

LC-MS CHARACTERIZATION AND CELL-BINDING PROPERTIES OF CHELATE MODIFIED SOMATROPIN

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Somatropin, a recombinant protein containing 191 amino acids, is derived from the endogenous human growth hormone, somatotropin. This protein is clinically used in children and adults with inadequate endogenous growth hormone to stimulate a normal bone and muscle growth. In addition, somatotropin is recently being investigated for the diagnosis and radiotherapy of certain hormonal cancers. In some of these cancers, over-expression of the human growth hormone receptor (hGHR) is described.

The modification of the protein with a chelating agent like NOTA (1,4,7-triazacyclononane-1,4,7-triacetic acid) allows the inclusion of metals coupled to the protein. The NOTA unit is selectively introduced on a lysine side chain.

As site-specific labelling is necessary to avoid active region interactions (1-16, 41-68, 103-119 and 167-175), characterization of the chelate-modified somatotropin is indispensable. Therefore, we have applied an enzymatic digestion procedure using trypsin, chymotrypsin and a combination of both enzymes. The resulting peptides were then monitored using HPLC-MSⁿ, allowing the investigation of the exact amino acid modifications. The use of a mixture of trypsin and chymotrypsin gave an enhanced information efficiency. Moreover, the intact protein, without enzymatic degradation, was analysed on a protein HPLC column using UV detection for quantification and ESI-MS/MS for characterization (Figure 1).

Based upon the HPLC-MSⁿ results of the digested somatotropin, the chelating molecule is mainly bound to a specific lysine amino acid that is located away from the receptor binding site [1]. Therefore, the cell-binding functionality of the characterized NOTA-somatropin is measured, using a HepG2 cell line (Figure 2).

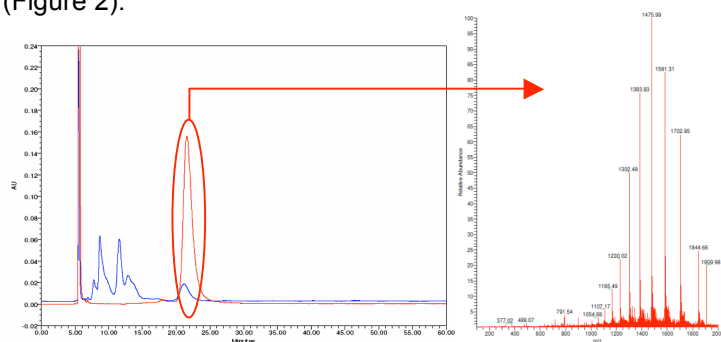


Figure 1: HPLC chromatogram (left) of somatotropin (red) and NOTA-modified somatotropin (blue), together with the mass spectrum (right) of somatotropin.

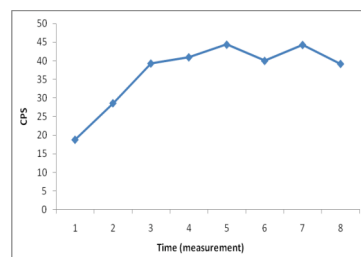


Figure 2: Cell binding results of NOTA-modified somatotropin.

References

- 1) Patent application GB1006285.9