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An Investigation into the Gastrointestinal Stability of Exenatide in the Presence of Pure Enzymes, Everted Intestinal Rings and Intestinal Homogenates

Yanan Sun, Mengshu Wang, Bingxue Sun, Feng Li, Shubo Liu, Yong Zhang, Yan Zhou, Yan Chen, and Wei Kong

*National Engineering Laboratory for AIDS Vaccine, School of Life Sciences, Jilin University; Changchun 130012, China; b Changchun BCHT Biopharm Co., Ltd.; Changchun 130012, China; and c Key Laboratory for Molecular Enzymology and Engineering, the Ministry of Education, School of Life Sciences, Jilin University; Changchun 130012, China.

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The purpose of this study was to investigate the gastrointestinal stability of exenatide to determine the key factor(s) contributing to peptide degradation during the oral delivery process. The effects of pH and various digestive enzymes on the degradation kinetics of exenatide were determined. Moreover, the degradation clearances of peptide were also examined using rat everted intestinal rings and intestinal homogenates from various intestinal locations. Exenatide was comparatively stable within a pH range of 1.2–8. However, obvious degradation was observed in the presence of digestive enzymes. The order of enzymes, in terms of ability to degrade exenatide, was chymotrypsin > aminopeptidase N > carboxypeptidase A > trypsin > pepsin. Chymotrypsin showed the greatest ability to degrade exenatide (half-life $t_{1/2}$, 5.784 × $10^{-2}$h), whereas aminopeptidase N and carboxypeptidase A gave $t_{1/2}$ values of 3.53 and 10.16h, respectively. The degradation of exenatide was found to be peptide concentration- and intestinal site-dependent, with a lower clearance in the upper part of the duodenum and the lower part of the ileum. When using intestinal homogenates as enzyme sources, the order, in terms of peptide degradation ability, was ileum > jejunum > duodenum. However, no significant difference was observed in the remaining peptide concentrations throughout 2h of incubation, which may be due to the involvement of cytosolic enzymes. These results revealed key factors contributing to peptide degradation, and suggest that the inhibition of chymotrypsin and site-specific delivery of exenatide might be advantageous in overcoming metabolic obstacles during its oral delivery.

Key words: exendin-4; enzymatic degradation; gastrointestinal stability; kinetics

Exenatide is a synthetic 39-amino-acid peptide amide with the same amino acid sequence as exendin-4 which was originally isolated from lizard venom, and the sequence of exenatide is shown in Fig. 1. It bears a 53% structural homology to glucagon-like peptide-1 (GLP-1) and can be used as a GLP-1 receptor agonist. In comparison with GLP-1, exenatide has a longer half-life due to its resistance to dipeptidyl peptidase IV degradation, and is mainly degraded and eliminated in the kidney after absorption into the systemic circulation. Exenatide was approved by the U.S. Food and Drug Administration (FDA) for the treatment of type 2 diabetes in 2005. Generally, the medication is administrated subcutaneously twice daily. More recently in 2012, exenatide-loaded extended-release microspheres were approved by the FDA, which allowed it to be injected once a week for the treatment of type 2 diabetes. Although this long-acting medication can significantly reduce the frequency of injections, the therapeutic application of exenatide is still limited due to patient noncompliance related to both the expense associated with injection and pain at the site of injection. Therefore, non-injection routes of administration for exenatide have been the focus of attention over the past several years. Among these, development of an oral dosage form of exenatide remains a particular challenge. Excessive enzymatic degradation in gastrointestinal tracts and poor penetration across the intestinal membrane are proved to be two primary barriers to oral delivery of protein and peptide therapeutics into systemic circulation. A previous study reported that the intraduodenal bioavailability of exenatide was 0.0053% of that by subcutaneous administration in rats. Our recent study revealed a limited apparent permeability coefficient of approximately $1 \times 10^{-7}$ cm/s for exenatide across a Madin Darby canine kidney (MDCK) cell monolayer, an in vitro model commonly used to assess drug intestinal absorption. However, the permeability of exenatide across the MDCK cell monolayer could be significantly improved by 2.2 to 11.9-fold through the addition of appropriate absorption enhancers without apparent cytotoxicity. Besides poor penetration, we hypothesized that enzymatic degradation along with oral delivery probably will also attribute to the abovementioned low bioavailability of exenatide. Thus, the addition of enzyme inhibitors would likely increase the bioavailability of exenatide and improve its oral efficiency. However, no detailed information has been reported on the enzymes involved, enzymatic degradation kinetics and effect of intestinal locations on the degradation of this peptide.

In this study, effects of pH and various digestive enzymes, such as pepsin, trypsin, chymotrypsin, aminopeptidase N and carboxypeptidase A, on the degradation kinetics of exenatide were examined to determine the key factor(s) contributing to peptide degradation during oral delivery process. Moreover, the degradation clearance of exenatide was also examined using rat everted intestinal rings and intestinal homogenates from various intestinal locations. The enzymes we selected are main digestive enzymes existing in gastrointestinal...
They are proved to be the greatest threat to many therapeutic peptides in the lumen of the gastrointestinal tract, which are followed by membrane-bound proteases. Cytoplasmic and lysosomal peptidases could be the third enzymatic barrier when therapeutic peptides is endocytosed by the epithelial cells. In this study, pepsid is secreted from stomach, while chymotrypsin, trypsin, and carboxypeptidase A are secreted from pancreas. Aminopeptidase N belongs to membrane-bound protease. For more membrane-bound proteases and cytoplasmic peptidases, inverted intestinal rings and intestinal homogenates were used as enzyme sources, respectively. This study was intended to provide essential information for improving the gastrointestinal stability of exenatide.

MATERIALS AND METHODS

Materials Exenatide was purchased from GL Biochem Co., Ltd. (Shanghai, China) with purity higher than 95% determined by high-performance liquid chromatography. Immobilized pepsid and immobilized trypsin were purchased from Pierce (Thermo Fisher Scientific, Rockford, IL, U.S.A.). Chymotrypsin from bovine pancreas, aminopeptidase N from porcine kidney, and immobilized carboxypeptidase A from bovine pancreas were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). All other chemical products were commercially available and of analytical grade or higher.

Peptide Degradation in Solutions with Different pH Levels Peptide at the final concentration of 100 μg/mL was incubated with 5mL pre-warmed (37℃) simulated gastric fluid solution (pH 1.2) or acetate buffer solutions or phosphate buffer solutions with different pH values (pH 4.0, 6.0, 8.0) for 4h. During this period, 100μL aliquots were taken every hour from the incubated solution. Subsequently, a 100μL aliquot of simulated gastric fluid solution or buffer solution was added to the incubation medium. Intact peptide concentrations in the aliquots were determined using a modified method as described by Kehler et al. The mobile phase consisted of a mixture of solvent A and solvent B (25:75, v/v): solvent A was a mixture of 5% (v/v) acetonitrile and 95% (v/v) of 0.1 M monopotassium phosphate (pH 2.0), and solvent B was a mixture of 40% (v/v) acetonitrile and 60% (v/v) of 0.1 M monopotassium phosphate (pH 2.0). The chromatography column was a Waters C4 column (4.6×150 mm, 3.5μm, Waters) and the temperature of the thermostatted column compartment was kept constant at 40℃. The flow rate was 1.0 mL/min, and detection wavelength was set up at 214 nm.

Peptide Degradation with Pure Enzymes Immobilized pepsid was suspended in 5mL pre-warmed (37℃) simulated gastric fluid solution (pH 1.2) prepared according to the Chinese Pharmacopoeia (2010). Immobilized trypsin, chymotrypsin, aminopeptidase N and immobilized carboxypeptidase A were suspended in 5mL pre-warmed (37℃) simulated intestinal fluid buffer (pH 6.8) prepared according to the Chinese Pharmacopoeia (2010). After a 30-min enzymatic activation period, peptide was added to the solution at the final concentration of 23.88 nmol/mL. At specified time intervals after the addition of peptide, 100μL aliquots were taken from the incubated solution, followed by centrifugation to remove the immobilized enzyme or dilution with an equal volume of ice-cold 1 M perchloric acid to stop the degradation by non-immobilized enzymes. Subsequently, a 100μL aliquot of simulated gastric or intestinal fluid solution was added to the incubation medium. Intact exenatide concentrations in the aliquots were determined using the method as described above after centrifugation. Each enzymatic degradation experiment was performed at least three times. The successful separation of intact peptide with its metabolites using above methods was further confirmed using a 5800 MALDI-TOF/TOF mass spectrometer (ABSciex, Foster City, CA, U.S.A.). For each sample, 2μL sample of separated intact proteins was spotted on the MALDI plate. After drying, 2μL of 5mg/mL of α-cyano-4-hydroxycinnamic acid solubilized in 50% acetonitrile and 0.1% trifluoroacetic acid were added. Spotted samples were submitted for data acquisition at a collision energy of 1eV with collision gas air at a pressure of about 1×10⁻⁶ Torr.

Peptide Degradation with Everted Intestinal Rings Wi-star male rats, weighing 230–260g, were obtained from the Experimental Animal Center of Norman Bethune College of Medicine (Jilin University, Changchun, China). The rats were maintained in accordance with the guidelines of the Animal Use Committee of Jilin University for the use and care of laboratory animals. Rats were housed in groups of 6–8 under a 12-h light/dark cycle and allowed food and water ad libitum. Everted intestinal rings were prepared as previously described. Briefly, male rats were fasted overnight (approximately 18h) before the experiment but had free access to water. After each rat was anesthetized with 1% pentobarbital sodium (4.0 mL/kg, intraperitoneal injection), a midline laparotomy was performed to expose the abdomen. The intestinal segments of interest were identified as the duodenum, jejunum and ileum as described by Bai. Each segment was further cut into the upper, middle and lower section each with a length of ca. 1 cm. The intestinal segments were immediately placed in pre-warmed (37℃) Krebs phosphate buffer (1.2 mM magnesium sulfate, 1.3 mM calcium chloride, 4.8 mM potassium chloride, 16.5 mM sodium phosphate, 120.8 mM sodium chloride, adjusted to pH 7.0 with hydrochloric acid) and carefully everted using a glass rod, and the outer connective tissue and inner food debris were removed by careful stirring. The everted intestinal rings were kept in 37℃ Krebs phosphate buffer containing exenatide at different concentrations (25, 50, 100 μg/mL). At specified time intervals after the addition of peptide, 100μL aliquots were taken from the incubated solution, centrifuged and diluted with an equal volume of ice-cold 1 M perchloric acid to stop the enzymatic degradation. After the removal of each sample, a 100μL aliquot of Krebs phosphate buffer was added to the incubation medium to maintain a constant volume. Intact peptide concentrations in the aliquots were determined in the same manner as for the above enzymatic degradation experiment. Each degradation experi-

Fig. 1. The Amino Acid Sequence of Exenatide
ment was performed at least three times.

Peptide Degradation in Intestinal Homogenates Intestinal segments of the duodenum, jejunum and ileum were identified and isolated as above, and then homogenates were prepared according to our previous report.\textsuperscript{13) Briefly, after washing in ice-cold saline, the intestinal tissue with wet weight of 70–80 mg was cut into small pieces and placed in 5 mL of phosphate buffer solution (pH 7.4) for processing in a tissue homogenizer in an ice bath. The homogenate was centrifuged at 10000 rpm for 10 min at 4°C, and the supernatant was collected. The total protein concentration in the supernatant was measured by ultraviolet spectroscopy at 280 nm. Supernatants of the intestinal tissue homogenates were further diluted to the appropriate concentration as necessary and incubated with peptide at the final concentration of 100 \(\mu\)g/mL at 37°C. At specified time intervals after the addition of peptide, 100 \(\mu\)L aliquots were taken from the incubated solution and diluted with an equal volume of ice-cold 80% acetonitrile aqueous solution to stop the enzymatic degradation. The solution was then centrifuged to remove cellular debris. After each sample was taken, a 100 \(\mu\)L aliquot of supernatant from the corresponding intestinal tissue homogenate was added to the incubation medium to maintain a constant volume. Intact peptide concentrations in the aliquots were determined as above. Each degradation experiment was performed at least three times.

Calculation of Degradation Clearances and Degradation Rate Constant The degradation clearance of peptide in the presence of pure enzymes, everted intestinal rings or intestinal homogenates was calculated from the total metabolized peptide amount ($\Sigma M$) in the incubation medium at various time points and the area under the peptide concentration–time curve ($AUC$) according to the relationship\textsuperscript{22,23)}, 

\[
\Sigma M = CL_{\text{deg}} \times AUC,
\]

where $CL_{\text{deg}}$ was calculated by linear regression analysis from plots of $\Sigma M$ versus $AUC$ at different time points using Origin software Version 6.5. The total metabolized peptide amount ($\Sigma M$) at various time points can be obtained by subtracting the amount of remained intact peptide (determined as above) in the incubation medium at various time points from the initial total peptide amount. In addition, the degradation patterns of exenatide under different experimental conditions were also analyzed through curve-fitting to obtain their degradation rate constant $k$ and half-life $t_{1/2}$. It should be noted that, if the peptide degradation follows first-order kinetics (that is, a constant fraction of peptide is degraded per unit time), the degradation clearance $CL_{\text{deg}}$ would be constant and positively correlated with the degradation rate constant $k$.\textsuperscript{24) Their relationship could be described by the equation: 

\[
CL_{\text{deg}} = V \times k,
\]

where $V$ is volume of peptide distribution. In this case, degradation clearance and degradation rate constant $k$ (and thus half-life $t_{1/2}$) are coordinative for further analysis.

Statistical Analysis Results were calculated as the mean±standard deviation (S.D.). Statistical comparisons were performed using the two-tailed unpaired Student’s $t$-test for independent data, and a $p$-value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Effects of pH on Exenatide Stability Luminal pH has been reported to be site-dependent and gradually increase along the intestine with a range from 1.1 to 8.0.\textsuperscript{25,26} Generally, gastric pH tends to be acidic, whereas pH in the distal small intestine is slightly alkaline. The changes in gastrointestinal pH may affect peptide solubility and stability, which in turn influences its bioavailability. Due to the hydrophilic characteristics of exenatide, the solubility is not expected to be the factor undermining its bioavailability. Therefore, the effect of pH on peptide stability was investigated to reveal its contribution to the degradation of exenatide. Effects of different pH values on the stability of exenatide for different incubation conditions were studied in simulated gastric liquid solutions (pH 1.2), acetate buffer solutions and phosphate buffer solutions with different pH values (4.0, 6.0, 8.0) during different incubation times. Data were presented as the mean±S.D., $n=3$.
tion times are shown in Fig. 2. Among these, percentages of the residual concentration of exenatide in solutions of pH 1.2 and 8.0 were slightly lower than those in solutions with the pH of 4.0 and 6.0 at all sampling points, indicating that the peptide was comparatively susceptible to acidic and alkaline environments. In addition, exenatide incubated with phosphate buffer solution with the pH of 6.0 remained at the highest concentration at all sampling points. However, no statistically significant difference in residual exenatide concentration was observed between any of the tested conditions. More than 95% of the original amount of exenatide remained in all solutions with different pH values during the 4-h incubation, suggesting that exenatide was comparatively stable within a pH range of 1.2–8.

Effects of Pure Enzymes on Exenatide Stability Besides luminal pH, numerous digestive enzymes present in the gastrointestinal tract can be great challenges for increasing peptide oral efficiency. Currently, no related study has been reported on exenatide in terms of the involved enzymes and enzymatic degradation kinetics. Such information hopefully would inform us on what kind of inhibitors is required and how to improve peptide stability during oral delivery of exenatide. For these reasons, the degradation kinetics of exenatide in the presence of various digestive enzymes were determined. The results are shown in Fig. 3. It should be noted that, in the present study, peptide degradation by dipeptidyl peptidase IV was not investigated because the resistance of exenatide to degradation by that enzyme has been demonstrated in previous reports.

In all experiments, obvious degradation of exenatide was observed when incubated with pepsin, trypsin, chymotrypsin, aminopeptidase N and carboxypeptidase A. Chymotrypsin was found to have the greatest ability among all enzymes to digest exenatide. The order of enzymes in terms of degradation ability to exenatide was chymotrypsin>aminopeptidase N>carboxypeptidase A>trypsin>pepsin. In order to further obtain quantitative information, $t_{1/2}$ values (half-life) were also extracted through curve-fitting, and the results are summarized in Table 1. The degradation pattern of exenatide in the presence of various enzymes followed pseudo first-order kinetics except the one with 0.2 U/mL pepsin, which can be described by the following equation: $\log C_t = \log C_0 - kt/2.303$, where $C_t$ is the concentration of the intact peptide (nmol/mL) at the incubation time $t$ (h), $C_0$ is the initial concentration of peptide, and $k$ is the pseudo-first-order rate constant. According to the rate constant $k$, the half-life $t_{1/2}$ can be calculated from the equation: $t_{1/2} = \ln 2/k$. In case of zero-order kinetics, the degradation pattern of exenatide can be described by the following equation: $Q_t = Q_0 - kt$, where $Q_t$ is the intact peptide.

Fig. 3. Degradation Kinetics of Exenatide (100 $\mu$g/mL) in the Presence of Pepsin, Trypsin, Carboxypeptidase A (CPA), Aminopeptidase N (APN) and Chymotrypsin

The concentrations of all enzymes are set at 0.2 U/mL. Data were presented as the mean±S.D., n=4. All error bars were displayed, and some are smaller than the symbols.

Table 1. Results of Correlation Coefficient (Adjusted $r^2$ Square) Fitted from Mathematical Models and the Calculated Half-Life $t_{1/2}$ of Exenatide (100 $\mu$g/mL) in the Presence of Various Enzymes and Different Intestinal Homogenates at 37°C

<table>
<thead>
<tr>
<th>Enzyme/intestine</th>
<th>First order kinetic model</th>
<th>Zero order kinetic model</th>
<th>$t_{1/2}$ (h)</th>
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<tbody>
<tr>
<td><strong>Enzymes</strong></td>
<td></td>
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<tr>
<td>Chymotrypsin (0.2 U/mL)</td>
<td>$y=-5.203x+1.285$</td>
<td>$y=-110.819x+18.424$</td>
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<td></td>
<td>$r^2=0.9544$</td>
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<tr>
<td>APN (0.2 U/mL)</td>
<td>$y=-0.0853x+1.372$</td>
<td>$y=-2.297x+21.391$</td>
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<td></td>
<td>$r^2=0.9979$</td>
<td>$r^2=0.9390$</td>
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<tr>
<td>CPA (0.2 U/mL)</td>
<td>$y=-0.0296x+1.347$</td>
<td>$y=-1.192x+21.959$</td>
<td>10.16</td>
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<td></td>
<td>$r^2=0.9612$</td>
<td>$r^2=0.9188$</td>
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<td>Trypsin (0.2 U/mL)</td>
<td>$y=-0.0243x+1.363$</td>
<td>$y=-1.052x+22.848$</td>
<td>12.38</td>
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<tr>
<td></td>
<td>$r^2=0.9770$</td>
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<tr>
<td>Pepsin (0.2 U/mL)</td>
<td>$y=-0.0177x+1.386$</td>
<td>$y=-0.836x+24.099$</td>
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<td></td>
<td>$r^2=0.9842$</td>
<td>$r^2=0.9930$</td>
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<td><strong>Intestinal sections</strong></td>
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<td>Duodenum</td>
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<td>Jejunum</td>
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<tr>
<td>Ileum</td>
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<tr>
<td></td>
<td>$r^2=0.9882$</td>
<td>$r^2=0.9558$</td>
<td></td>
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*The value of half-life was calculated with zero-order rate constant, and other half-lives were calculated with first-order rate constants due to the higher $r^2$ squares and thus better fit quality.
amount (nmol) at the incubation time $t$ (h), $Q_0$ is the initial amount of peptide, and $k$ is the zero-first rate constant. The half-life $t_{1/2}$ can be calculated from the equation: $t_{1/2}=\frac{Q_0}{k}$. Chymotrypsin was found to yield a half-life of $5.784\times10^{-3}$ h at 0.2 U/mL which was followed by aminopeptidase N (3.53h) and carboxypeptidase A (10.16h). In contrast, both pepsin (0.2 U/mL) and trypsin (0.2 U/mL) showed longer half-lives of 14.28 and 12.38h, respectively, indicating weaker degradation abilities to exenatide. The above results are in agreement with those of degradation clearance analysis which is shown in Fig. 4. The agreement could be explained by the relationship between the degradation clearance and the degradation rate constant as mentioned in Materials and Methods.

Exenatide Degradation in the Presence of Everted Intestinal Rings Peptides, such as thymopoietin oligopeptides, luteinizing hormone releasing hormone (LHRH) and other model peptides have been reported to be rapidly degraded by different enzymes in the intestine. Typically, the degradation of these peptides were found to be intestinal site-dependent, whereas others such as insulin-like growth factor I displayed similar clearance rates in flushing solutions from the jejunum, ileum and colon. In order to further reveal the peptide stability in different intestinal segments, degradation kinetics of exenatide in the presence of everted intestinal rings from different locations were investigated. Here, everted intestinal rings from different regions were used as enzyme sources as previously reported, which mainly involved enzymes present on the brush border membrane. The degradation clearances of exenatide incubated with various intestinal rings are shown in Fig. 5.

The degradation of exenatide was found to be dependent on both the peptide concentrations and intestinal sites. The order of intestinal sites in terms of degradation ability to exenatide was jejunoileal junction > middle ileum > middle jejunum > duodenum > lower ileum. At the low peptide concentration of 25 µg/mL, clearances of exenatide at the middle segments of the jejunum and ileum appeared to be higher than that of the duodenum ($p<0.05$). In addition, the degradation clearance of exenatide at the upper segment of the duodenum was significantly lower than that of the middle segments of the duodenum ($p<0.05$). The same phenomenon also was observed at the jejunum. However, with respect to various segments of the ileum, the lower segment displayed the lowest degradation clearance compared with those of the upper and middle segments ($p<0.05$). When the concentration of exenatide was increased to 50 µg/mL, the peptide clearance at the middle segments of the jejunum and ileum still appeared to be higher than that of the duodenum ($p<0.05$). However, there was no statistically significant difference between the upper, middle and lower segments of the duodenum and ileum. In the case of the jejunum, the upper segment displayed a lower clearance than the middle and lower segments. With the further increase of peptide concentration up to 100 µg/mL, no significant difference was determined between the middle segments of all everted intestinal rings.

The above intestinal site-dependent degradation profile of exenatide is partly similar to those of LHRH, thymopoietin oligopeptides and acetylneurotensin. However, in the above cited reports, various intestinal segments were not investigated in detail as described in the present study. The above results suggested that the upper part of the duodenum and lower part of the ileum may be used as potential delivery sites of exenatide due to their slower clearance in all intestinal segments. However, the lower part of the ileum may be more advantageous due to its longer dimension compared with the upper part of the duodenum. In addition, the decreased degradation clearance of exenatide with increasing peptide concentrations suggested increasing the peptide concentration at a local site would benefit the oral delivery of exenatide.

Exenatide Degradation in the Presence of Intestinal Homogenates Our previous study revealed that exenatide was transported across the MDCK cell monolayer mainly by a passive paracellular pathway. However, a possible endocytic pathway across the intestinal mucosa still could not be excluded. In this study, intestinal homogenates from the duodenum, jejunum and ileum of rats were used, which involved not only the brush border membrane peptidases, but also the cytosolic enzymes of intestinal enterocytes. The degradation kinetics of exenatide in the presence of intestinal homogenates from different locations are shown in Fig. 6 and the degrada-
tion rate constant $k$ and half-life $t_{1/2}$ were also calculated and summarized in Table 1. It should be noted that no saturation phenomenon of enzymatic activity was observed as all degradation pattern of exenatide in the presence of various intestinal homogenates followed pseudo first-order kinetics (Table 1). The order of intestinal homogenates in terms of degradation ability to exenatide was ileum > jejunum > duodenum, which is partly in agreement with the above results of everted intestinal rings. However, no significant difference was observed between the different intestinal homogenates in the remaining concentrations of exenatide in all time points during 2h of incubation, which is presumably due to the involvement of cytosolic enzymes from intestinal enterocytes. It is well known that everted intestinal rings as enzyme source generally involve brush-border membrane peptidase, but not cytosolic enzymes.\(^{20,30}\) Although lysosomal enzymes may exist in the intestinal homogenates, they probably will not contribute to the peptide degradation due to lack of acidic environment. The above result suggests that exenatide may be subjected to cytosolic degradation in the case that endocytic process happens fast and drug is exposed to cytoplasm. However, when endocytic process is the rate-limiting step in drug transportation, exenatide is expected to suffer extensive degradation, resulted from the enzymes existing in both brush-border membrane and intestinal juice before uptake into intestinal epithelial cells, as evidenced in this study. Further investigation is needed to clarify the effect of endocytic process on drug degradation and involved cytosolic enzymes when endocytosis is proved to be the primary route of peptide across the intestinal mucosa.

CONCLUSION

In this study, gastrointestinal stabilities of exenatide were investigated in the presence of various gastrointestinal enzymes, everted intestinal rings and intestinal homogenates from different locations. The results showed that exenatide was comparatively stable in solutions with pH ranging from 1.2–8. However, exenatide was found to be digested rapidly by both chymotrypsin, whereas it was less susceptible to degradation by pepsin and trypsin. In the presence of rat everted intestinal rings, the degradation of exenatide displayed peptide concentration- and intestinal site-dependent properties with the lower degradation clearance rates in the upper part of the duodenum and lower part of the ileum. The study using intestinal homogenates as enzyme sources also revealed an intestinal site-dependent degradation of exenatide. However, no statistically significant difference was found between duodenum, jejunum and ileum in the remaining concentrations of exenatide in all time points during 2h of incubation. Findings of this study suggest that combination of chymotrypsin inhibitors with site-specific delivery might bypass the metabolic barrier during the oral delivery of exenatide.

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Conflict of Interest The authors declare no conflict of interest.

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