation of labeled galactose into glycoproteins was 80 dpm/20 islets/2 hr and that into glycolipids was 92 dpm/20 islets/2 hr. When islets were pretreated with either the α or the β anomer at 16.7 mM concentration for 5 min at 37 °C, neither anomer had a significant effect on the incorporation of labeled galactose into glycoproteins and glycolipids (Fig. 1). The incorporation of labeled galactose was a linear function of time up to 120 min (not illustrated).

The effect of various concentrations of alloxan on the incorporation of UDP-galactose is summarized in Fig. 2. The incorporation of labeled galactose into glycoproteins and glycolipids was not significantly affected by 1.25 mM alloxan. Alloxan at either 6.25 or 31.2 mM significantly diminished the incorporation of UDP-galactose to 39.5 or 59.3% of the control value for glycoproteins, and 26.0 or 62.3% of the control value for glycolipids (Fig. 2).

Figure. 3A shows that the presence of the α anomer at 16.7 mM produced a significant protection against the inhibitory effect of alloxan at 31.2 mM, in contrast to the β anomer; the presence of the α anomer restored the incorporation into glycoproteins to 132.6% and that into glycolipids to 222.2% of the values for alloxan-treated islets. In the presence of 6.25 mM alloxan, the α anomer slightly reduced the inhibition by alloxan of the incorporation into glycolipids but not into glycoproteins, while the β anomer had no effect (Fig. 3B).

SDS-polyacrylamide gel eletrophoresis demonstrated label distribution into islet com-

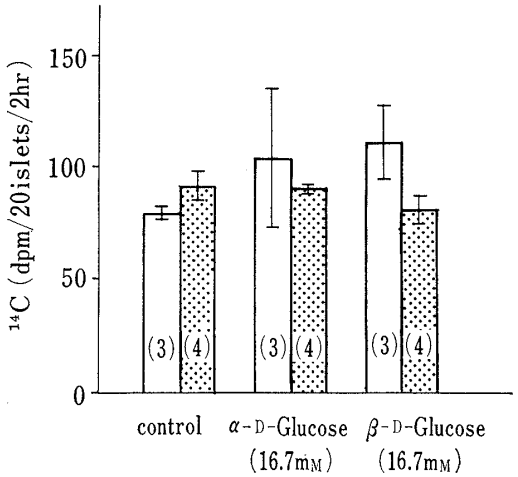

Fig. 1. Effect of the Anomers of D-Glucose on the Incorporation of UDP-[U-^{14}C]galactose

Islets were pretreated with medium containing α anomer or β anomer of D-glucose at 37° for 5 min and then incubated in a Tris buffer containing UDP-[U-^{14}C]galactose at 37° for 120 min. The open columns represent the incorporation into glycoproteins and the dotted columns represent that into glycolipids.

Each value is the mean (±SEM) of n experiments (in parentheses).

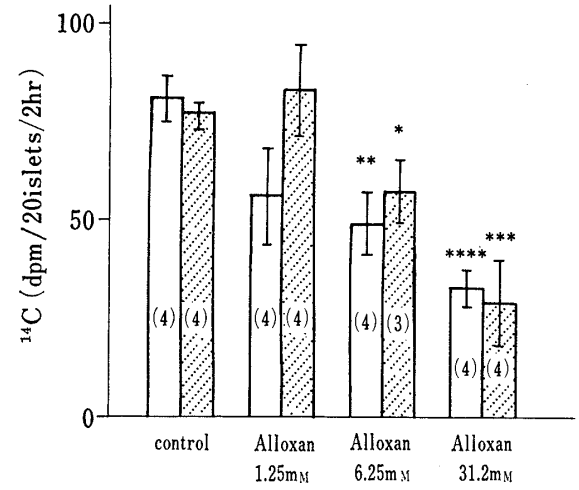

Fig. 2. Effects of Various Concentrations of Alloxan on the Incorporation of UDP-[U-^{14}C]galactose

Islets were pretreated with media containing 1.25 mM, 6.25 mM and 31.2 mM alloxan at 37° for 5 min and then incubated in a Tris buffer containing UDP-[U-^{14}C]galactose at 37° for 120 min. Control islets was incubated in a glucose-free medium at 37° for 5 min. The open columns represent the incorporation into glycoproteins and the dotted columns represent that into glycolipids.

Each value is the mean (±SEM) of n experiments (in parentheses). Statistical comparison was performed against the control. $*p < 0.05$, $**p < 0.02$, $***p < 0.01$, $****p < 0.001$.

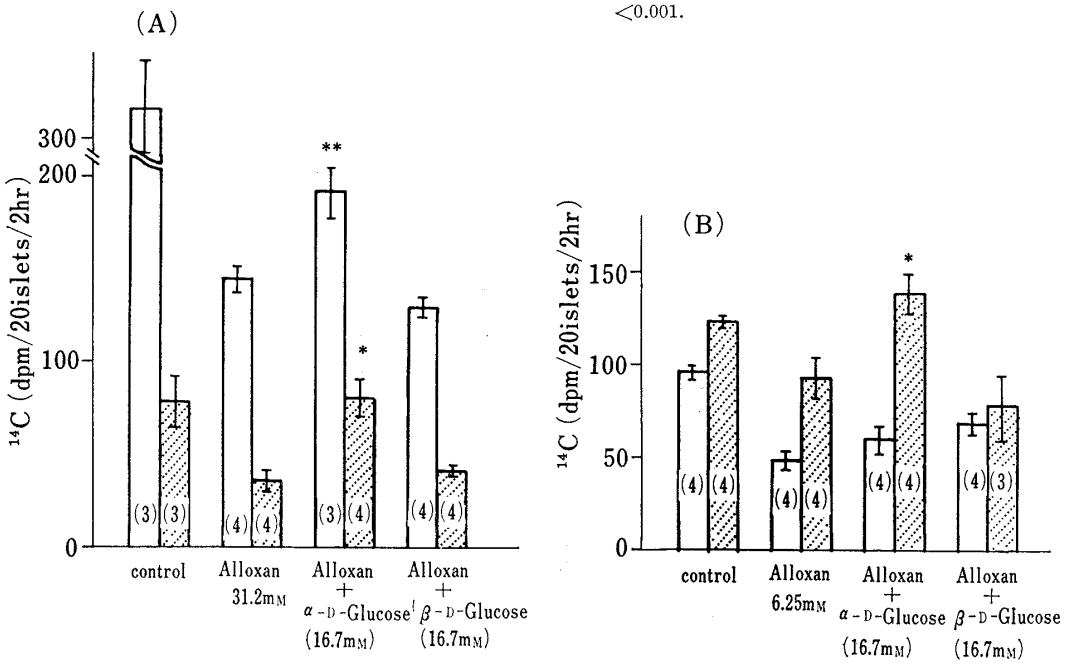

Fig. 3. Effect of Alloxan plus the Anomers of D-Glucose on the Incorporation of UDP-[U-^{14}C]galactose

Islets pretreated with 31.2 mM alloxan and 31.2 mM alloxan plus the anomers of D-glucose at 16.7 mM concentration at 37° for 5 min (A) and islets pretreated with 6.25 mM alloxan and 6.25 mM alloxan plus the anomers of D-glucose at 16.7 mM concentration (B), were incubated in Tris buffer containing UDP-[U-^{14}C]galactose at 37° for 120 min. The radioactivities incorporated into glycoproteins and glycolipids were determined as described in "Meterials and Methods." Control islets in each experiment were pretreated with a glucose-free medium at 37° for 5 min. The open columns represent incorporation into glycoproteins and the dotted columns represent that into glycolipids. Each value is the mean (±SEM) of n experiments (in patentheses). Statistical comparison was performed against the alloxan-treated islets. $*p < 0.05$, $**p < 0.02$.

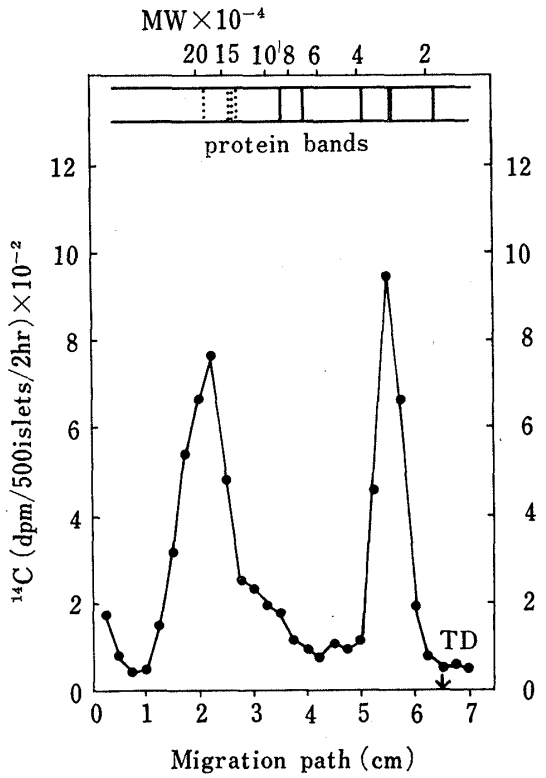

Fig. 4. Electrophoretic Analysis of Islets labeled with UDP-[U-^{14}C]galactose

This graph shows the distribution of islet components with labeled galactose on SDS-polyacrylamide gel electrophoresis. Five hundred islets were incubated with UDP-[U-^{14}C]galactose as described in "Materials and Methods."

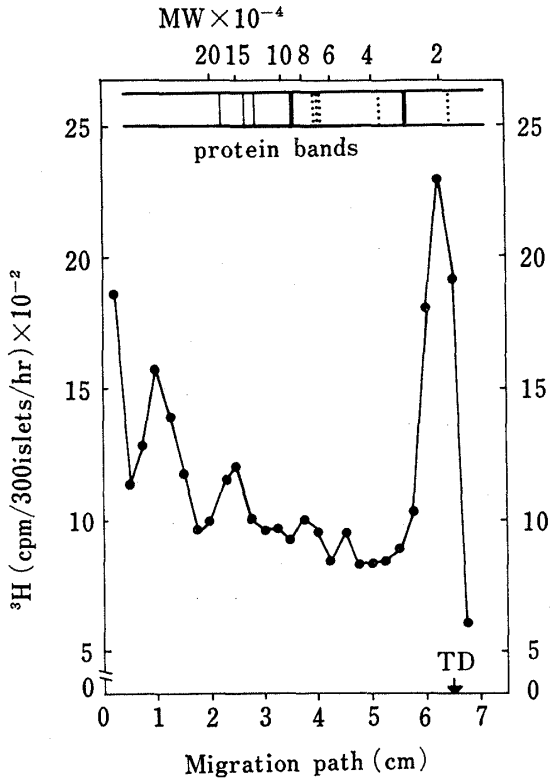

Fig. 5. Electrophoretic Analysis of Islets labeled with Galactose Oxidase and NaB^3H_4

This graph shows the distribution of islet components labeled with galactose oxidase and NaB^3H_4 on SDS-polyacrylamide gel electrophoresis. Three hundred islets were incubated with galactose oxidase (25 units/ml) and NaB^3H_4 (0.5 μCi/300 μl) at 20° for 60 min.

TABLE I. Effects of Alloxan and Alloxan plus D-Glucose Anomers on Glucose Utilization

	Concn (mM)	Glucose utilization (pmol/islet/hr)
Control	—	7.7±0.6(5)
Alloxan	1.25	4.8±0.3(3)[a]
	6.25	3.8±1.5(3)[a]
	31.20	2.9±1.4(4)[a]
Control	—	8.6±2.5(4)
Alloxan	31.20	2.4±0.9(6)[a]
Alloxan+16.7 mM α-D-Glucose	31.20	6.1±0.6(4)
Alloxan+16.7 mM β-D-Glucose	31.20	6.8±0.6(3)

Each batch of 5 islets was incubated in 100 μl of 2 mM [5-^{3}H]glucose (1.5 μCi/mmol) for 2 hr at 37°. Each value is the mean (±SEM) of n experiments (in parentheses). Statistical comparison was performed against the control.
a) $p<0.02$.

ponents having molecular weights of approximately 163000, 72000 to 123000 and 27000. The protein staining bands are shown in the upper part of the figure (Fig. 4).

As depicted in Fig. 5, the combination of galactose oxidase and NaB^3H_4 appeared to label the same glycoproteins and glycolipids as UDP-galactose (Fig. 5).

Rates of glucose utilization in intact and alloxan-treated islets are summarized in Table I. When islets were pretreated with 31.2 mM alloxan, glucose utilization significantly decreased

to 37% of the control, while the presence of the anomers largely abolished the inhibitory effect of alloxan to similar extents (Table I).

Discussion

Alloxan is known to induce diabetes in animals, but the exact mechanism by which alloxan destroys pancreatic B cells remains to be elucidated.

As is well known, galactosyltransferases can catalyze the transfer of sugars from nucleotide sugars to acceptor molecules located on the cell surface, and the enzymes are also a part of cell surface receptors functioning in cellular adhesion.[12] Since nucleotide sugars are substrates specific for cell surface glycosyltransferases, a study of the changes in the incorporation of UDP-[U-^{14}C]galactose caused by alloxan should cast light on the mechanism of alloxan action on the membrane of islets.

The incorporation of UDP-[U-^{14}C]galactose into glycoproteins of non-treated islets was 80 dpm/20 islets/2 hr and that into glycolipids was 92 dpm/20 islets/2 hr. The finding that islets incorporated galactose from UDP-galactose suggests that galactosyltransferases may exist on the plasma membrane of islets, as observed in other types of cells,[13,14] since nucleotide sugars not pass the cell membranes.[5] However, in order to rule out the possibility that UDP-galactose freely penetrates the cell surface of islets, we first compared the incorporation of D-[U-^{14}C]galactose with that of UDP-[U-^{14}C]galactose, since D-galactose can be converted to UDP-galactose in islets and then incorporated. Incorporation of UDP-[U-^{14}C]galactose was at least 80 dpm/20 islets /2 hr, the maximum being 320 dpm/20 islets/2 hr, while that of D-[U-^{14}C]galactose exceeded 300 dpm/20 islets/2 hr in all preparations of islets. It thus seems likely that UDP-galactose either cannot penetrate the cell surface or is much less able to penetrate it than D-galactose. The present view that the cell surface of islets possesses galactosyltransferases seems to be supported by the observation that glycoproteins and glycolipids labeled by the combination of galactose oxidase and NaB^3H_4, appeared in the same regions as those labeled with UDP-galactose on SDS-polyacrylamide gel electrophoresis.

On the other hand, it is evident that alloxan markedly inhibited the incorporation of UDP-[U-^{14}C]galactose into glycoproteins and glycolipids and that the α anomer is more effective than the β anomer in overcoming the action of alloxan. These results seem to support the view that the α anomer and alloxan may compete for a common site on the membrane.[15,16]

The concentrations of alloxan causing inhibition of the galactosyltransferase activities, 6.25 and 31.2 mM, are relatively high as compared to the concentrations at which alloxan inhibits insulin release and biosynthesis. However, the data on glucose utilization demonstrated that islets are viable even if treated with alloxan at 31.2 mM.

Recently, Orci *et al.*[17] (1976) demonstrated that alloxan at 6.25 mM, a relative high concentration, produced a significant decrease of membrane associated particles in rat islets and that D-glucose prevented the ultrastructural alteration of the membrane. Therefore, it seem likely that the action of alloxan may be associated with a change of morphological organization including the fluidity of the membrane structure, leading to the inhibition of galactosyltransferase activity.

The present data indicate that pancreatic islets of the rat can transfer galactose from UDP-galactose and that alloxan inhibits the activity of the galactosyltransferases.

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