In Vitro Stability of GLP-1 and its Cleaved Metabolites in Human Serum and Plasma

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Introduction
Glucagon-like-peptides, GLP-1(7-37) and GLP-1(7-36) amide, stimulate insulin-release in a glucose-dependent manner, and may have a therapeutic value in treatment of type II diabetes (Beggs & Drucker 2007). However, GLP-1 is a substrate of dipeptidyl peptidase IV (DPP IV) which cuts and removes the N-terminal two residues of GLP-1 peptide (Wendelborg et al 1998). Like other insulinase plasma peptides (Yi et al 2007 and 2008), stabilization of GLP-1 as a biomarker under ex vivo conditions is critical for its clinical applications.

We investigated the stability of spiked GLP-1 peptides in blood samples to assess the effect of the endogenous and protease inhibitors. The peptides included: GLP-1(7-37), GLP-1(7-36) amide, and stable isotopically labeled peptide GLP-1(7-36). We reported that the loss of the full-length GLP-1 peptides was caused by the digestion of not only DPP IV activity but also an unidentified carboxypeptidase activity which removed the C-terminal amino acids residue. We also observed the further digestion of the GLP-1 fragments due to the intrinsic peptidase activities. Time-course kinetic analysis indicated that the substrate specificity of the intrinsic peptidase activity was related to sample types, and serum intrinsic peptidase activity was broader than plasma samples.

Methods and Procedure
Blood Collection and Plasma/Serum Preparation
- Human blood from healthy individuals was directly drawn into a variety of plasma and serum tubes
- Blood was processed as previously described (Yi et al 2007)

GLP-1 Peptides
- GLP-1 (7-37): H4547T/GSD/LEQAEQKVRK-VH/LRSG-GSH
- GLP-1 (7-36): H4547T/GSD/LEQAEQKVRK-VH/LRSG-GSH
- GLP-1 (7-36) amide: H4547T/GSD/LEQAEQKVRK-VH/LRSG-GSH
- GLP-1 (7-36) G36A: H4547T/GSD/LEQAEQKVRK-VH/LRSG-GSH
- GLP-1 (7-36) G37: H4547T/GSD/LEQAEQKVRK-VH/LRSG-GSH

Blood drawn into New Generation Stabilizer (NGS), BD P700*, EDTA, Citrate, Heparin plasma and serum tubes
- All blood samples were collected from same subjects
- Monitoring the time course changes of peptide fragments

MALDI-TOF MS
- Performed on an Ultraflex II MALDI-TOF MS (Bruker-Daltonics) as described previously (Yi et al 2008)
- The final spectrum was obtained from accumulation of 30 qualified spectra; each of these was from 100 laser shots under fixed laser power
- The time-course experiments were calibrated externally

Stability Analysis
(Yi et al 2006)
- Peptide stabilization was monitored by MALDI-TOF mass spectrometer, each of these was from 100 laser shots under fixed laser power
- The sample sites targeted by the laser was moved automatically after each of 108 shots to prevent over heating
- The final spectrum was calibrated externally

Validity Analysis
(Yi et al 2006)
- The peptide stabilization was validated in a parallel set: 1x 1h - 1x 24h, suggesting that degradation occurred according to a first-order reaction
- The peptide half-life is determined by 1x 24h - 1x 1h

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Substrate Specificity Versus Sample Types

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Serum</th>
<th>Plasma</th>
<th>EDTA</th>
<th>Citrate</th>
<th>Heparin</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLP-1 (7-37)</td>
<td>1.7 h</td>
<td>&gt; 3 D</td>
<td>&gt; 3-4 D</td>
<td>&gt; 3-4 D</td>
<td>&gt; 3-4 D</td>
</tr>
<tr>
<td>GLP-1 (7-36) amide</td>
<td>4.0 h</td>
<td>12.8 h</td>
<td>4.2 h</td>
<td>3.9 h</td>
<td>&gt; 3-4 D</td>
</tr>
<tr>
<td>GLP-1 (7-36) G36A</td>
<td>5.5 h</td>
<td>&gt; 3 D</td>
<td>&gt; 3-4 D</td>
<td>&gt; 3-4 D</td>
<td>&gt; 3-4 D</td>
</tr>
<tr>
<td>GLP-1 (7-36) G37</td>
<td>7.0 h</td>
<td>&gt; 3 D</td>
<td>&gt; 3-4 D</td>
<td>&gt; 3-4 D</td>
<td>&gt; 3-4 D</td>
</tr>
</tbody>
</table>

GLP-1 in Serum
- - The half-life (stability) of three GLP-1 peptides (G37, G36A, AG36) are 2.0, 4.0, and 1.7 hours, respectively

GLP-1 in EDTA
- - GLP-1 (7-37) and GLP-1 (7-36) amide are more stable in EDTA plasma than in serum, verifying our previous reported results that the positively charged residue can provide more stability of the GLP-1 peptide
- - The short half-life of three GLP-1 peptides suggests that extracellular instability of GLP-1 in ex vivo serum sample

Results

GLP-1 Peptides Are Stabilized in the Inhibited Plasma Samples
- - Three GLP-1 peptides were not stable in EDTA samples, however, they were stabilized in both P700 and NGS samples with better stability in NGS samples

Mechanistic Analysis of GLP-1 Peptide in Serum and Plasma Samples
- - The stability of G36A in EDTA, P700, and NGS samples was compared
- - GLP-1 peptides were not stable in EDTA samples, however, they were stabilized in both P700, and NGS samples with better stability in NGS samples

Conclusions
- - GLP-1 activity was also observed in all three traditional plasma samples including EDTA, Citrate, and Heparin plasma samples
- - As shown in Table 1, both G37 and AG36-Fn were easily detected
- - Both DD-peptides were more stable in plasma samples than in serum samples
- - GLP-1 (7-36) amide is more stable than AG36-2N in both serum and plasma samples
- - The carboxypeptidase-caused fragment was not detected in plasma samples
- - GLP-1 activity was also observed in all three traditional plasma samples including EDTA, Citrate, and Heparin plasma samples

References
Baggio, L., and Drucker, D. J. Diabetes Metabolism 2007, 32:213-2137

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