

Pharmacy 493

Protein Formulation & Delivery

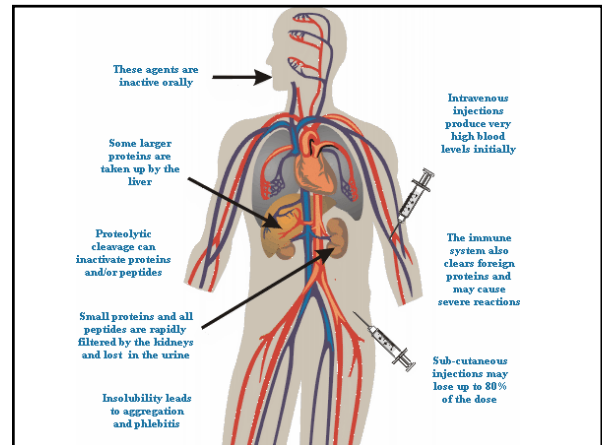
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The Problem with Proteins (*in vivo - in the body*)

- Elimination by B and T cells
- Proteolysis by endo/exo peptidases
- Small proteins (<30 kD) filtered out by the kidneys very quickly
- Unwanted allergic reactions may develop (even toxicity)
- Loss due to insolubility/adsorption

Today's lecture notes are available at:

<http://redpoll.pharmacy.ualberta.ca>



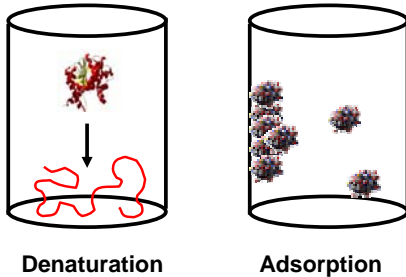
The Problem with Proteins

- Very large and unstable molecules
- Structure is held together by weak noncovalent forces
- Easily destroyed by relatively mild storage conditions
- Easily destroyed/eliminated by the body
- Hard to obtain in large quantities

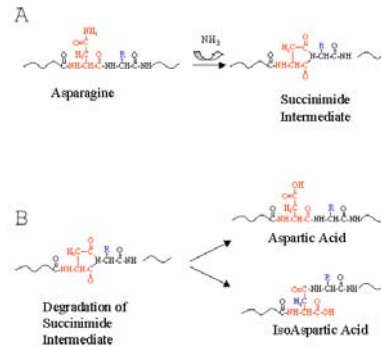
The Problem with Proteins (*in vitro - in the bottle*)

- | Noncovalent | Covalent |
|-----------------|----------------------|
| • Denaturation | • Deamidation |
| • Aggregation | • Oxidation |
| • Precipitation | • Disulfide exchange |
| • Adsorption | • Proteolysis |

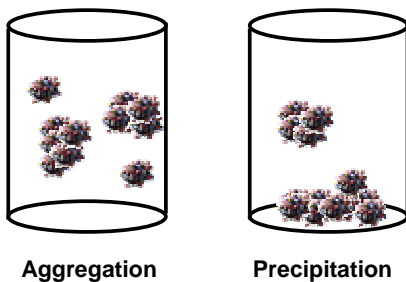
Noncovalent Processes



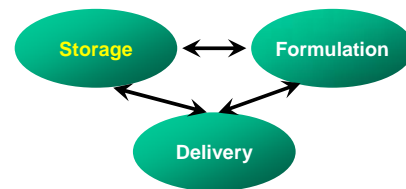
Deamidation



Noncovalent Processes



How to Deal with These Problems?



Pharmaceutics

Covalent Processes

- Deamidation - conversion of Asn-Gly sequences to α -Asp-Gly or β -Asp-Gly
- Oxidation - conversion RSR' to RSOR', RSO₂R' or RSO₃R' (Met & Cys)
- Disulfide exchange - RS⁻ + R'S-SR'' goes to RS-SR'' + R'S⁻ (Cys)
- Proteolysis - Asp-Pro, Trypsin (at Lys) or Chymotrypsin (at Phe/Tyr)

Storage - Refrigeration

- Low temperature reduces microbial growth and metabolism
- Low temperature reduces thermal or spontaneous denaturation
- Low temperature reduces adsorption
- Freezing is best for long-term storage
- Freeze/Thaw can denature proteins

Storage - Packaging

- Smooth glass walls best to reduce adsorption or precipitation
- Avoid polystyrene or containers with silanyl or plasticizer coatings
- Dark, opaque walls reduce hv oxidation
- Air-tight containers or argon atmosphere reduces air oxidation

Freeze Drying

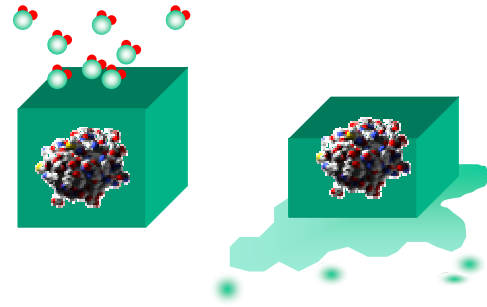


- Freeze liquid sample in container
- Place under strong vacuum
- Solvent sublimates leaving only solid or nonvolatile compounds
- Reduces moisture content to <0.1%

Storage - Additives

- Addition of stabilizing salts or ions (Zn⁺ for insulin)
- Addition of polyols (glycerol and/or polyethylene glycol) to solubilize
- Addition of sugars or dextran to displace water or reduce microbe growth
- Use of surfactants (CHAPS) to reduce adsorption and aggregation

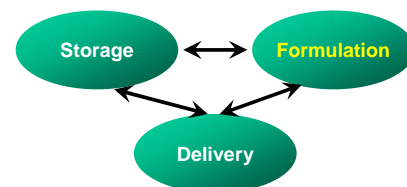
Sublimation vs. Melting



Storage - Freeze Drying

- Only cost-effective means to prepare solid, chemically active protein
- Best for long term storage
- Removes a considerable amount of water from protein lattice, so much so, that some proteins are actually deactivated

Protein Pharmaceuticals



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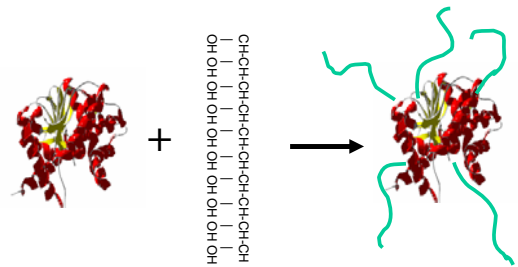
Site Directed Mutagenesis

- Allows amino acid substitutions at specific sites in a protein
- i.e. substituting a Met to a Leu will reduce likelihood of oxidation
- Strategic placement of cysteines to produce disulfides to increase Tm
- Protein engineering (size, shape, etc.)

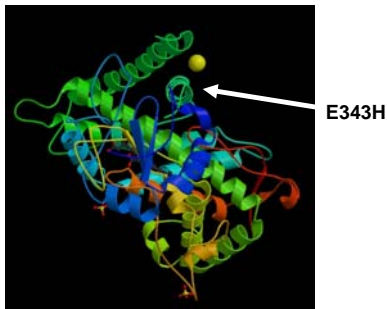
Protein Formulation

- Protein sequence modification (site directed mutagenesis)
- PEGylation
- Proteinylation
- Microsphere/Nanosphere encapsulation
- Formulating with permeabilizers

PEGylation



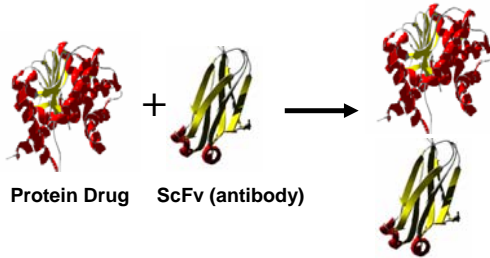
Site Directed Mutagenesis



PEGylation

- PEG is a non-toxic, hydrophilic, FDA approved, uncharged polymer
- Increases *in vivo* half life (4-400X)
- Decreases immunogenicity
- Increases protease resistance
- Increases solubility & stability
- Reduces depot loss at injection sites

Proteinylation



Encapsulation

- Process involves encapsulating protein or peptide drugs in small porous particles for protection from “insults” and for sustained release
- Two types of microspheres
 - nonbiodegradable
 - biodegradable

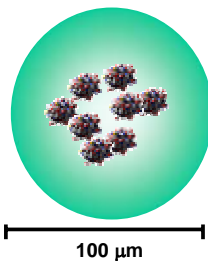
Proteinylation

- Attachment of additional or secondary (nonimmunogenic) proteins for in vivo protection
- Increases in vivo half life (10X)
- Cross-linking with Serum Albumin
- Cross-linking or connecting by protein engineering with antibody fragments

Types of Microspheres

- **Nonbiodegradable**
 - ceramic particles
 - polyethylene co-vinyl acetate
 - polymethacrylic acid/PEG
- **Biodegradable (preferred)**
 - gelatin
 - polylactic-co-glycolic acid (PLGA)

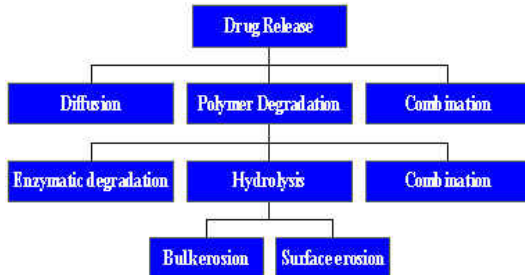
Microsphere Encapsulation



Microsphere Release

- **Hydrophilic (i.e. gelatin)**
 - best for burst release
- **Hydrophobic (i.e. PLGA)**
 - good sustained release (esp. vaccines)
 - tends to denature proteins
- **Hybrid (amphipathic)**
 - good sustained release
 - keeps proteins native/active

Release Mechanisms



Permeabilizers (Adjuvants)

- Salicylates (aspirin)
- Fatty acids
- Metal chelators (EDTA)
- Anything that is known to “punch holes” into the intestine or lumen

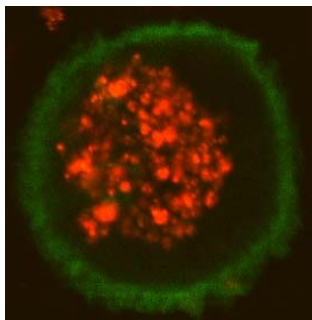
Nanoparticles for Vaccine Delivery

- Mimic pathogen surface characteristics
- Antigen for controlled delivery within Dendritic Cells
- Selective activation of cytokine genes in Dendritic Cells
- Applications in Therapeutic Vaccines (e.g., cancer, AIDS, HBV, HCV)

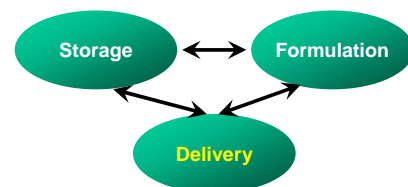
Protein Formulation

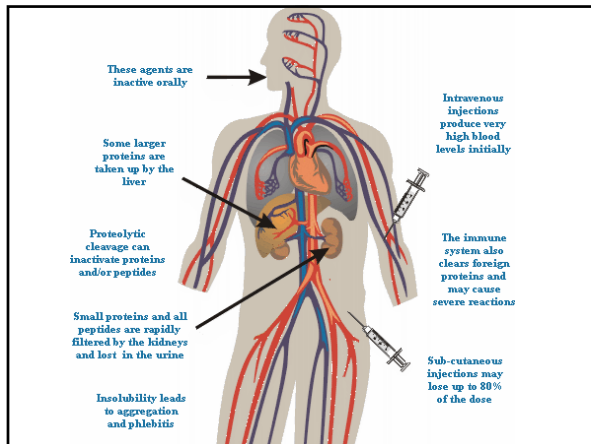
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Polymeric Nanoparticle Uptake by Human DCs: Confocal Image



Protein Pharmaceuticals





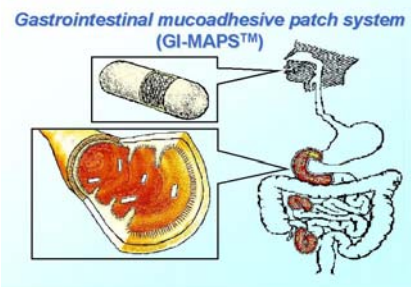
Parenteral Delivery

- Route of delivery for 95% of proteins
- Allows rapid and complete absorption
- Allows smaller dose size (less waste)
- Avoids first pass metabolism
- Avoids protein “unfriendly zones”
- **Problems with overdosing, necrosis**
- **Local tissue reactions/hypersensitivity**
- **Everyone hates getting a needle**

Routes of Delivery

- Parenteral (injection)
- Oral or nasal delivery
- Patch or transdermal route
- Other routes
 - Pulmonary
 - Rectal/Vaginal
 - Ocular

Patch Delivery



Parenteral Delivery

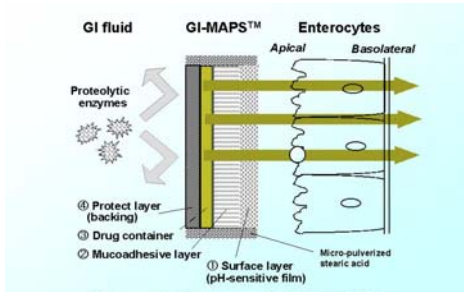


- Intravenous
- Intramuscular
- Subcutaneous
- Intradermal

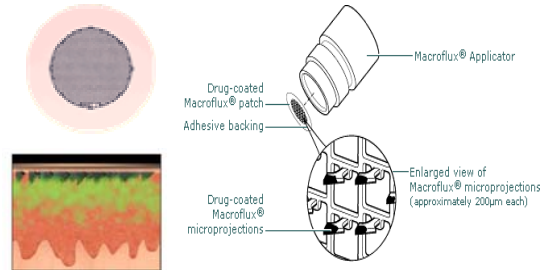
Mucoadhesive Patch

- Adheres to specific region of GI tract
- Ethylcellulose film protects drugs from proteolytic degradation
- Composed of 4 layers
 - Ethylcellulose backing
 - Drug container (cellulose, citric acid)
 - Mucoadhesive glue (polyacrylic acid/PEG)
 - pH Surface layer (HP-55/Eudragit)

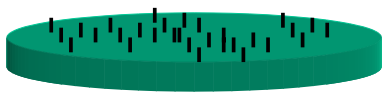
Patch Delivery



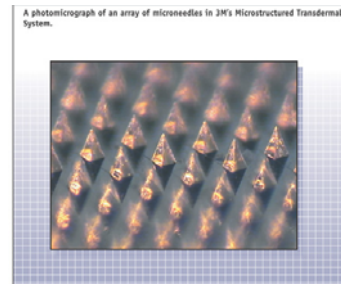
MacroFlux Transdermal Patch



Transdermal Patches



Close-up of Patch Pins



Transdermal Patches

- Proteins imbedded in a simple matrix with appropriate additives
- Patch is coated with small needles that penetrate the dermal layer
- Proteins diffuse directly into the blood stream via capillaries
- Less painful form of parenteral drug delivery

Summary

- Protein pharmaceuticals are (and will be) the most rapidly growing sector in the pharmaceutical repertoire
- Most “cures” for difficult diseases (Alzheimers, cancer, MS, autoimmune diseases, etc.) will probably be found through protein drugs

Summary

- BUT Proteins are difficult to work with
- Most protein delivery is via injection
- Newer methods are appearing
- Oral delivery using “smart materials” is looking promising
- By 2007 many more protein drugs will be taken orally