Mapping insulin non-covalent interactions with natural polysaccharides by hydrogen/deuterium exchange mass spectrometry

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RATIONAL: Drug development efforts involving therapeutic peptides or proteins strongly lead optimization of drug delivery, drug stability, solubility and functionality. The key feature of controlled drug delivery is the use of biocompatible polymers able to interact via non-covalent bonds with an active principle through multiple functional groups. Here amide hydrogen/deuterium exchange (HDX) mass spectrometry was employed to localize insulin dynamics induced by interactions with three natural polysaccharides, i.e. chitosan (CH), sodium alginate (ALG) and chondroitin sulfate (CS).

METHODS: LTQ-Orbitrap continuous-labelling mass spectra were collected by diluting insulin stock solution (10 mM in 0.1% formic acid) to a final concentration of 0.1 mM in D₂O containing 1 mM deuterated ammonium acetate (final pH .6) (insulin:polysaccharide ratio 1:2, w/v). For peptide mapping, deuterated samples were quenched after 0.5, 30, 60, 120 minutes exchange by adding HCl (pH ) and digested with pepsin before LC–MS/MS analysis.

RESULTS: Differences in the insulin backbone dynamics in the presence of the three polysaccharides were highlighted by monitoring peptic peptides at different time points. No significant differences were observed in the presence of CH, whereas the negatively charged ALG and CS were able to induce significant conformational variations at the B-chain level resulting in more protection against H/D exchange. The A-chain interacted only with CS reducing the protein mobility on a long time scale (120 min). HDX data evidenced heterogeneous insulin dynamics in the presence of ALG and CS.

CONCLUSIONS: The studies reported here demonstrated the capabilities of mass spectrometry techniques and HDX methods to obtain useful information toward the flexibility and the behavior of native insulin in the presence of natural polysaccharides, and could provide insights to study the behavior of pharmaceutical formulations. Copyright © 2016 John Wiley & Sons, Ltd.