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UNIVERSITEIT GENT

FACULTY OF PHARMACEUTICAL SCIENCES

HPLC-MS characterisation of chelate modified somatropin

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INTRODUCTION

Somatropin is a recombinant human growth hormone, consisting of 191 amino acids [1]. This protein is clinically used in children and adults with inadequate endogenous growth hormone to stimulate a normal bone and muscle growth. In addition, somatropin is currently being investigated for the diagnosis and radiotherapy of certain hormonal cancers. The modification of the protein with the chelating agent NOTA (1,4,7-triazacyclononane-1,4,7-triacetic acid) allows the inclusion of metals coupled to the protein for diagnostic (*e.g.* ^{68}Ga) or therapeutic (*e.g.* ^{90}Y) purposes. The NOTA unit is selectively introduced on a lysine side chain. This yields 9 possible labelling sites for somatropin:
FPTIPLSRLFDNAMLRAHRLHQLAFDTYQEFEEAYIPK^{38}EQK^{41}YSFLQNPQTSLCFSESIPTPSNREETQQK^{70}SNLELLRISLLLIQSWLEPVQFLRSVFANSLV
YGASDSNVYDLLK^{115}DLEEGIQTLMGRLEDGSPRTGQIFK^{140}QTYSK^{145}FDTNSHNDDALLK^{158}NYGLLYCFRK^{168}DMDK^{172}VETFLRIVQCRSVEGSCGF
The Ph. Eur. describes the selective cleavage of somatropin, using trypsin, as an identification test with a coverage of 95% [1]. As we were only interested in the modified lysine amino acids, we have investigated different immobilized proteolytic enzymes: trypsin, chymotrypsin and *Staphylococcus aureus* V-8 proteases. The resulting peptides were then monitored using HPLC-MS^2, allowing the characterisation of the modified protein.

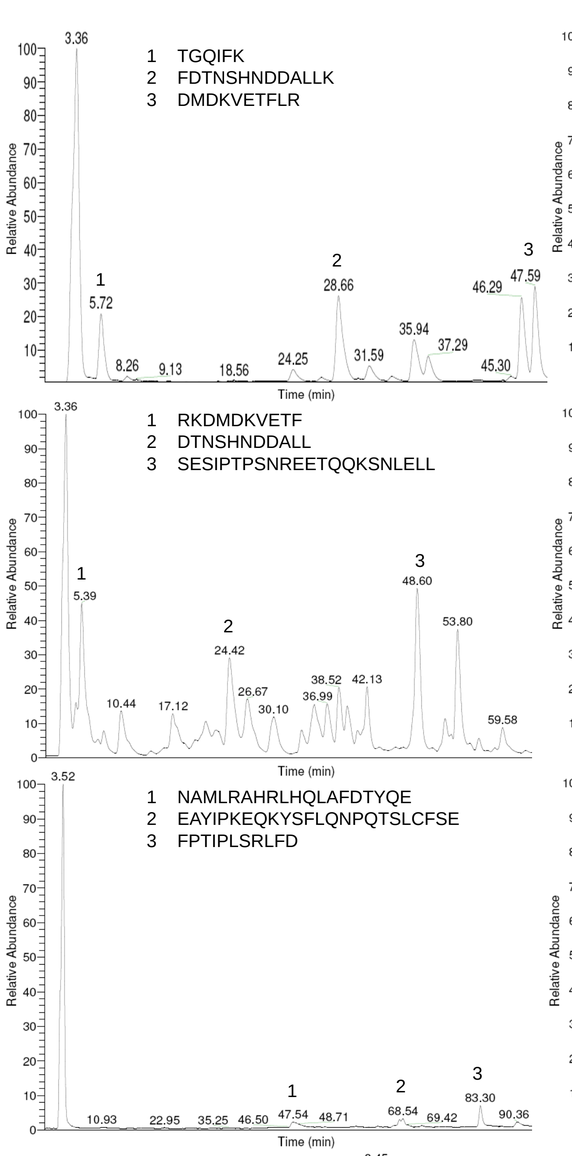
EXPERIMENTAL

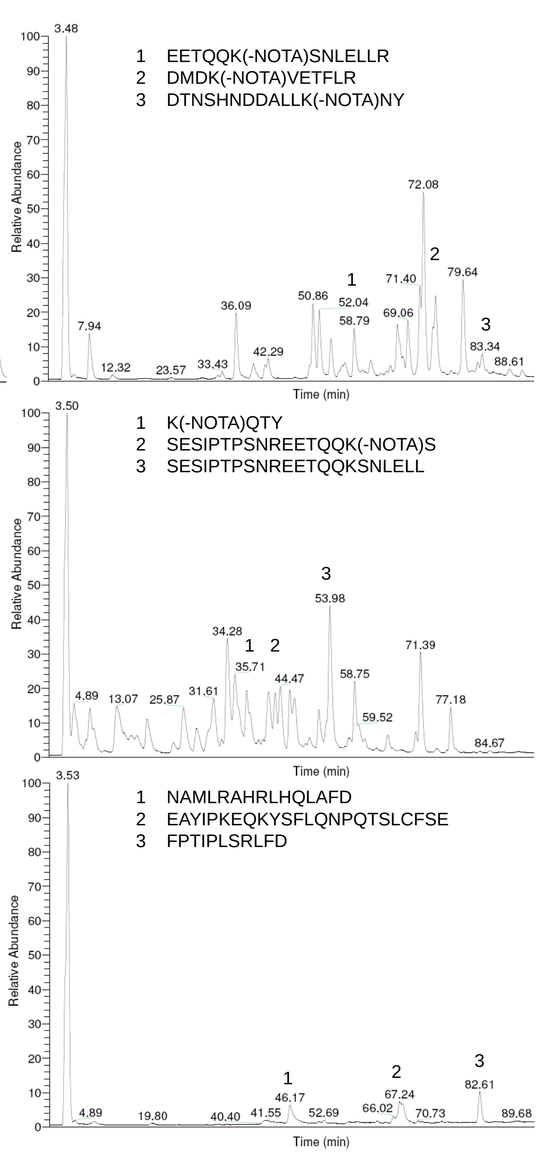
After p-SCN-Bn-NOTA labelling of somatropin, the cysteine residues were reduced and alkylated using dithiothreitol (DTT) and iodoacetamide, respectively. The solution was then desalted, using a PD-10 desalting column. Subsequently, an aliquot of the solution was incubated with trypsin (4 hours, 37°C), chymotrypsin (24 hours, 37°C) and *Staphylococcus aureus* V-8 (18 hours, 37°C) proteases. In addition, a mixture of trypsin and chymotrypsin was added to the protein and incubated for 24 hours at 37°C.

RESULTS and DISCUSSION

Unlabelled | **NOTA-labelled**

Trypsin protease:
- Serine protease
- Cleaves at carboxyl side of K and R
- Theoretically: 21 fragments for somatropin





Lysine coverage:
- Unlabelled: 100%
- NOTA-labelled: 100%,
  4 NOTA-modified Lys:
  Lys^{70} (63.84%), Lys^{158} (26.40%), Lys^{140} (4.42%), Lys^{172} (5.34%)

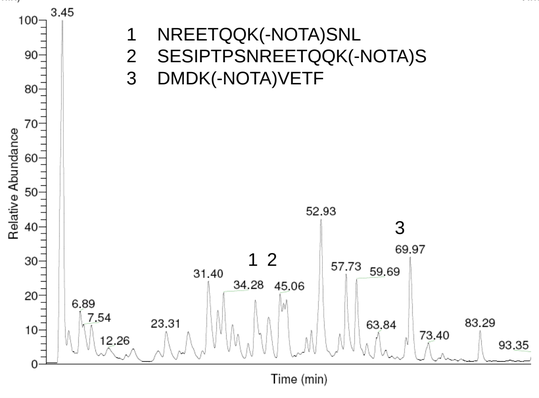
Chymotrypsin protease:
- Serine protease
- Cleaves at carboxyl side of F, Y and W
- Theoretically: 21 fragments for somatropin

Lysine coverage:
- Unlabelled: 89%
- NOTA-labelled: 67%,
  2 NOTA-modified Lys:
  Lys^{70} (79.58%), Lys^{140} (20.42%)

Staphylococcus aureus V-8 protease:
- Serine protease
- Cleaves at carboxyl side of E and D
- Theoretically: 12 fragments for somatropin

Lysine coverage:
- Unlabelled: 22%
- NOTA-labelled: 22%,
  no NOTA-modified Lys found

➡ Mixture: trypsin + chymotrypsin
NOTA-labelled peptide HPLC:



Lysine coverage of NOTA-labelled: 89%,
3 NOTA-modified Lys:
Lys^{70} (59.37%), Lys^{140} (18.06%), Lys^{172} (22.57%)

CONCLUSIONS

Staphylococcus aureus V-8 protease digestion of somatropin is unsuitable for localisation of modified lysine amino acids, as the lysine coverage is inadequate. Chymotrypsin and trypsin digestion are thus the most appropriate methods for establishing the NOTA-attached lysine amino acids. A combination of these enzymes is useful to discover the most abundant modified lysine amino acid, namely Lys^{70}.

REFERENCES

[1] European Pharmacopoeia 6.0, 01/2008:0951.