Synthesis of an organoinsulin molecule that can be activated by antibody catalysis

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We have developed a methodology of prodrug delivery by using a modified insulin species whose biological activity potentially can be regulated *in vivo*. Native insulin was derivatized with aldol-terminated chemical modifications that can be selectively removed by the catalytic aldolase antibody 38C2 under physiologic conditions. The derivatized organoinsulin (insulin^D) was defective with respect to receptor binding and stimulation of glucose transport. The affinity of insulin^D for the insulin receptor was reduced by 90% in binding studies using intact cells. The ability of insulin^D to stimulate glucose transport was reduced by 96% in 3T3-L1 adipocytes and by 55% in conscious rats. Incubation of insulin^D with the catalytic aldolase antibody 38C2 cleaved all of the aldol-terminated modifications, restoring native insulin. Treatment of insulin^D with 38C2 also restored insulin^D's receptor binding and glucose transport-stimulating activities *in vitro*, as well as its ability to lower glucose levels in animals *in vivo*. We propose that these results are the foundation for an *in vivo* regulated system of insulin activation using the prohormone insulin^D and catalytic antibody 38C2 with potential therapeutic application.

A number of examples exist in which pharmaceutically active compounds are administered as prodrugs. The prodrug has little or no biologic activity but is converted to an active compound *in vivo*. The site of activation can be extra- or intracellular and typically involves enzymatic cleavage of the prodrug to yield the active agent. Shabat *et al.* (1) have reported a system in which a catalytic antibody is used to convert a prodrug into an active compound. The process utilizes the catalytic aldolase antibody 38C2 to specifically recognize and cleave aldol-terminated linkers in the prodrug. The aldol-terminated linkers functionally mask the drug until they are cleaved by the aldolase antibody, regenerating the original drug molecule and restoring its biologic activity. There is no known enzyme that can mimic the catalytic action of the aldolase antibody 38C2, and the background rate of linker hydrolysis is negligible. Thus, unmasking and activation of aldol-derivatized prodrugs can be catalyzed only by 38C2. This strategy has been used successfully with the anticancer drugs doxorubicin, camptothecin (1), and etoposide (2); the prodrugs of these agents were therapeutically activated by the catalytic antibody. A previous study (1) also has shown that antibody 38C2 is stable after *in vivo* administration in mice ($t_{1/2} = \approx 12$ days).

It is now well established that in patients with diabetes mellitus, the degree of hyperglycemia is directly proportional to the incidence and severity of diabetic complications (3, 4). Therefore, excellent glycemic control is the goal of antidiabetic therapy, and exogenous insulin is the mainstay of therapy in all type 1 diabetes patients, as well as in many patients with type 2 diabetes. Insulin is available in short-, medium-, and long-acting preparations, and various mixtures and combinations are used in the clinical setting. Basal insulin replacement, particularly in type 1 diabetes, has been shown to have important therapeutic benefits, contributing substantially to the attainment of glycemic control. Unfortunately, basal insulin must be provided by continuous s.c. insulin administration through an insulin pump, and this procedure is both costly and often poorly accepted by patients. These issues have spurred an effort to design slow-release, long-acting insulin formulations that could be used to simulate basal insulin replacement. However, no suitable preparation is yet available.

In the current study, we have analyzed the functional properties of native insulin compared with an aldol-derivatized organoinsulin prohormone before and after incubation with the catalytic aldolase antibody 38C2. Derivatized organoinsulin (insulin^D) exhibits markedly diminished receptor binding and biologic activity both *in vitro* and *in vivo*. When the insulin^D is incubated with 38C2, native insulin is regenerated that displays restored receptor-binding affinity as well as normal *in vitro* and *in vivo* biologic activity. These facts raise the possibility that an aldol-derivatized insulin prodrug in combination with the use of a catalytic antibody may be useful in the treatment of diabetes mellitus. Generation of a prodrug form of a peptide may represent a new avenue of therapeutics, because previous prodrug efforts have focused largely on small organic molecules.

Materials and Methods

Cell Lines and Materials. Rat 1 fibroblasts stably expressing the human insulin receptor (HIRcB cells) were grown in DMEM-Ham's F-12, 2 mM Glutamax (both from Life Technologies, Rockville, MD), 10% (vol/vol) FCS, 0.5% Gentimicin (both from Omega Scientific, Tarzana, CA), and 500 nM methotrexate (Sigma). 3T3-L1 adipocytes were differentiated and maintained as described (5). Human insulin and ^{125}I human insulin were gifts from Lilly Research Laboratories (Indianapolis). Tetramethylrhodamine B isothiocyanate (TRITC)-conjugated phalloidin was obtained from Sigma.

Derivatization of Insulin. The generic amine-masking linker (Fig. 1*A*, compound 1) was synthesized as described (1). Seventy-two milligrams (12 μmol) of recombinant human insulin (Sigma) were dissolved in 3.0 ml of reagent grade DMSO (Aldrich). To this mixture was added 500 μl of compound 1 (0.4 M in ethanol, three equivalents with respect to primary amines). This mixture was allowed to stir overnight.

Isolation and Characterization of Individual Species of Modified Insulin. The organoinsulin preparation was characterized by matrix-assisted laser desorption ionization–time of flight (MALDI-ToF) mass spectrometry using α-cyanohydroxycinnamic acid as the matrix and found to be a mixture of modified

Abbreviations: insulin^D, derivatized organoinsulin; MALDI-ToF, matrix-assisted laser desorption ionization–time of flight.

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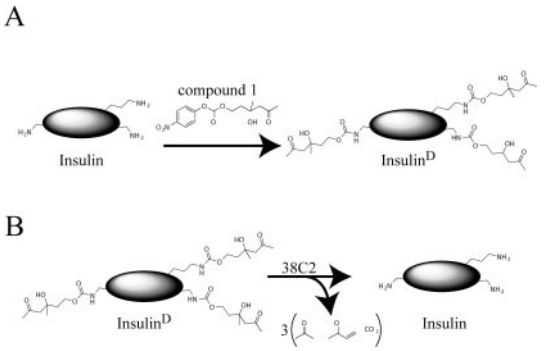

Fig. 1. Derivatization of the primary amines by the masking linker. (*A*) Structure of the aldol-terminated masking linker (compound 1) and modification of the primary amines on insulin are shown schematically. (*B*) Schematic of the catalytic removal of an aldol-terminated protein modification by the aldolase antibody 38C2.

insulins, with each peptide bearing between two and six masking groups (Fig. 2*B*). To determine the biological activity of a single insulin^D, the mixture of prepared insulin species was separated with analytical scale reverse-phase HPLC (Solvent A, 0.1% TFA in H_2O; solvent B, 0.08% TFA in CH_3CN; Fig. 2*C*, with a gradient of 0–100% solvent B over 50 min on a 300A C18 column). This gradient was used on a preparative scale, and fractions were collected for the six most abundant species. The purity of each fraction was determined by analytical HPLC. The collected fractions were lyophilized and resuspended in 5.0 ml of 0.01 N HCl. The concentration of each fraction was determined spectrophotometrically and confirmed by both bicinchoninic acid assay (Pierce) and integration of the HPLC trace at 280 nm. A 20-μl aliquot of each fraction was desalted with ZipTips (Millipore) and characterized by MALDI-ToF mass spectrometry. The most abundant fraction recovered contained 54% of the total insulin used and was found to be the Tris-modified species, insulin^D (Fig. 2 *D* and *E*).

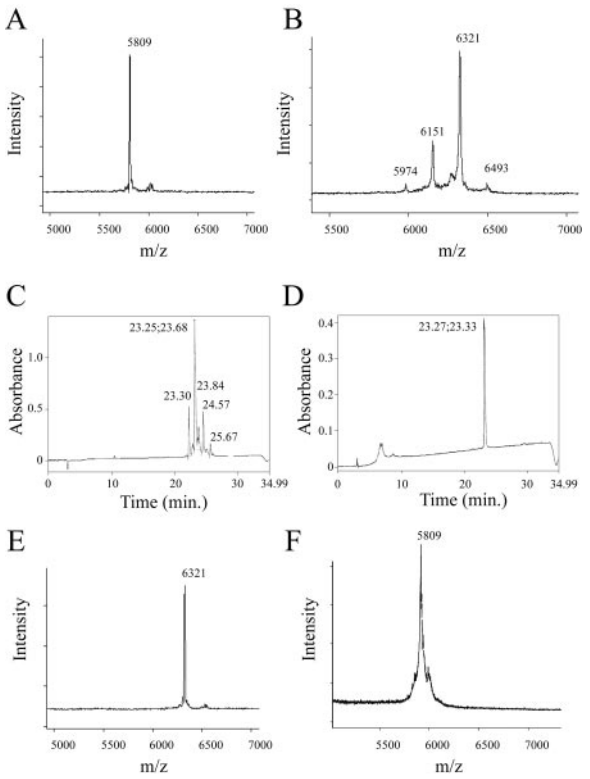

Fig. 2. Physical analysis of insulin/insulin^D preparations. (*A*) MALDI-ToF mass spectrum of insulin [m/z (M + H$^+$) = 5,809 Da]. (*B*) MALDI-ToF mass spectrum of the mixture of insulins produced by chemical addition of compound 1. The peaks are separated by the additive mass of a single linker ($\Delta m/z$ = 172 Da). (*C*) Analytical scale reverse-phase HPLC of the insulin reaction mixture monitored at λ = 280 nm. (*D*) Analytical scale reverse-phase HPLC of the purified insulin species with retention time = 23.29 min (insulin^D). (*E*) MALDI-ToF mass spectrum of insulin^D indicating this species bears three linkers [m/z (M + H$^+$) = 6,325 Da]. (*F*) MALDI-ToF mass spectrum of insulin^D after 72-h incubation with antibody 38C2 [m/z (M + H$^+$) = 5,809 Da]. The linkers are quantitatively removed after this treatment.

Characterization of Aldol-Derivatization Sites on Insulin. A series of chemical and enzymatic assays were performed on the isolated material to determine the location of the three linkers on insulin^D. Because the linker is added as an activated carbonate, only nucleophilic groups on the peptide can react. Although insulin contains six cysteines, they are involved in disulfide bonds and are not accessible to modification. The remaining sites for modification, then, are the amines (N termini and the ε-amino group of Lys) and alcohols (Ser, Thr, and Tyr).

First, a series of thermostability tests were performed that showed a population containing 3–8× modified insulin would revert to a Tris-modified insulin upon incubation at 37°C for 1 week. Further incubation of this material or insulin^D did not result in additional linker loss. The remaining modifications were expected to be on the amines because carbamate linkages are significantly more resistant to hydrolysis than carbonates. Second, reduction of insulin^D with DTT followed by alkylation with iodoacetamide MALDI-ToF mass spectrometry revealed the presence of a single linker on the A-chain and two linkers on the B-chain. Third, digestion of insulin with carboxypeptidase Y reveals a sequence ladder of the seven C-terminal amino acids on insulin's B-chain. MALDI-ToF mass spectrometry revealed that a single, thermo-stable linker was attached to the C-terminal tetrapeptide, TPKT. The A chain of insulin is not a substrate for carboxylpeptidase Y because of the extensive disulfide bonds between the two chains. Finally, the Tris-modified insulin was not accessible to modification by fluorescein isothiocyanate. This fact indicates that insulin's three amines were blocked. Edman degradation chemistry cleaved all linkers from insulin, and, thus, we were unable to demonstrate conclusively the presence of the linker on both of insulin's N termini.

Incubations with Catalytic Antibody 38C2. Native insulin or insulin^D (17.2 μM in PBS with 0.3% BSA) was incubated either alone or in the presence of catalytic antibody 38C2 (73 μM) at 37°C for 72 h (Fig. 2*F*). Preparations were kept at 4°C after incubations.

Binding Studies. HIRcB cells were grown to ≈25% confluency. Cells were rinsed two times with cold Hepes/Salts buffer (10 mM Hepes, pH 7.4/2.5 mM NaH_2PO_4/130 mM NaCl/4.7 mM KCl/1.2 mM $MgSO_4$/2.5 mM $CaCl_2$) with 1% BSA. Cells were incubated for 6 h at 12°C in the presence of 0.2 ng/ml [^{125}I]insulin tracer and varying concentrations of each insulin preparation, as indicated. After the binding incubation, cells were rinsed three times with ice-cold PBS and lysed with 1 N NaOH. Binding of the [^{125}I]insulin tracer was calculated by the counts per min measured in each lysate and is expressed as a percentage of total counts bound in the absence of cold insulin. Specific binding was determined by subtracting the cpms bound to cells incubated with tracer and 10 μg/ml cold insulin (nonspecific counts).

2-Deoxyglucose Uptake in 3T3-L1 Adipocytes. At 10 days after differentiation, 3T3-L1 adipocytes were stimulated with varying concentrations of each insulin preparation for 20 min at 37°C. Glucose transport was determined by the addition of 0.1 mM

2-deoxyglucose containing 0.2 μCi of 2-[^{3}H]deoxyglucose, as described (5). Nonspecific uptake was assessed with 0.1 mM L-glucose containing 0.2 μCi of L-[^{3}H]glucose. The reaction was stopped after 10 min by aspiration, and extracellular glucose was removed by four washes with ice-cold PBS. Cells were lysed with 1 N NaOH, and glucose uptake was quantitated by scintillation counting. Samples were normalized for protein content by Bio-Rad protein assay.

Animals. Male Wistar rats (Simonsen, Gilroy, CA) weighing 307 ± 7 g were received at 12 weeks of age and housed individually under controlled light (12-h light/12-h dark cycle) and temperature conditions. Animals had access to food and water *ad libitum*. All procedures were performed in accordance with the Guide for Care and Use of Laboratory Animals of the National Institutes of Health, and were approved by the University of California, San Diego Animal Subjects Committee.

Animal Study Design. To test the ability of insulin^D to lower arterial glucose concentration, rats were randomly divided into three experimental groups, and subjected to an insulin-toleranced test using one of three insulin preparations: control, native insulin (n = 5), insulin^D (n = 5), or insulin^D + antibody 38C2 (n = 6).

Surgery and Insulin Tolerance Test Procedure. Several days after receiving the animals to the vivarium, rats were chronically cannulated under single-dose anesthesia (42 mg of ketamine HCl per kg of body weight, 5 mg of xylazine per kg of body weight, and 0.75 mg of acepromazine maleate per kg of body weight, administered i.m.). A cannula (Intramedic polyethylene tubing PE-50, Clay Adams) was placed in the carotid artery for insulin injection and arterial blood sampling. The cannula was tunneled s.c., exteriorized at the back of the neck, encased in silastic tubing (0.2 cm i.d.), and sutured to the skin. Animals were allowed 3 days of recovery from surgery to regain body weight.

Six hours before the insulin tolerance test, food was withdrawn from the cage. All animals were exposed to the same general insulin-tolerance test protocol. Sixty minutes before the experiment, animals were weighed and placed into a modified metabolic chamber. After 60 min of acclimatization to the metabolic chamber, a basal sample was drawn at 0 min. Subsequent to basal sampling, 300 mU per kg of body weight of either native insulin, insulin^D, or insulin^D pretreated with catalytic aldolase antibody 38C2 was injected slowly into the carotid artery. After insulin injection, the carotid cannula was flushed with 200 μl of heparinized saline (100 units per ml) to ensure proper mixture of insulin with the arterial circulation.

Small blood samples (50 μl) were drawn at 5, 10, 20, and 30 min to assess the rate of decline in blood glucose. Subsequent samples were taken at 45, 60, 90, and 120 min to assess the degree of recovery of blood glucose as compared with the mean basal concentration. All blood samples drawn from the carotid artery were immediately centrifuged and plasma-analyzed for glucose. After the terminal blood sample at 120 min, animals were euthanized with a lethal dose of Nembutal (100 mg per kg of body weight, i.v.). Plasma determination of glucose was assayed by the glucose oxidase method (YSI 2300, Yellow Springs Instruments).

Actin Localization. HIRcB cells were grown on coverslips to ≈25% confluency and serum-starved in DMEM-low glucose + 0.1% BSA for 16 h. Cells were stimulated with the indicated insulin preparations at 60 ng/ml for 20 min at 37°C. Cells were fixed in 3.7% (wt/vol) formaldehyde/PBS for 10 min, permeabilized in 0.1% Triton X-100/PBS for 15 min, and washed two times with PBS. Coverslips were stained with TRITC-phalloidin for 45 min to detect polymerized actin. Coverslips were rinsed with PBS and water and then mounted on slides with Gelvatol. Actin rearrangement was scored per cell by the disappearance of stress fibers. Coverslips were scored blindly; 100–200 cells were scored per random field. Cells were inspected with a Zeiss Axiophot fluorescence microscope (Zeiss).

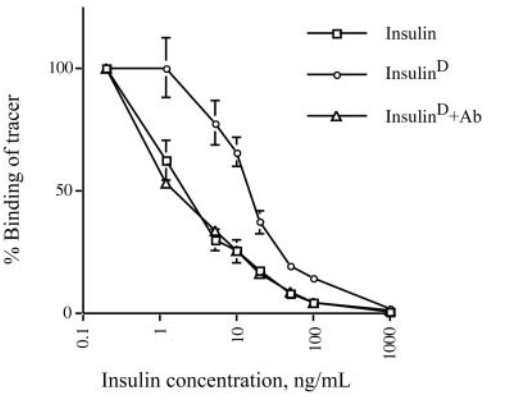

Fig. 3. Ability of insulin/insulin^D preparations to competitively inhibit [^{125}I]insulin binding. HIRcB cells (≈10^5 per sample) were incubated with 0.2 ng/ml [^{125}I]insulin tracer and the indicated concentration of insulin (□), insulin^D (○), insulin^D + antibody 38C2 (△), as described in *Methods*. Data are expressed as the percent of maximal tracer bound in the absence of cold insulins. Data are the average of 4–5 experiments done in duplicate ± SEM.

Results

Derivatization of Native Insulin. Native insulin was subjected to aldol derivatization as described in *Methods*. The derivatizing linker (compound 1) used in our studies reacts principally with the primary amines in a polypeptide to form a carbamate (Fig. 1*A*). The insulin molecule consists of two polypeptide chains and, thus, has two N-terminal primary amines. Insulin has an additional primary amine on Lys at position B29. Derivatization can also occur at hydroxyl-containing residues. Insulin preparations, before and after derivatization, were analyzed by mass spectrometry (Fig. 2 *A* and *B*). The insulin^D consists of a pool of derivatized peptides with between two and six modifications per insulin molecule (Fig. 2*B*). This mixture was amenable to separation by reverse-phase HPLC (Fig. 2*C*), and the purified material (Fig. 2*D*) was found to contain three linkers (Fig. 2*E*). All modifications were removed after 72 h of incubation with catalytic aldolase antibody 38C2, as evidenced by a single mass spectra peak representing the molecular mass of native insulin (Fig. 2*F*).

Insulin^D Exhibits Reduced Affinity for the Insulin Receptor. The insulin receptor-binding affinity of native insulin and insulin^D before and after incubation with catalytic aldolase antibody 38C2 was measured in HIRcB cells (rat 1 fibroblasts that stably express the human insulin receptor). The binding affinity of each preparation was measured by the dose-response displacement of an ^{125}I-labeled insulin tracer; results are shown in Fig. 3. The half-maximal displacement concentration for insulin^D was 16 ng/ml compared with 1.9 ng/ml for native insulin. This result represents a 90% decrease in the receptor-binding affinity of insulin^D. In contrast, when insulin^D was pretreated with antibody 38C2, the ability of the treated insulin^D preparations to bind to insulin receptor was restored to normal.

Defective Stimulation of Glucose Uptake by Insulin^D. To quantitate the ability of insulin^D to initiate signal-transduction pathways in insulin-sensitive cells, we measured two downstream effects of insulin, glucose transport and actin rearrangement. Glucose uptake stimulated by insulin and insulin^D, either before or after

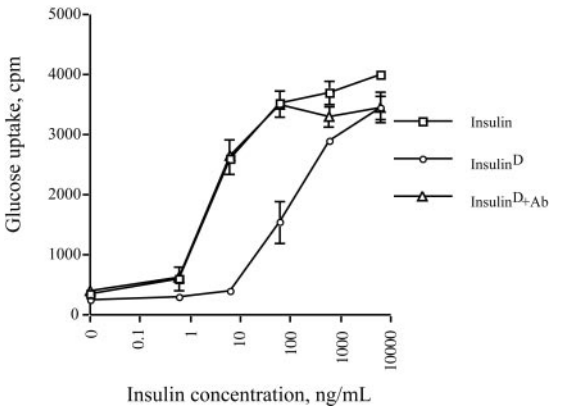

Fig. 4. Glucose uptake stimulated by insulin/insulin^D preparations. 3T3-L1 adipocytes (10 days after differentiation) were stimulated with the indicated insulin preparations for 20 min at 37°C. 2-deoxyglucose uptake was quantitated as described in *Methods*. Each insulin preparation was assayed in a dose-responsive manner: insulin (□), insulin^D (○), insulin^D + antibody 38C2 (△).

incubation with catalytic aldolase antibody 38C2, was measured by using 3T3-L1 adipocytes. As shown in Fig. 4, insulin^D exhibited defective stimulation of glucose transport with a half-maximal effect reached at 120 ng/ml vs. 4.2 ng/ml for native insulin (Fig. 4). This change in half-maximal activity is equivalent to a 96% decrease in glucose-transport sensitivity. Comparable to the results for receptor-binding affinity in HIRcB cells, the effect of 38C2-treated insulin^D to stimulate glucose transport was indistinguishable from native insulin.

Defective Stimulation of Actin Rearrangement by Insulin^D. Binding of native insulin to the insulin receptor initiates a signaling cascade that causes actin rearrangement, e.g., stress fiber breakdown and membrane ruffling (6). Actin rearrangement stimulated by insulin^D was 18% of that stimulated by native insulin (Fig. 5); this defect was reversed by preincubation of insulin^D with 38C2.

Defective Stimulation of Glucose Disposal *in Vivo*. The *in vivo* effectiveness of the different insulin preparations was assessed in male Wistar rats by insulin-tolerance tests, which measure the ability of insulin to induce a drop in blood glucose because of the

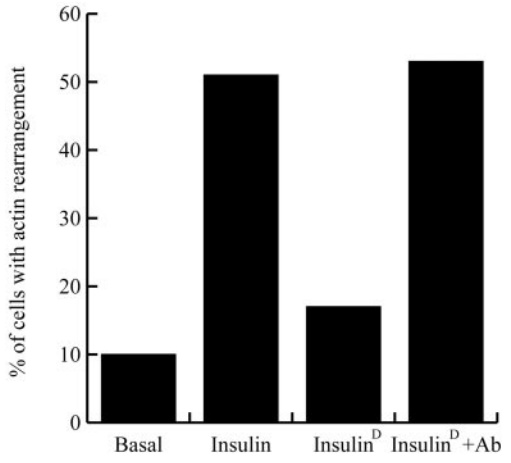

Fig. 5. Actin rearrangement stimulated by insulin/insulin^D. HIRcB cells grown on coverslips were serum-starved for 16 h before stimulation with 60 ng/ml of the indicated insulin preparation for 20 min at 37°C. Cells were fixed, stained, and scored for actin rearrangement, as described in *Methods*.

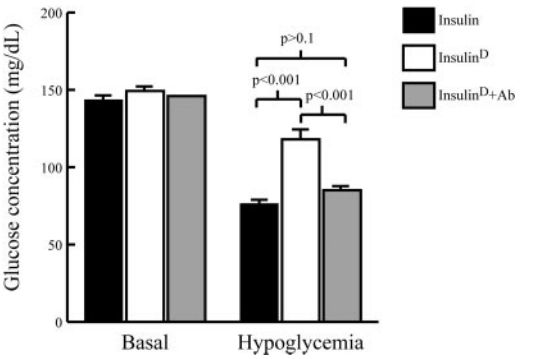

Fig. 6. Insulin/insulin^D tolerance in rats. Male Wistar rats were subjected to insulin-tolerance tests and blood-glucose analyses, as described in *Methods*. Blood-glucose values corresponding to basal and maximal hypoglycemic (10 min after insulin injection) levels are shown.

combined effects of inhibition of hepatic glucose production and stimulation of overall glucose disposal. Blood glucose levels were determined before and after a bolus injection with the indicated insulin preparations, as described in *Methods*. Maximal hypoglycemia occurred at ≈10 min. after injection for each insulin preparation. The results of these studies are shown in Fig. 6. Native insulin injection caused a maximal 47% reduction in blood glucose. In contrast, insulin^D only induced a maximal 21% reduction in blood glucose. Thus, insulin^D was 55% less effective than native insulin. This defect was reversed completely by pretreatment of insulin^D with catalytic aldolase antibody 38C2 before injection.

Discussion

Insulin is the principal hormone controlling glucose homeostasis, and exogenous insulin administration is the mainstay of therapy in all patients with type 1 diabetes and in many with type 2 diabetes. However, current insulin administration modalities are inadequate substitutes for normal insulin secretion by the pancreatic β-cells. An insulin prohormone that has little or no functional activity but can be selectively and fully activated *in vivo* could be a useful therapeutic agent for patients with defective pancreatic insulin secretion. Accordingly, an organoinsulin prohormone—designated insulin^D—was synthesized with aldol-terminated chemical modifications and has only a fraction of the biologic activity of native insulin. Insulin^D regains its full functional activity after incubation with the catalytic aldolase antibody 38C2, which specifically removes the aldol-terminated modifications. We propose that this system to chemically activate insulin by antibody catalysis has potential for therapeutic applications.

The current study describes the structural and functional characteristics of prohormone organoinsulin with aldol-terminated modifications. The design of insulin^D is based on the chemistry of linker-masking of primary amine groups of polypeptides, similar to that described (1). Additionally, the aldol-derivatized insulin^D can be reverted to native insulin by incubation with the specific aldolase-like catalytic antibody 38C2. This antibody has been studied extensively and was originally generated by reactive immunization (1, 7, 8). Catalytic antibody 38C2 was selected for its ability to catalyze a broad range of aldol-based reactions at rates similar to those of endogenous aldolase enzymes and at physiological temperature and pH. Antibody 38C2 has the unique ability to catalyze cleavage of the tertiary aldol linkers (9) on aldol-derivatized insulin^D, as this specific cleavage reaction is not catalyzed by any known endogenous enzyme. The unique catalytic properties of antibody 38C2 (10) and its recent humanization (11) make it an

interesting tool for therapeutic design. A strategy of catalytic antibody 38C2-mediated prodrug therapy has been described (1, 12) as a means of regulating and restricting drug activity to the relevant site of need and preventing drug toxicity elsewhere. In theory, one could administer catalytic antibody, which would persist *in vivo* for many days based on the prolonged 12-day half-life that has been demonstrated. In parallel, one could give insulin^D, which then would be converted to native insulin *in vivo* for a prolonged interval of time. A full understanding of the kinetics of this system along with the administration of appropriate doses of both components could result in slow and sustained *in vivo* production of native insulin, which could mimic endogenous basal insulin secretion.

In 1953, Mills (13) first demonstrated that insulin's biological activity could be attenuated by covalent modification of its primary amines. The introduction of steric bulk alters both the structure and dynamics of insulin's receptor-binding surface. We have shown that binding of insulin^D to the insulin receptor is severely inhibited by the presence of the aldol-terminated modifications. Pretreatment of insulin^D with catalytic aldolase antibody 38C2 regenerates native insulin, as indicated by mass spectroscopy, as well as its full insulin receptor-binding activity. The fact that insulin^D does not bind well to the insulin receptor is significant clinically, because plasma clearance of insulin^D will be decreased and circulating levels of this prohormone should be well maintained until antibody catalysis occurs *in vivo*.

Insulin^D has significantly reduced biological activity, which is reflective of its insulin receptor-binding properties. The ability of insulin^D to initiate two downstream effects of insulin signal transduction in insulin-sensitive cells, namely, glucose transport and actin rearrangement, was studied. Insulin^D exhibited dramatically reduced potency for activating both phenomena. Again, pretreatment of insulin^D with catalytic aldolase antibody 38C2 reversed completely these inhibitory effects of derivatization.

Lastly, the ability of insulin^D to activate glucose metabolism by conducting insulin-tolerance tests in conscious rats was examined. The hypoglycemic response generated by administration of insulin^D was greatly compromised compared with that generated by native insulin. Administration of a preparation of insulin^D that had been pretreated with catalytic aldolase antibody 38C2 led to a hypoglycemic response that was the same as that of native insulin.

We have demonstrated that the catalytic antibody-mediated prodrug strategy incorporating insulin^D and catalytic aldolase antibody 38C2 is a practical method for regulated activation of native insulin. Application of this strategy to *in vivo* studies is possible because circulating serum levels of antibody 38C2 are sustained for a significant amount of time in mice ($t_{1/2} = \approx 12$ days) as is its aldolase activity (1). Importantly, antibody 38C2 catalyzes the conversion of insulin^D to native insulin at physiological temperature and pH, and the recent humanization of this antibody clone could allow its therapeutic administration in combination with insulin^D to be tested (11). These studies illustrate new possibilities to link the vast resources of organic chemistry with protein chemistry. Because organic chemistry is not limited by a genetic code or a specified order of amino acids, as is protein chemistry, one can use organic chemistry to engineer an almost limitless array of modifications into therapeutic proteins. Until now, the utility of such an approach has not been obvious, because it has not been possible to reverse readily these chemical modifications *in vivo*. Here, we show that a catalytic antibody can readily and quantitatively reverse organically modified insulin. The modified organoinsulin has very little biologic activity, but antibody catalysis releases intact, fully active, insulin. This result creates the possibility of introducing chemical modifications into therapeutic proteins, such as insulin, which will alter the half-life, distribution, biologic activity, or other properties in such a way as to achieve a therapeutic advantage when combined with *in vivo* antibody-mediated catalysis. In addition, the rates of catalysis can be controlled over a wide spectrum by altering the amounts of antibody or substrate, or by modifying the organoprotein. For example, one could modify a therapeutic protein to create a very stable formulation that is only slowly susceptible to antibody catalysis, yielding a sustained-release therapeutic. Studies addressing the feasibility of *in vivo* prodrug antibody catalysis will be of very great interest.

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