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Alloxan is an inhibitor of the enzyme O-linked *N*-acetylglucosamine transferase☆

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Abstract

We have previously shown that diabetogenic antibiotic streptozotocin (STZ), an analog of *N*-acetylglucosamine (GlcNAc), inhibits the enzyme *O*-GlcNAc-selective *N*-acetyl-β-D-glucosaminidase (*O*-GlcNAcase) which is responsible for the removal of *O*-GlcNAc from proteins. Alloxan, another β-cell toxin is a uracil analog. Since the *O*-GlcNAc transferase (OGT) uses UDP–GlcNAc as a substrate, we investigated whether alloxan might interfere with the process of protein O-glycosylation by blocking OGT, a very abundant enzyme in β-cells. In isolated pancreatic islets, alloxan almost completely blocked both glucosamine-induced and STZ-induced protein O-GlcNAcylation, suggesting that alloxan indeed was inhibiting (OGT). In order to show definitively that alloxan was inhibiting OGT activity, recombinant OGT was incubated with 0–10 mM alloxan, and OGT activity was measured directly by quantitating UDP–[^{3}H]-GlcNAc incorporation into the recombinant protein substrate, nucleoporin p62. Under these conditions, OGT activity was completely inhibited by 1 mM alloxan with half-maximal inhibition achieved at a concentration of 0.1 mM alloxan. Together, these data demonstrate that alloxan is an inhibitor of OGT, and as such, is the first OGT inhibitor described.

Keywords: Alloxan; Islets; O-glycosylation; *O*-GlcNAc transferase

Many nuclear and cytoplasmic proteins are covalently modified by the linkage of monosaccharide *N*-acetylglucosamine (GlcNAc) to serine or threonine, a reaction catalyzed by *O*-GlcNAc transferase (OGT) [1–4]. This enzyme serves vital purposes in the cell, and a homozygous knock-out of the enzyme is lethal at the one cell stage [5]. The list of fundamental cellular processes in which OGT is involved is growing. The enzyme modifies most RNA polymerase II transcription factors [6], and O-GlcNAc modification blocks Sp1 protein–protein interactions [7] and transcriptional activation [8]. For RNA polymerase II, Sp1, and probably other modified proteins, the cyclical addition and removal of *O*-GlcNAc are vital for the biological function. Proteasomal degradation of Sp1 is also blocked by O-GlcNAc modification [9] of a proteasomal component [10,11] suggesting that OGT may link the nutritional status of the cell to the stability of intracellular proteins normally degraded by this organelle. OGT may also function as part of a complex of proteins involved in the repression of specific gene transcription (Yang and Kudlow, personal communication).

OGT is most abundant in pancreatic β-cells [12–14]. Hyperglycemia in vivo results in a marked increase in protein O-glycosylation selectively in β-cells but not in pancreatic acinar cells [15]. In isolated islets, a predominant 135 kDa protein is O-glycosylated in response to high glucose and glucosamine [16], and this protein

☆ *Abbreviations*: STZ, streptozotocin; GlcNAc, *N*-acetylglucosamine; OGT, *O*-GlcNAc transferase; *O*-GlcNAcase, *O*-GlcNAc-selective *N*-acetyl-β-D-glucosaminidase; HBSS, Hanks' balanced salt solution; KRB, Krebs'-HEPES buffer; GlcN, glucosamine; GalNAc, *N*-acetylgalactosamine.

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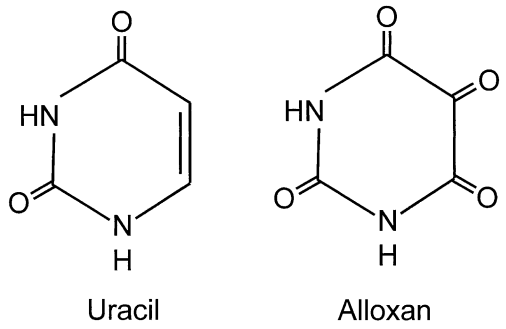

Fig. 1. The chemical structures of uracil and alloxan. It is readily apparent that alloxan is an analog of uracil.

preliminarily appears to be OGT itself [16–19]. When the activity of OGT is unopposed due to the non-competitive blockade of the *O*-GlcNAcase enzyme by the GlcNAc analog streptozotocin [20], β-cell death ensues both via apoptosis and a more rapid means [15,18,21]. The high abundance of OGT in β-cells [12–14] coupled with increased sensitivity of islet *O*-GlcNAcase to STZ [18] appears to explain the selective β-cell toxicity of STZ.

In light of these findings, we have investigated whether the diabetogenic agent alloxan also interferes with the process of O-glycosylation in β-cells. Because alloxan is derived from uracil (Fig. 1) and OGT uses UDP–GlcNAc as a substrate, we tested the idea that alloxan may interfere with O-GlcNAcylation by being an inhibitor of OGT, possibly through an interaction of alloxan with the UDP-binding domain in OGT. Since OGT is required for cell viability [5] and is so abundant in β-cells [12–14], inhibition of OGT by alloxan might explain how this drug is relatively specific for β-cells. In the manuscript we show that in isolated pancreatic islets, alloxan blocks both glucosamine and STZ-induced protein O-glycosylation, suggesting that alloxan indeed inhibits OGT. Furthermore, when recombinant OGT was incubated with alloxan, OGT activity was inhibited in a dose-dependent manner with complete inhibition at a concentration of 1 mM alloxan and half-maximal inhibition at a concentration of 0.1 mM alloxan. These data confirm that alloxan inhibits OGT activity, and as such, is the first OGT inhibitor to be described.

Materials and methods

Islet isolation. In a typical experiment, islets were isolated aseptically from 3 to 4 male Sprague-Dawley rats as previously described [16–19]. Islets were isolated using Hanks balanced salt solution (HBSS) and Ficoll supplemented with 5.5 mM glucose, 1 mM L-glutamine, penicillin (25 U/ml), and streptomycin (25 U/ml). During surgery, the common bile duct of each pancreas was cannulated, and the pancreas was inflated with 10–20 ml of HBSS. The distended pancreas was excised, and lymph nodes, fat, blood vessels, and bile ducts were removed under a stereo-dissecting microscope. Tissue was chopped extensively, rinsed 5–6 times with HBSS, and digested with collagenase P (3 mg/ml tissue) at 37 °C for 3–4 min. Digested tissue was rinsed 3–4 times with HBSS. Islets were purified on a discontinuous Ficoll gradient consisting of 27%, 23%, 20.5%, and 11% Ficoll in (25 mM) HEPES–HBSS buffer. Islets were harvested and washed once with HBSS and six times in the appropriate incubation buffer. This isolation procedure typically provided 400–500 islets per rat, which were used as described below.

Incubation of islets for protein O-glycosylation. Freshly isolated islets were washed six times Krebs'-HEPES buffer (25 mM HEPES, pH 7.40, 115 mM NaCl, 24 mM $NaHCO_3$, 5 mM KCl, 2.5 mM $CaCl_2$, and 1 mM $MgCl_2$) supplemented with 0.1% bovine serum albumin, 3 mM glucose, and 1 mM L-glutamine. Islets (100/condition) were placed into siliconized 16 × 100-mm round-bottom, screw-cap tubes. Pre-incubation and all subsequent incubations were performed at 37 °C under an atmosphere of 95% O_2/5% CO_2. After pre-incubation buffer was aspirated, islets were incubated with Krebs'-HEPES buffer supplemented with the appropriate compound. At the end of the experiment, supernatants were removed from the tubes, and islets were processed as described below.

Processing of islets for immunoprecipitation of O-glycosylated proteins. At the end of the experiment, supernatant was removed from the tubes. One milliliter of ice-cold lysis buffer (50 mM HEPES, pH 7.40, 150 mM NaCl, 1% Triton X-100, 5 mM EDTA, 5 mM EGTA, 20 mM NaF, 20 mM $Na_4P_2O_7$, 1 mM $NaVO_4$, 1 mg/ml bacitracin, 1 mM phenylmethylsulfonylfluoride (PMSF), 1 μg/ml leupeptin, and 1 μg/ml aprotinin) was added to each tube. After vortexing for 30 s, tubes were placed on ice for 30 min, and vortexed for an additional minute before transfer to 1.5-ml conical screw-cap Eppendorf tubes. All subsequent immunoprecipitation steps were performed at 4 °C. Samples were centrifuged at 10,000*g* for 15 min. The supernatant was transferred to a second 1.5-ml conical Eppendorf tube, and O-glycosylated proteins were immunoprecipitated for 2 h on a rocker with 1 μl of mouse monoclonal RL2 antibody, expressed from a hybridoma (RL2 antibody is also commercially available from Affinity Bioreagents, Golden, CO). This antibody selectively binds O-glycosylated protein, and immunoprecipitation of O-glycosylated proteins by RL2 is specifically blocked by GlcNAc [18]. After 2 h, 20 μl of protein A trisacryl beads (Pierce) pre-adsorbed with 20 μg of rabbit anti-mouse antibody (Sigma) was added to the tubes and the incubation was continued overnight. At the end of the incubation, beads were washed once with Wash Buffer 1 (150 mM NaCl, 10 mM HEPES, pH 7.40, 1% Triton X-100, and 0.1% SDS) and once with Wash Buffer 2 (10 mM HEPES, pH 7.40, 1% Triton X-100, and 0.1% SDS). After the final washing step, 25 μl of 2× sample buffer (100 mM Tris, pH 6.80, 4% SDS, 20% glycerol, and 20 μg/l bromophenol blue) was added to each tube. Samples were vortexed for 30 s, boiled for 5 min, vortexed for an additional 30 s, and stored at −20 °C prior to subsequent analysis.

Western blotting. Samples were loaded onto 7.5% SDS–polyacrylamide gels. Colored molecular weight markers (Amersham) were run on each gel. Proteins were separated for 1 h at 175 V at room temperature using a Bio-Rad Mini-PROTEAN II dual slab cell. Proteins were transferred to ECL nitrocellulose paper (Amersham) for 1.5 h (100 V, 4 °C). Nitrocellulose blots were blocked for 1 h at room temperature in a blocking buffer (5% bovine serum albumin in 10 mM Tris, pH 7.40, 150 mM NaCl, 0.1% sodium azide, and 0.05% Tween 20). After blocking, the blots were probed with RL2 antibody (1:1000 dilution in blocking buffer) for 1 h at room temperature. Blots were washed six times (5 min each) with TBST (10 mM Tris, pH 7.40, 150 mM NaCl, and 0.05% Tween 20). After washing, blots were probed with horseradish peroxidase conjugated sheep anti-mouse antibody (Amersham) at a 1:1500 dilution in TBST for 1 h at room temperature. Blots were washed again and developed with ECL reagent (Amersham). After air-drying, blots were exposed to Bio-Max X-ray film (Kodak). For each experiment, the intensity of the control sample was set at 100% and all other results were expressed as a percent of control.

OGT assay. The OGT cDNA (a gift from G.W. Hart, Baltimore) was cloned in frame downstream of GST into the pGEX vector and transformed into the BL-21 strain of *E. coli*. Expression of the GST–OGT fusion protein was induced with IPTG, and the enzyme was purified using 10 μl glutathione beads per 50 μl *E. coli* BL-21 lysate

containing recombinant GST–OGT for each of the indicated reactions. Recombinant p62 nucleoporin protein [22] was expressed in BL-21 cells and purified as previously described [23]. The beads were then mixed with the indicated concentration of alloxan, 0.5 μCi UDP–[^{3}H]-GlcNAc and 100 ng of recombinant p62 in OGT activity assay buffer (50 mM Tris–HCl, 12.5 mM $MgCl_2$, and 1 mM DTT). The reaction mixture was incubated at 22 °C for 90 min. The reaction was stopped by adding equal volume of 2× sample buffer and boiling for 5 min. The proteins were separated by 10% SDS–PAGE, which was stained with Coomassie blue to confirm equal loading of protein. Subsequently [H^3]–GlcNAc incorporated into p62 was determined by fluorography.

Alloxan treatment of recombinant OGT. Recombinant OGT was incubated in the presence or absence of 5 mM alloxan for 30 min at 37 °C. Afterward, the reaction was stopped by addition of 2× sample buffer supplemented with 1 mM DTT. Samples were boiled for 5 min and run on 1 mm-thick 7.5% SDS–PAGE gels, which had been pre-run at 175 V for 10 min in order to eliminate reactive groups in the polyacrylamide. Colored molecular weight markers (Amersham) were run on a non-adjacent lane of the gel. Following electrophoresis for 1 h at 175 V, the gel was washed twice with distilled water, stained for 1 h with Coomassie stain, and detained for 3 h with several rinses of destaining solution. Afterward, the gel was washed twice with distilled water. Coomassie-stained bands corresponding to alloxan-treated and untreated OGT were cut out of the gel and stored at −20 °C for subsequent analysis.

In-gel protein digestion. A standard in-gel procedure was utilized [24] and adapted for use on a Tecan robot. OGT bands visualized with Coomassie blue were excised, cut into 3–4 pieces and destained by extensive washing with 50% acetonitrile, 50 mM NH_4HCO_3. Protein was reduced in the gel with 50 μl of 50 mM DTT in 50mM NH_4HCO_3 for 60 min at 37 °C. The DTT solution was removed and replaced with 50 μl of 100 mM iodoacetamide in 50 mM NH_4HCO_3 for 15 min at 37 °C. The alkylating solution was removed, gel pieces were washed once with 100 mM NH_4HCO_3, then with acetonitrile, and dried by placing the gel pieces at 37 °C for 10 min. Dried gel pieces were swollen in 10 μl of trypsin (33 μg/ml in 50 mM NH_4HCO_3, and pH 8.0), and digested overnight at 37 °C. After digestion, peptides were extracted and modified with 1 M *O*-methylisourea in 100 mM Na_2CO_3 (adjusted to pH 10 with 5 N NaOH) for 1 h at 37 °C. Peptide extracts were desalted on C-18 Zip tips (Millipore, Bedford, MA) by washing with 0.1% trifluoroacetic acid and eluting with 6 μl of 50% acetonitrile and 0.05% trifluoroacetic acid.

MALDI-TOF mass spectrometry analysis of recombinant OGT. MALDI-TOF spectra of alloxan-treated and untreated OGT were obtained on a Perceptive Biosystems Voyager DE Pro mass spectrometer. An aliquot (1 μl) of the desalted tryptic peptide mixture prepared above was mixed with matrix (α-cyano 4-hydroxy cinnamic acid) on the sample target and allowed to dry before collecting data. The instrument was calibrated with a mixture of angiotensin 1, substance P, and ACTH (Sigma). Internal calibration was achieved using the trypsin autolysis products with protonated masses of 842.5 and 2253.12 (modified with *O*-methylisourea).

Data analysis. Films were photographed using a digital camera. Intensities of bands were quantitated using the program NIH Image. Results are expressed as the mean ± SEM, using the Windows-compatible version 2.98 of the program FigP (Biosoft, St. Louis, MO). Statistical analysis was performed using the same program. Data were analyzed by one-way analysis of variance followed by multiple comparisons between the means using the least significant difference test. A probability of $p < 0.05$ was considered to indicate statistical significance.

Results

We and others have previously shown that STZ, an analog of GlcNAc, increases protein O-glycosylation in pancreatic β-cells by blocking *O*-GlcNAcase in these cells, thereby allowing unopposed O-GlcNAcylation of β-cell proteins [15–18]. We have attributed STZ's toxicity to this property of interfering with O-GlcNAc metabolism in β-cells. Since β-cells contain the highest level of OGT of any known tissue [12–14] and appear to express a form of *O*-GlcNAcase with higher affinity for STZ than in the brain [18], they are particularly sensitive to STZ. An even older β-cell-specific toxin is alloxan, a uracil analog (Fig. 1) whose β-cell specificity and antineoplastic properties have not been completely elucidated [25]. Based on the idea that β-cells are particularly sensitive to interference with O-GlcNAc metabolism, we developed the notion that alloxan may also exhibit β-cell toxicity because it interferes with O-GlcNAc metabolism. Since alloxan is structurally related to uracil and there is a uracil moiety in UDP–GlcNAc, the substrate for OGT, the possibility that alloxan interferes with the activity of OGT was investigated.

To determine the effect of alloxan on O-GlcNAc metabolism in β-cells under 'fasting' glucose conditions, isolated islets were incubated for 60 min in 3 mM glucose, 3 mM glucose + 5 mM STZ, or 3 mM glucose + 5 mM alloxan. Then, supernatant was removed and protein O-glycosylation quantitated using RL2 anti-*O*-GlcNAc antibody for immunoprecipitation and subsequent Western blotting of O-glycosylated proteins. Under these conditions, streptozotocin caused a $460.8 \pm 91.5\%$ increase in protein O-glycosylation ($p < 0.05$ vs. control), consistent with our previously reported results [18–20], while alloxan was without significant effect (Fig. 2).

Next, islets were pre-incubated in the presence or absence of 5 mM alloxan and subsequently stimulated with either 15 mM glucosamine or 5 mM STZ, both of

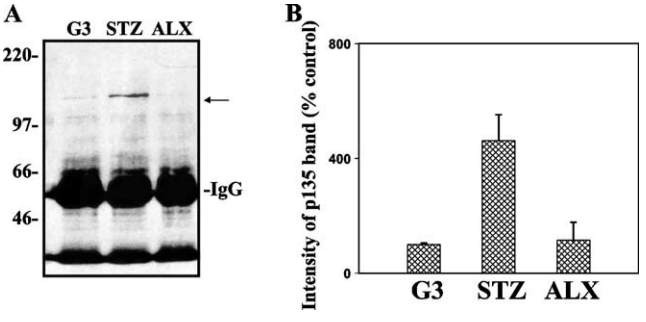

Fig. 2. In contrast to STZ, alloxan does not stimulate O-glycosylation. (A) To determine if alloxan could induce O-glycosylation, islets (100/condition) were incubated for 60 min in 3 mM glucose (G3), 3 mM glucose + 5 mM STZ (STZ), or 3 mM glucose + 5 mM alloxan. At the end of the experiment, supernatant was removed from the tubes, and O-glycosylated proteins were immunoprecipitated with RL2 antibody, separated, and transferred to nitrocellulose for RL2 Western blotting. (B) Data corresponding to (A) in which the intensity of the band is shown as the mean ± SEM from four independent sets of observations from two separate experiments (two independent sets of observations per experiment).

which we have previously shown cause increased O-glycosylation [16]. As Figs. 3 and 4 demonstrate, pre-exposure to alloxan almost completely blocked both STZ and glucosamine-induced O-glycosylation. While STZ increased O-glycosylation by 624.1 ± 173.6% in the absence of alloxan ($p < 0.05$ vs. control), pre-incubation with alloxan reduced STZ-induced O-glycosylation to 169.1 ± 58.6% of control. Likewise, while glucosamine increased O-glycosylation by 319.6 ± 30.8% in the absence of alloxan ($p < 0.05$ vs. control), pre-incubation with alloxan reduced glucosamine-induced O-glycosylation to 105.7 ± 29.9% of control. Together, these data show that alloxan prevents the flux of glucose or glucosamine into *O*-GlcNAc in β-cells. This result is compatible with alloxan blocking the activity of OGT.

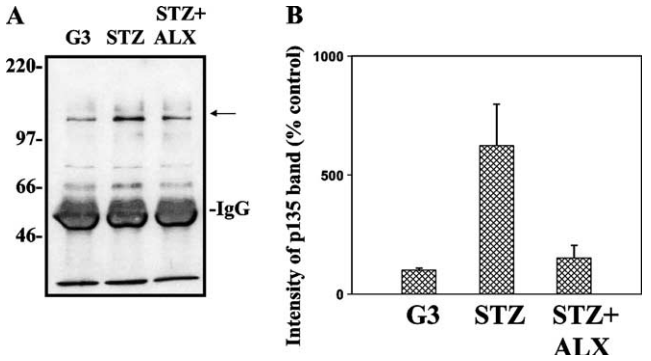

Fig. 3. Alloxan blocks STZ-induced islet O-glycosylation. (A) To determine if alloxan prevents STZ-induced islet O-glycosylation, islets (100/condition) were pre-incubated for 30 min in 3 mM glucose (G3), or 3 mM glucos + 5 mM alloxan. Islets were then incubated for 60 min in 3 mM glucose (G3) or 3 mM glucose + 5 mM STZ (STZ). At the end of the experiment, supernatant was removed from the tubes, and O-glycosylated proteins were analyzed as in Fig. 2. (B) Data corresponding to (A) in which the intensity of the band is shown as the mean ± SEM from eight independent sets of observations from two separate experiments (four independent sets of observations per experiment).

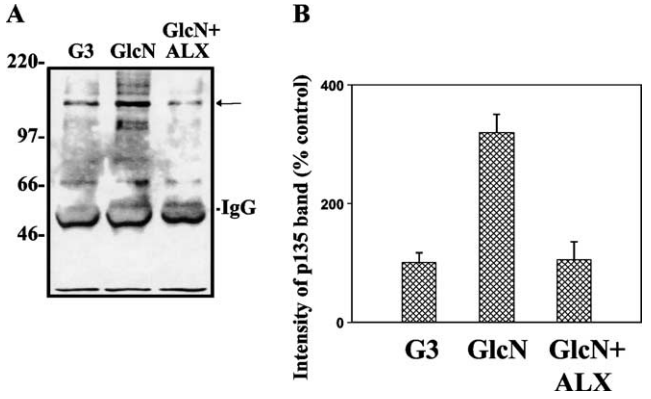

Fig. 4. Alloxan blocks glucosamine-induced O-glycosylation. (A) To determine if alloxan prevents glucosamine-induced islet O-glycosylation, islets (100/condition) were pre-incubated for 30 min in 3 mM glucose (G3), or 3 mM glucose + 5 mM alloxan. Islets were then incubated for 60 min in 3 mM glucose (G3) or 3 mM glucose + 15 mM glucosamine (GlcN). At the end of the experiment, supernatant was removed from the tubes, and O-glycosylated proteins were analyzed as in Fig. 2. (B) Data corresponding to (A) in which the intensity of the band is shown as the mean ± SEM from six independent sets of observations from two separate experiments (two independent sets of observations in one experiment and four in the other).

In order to determine if alloxan is an inhibitor of OGT, recombinant OGT was expressed and treated with the indicated concentrations of alloxan in vitro (Fig. 5). The OGT activity was measured directly by quantitating OGT-catalyzed [H^3]-GlcNAc incorporation into recombinant nucleoporin p62 [24], a substrate of OGT [26]. OGT activity was completely inhibited by 1 mM alloxan with half-maximal inhibition of OGT activity at a concentration of 0.1 mM alloxan. These data thus confirmed that alloxan is indeed an OGT inhibitor.

In light of the previous observation by Lenzen and colleagues [27] that alloxan may block β-cell glucokinase by oxidation of SH groups in the enzyme, we further investigated the mechanism of alloxan-induced OGT

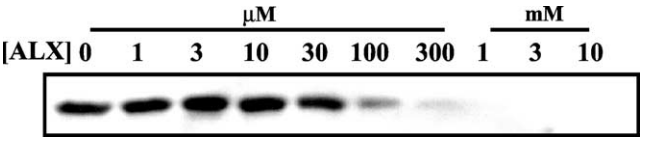

Fig. 5. Alloxan inhibits recombinant OGT activity. Equal quantities of GST–OGT bound to glutathione beads were incubated with the indicated concentrations of alloxan in the presence of UDP–[^{3}H]-GlcNAc and p62 in OGT activity assay buffer (50 M Tris–HCl, pH 7.5, 12.5 mM $MgCl_2$, and 1 mM DTT) at 22 °C for 90 min. The proteins in the assay were separated by SDS–PAGE on a 10% gel, stained with Coomassie blue and the radioactivity incorporated into p62 was observed by fluorography. Equal loading of p62 and GST–OGT was confirmed by Coomassie blue staining (not shown).

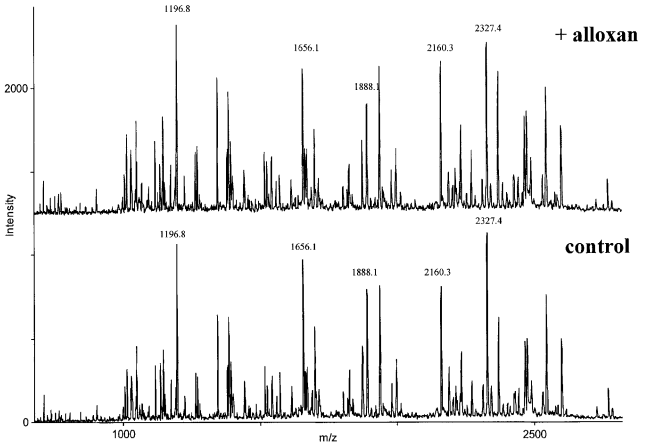

Fig. 6. MALDI-TOF mass spectrometry analysis of recombinant OGT. Recombinant OGT was first incubated in the presence or absence of 5 mM alloxan. Afterward, MALDI-TOF spectra of alloxan-treated and untreated OGT were obtained as described in Materials and methods. Data are representative of two separate experiments.

inhibition. To determine how alloxan inhibits OGT, recombinant OGT was prepared and treated in the presence or absence of 5 mM alloxan. Afterward, MALDI-TOF mass spectrometry analysis of alloxan-treated and untreated OGT was performed in order to find an OGT peptide that was covalently modified by alloxan. Fig. 6 shows the MALDI-TOF spectra of alloxan-treated and untreated OGT. There were no appreciable differences of the two spectra, consistent with the idea that alloxan may exert its inhibition through SH group oxidation rather than by alkylation of the enzyme [27].

Discussion

The above data demonstrate that alloxan is an inhibitor of the enzyme OGT. Whether this mechanism is completely responsible for the β-cell-specific toxicity of this agent is not clear because alloxan might be capable of inhibiting other critical enzymes that are required for β-cell viability. For example, alloxan has also been shown to inhibit the β-cell glucokinase by oxidation of free SH groups in this enzyme [27]. In addition, alloxan, because of its uracil-like structure, might also inhibit other enzymes that recognize uracil moieties. The enzymes involved in N-glycosylation in the endoplasmic reticulum and Golgi that use UDP-sugars as substrates or the enzymes that charge these sugars with UDP might also be targets for alloxan. It is tempting, however, to assign alloxan's β-cell toxicity to its ability to inhibit OGT since O-GlcNAc metabolism in β-cells appears to be particularly important. β-Cells express the highest levels of OGT known in the body [12–14] and interference with this pathway by blocking the *O*-GlcNAcase with STZ does lead to β-cell specific death either by apoptosis [17] or other means [18,21].

On the surface, it appears paradoxical that while excess *O*-GlcNAc, as caused by STZ, kills β-cells, a deficiency of this modification, as might be caused by OGT blockade, also kills these cells. Empirically, this bimodal toxicity is well documented. Not only are β-cells killed by STZ via this pathway, but transgenic *C. elegans* overexpressing OGT are also non-viable [2]. Conversely, mouse embryonic stem cells in which the OGT gene is knocked out are also non-viable [5]. We can rationalize these empiric facts by noting that the O-GlcNAc modification may be involved in cell signaling [28]. Clearly, there is no signal without the dichotomous nature of this modification. Examination of specific protein examples where the O-GlcNAc modification is thought to play a role demonstrates the dichotomous need for both an on and off reaction. In the case of RNA polymerase II, it is believed that the transition of this enzyme from the initiation state of mRNA transcription to the elongation state requires the cycling of the O-GlcNAc modification [29]. For Sp1, we have postulated that the monomeric nascent transcription factor or its storage form must be glycosylated while the multimeric active form bound to DNA must have the modification removed. In these cases the failure to transition from one state to another by the cyclical addition or removal of *O*-GlcNAc would have serious outcomes on the functions of these vital proteins.

Understanding exactly how alloxan inhibits OGT is also complicated by the fact that the structural basis for the manifold activities of OGT has not been completely elucidated. The N-terminal half of the molecule contains multiple tetratricopetide repeats (TPR), which vary depending on the species [1–4]. This domain arose early in eukaryotic evolution and in yeast functions as a transcriptional repressor in a manner independent of the glycosyl transferase enzymatic activity (SSN6-Tup1) [30,31]. It was our hope that alloxan might modify OGT at the UDP–GlcNAc binding site so that we might be able to identify in the C-terminal region of the protein the sequence of the enzyme that recognizes the uracil moiety in UDP–GlcNAc. Our MALDI-TOF data, however, are inconclusive as to how alloxan actually inhibits OGT. There were no appreciable differences in the spectra of alloxan-treated and untreated OGT. This finding suggests that alloxan may inhibit OGT via SH group oxidation rather than alkylation, consistent with the mechanism proposed by Lenzen and colleagues [27].

In many ways, the field of protein O-glycosylation is in its infancy. OGT itself was first cloned and characterized only a few years ago [1–5], and *O*-GlcNAcase, the enzyme responsible for removing *O*-GlcNAc from protein was cloned and characterized only last year [32,33]. Our past and present data provide additional insight into this emerging pathway and suggest that it may be involved in both alloxan and streptozotocin-induced β-cell toxicity as well as contributing to β-cell glucotoxicity. Furthermore, our description of alloxan as the first reported OGT inhibitor provides a means by which to further investigate OGT structure–function relationships.

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