



Enzyme Replacement Therapies: Regulatory Science Perspectives

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Classes of regulated products

- Enzyme Replacement Therapy
- Oncology
- Anticoagulants
- Anti-infectives
- Others

Products complexity

- Large macromolecules
- Post-translational modifications
- Additional modifications artificially introduced to design a molecule with the desired therapeutic characteristics
- Complex mixtures rather than purified recombinant proteins
- Naturally derived products, only partially characterized

General considerations

- Several guidance documents are available on manufacturing of biotechnology products
- Specific regulatory science issues pertaining to therapeutic enzymes

Enzymes and enzymes replacement therapies: emerging regulatory issues

- Identification of critical quality attributes relevant for clinical safety and efficacy
 - Control strategy
 - Stability
- Potency assays measurements
- Immunogenicity assays

Determination of critical structural quality attributes

Product characteristics relevant for clinical safety and efficacy

- Biological activity of the enzyme
- Target substrate localization
 - Bloodstream or restricted environments (i.e. gut)
 - Target cells or tissues (i.e. macrophages, skeletal muscle)
- Clearance
 - Glycosylation
 - PEGylation

Biological activity considerations

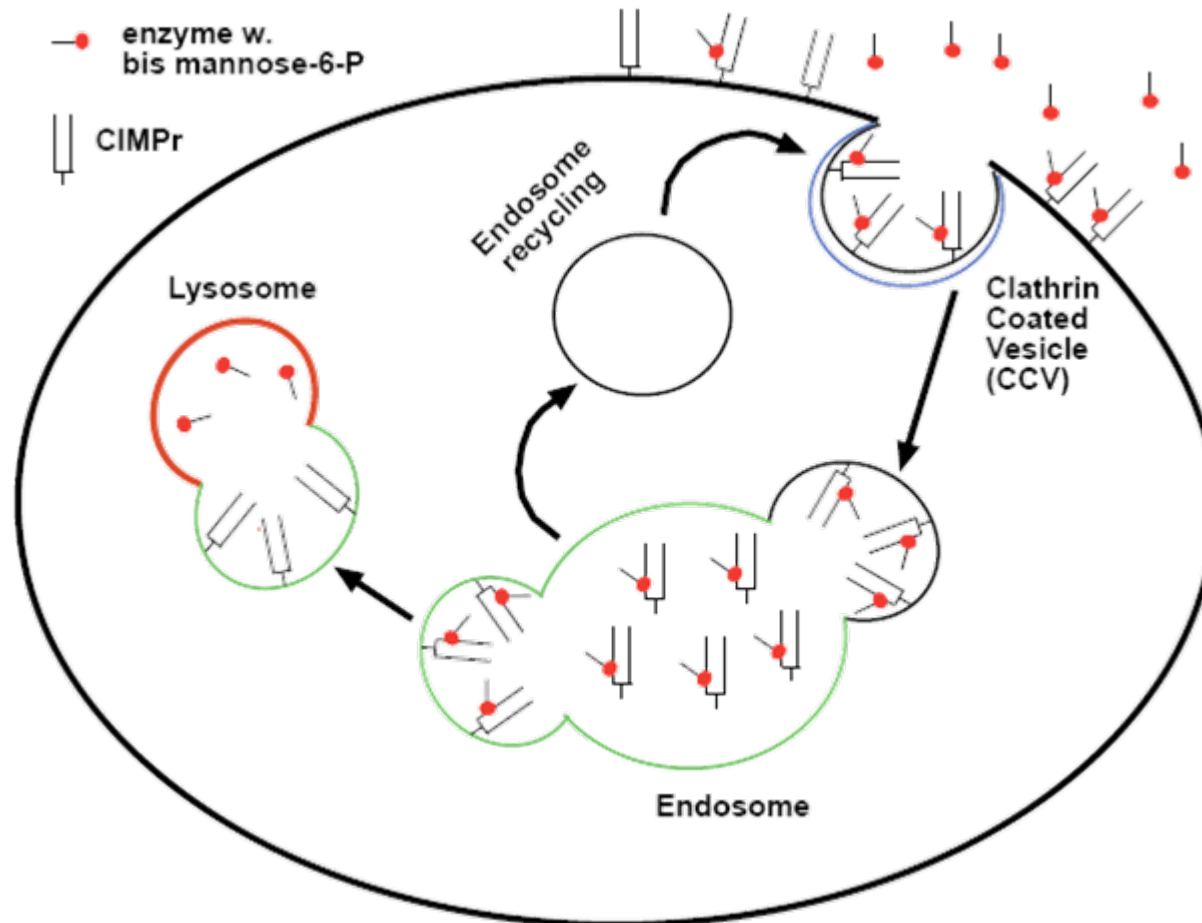
- Specific residues required for full activity (i.e conversion to formylglycine)
- Residues required for targeting of the therapeutic enzymes (i.e. specific sugar residues)
- Complex glycan chains may have an impact on bioavailability
- Chemical modifications to enhance half-life (PEGylation)
- Cofactors may be required for activity
 - Metals
 - Accessory proteins

Biological Characterization

- Knowledge of how a quality attribute impacts on safety or efficacy may change the risk profile
 - Attribute is not so critical (e.g., Deamidation, in some cases) and does not need to be specified
 - Attribute is more critical than anticipated and the current control strategy does not provide sufficient assurance of product quality

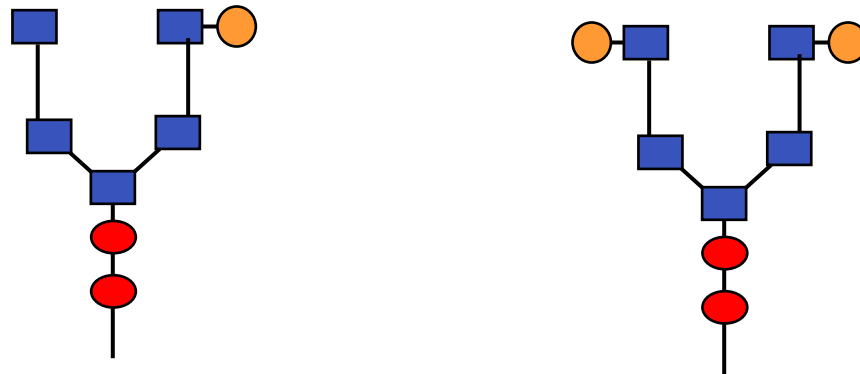
Complex Biological Activities

Enzyme Replacement Therapies



High Mannose Oligosaccharides

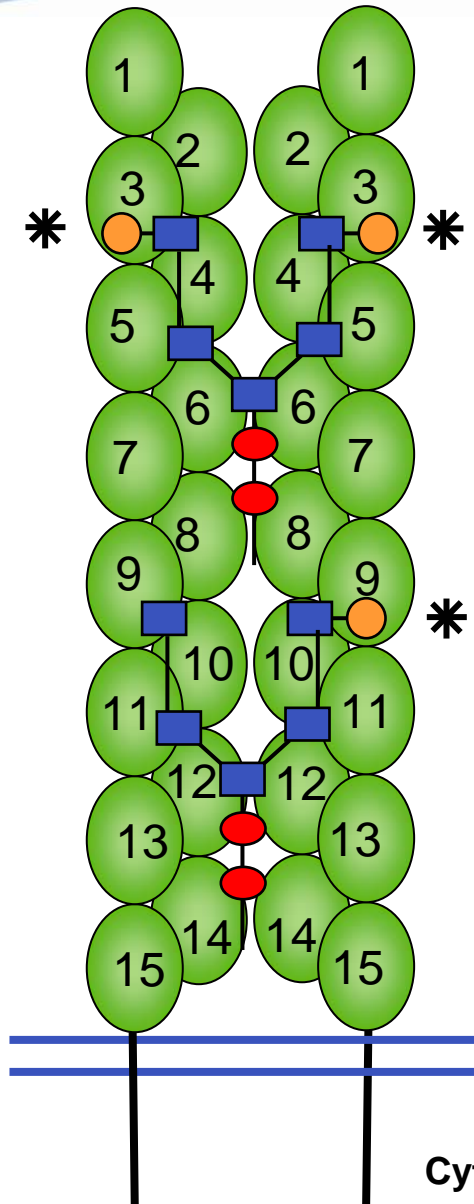
- Mannose 6 phosphate binds to cation independent M6P receptor and is taken up into the cell



- This oligosaccharide is critical for many enzyme replacement therapies but can also be found on other therapeutic proteins

cIMPR

Extracellular domains



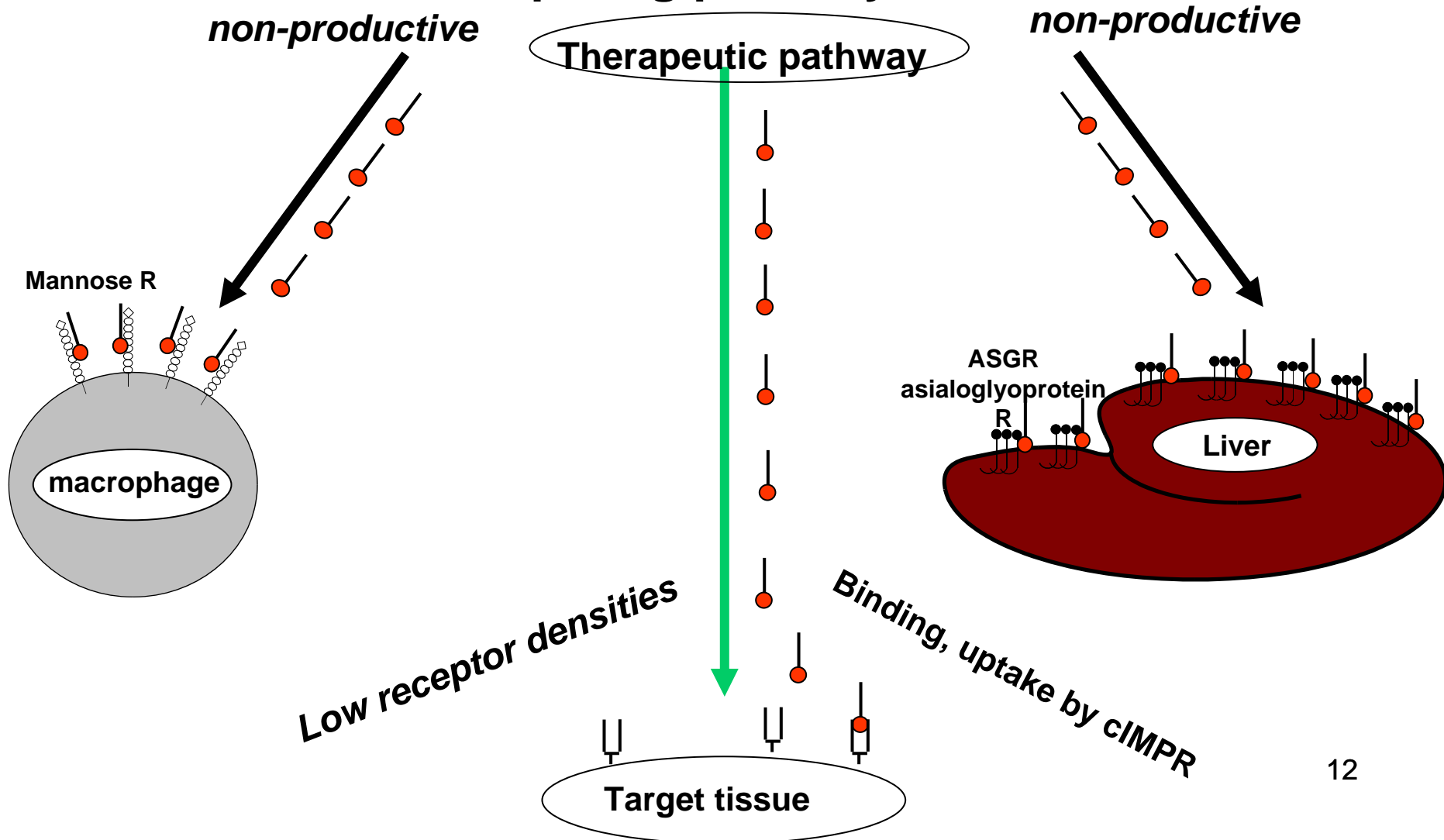
bis M6P
- two phosphates
*100-1000 x
higher affinity
than M6P*

M6P
-one phosphate

Cell membrane

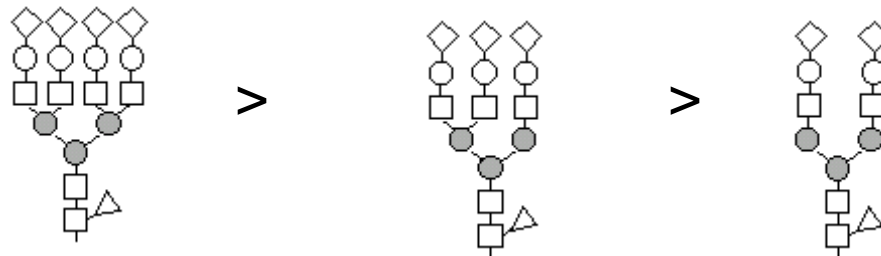
Cytoplasmic tail

Enzyme Replacement Therapies (ERTs) encounter competing pathways



Complex N-linked Glycans

- The ability to measure antennary species complex has led to a better understanding of their functionality
- Tetraantennary species appear to contribute more to overall bioavailability of a protein product than triantennary structures



- Small changes in tetraantennary content can have a significant impact on bioavailability and potentially on product safety or efficacy, raising concerns regarding the need to monitor during routine manufacturing

Glycan structures: control strategies

- Exposed galactose residues enhance liver uptake of the product through interaction with the Asialoglycoprotein receptor
 - Emphasis on ensuring SA occupancy on the tri and tetra antennary structures rather than simply SA content
- Distribution of antennary structures and particularly synergistic effects when they appear on the same molecule may be important.
 - Highest charged species have the greatest bioavailability
- Detailed glycan structures
 - Phosphate on terminal or penultimate mannose residues

Potency assays

- Potency is a critical element in ensuring safety and efficacy in clinical setting
- A potency assay for a therapeutic enzyme should reflect the proposed *in vivo* mechanism of action
 - Enzyme activity-based assays
 - Cell-based assays
 - Receptor binding assays

Assessment of biological activity: cell based assays/receptor binding

- Therapeutic enzyme uptake by cells
- Reduction in the accumulated substrate
- Receptor binding affinity measurements (i.e Biacore)

Biological activity assays: how and when

- May be substituted in early development by “surrogate” assays (i.e. Mannose 6-Phosphate content)
- Should be developed and introduced in the control and stability strategies by Phase III
- Necessary for product characterization and assessment of comparability following post-marketing changes

Assessment of biological activity: enzyme activity assays

- Enzyme activity measurements
 - Specific activity
 - Kinetic parameters
- Substrate choice
 - Surrogate
 - Physiologically relevant

Enzyme activity assays: substrate choice

- The substrate in enzymatic assay should be structurally similar to the physiological target
- Surrogate substrate:
 - May contain a moiety easily detected in the product
 - May have limited structural similarity to the target
 - Might be easier to validate the assay
 - How relevant is the information obtained?
- Physiologically relevant substrate:
 - Purified from a natural source or chemically synthesized
 - Consistency and availability, detection systems, assay precision

Surrogate versus physiologically relevant substrate

- Use of physiologically relevant substrates in product development
 - Release
 - Stability
 - Comparability
- Use of surrogate substrates versus physiologically relevant substrates

Why physiologically relevant substrates?

- Give assurance on the clinical efficacy of the enzyme
- Can be more sensitive to structural changes in the enzyme following modifications in the manufacturing process

Enzyme activity assays: specific activity

- Specific activity measurements are typically conducted under optimal reaction conditions
 - Saturating substrate
 - May not be sensitive to structural changes

Enzyme activity assays: kinetic parameters

- Kinetic parameters in an enzyme reaction are sensitive measures of enzyme activity and structural integrity
 - By Phase III, K_m and k_{cat} measurements using a physiologically relevant substrate should be included in the release and stability program.

Control and stability strategies: Example 1

- Target substrate is extracellular (i.e. bloodstream, gut)
 - Potency measurements
 - Specific activity during development
 - Kinetic parameters using a physiologically relevant substrate by Phase III
 - Assays that measure additional critical quality attributes identified during development

Control and stability strategies: Example 2

- Target substrate is intracellular (i.e. accumulated in lysosome)
 - Biological activity assays
 - In early development, surrogate assays may be used (i.e. control of Mannose, Mannose-6 Phosphate or Bis-Mannose-6 Phosphate)
 - By Phase III, cellular uptake and substrate degradation, receptor binding
 - Potency assays
 - Specific activity in early development
 - Kinetic parameters using physiologically relevant substrates by Phase III
 - Assays that measure additional critical quality attributes identified during development

Immunogenicity assays for therapeutic enzymes

- Either foreign or human proteins can elicit an immune response with clinical consequences
- Appropriate assays must be developed to monitor antibody titer and neutralization potential
 - Neutralization assays need to be developed keeping in mind the biological activity of the therapeutic enzyme
 - Neutralization of enzyme activity
 - Neutralization of receptor binding
 - Neutralization of cellular uptake/substrate degradation