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Transport studies of insulin across rat jejunum in the presence of chicken and duck ovomucoids

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Abstract

Our aim was to evaluate the transport of insulin across rat jejunum in the presence of ovomucoids and to assess the effect of ovomucoids on intestinal tissue by studying the permeation of a lipophilic and a hydrophilic marker. Rat jejunal segments were mounted in a side-by-side diffusion chamber filled with Krebs bicarbonate buffer, bubbled with 95 % O_2/5 % CO_2 at a fixed flow rate and maintained at 37°C. The permeation of insulin, a lipophilic marker ([7-^{3}H] testosterone) and a hydrophilic marker (D-[1-^{14}C] mannitol) was evaluated in the presence of 0.5–1.5 μM duck ovomucoid (DkOVM) or chicken ovomucoid (CkOVM). For stability and permeation of insulin in the presence of α-chymotrypsin, an enzyme-to-inhibitor ratio of 1:1 and 1:2 was used. In the absence of α-chymotrypsin, the permeability coefficient (P_{app}) of insulin at pH 7.4 was $0.922 \pm 0.168 \times 10^{-7}$ cm s^{-1}, which decreased with increasing concentrations of DkOVM or CkOVM. Conversely, the permeation of the hydrophilic and lipophilic marker increased with increasing concentrations of CkOVM and DkOVM. In stability studies, the percentage of drug remaining was found to be 2-fold higher at the 1:2 ratio than with the 1:1 ratio of enzyme to inhibitor. This was in agreement with the 2-fold increase in flux values of insulin in the presence of α-chymotrypsin and DkOVM at the 1:2 ratio of enzyme to inhibitor. The decrease in permeation of insulin in ovomucoids was unexpected. Marker transport studies indicated that ovomucoids have the potential to modulate transcellular and paracellular permeability. The flux enhancement of insulin in the presence of α-chymotrypsin and DkOVM is encouraging. The use of ovomucoids offers potential to enhance oral delivery of insulin and warrants further investigation.

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Introduction

Enzymatic degradation and epithelial permeability of proteins across the gastrointestinal tract are two important factors that affect their oral bioavailability. Insulin is one of the most widely studied proteins for oral delivery. It has been demonstrated that insulin can be absorbed in the gastrointestinal lumen when protected by appropriate concentrations of enzyme inhibitor (Bendayan et al 1990).

Enzymatic degradation of insulin in the gastrointestinal tract is mediated by α-chymotrypsin and trypsin in the lumen (Ginsburg & Schachman 1960; Young & Carpenter 1961; Schilling & Mitra 1991) and insulin-degrading ezyme (IDE) in the gut wall (Bai & Chang 1996; Chang et al 1997). Consequently, inhibitors of these enzymes have the potential to enhance the oral delivery of insulin. Enzyme inhibitors have been evaluated for oral delivery by in-vitro intestinal studies, in-situ

perfusion and in-vivo bioavailability studies. The reported enhancers have improved delivery of insulin by increasing its stability in the presence of gastrointestinal enzymes or modulating the transport by modification of the cell membrane and junctional integrity. Some examples of inhibitors evaluated include aprotinin, bowman birk inhibitor, trypsin inhibitor, bacitracin, *N*-ethylmaleimide, 1,10-phenanthroline and FK-448 (Fujii et al 1985; Yokoo et al 1988; Morishita et al 1992; Ziv et al 1994; Bai & Chang 1996; Robert et al 1996; Langguth et al 1997).

Ovomucoids represent a new class of enzyme inhibitors derived from the egg white of avian species. Extensive reviews entailing their source, active domains and mechanism of inhibitory action can be found elsewhere (Laskowski & Kato 1960). Briefly, avian ovomucoids are present in egg white of avian species and account for 10% (approx.) of egg-white proteins. Their inhibitory activity is dependent on the species from which they are isolated. They inhibit pancreatic enzymes by binding to the corresponding enzymes through their reactive site. Since they inhibit digestive enzymes such as bovine trypsin and bovine α-chymotrypsin, they might be useful as absorption enhancers for oral delivery of proteins in general, and insulin in particular. Ovomucoids are glycoproteins and contain carbohydrates of the *N*-acetylgalactosamine-Asn type and differ in composition depending on the species (Beeley 1971, 1976; Yamashita et al 1984). The carbohydrate group mediates adsorption to the natural lectins present on the surface of the cell. Applications of ovomucoids include immobilization of enzymes on polymers for use in the adsorption of proteolytic enzymes from blood (Plate et al 1993) and as stationary phase in HPLC columns for chiral recognition (Haginaka et al 1995).

The stability of insulin in the presence of chicken ovomucoid (CkOVM) and duck ovomucoid (DkOVM) against α-chymotrypsin- and trypsin-mediated degradation has been studied in our laboratory (Agarwal et al 2000). The focus of this study was to evaluate the role of ovomucoids as absorption modifiers in the oral delivery of insulin. The first objective was to study the effect of various concentrations of CkOVM and DkOVM on the in-vitro intestinal permeation of insulin. The second was to evaluate the effect of DkOVM and CkOVM on the junctional integrity of cell membranes. This was accomplished by studying the permeation of a lipophilic ([7-^{3}H] testosterone) and a hydrophilic (D-[1-^{14}C] mannitol) marker at various concentrations of DkOVM or CkOVM. The third objective was to study the flux of insulin in the presence of α-chymotrypsin plus DkOVM.

Materials and Methods

Materials

Recombinant human insulin was obtained from the Intergen Company, Purchase, NY. Chicken ovomucoid (Type II-O; CkOVM), D-[1-^{14}C] mannitol (specific activity, 43 mCi mmol^{-1}; radiochemical purity, 99.3%), [1,2,5,7-^{3}H] testosterone (specific activity, 100 Ci mmol^{-1}; radiochemical purity, 95.6%) and α-chymotrypsin (Type VII, TLCK treated) from bovine pancreas were purchased from Sigma Chemical Company (St Louis, MO). Duck ovomucoid (DkOVM) was a sample from Dr Laskowski Jr, Department of Chemistry, Purdue University. All other chemicals were of reagent grade and were used as received.

Isolation of rat jejunal segments

Male Sprague-Dawley rats, 200–300 g, were used for the permeability experiments. The intestine was excised and the jejunum was isolated by a reported method (Asada et al 1995). Briefly, the duodenal and ileal segments were removed from top and bottom (13 cm on either side) and the residual intestine was designated as jejunum. In our study, the central part of the jejunum was used. A side-by-side diffusion apparatus from Trega Biosciences (San Diego, CA) was used. The jejunal segments were mounted without stripping on a pre-heated acrylic half-cell and the cell assembly was then placed in a heated block after joining the other half-cell. The exposed surface area was 1.78 cm^2 and the reservoir volume was 6 mL. The donor and receiver compartments were immediately filled with pre-warmed oxygenated Krebs bicarbonate buffer adjusted to pH 7.4 with NaOH or HCl. The mucosal and serosal buffers consisted of 40 mM mannitol and 40 mM glucose, respectively. Both donor and receiver media had an osmotic pressure of 290–300 mOsm kg^{-1}, verified with an Osmometer (Osmette A, Precision Systems Inc, Natick, MA). Mannitol equalized the osmotic load between the mucosal and apical buffers and glucose helped to maintain tissue viability. The buffer was circulated by a gas lift (95% O_2–5% CO_2). The flow rate of gas lift was adjusted to 10 ± 2 mL min^{-1} using a flow meter (ADM 1000, J&W Scientific, Folsom, CA). The tissues were equilibrated for 10 min before the drug solution was added.

Transport studies

Stock solutions of insulin, inhibitors and enzyme were prepared in mucosal buffer. After equilibration for 10 min, drug and inhibitor solution were added on the

mucosal side so that the final concentration of insulin was 100 μM and that of inhibitors was 0–1.5 μM. For the marker studies, the concentration of mannitol in the donor compartment was $3.5 \times 10^{-5}\mu$M and testosterone was 4×10^{-2} μM. The integrity of the tissues was determined by calculating the permeability coefficient (P_{app}) of mannitol. For α-chymotrypsin studies, the enzyme was added immediately after the addition of drug and inhibitor solution to achieve a final concentration of 0.5 μM. Samples (1 mL) were taken from the serosal side at various times up to 180 min and replaced with fresh transport medium. Samples (10 μL) were taken from the mucosal side at the beginning and end of the experiment and analysed by the HPLC method discussed below. Receiver-compartment insulin was analysed using a solid-phase radioimmunoassay method (Coat A Count, DPC, Los Angeles, CA). The logit–log graph of percent bound vs concentration was used to interpolate values of unknown concentrations. The radioactive samples were analysed using a Beckman LSC6000K liquid scintillation counter.

Stability studies in the presence of duck ovomucoid

Insulin solutions (100 μM) were incubated at 37°C in Krebs bicarbonate buffer. The degradation profiles were generated in the presence of 0.5 μM α-chymotrypsin over a period of 3 h that served as controls. DkOVM was evaluated for its efficiency against enzyme-mediated degradation of insulin at enzyme-to-inhibitor ratios of 1:1 and 1:2. Stability studies were also carried out by incubating insulin with DkOVM in the absence of α-chymotrypsin. Insulin and enzyme solutions were incubated for 15 min at 37°C before starting the experiments. Samples were taken up to 180 min and immediately diluted with cold 1% TFA (trifluoroacetic acid)–TRIS to reduce the pH to 2.5. The samples were analysed by the RP-HPLC method reported below.

Analytical method

Insulin samples from the permeability and stability studies were analysed by an RP-HPLC method. Compounds were separated on a C_{18} Vydac 218MS54 column (4.6 × 250 mm) with a pore size of 300 Å and particle size of 5 μm. The mobile phase consisted of 0.05% v/v TFA–water (A) and 0.05%v/v TFA–acetonitrile (B). The gradient conditions were 27% B for 4 min and 27–36% B for the next 11 min at a flow rate of 1 mL min^{-1}. The detection wavelength was 210 nm. Under these conditions, the retention time of insulin was 10.8 min.

Data analysis

Apparent permeability coefficients (P_{app}) of insulin, D-[1-^{14}C] mannitol and [7-^{3}H] testosterone in the presence and absence of CkOVM, DkOVM and α-chymotrypsin were calculated using equation 1:

$$P_{app} = (1/AC_0)(dM/dt) \quad (1)$$

where dM/dt is the flux across the intestinal membrane (mIU min^{-1} or μCi min^{-1}), A is the surface area of the membrane (1.78 cm^2) and C_0 is the initial drug concentration (100 μM). The results of experiments performed at least in triplicate are presented as mean ± s.e.m. Statistical differences between permeability in the presence of DkOVM and CkOVM and the means were determined by one-way analysis of variance. The criterion for statistical significance was $P < 0.05$.

Results and Discussion

Influence of DkOVM and CkOVM on the jejunal permeability of insulin

The selection of jejunal segments for evaluating permeability of the intestine to insulin was based on a report suggesting that there is only a slight difference in permeability between the jejunum and ileum (Asada et al 1995) plus the fact that the jejunum is the longest, as well as the largest, section of small intestine through which absorption occurs. The concentrations of insulin and inhibitors selected were representative and are comparable with the ratio used in an earlier stability study (Agarwal et al 2000). CkOVM and DkOVM purity was greater than 90% and molecular weights of 27 kD and 28 kD, respectively, were used for all calculations (Rhodes et al 1960). Mannitol flux is a convenient and relatively sensitive measure of the integrity and permeability of the intestinal layer (Marks et al 1991), reflecting the resistance across the tight junctions and not the cell membrane. The P_{app} value of mannitol calculated from rat jejunum was found to be $3.465 \pm 0.251 \times 10^{-6}$ cm s^{-1}. This value is in agreement with values reported using the same apparatus (Grass & Sweetana 1988), suggesting that the integrity of the tissue was maintained throughout the duration of the studies.

Table 1 Permeation of insulin across rat jejunum in the presence of CkOVM and DkOVM at various concentrations

Solution in donor compartment	Permeability coefficient ($P_{app} \times 10^7$ cm s^{-1})	Ratio
Insulin 100 μM	0.922 ± 0.168	1.0
Insulin 100 μM +		
DkOVM 0.5 μM	0.491 ± 0.022	0.54
DkOVM 1.0 μM	0.321 ± 0.089	0.35
DkOVM 1.5 μM	0.066 ± 0.043	0.07
Insulin 100 μM +		
CkOVM 0.5 μM	0.206 ± 0.047	0.22
CkOVM 1.0 μM	0.291 ± 0.095	0.32
CkOVM 1.5 μM	0.018 ± 0.008	0.02

Data shown are mean ± s.e.m. of at least three experiments. The permeability values shown are significantly different from controls at $P < 0.05$.

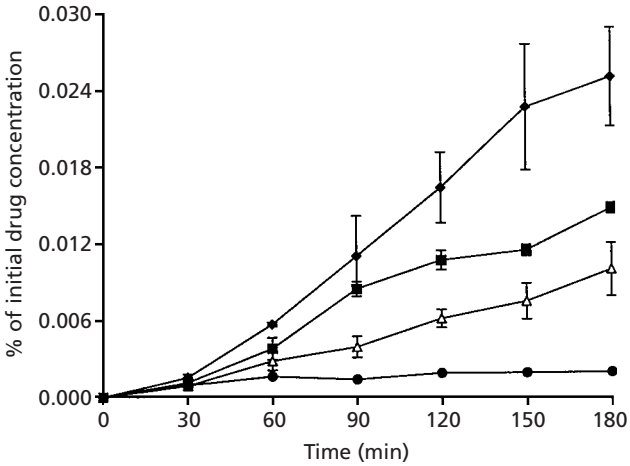

Figure 1 Cumulative amount (% of dose) of insulin permeating rat jejunum (% of dose) vs time in the absence of DkOVM (◆) and at DkOVM concentrations of 0.5 μM (■), 1.0 μM (△) and 1.5 μM (●), respectively. Data represents mean ± s.e.m., n = 3.

The P_{app} calculated for insulin, under conditions identical to those in the mannitol study, was 0.922 ± 0.168 × 10^{-7} cm s^{-1} (Table 1). This is less than the values of 5 ± 2 × 10^{-7} cm s^{-1} (Schilling & Mitra 1990) and 12.27 ± 1.73 × 10^{-7} cm s^{-1} (Asada et al 1995) reported for P_{app} of insulin from rat jejunum. A recent study estimated the apical epithelial permeability of insulin to be 0.32 × 10^{-7} cm s^{-1} (Stoll et al 2000). However, the value obtained in our laboratory is used as reference for the evaluation of insulin permeation in the presence of ovomucoids. The variation in permeability coefficients may be attributed to differences in apparatus, tissue preparation, concentrations studied, analytical method employed and the duration of study. The transport of insulin across rat jejunum decreased in the presence of both the inhibitors (Figure 1, Table 1). The P_{app} of insulin in the presence of DkOVM decreased in a concentration-dependent manner (Figure 1). With 1.5 μM DkOVM, the P_{app} was 0.066 ± 0.043 × 10^{-7} cm s^{-1}, representing a substantial decrease when compared with the control value for insulin (Table 1). The corresponding permeability ratio had actually decreased from 0.54 at 0.5 μM to 0.07 at 1.5 μM DkOVM in insulin solution. Similarly, the permeability ratio decreased from 0.22 to 0.02 when the CkOVM concentration in insulin solution increased from 0.5 to 1.5 μM (Table 1). This study indicates that there are differences in transjejunal permeation of insulin with the type of ovomucoid used (DkOVM vs CkOVM).

Table 2 Permeability coefficients of [7-^{3}H] testosterone and D-[1-^{14}C] mannitol during transport across rat jejunum in the presence of DkOVM and CkOVM

Solutions in donor compartment	[^{3}H] testosterone		[^{14}C] Mannitol	
	Permeability coefficient ($\times 10^6$ cm s^{-1})	Ratio	Permeability coefficient ($\times 10^6$ cm s^{-1})	Ratio
Control	19.750 ± 0.310	1.0	3.465 ± 0.251	1.0
DkOVM 0.5 μM	23.354 ± 1.955	1.18	5.702 ± 0.764	1.65
DkOVM 1.0 μM	26.015 ± 1.341*	1.31	5.958 ± 0.944	1.72
DkOVM 1.5 μM	31.858 ± 1.897*	1.61	8.278 ± 1.321*	2.39
Control	19.750 ± 0.310	1.0	3.465 ± 0.251	1.0
CkOVM 0.5 μM	21.667 ± 0.672	1.1	4.495 ± 0.784	1.30
CKOVM 1.0 μM	23.256 ± 2.623	1.18	6.320 ± 1.100*	1.83
CkOVM 1.5 μM	26.453 ± 2.278*	1.34	11.716 ± 0.284*	3.38

Data shown are mean ± s.e.m. of at least three experiments. *$P < 0.05$ vs controls.

It has been found, using an immunohistochemical method, that insulin is absorbed trans-cellularly from enterocytes (Bendayan et al 1994). The uptake of insulin from hepatocytes and adipocytes is by a receptor-mediated process (Sonne 1988). The location of receptors for insulin at the enterocyte level has been established (Bergeron et al 1980; Pillion et al 1985; Gingerich et al 1987); reports suggest the presence of insulin receptors on enterocytes at both the apical and basolateral side. A recent report suggests the presence of insulin receptors on the apical side in the small-intestinal region in rats (Saffran et al 1997). The decrease in transport of insulin in the presence of ovomucoids may be explained in part by the action of insulin on adipocytes. It is hypothesized that binding of insulin to the adipocyte plasma membrane activates a membrane protease that results in the formation of a soluble factor that stimulates pyruvate dehydrogenase activity (Seals & Czech 1980, 1981, 1982; Czech et al 1984). This activation is blocked by trypsin-like proteases, such as ovomucoid and soybean trypsin inhibitor, which prevent the interaction of the protease with its endogeneous membrane substrate. If a similar event happened at the junction of enterocyte cells, insulin would not be able to bind to its receptors and get transported. This may be the reason for reduced permeation of insulin at the enterocyte level.

Influence of DkOVM and CkOVM on permeation of mannitol and testosterone

Markers have been used to assess cell damage by absorption enhancers and enzyme inhibitors (Bai et al 1995; Gotoh et al 1996). Parameters such as trans epithelial electrical resistance and transport of progesterone, testosterone, mannitol and phenol red have been used to assess the damage potential of absorption modifiers on cells. The influence of CkOVM and DkOVM on the integrity of cell membranes and junctions was evaluated by studying the permeation of a lipophilic and a hydrophilic marker. The cumulative percentage of testosterone (a lipophilic marker) transported across the rat jejunum increased in the presence of DkOVM and CkOVM in a concentration-dependent manner (Figure 2). The P_{app} value increased by 1.61 fold and 1.34 fold in the presence of 1.5 μM DkOVM and CkOVM, respectively (Table 2). The cumulative percentage of mannitol (a hydrophilic marker) transported also increased in the presence of DkOVM and CkOVM in a concentration-dependent manner (Figure 3). The P_{app} value of mannitol increased by 2.39 fold and 3.38 fold in the presence of 1.5 μM DkOVM and CkOVM, respectively (Table 2). Differences in the permeation of testosterone and mannitol were found to be dependent on the type of ovomucoid used (DkOVM vs CkOVM).

The increase in trans-jejunal permeation of the lipophilic and hydrophilic markers indicates that ovomucoids bring about changes in the epithelial cells in a concentration-dependent manner. The changes may be due to increase in the fluidity of the cell membrane and tight junctional integrity between the cells. This would cause the permeation of testosterone and mannitol to increase when compared with control values. The increase in permeability coefficients of testosterone and mannitol may be explained by the role of lectin-type binding to the mucosal surfaces. The binding of poly-

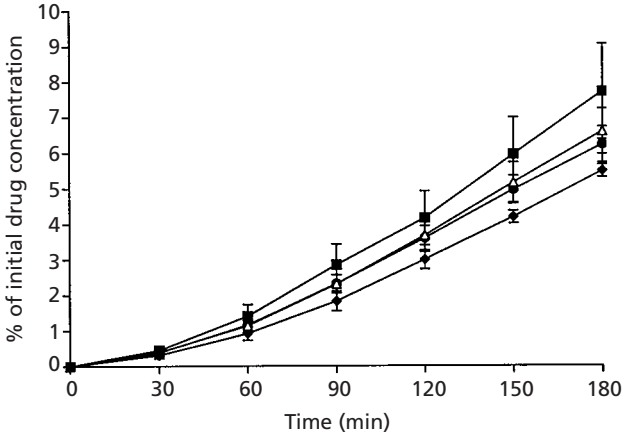

Figure 2 Cumulative amount (% of dose) of [7-^{3}H] testosterone (◆) permeating rat jejunum vs time in the presence of DkOVM at concentrations of 0.5 μM (●), 1.0 μM (△) and 1.5 μM (■), respectively. Data epithelial electrical resistance

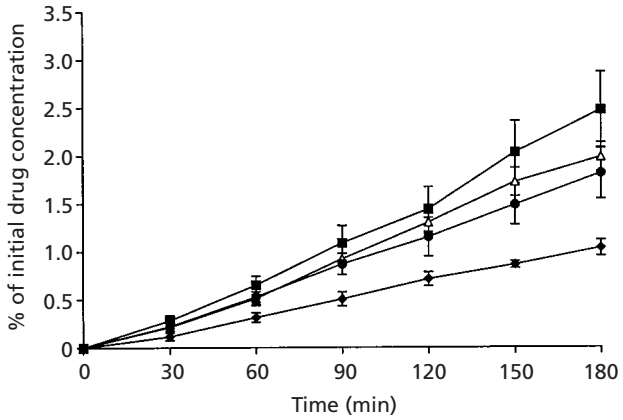

Figure 3 Cumulative amount (% of dose) of D-[1-^{14}C] mannitol (◆) permeating rat jejunum vs time in the presence of DkOVM at concentrations of 0.5 μM (●), 1.0 μM (△) and 1.5 μM (■), respectively. Data represents mean ± s.e.m., n = 3

saccharides to lectins is documented in literature (Rihova et al 1992; Palomino 1994). Damage to cells due to binding of lectins to the bound sugars on the cell membrane has been observed. Lectins present in wheat-germ agglutinin bind to the sugar molecules on the surface of cells and causes damage to the cells (Lorenzsonn & Olsen 1982; Sjolander et al 1986) and kidney-bean lectin affects the function of the entire gastrointestinal tract (Bardocz et al 1995). Ovomucoids have a glycoprotein portion that is assumed to interact with the natural lectins on the mucosal surface of the intestine (Valuev et al 1999). This may also initiate damage to the cells, as does the process of lectin binding to natural sugars.

Insulin stability and permeation in the presence of α-chymotrypsin and DkOVM

Insulin is likely to encounter the degrading effect of luminal enzymes during absorption in-vivo. During the preparation of tissue for mounting on the diffusion chamber, the enzymes are washed off (Yamamoto et al 1994). To simulate the scenario of absorption in the presence of enzymes, the flux of insulin across rat jejunum was also evaluated in the presence of α-chymotrypsin and DkOVM. Brush-border enzymes such as aminopeptidases do not degrade insulin (Schilling & Mitra 1990) and were not evaluated for their effect on stability and transport. Stability studies were performed with DkOVM only as it inhibits both trypsin- and α-chymotrypsin-mediated degradation of insulin, while CkOVM inhibits only trypsin-mediated degradation of insulin (Agarwal et al 2000).

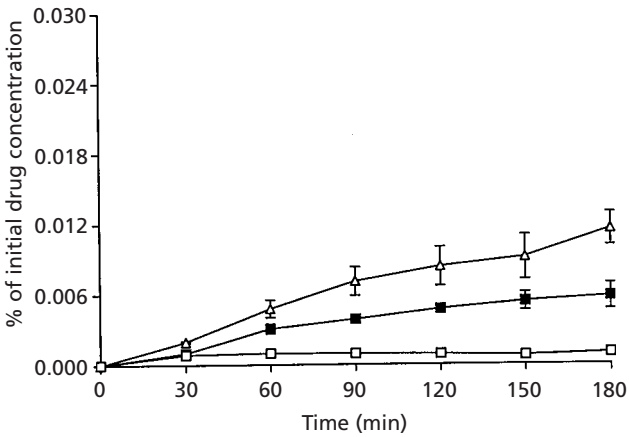

Figure 4 Cumulative amount of insulin permeating rat jejunum (% of dose) vs time in the presence of α-chymotrypsin without (□) or with DkOVM at an enzyme-to-inhibitor ration of 1:1 (■) or 1:2 (△). Data represents mean ± s.e.m., n = 3.

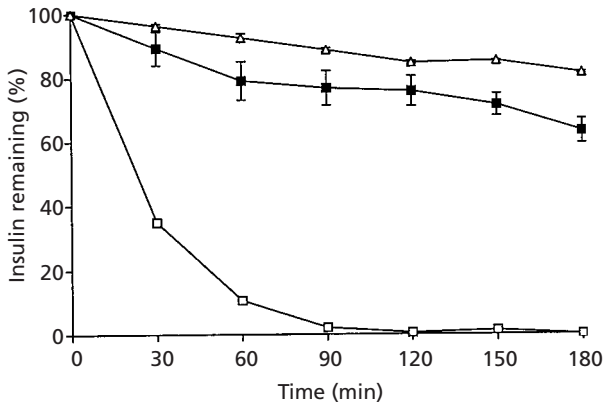

Figure 5 Chymotrypsin-mediated degradation of insulin as a function of time in the absence (□) or presence of DkOVM at an enzyme-to-inhibitor ratio of 1:1 (■) or 1:2 (△). Data represents mean ± s.e.m., n = 3.

For comparison purposes, the flux of insulin was calculated instead of permeability as the concentration of insulin in the donor compartment changed with time due to α-chymotrypsin-mediated degradation. The cumulative percentage of insulin crossing the jejunum in the presence of α-chymotrypsin was found to be negligible (Figure 4). This was expected since there are several reports of insulin degradation being caused by this enzyme. However, the percentage of insulin transported was found to increase as a function of increasing concentration of DkOVM (Figure 4). The flux values of insulin calculated at enzyme-to-inhibitor ratios of 1:1 and 1:2 were, respectively, 1.70 ± 0.46 ng cm^{-2} s and 3.44 ± 0.59 ng cm^{-2} s. The flux value at an enzyme-to-inhibitor ratio of 1:2 was 2-fold higher than at the 1:1 ratio and significantly higher than the control value. Under conditions simulating the donor compartment concentrations with an enzyme-to-inhibitor ratio of 1:1 and 1:2, the percentage of insulin remaining at the end of 3 h was 63.34 ± 3.83 and 81.53 ± 0.34, respectively (Figure 4). Assuming a linear degradation rate of insulin at the 1:2 ratio of enzyme to inhibitor, the rate of degradation was 6.66 % h^{-1}. This was twice as low as the rate of degradation of insulin at the 1:1 ratio (12.22 % h^{-1}). This 2-fold reduction in degradation of insulin could explain the 2-fold enhancement of its flux when the enzyme-to-inhibitor ratio increased from 1:1 to 1:2.

In the presence of α-chymotrypsin and DkOVM, the events that are occurring simultaneously include enzyme-mediated insulin degradation and permeation of insulin across the jejunum. In the absence of DkOVM, there is extensive degradation of insulin. This is evident from the negligible value of insulin (%) remaining at the

end of 3 h in the stability experiments. When DkOVM is added it binds to α-chymotrypsin and slows the degradation of insulin (Figure 5). Consequently, the flux of insulin increases due to the increased amount of insulin in the donor compartment in the presence of the enzyme inhibitor.

Ovomucoids represent attractive absorption modifiers for the oral delivery of proteins due to their inhibitory action towards enzymes present in the gut and binding to natural lectins on the mucosal cells through their carbohydrate moiety. In this investigation it was found that ovomucoids decreased the transjejunal permeation of insulin, increased the permeation of a hydrophilic and a lipophilic marker and increased the permeation of insulin in the presence of α-chymotrypsin.

The decrease in permeation of insulin across the rat jejunum in the presence of ovomucoids was unexpected. It would be interesting to explore the consequences of insulin binding to enterocytes in the presence of ovomucoids, thus allowing direct comparison with the literature on insulin binding to adipocytes in the presence of ovomucoids. Unfortunately this is beyond the scope of our investigation. The steric hindrance of insulin, by the large ovomucoid molecule, is also possible during transport. Such hindrance was not observed when mannitol and testosterone were used as markers. This study has demonstrated the increased flux of insulin across rat jejunum in the presence of α-chymotrypsin and DkOVM. Preparation of an oral dosage form of insulin containing ovomucoids and studies on their bioavailability are in progress.

Conclusions

Oral delivery of insulin has several limitations including slow permeation across the gastrointestinal tract and enzymatic degradation by α-chymotrypsin and other enzymes. For the realistic use of insulin by the oral route, its enzymatic degradation should be reduced in the gastrointestinal lumen to provide higher concentrations for transport and absorption. The use of stability-enhancing agents and absorption modifiers provides the opportunity to overcome these limitations. The two inhibitors evaluated in this study, DkOVM or CkOVM, decreased the permeability of the intestine to insulin when used alone. However, when α-chymotrypsin was used to degrade the insulin, DkOVM slowed down the degradation and enhanced the flux of insulin. Ovomucoids have also shown the ability to modulate the mucosal barrier of the small intestine. This may also be exploited to enhance the absorption of the compound under study. Thus, DkOVM offers potential to enhance the oral bioavailability of insulin.

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