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Protein and peptide drug formulation : the way forward

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Advances in biotechnology are enabling the production of complicated peptides and proteins in sufficient quantities to enable their general use as medicines. Like all drugs, these products need to be formulated and satisfy the requirements of licensing agencies

However, compared to the relatively small organic molecules currently prevalent, proteins/ peptides present challenging issues :

1. **Molecular shape** : certainly controls biological activity and will have repercussions for physical properties (pK's, solubility, formulation ...etc). Spectroscopy must play an important role here.
2. **Chemical integrity and stability** : in some respects a protein/peptide can be considered to be the sum of many chemical species (the individual residues). Each residue needs to be verified (avoiding amongst other things phosphorylation or sulphation of tyrosines, succinimide formation), sequences need to be verified, disulphide identity and linking needs to be established, chirality of every residue ensured particularly if D-amino acids are involved. These factors require access to sophisticated analytical methods including separation techniques (HPLC & CZE), mass spectrometry and spectroscopy.

This communication concerns three case studies : Human calcitonin, Hirudin and Neuropeptide Y. Spectroscopic and chromatographic data will be presented and the relevance to drug formulation outlined.

Hirudin

Hirudin (65 amino acids) extracted from the saliva of the medicinal leech is an excellent blood anticoagulant. Molecular shape, chemical & physical properties have been determined by spectroscopy, mass spectrometry, HPLC & CZE.

Molecular shape : β-sheet core with 16 amino acid tail. Thrombin inhibition involves docking of the hirudin core and the tail wrapping around thrombin. The tail carries 8 carboxyl groups – pH & charges control chemistry. A mapping of the 33 pK's provides a rationale to help understand physical properties.

Chemical stability : two Asp-Gly sequences in conformationally *floppy* regions are susceptible to succinimide and isoAsp formation. Freeze-drying, the presence of sugars or metal ions control this process.

Human Calcitonin (hCT)

hCT is an important Ca^{2+} control, 32 amino acid peptide hormone. In non-polar media, hCT adopts an α-helix N-terminus and a *floppy* C-terminus. Fresh hCT in aqueous solution has a dynamic shape (no specific order); the solution ages to form fibrils and gels with α-helix N-termini and tails intermolecularly associated through a β-sheet. Control of fibrillation :

i. Explains apparent anomaly of low hCT bioactivity.
ii. Provides a stable formulation.

There are significant spectroscopic/shape changes with species/sequence variation.

Neuropeptide Y (NPY)

NPY is an important 36 amino acid peptide neurotransmitter found in the CNS. The α-helix observed in solution is the result of self-association (K_{ass}~5.10^5). Species variants (sequence changes) (and pH) severely affect self-association and the pK of His26. At biological levels, NPY will exist as a monomer with little ordered conformation. Ions help solubilise NPY in non-aqueous media.

Moral

Every protein/peptide needs to be taken on its own merits. Molecular shape and self-association are important features. Remember, a single amino acid change in a whole virus can have critical immunological effects.

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