SUPPLEMENTARY DATA

Polymer Masked-UnMasked Protein Therapy: Identification of the Active Species After Amylase-activation of Dextrin-Colistin Conjugates.

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**Table S1.** Characteristics of dextrin-colistin conjugates used for studying the effect of the succinoylation rate on the released species.

<table>
<thead>
<tr>
<th>Conjugate</th>
<th>$M_w$ (g/mol) ($M_w/M_n$)</th>
<th>Degree of succinoylation (mol %)</th>
<th>Protein content (%)</th>
<th>Molar ratio (dextrin:colistin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextrin-colistin 1%</td>
<td>9,000 (1.7)</td>
<td>1.0</td>
<td>5.2</td>
<td>3.2:1</td>
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<tr>
<td>Dextrin-colistin 2.5%</td>
<td>9,500 (1.7)</td>
<td>2.5</td>
<td>9.7</td>
<td>1.6:1</td>
</tr>
<tr>
<td>Dextrin-colistin 7.5%</td>
<td>13,000 (2.2)</td>
<td>7.5</td>
<td>21.0</td>
<td>0.7:1</td>
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</table>
Fig. S1: HPAEC-PAD analysis of maltose oligomer standards (0.01 mg/mL) and oligosaccharides released from intact and amylase-degraded dextrin-colistin conjugates (1 mg/mL). Degradation was performed by incubation of dextrin-colistin conjugate (3 mg/mL colistin base in PBS, pH 7.4) with amylase (100 IU/L) for 48 h at 37°C.
<table>
<thead>
<tr>
<th>Sample</th>
<th>RT (min)</th>
<th>Observed mass</th>
<th>Calculated mass</th>
<th>Species / Adducts</th>
<th>Molecular formula</th>
<th>Molecular Weight</th>
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<tr>
<td>Collistin</td>
<td>5.6</td>
<td>1155.76</td>
<td>Colistin B [M+H]+</td>
<td>Colistin B[M+H]+</td>
<td>C₉₉H₃₄N₃₉O₇</td>
<td>1154.76</td>
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<tr>
<td></td>
<td>5.8</td>
<td>1162.77</td>
<td>Colistin B [M+2H]+</td>
<td>C₉₉H₃₄N₃₉O₇</td>
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<td></td>
<td>6.5</td>
<td>556.17</td>
<td>DP7 [M+H]+</td>
<td>C₉₉H₃₄N₃₉O₇</td>
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<td>7.2</td>
<td>677.18</td>
<td>DP8 [M+H]+</td>
<td>C₉₉H₃₄N₃₉O₇</td>
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<td></td>
<td>7.3</td>
<td>758.23</td>
<td>DP9 [M+H]+</td>
<td>C₉₉H₃₄N₃₉O₇</td>
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<td></td>
<td>7.6</td>
<td>893.22</td>
<td>DP10 [M+H]+</td>
<td>C₉₉H₃₄N₃₉O₇</td>
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<td></td>
<td>8.9</td>
<td>920.24</td>
<td>DF11 [M+H]+</td>
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<tr>
<td></td>
<td>9.1</td>
<td>1001.25</td>
<td>DF12 [M+H]+</td>
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<td></td>
<td>9.5</td>
<td>1082.27</td>
<td>DF13 [M+H]+</td>
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<td></td>
<td>10.9</td>
<td>1126.16</td>
<td>DF14 [M+H]+</td>
<td>C₉₉H₃₄N₃₉O₇</td>
<td>1154.76</td>
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**Table S2:** Identification of the detected species from UPLC-QTOF-MS analysis of colistin sulfate and fractions F6, F7 and F8.
**Fig. S2:** QTOF-MS spectra of the isolated peaks from colistin with a retention of A. 5.6 min and B. 6.4 min.
**Fig. S3**: QTOF-MS spectra of the isolated peaks from F8 with a retention time of A. 1.2 min (m/z = 500 to 800), B. 1.2 min (m/z = 600 to 1000), C. 1.2 min (m/z = 900 to 1400), D. 7.2 min, E. 7.4 min, F. 7.6 min, G. 7.7 min, H. 7.8 min, I. 7.9 min, J. 8.1 min, K. 8.4 min, L. 8.6 min, M. 8.9 min, N. 9.2 min, O. 9.4 min, P. 9.5 min, Q. 9.7 min, R. 9.9 min, S. 10.1 min and T. 10.5 min.
Fig. S3: continued.
Fig. S3: continued.
Fig. S4: QTOF-MS spectra of the isolated peaks from F7 with a retention time of A. 1.1 min (m/z = 300 to 800), B. 1.1 min (m/z = 750 to 1200), C. 1.1 min (m/z = 1100 to 1600), D. 7.5 min, E. 7.9 min (m/z = 400 to 600) and F. 7.9 min (m/z = 600 to 900).
**Fig. S5:** QTOF-MS spectra of the isolated peaks from F6 with a retention time of A. 1.1 min (m/z = 100 to 900), B. 1.1 min (m/z = 900 to 1500), C. 1.1 min (m/z = 1500 to 2000), D. 7.9 min (m/z = 743), E. 7.9 min (m/z = 743), F. 7.9 min (m/z = 797), G. 7.9 min (m/z = 802), H. 7.9 min (m/z = 851), I. 7.9 min (m/z = 856), J. 7.9 min (m/z = 910), K. 7.9 min (m/z = 964), L. 7.9 min (m/z = 1018), M. 7.9 min (m/z = 1072) and N. 7.9 min (m/z = 1126).
Fig. S5: continued.
Fig. S6: UPLC-QTOF-MS chromatograms (Base Peak Intensity, BPI) of amylase-degraded dextrin-colistin conjugates (0.2 mg/mL colistin base) containing dextrin with 1.0, 2.5 and 7.5 mol% succinoylation. Degradation was performed by incubation of dextrin-colistin conjugate (3 mg/mL colistin base in PBS, pH 7.4) with amylase (100 IU/L) for 48 h at 37°C.
**Fig. S7**: Zoom view of UPLC-QTOF-MS chromatograms (Base Peak Intensity, BPI) between 7 and 13.5 min of amylase-degraded dextrin-colistin conjugates (0.2 mg/mL colistin base) containing dextrin with 1.0, 2.5 and 7.5 mol% succinoylation. Degradation was performed by incubation of dextrin-colistin conjugate (3 mg/mL colistin base in PBS, pH 7.4) with amylase (100 IU/L) for 48 h at 37°C.
**Table S3:** Identification of the major detected species from UPLC-QTOF-MS analysis of amylase-degraded dextrin-colicin conjugates containing dextrin with 1.0, 2.5 and 7.5 mol% succinoylation.

<table>
<thead>
<tr>
<th>Species</th>
<th>Adducts</th>
<th>RT (min)</th>
<th>Observed mass</th>
<th>Calculated mass</th>
<th>Molecular Formulae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colistin B + linker</td>
<td>[M+3H]^{3+}</td>
<td>7.4 / 7.6</td>
<td>419.27</td>
<td>419.26</td>
<td>C_{56}H_{102}N_{16}O_{16}</td>
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<tr>
<td>Colistin A + linker</td>
<td>[M+3H]^{3+}</td>
<td>8.1 / 8.4</td>
<td>423.93</td>
<td>423.94</td>
<td>C_{57}H_{104}N_{16}O_{16}</td>
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<tr>
<td>Colistin B + linker - H$_2$O</td>
<td>[M+3H]^{3+}</td>
<td>7.1 / 7.2 / 7.8 / 7.9</td>
<td>413.26</td>
<td>413.26</td>
<td>C_{56}H_{100}N_{16}O_{15}</td>
</tr>
<tr>
<td>Colistin A + linker - H$_2$O</td>
<td>[M+3H]^{3+}</td>
<td>7.9 / 8.5 / 8.6</td>
<td>417.93</td>
<td>417.93</td>
<td>C_{57}H_{102}N_{16}O_{15}</td>
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<tr>
<td>Colistin B + 2*linker - H$_2$O</td>
<td>[M+3H]^{3+}</td>
<td>8.9 / 9.2 / 9.4 / 9.7 / 9.9</td>
<td>446.60</td>
<td>446.60</td>
<td>C_{60}H_{104}N_{16}O_{18}</td>
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<tr>
<td>Colistin A + 2*linker - H$_2$O</td>
<td>[M+3H]^{3+}</td>
<td>10.1 / 10.6</td>
<td>451.27</td>
<td>451.27</td>
<td>C_{61}H_{106}N_{16}O_{18}</td>
</tr>
<tr>
<td>Colistin B + 2*linker - 2H$_2$O</td>
<td>[M+3H]^{3+}</td>
<td>8.9 / 9.5 / 9.6 / 9.7 / 10.1</td>
<td>446.60</td>
<td>446.60</td>
<td>C_{60}H_{102}N_{16}O_{17}</td>
</tr>
<tr>
<td>Colistin A + 2*linker - 2H$_2$O</td>
<td>[M+3H]^{3+}</td>
<td>10.2 / 10.3 / 10.5 / 10.9</td>
<td>445.27</td>
<td>445.27</td>
<td>C_{61}H_{104}N_{16}O_{17}</td>
</tr>
<tr>
<td>Colistin B + 3*linker - H$_2$O</td>
<td>[M+2H]^{2+}</td>
<td>11.3</td>
<td>719.40</td>
<td>719.40</td>
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<td>Colistin A + 3*linker - H$_2$O</td>
<td>[M+2H]^{2+}</td>
<td>12.0</td>
<td>726.41</td>
<td>726.41</td>
<td>C_{65}H_{110}N_{16}O_{21}</td>
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<tr>
<td>Colistin B + 3*linker - 2H$_2$O</td>
<td>[M+2H]^{2+}</td>
<td>11.2 / 11.4 / 11.6 / 11.7 / 11.8 / 11.9 / 12.1</td>
<td>710.40</td>
<td>710.40</td>
<td>C_{64}H_{106}N_{16}O_{20}</td>
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<td>Colistin A + 3*linker - 2H$_2$O</td>
<td>[M+2H]^{2+}</td>
<td>12.6 / 12.7</td>
<td>717.41</td>
<td>717.40</td>
<td>C_{65}H_{108}N_{16}O_{20}</td>
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<tr>
<td>Colistin B + 3*linker - 3H$_2$O</td>
<td>[M+2H]^{2+}</td>
<td>11.6 / 12.1 / 12.2 / 12.4</td>
<td>701.39</td>
<td>701.39</td>
<td>C_{64}H_{104}N_{16}O_{19}</td>
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<tr>
<td>Colistin A + 3*linker - 3H$_2$O</td>
<td>[M+2H]^{2+}</td>
<td>12.8 / 12.9 / 13.1</td>
<td>708.40</td>
<td>708.40</td>
<td>C_{65}H_{106}N_{16}O_{19}</td>
</tr>
</tbody>
</table>
**Fig. S8:** A. Biofilm formation assay showing LIVE/DEAD® (green and red colors respectively) stained CLSM images of *E. coli* biofilms (aerial and side view, scale bar = 40 µm) grown for 24 h in the presence of fractions F6, F7 and F8 at 0.25 µg/mL colistin base (equivalent to 2× fraction F8’s MIC). B. COMSTAT image analysis of biofilm CLSM z-stack images. Data represents mean ± SD; n = 3. Significant difference is indicated by *, where *p < 0.05, compared to untreated control.