

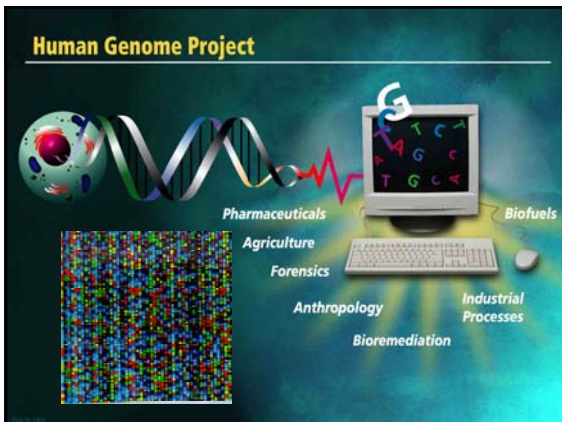
Pharmacy 360

Protein Formulation & Delivery

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Today's lecture notes are available at:

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



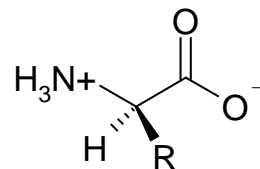
The Human Genome Project

- First Draft completed on June 26, 2000
- 3,260,000,000 bp on 24 chromosomes
- 3,201,762,515 bases sequenced (98%)
- 23,531 - 31,609 genes (predicted)
- All FDA approved drugs target just 417 different proteins

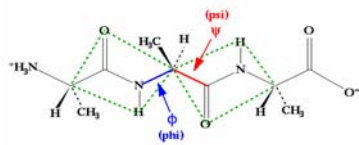
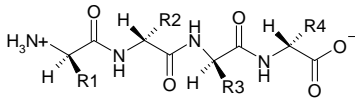
Proteins

- Polypeptides composed of covalently linked amino acids
- Polypeptides with <40 amino acids are called peptides
- Polypeptides with >40 amino acids are called proteins
- Function of a protein determined by its non-covalent 3D structure

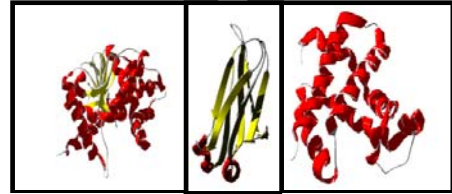
Amino Acids



Polypeptides



Protein Structure



Lactate
Dehydrogenase:
Mixed α / β

Immunoglobulin
Fold: β

Hemoglobin B
Chain: α

Protein Pharmaceuticals

- >200 FDA approved protein drugs (<http://www.biopharma.com/list.html>)
- >30% are recombinant (rDNA) proteins
- Protein pharmaceutical sales currently approach \$39 billion/yr
- By 2005 they are expected to reach \$43 billion/yr

Classes of Protein Pharmaceuticals

- **Vaccines** (peptides, parts of proteins, killed bacteria)
- **Peptides** (oxytocin, pitocin)
- **Blood products** (Factor X, Factor VIII, gamma globulin, serum albumin)
- **Recombinant therapeutic proteins** (herceptin, humulin, alferon, etc.)

Vaccines

- **Diphtheria** (*Corynebacterium diphtheriae*) - diphtheria toxin
- **Tetanus** (*Clostridium tetani*) - tetanus toxin
- **Whooping cough** (*Bordetella pertussis*) - acellular extract



Tetanus Toxin
HC Fragment

Therapeutic Proteins



- **Insulin** (diabetes)
- **Interferon β** (relapsing MS)
- **Interferon γ** (granulomatous)
- **TPA** (heart attack)

Therapeutic Proteins

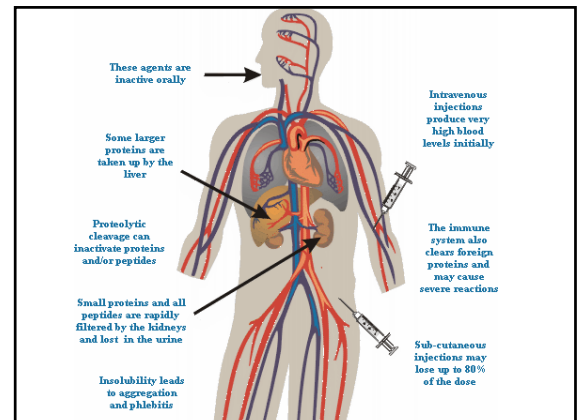
- Actimmune (If γ)
- Activase (TPA)
- BeneFix (F IX)
- Betaseron (If β)
- Humulin
- Novolin
- Pegademase (AD)
- Epogen
- Regranex (PDGF)
- Novoseven (F VIIa)
- Intron-A
- Neupogen
- Pulmozyme
- Infergen

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 vivo - in the body*)

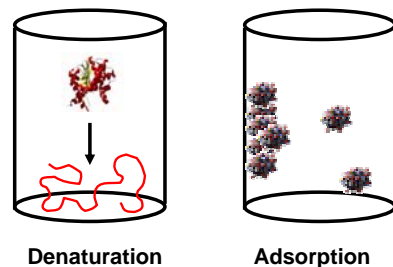
- Elimination by B and T cells
- Proteolysis by endo/exo peptidases
- Small proteins (<30 kD) filtered out by the kidneys very quickly
- Unwanted allergic reactions may develop (even toxicity)
- Loss due to insolubility/adsorption



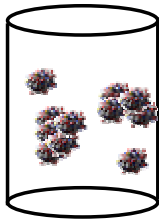
The Problem with Proteins (*in vitro - in the bottle*)

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

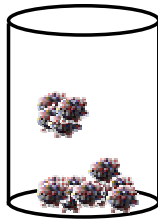
Noncovalent Processes



Noncovalent Processes



Aggregation

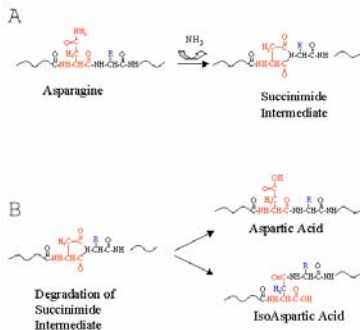


Precipitation

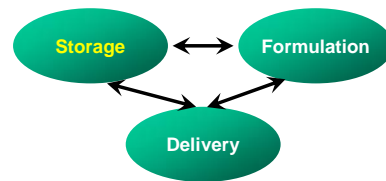
Covalent Processes

- 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)

Deamidation



How to Deal with These Problems?



Pharmaceutics

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

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

Storage - Freeze Drying

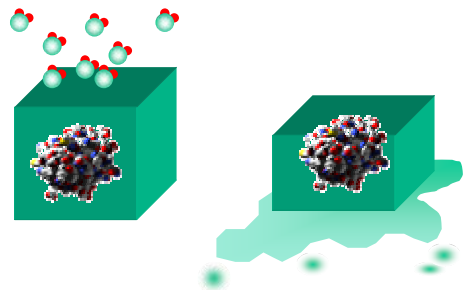
- Only cost-effective means to prepare solid, chemically active protein
- Best for long term storage
- Removes a considerable amount of water from protein lattice, so much so, that some proteins are actually deactivated

Freeze Drying

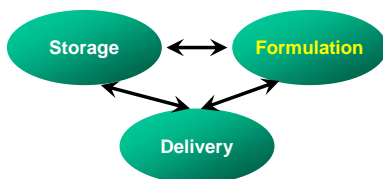


- Freeze liquid sample in container
- Place under strong vacuum
- Solvent sublimates leaving only solid or nonvolatile compounds
- Reduces moisture content to <0.1%

Sublimation vs. Melting



Protein Pharmaceuticals



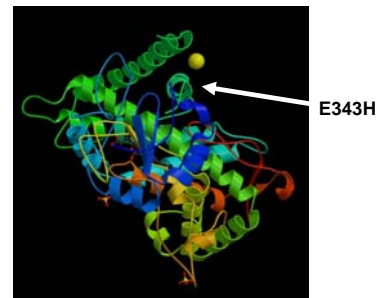
The Problem with Proteins (*in vivo*)

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Protein Formulation

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

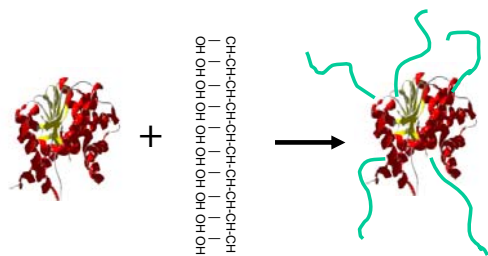
Site Directed Mutagenesis



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.)

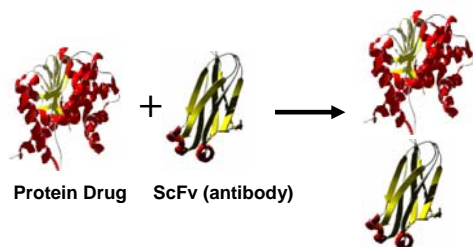
PEGylation



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

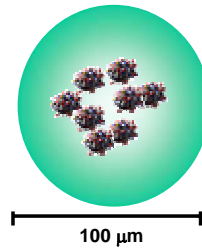
Proteinylation



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

Microsphere Encapsulation



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

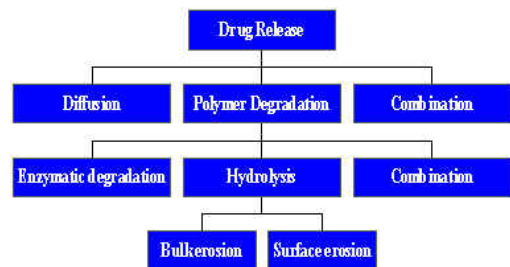
Types of Microspheres

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

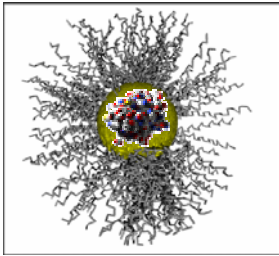
Microsphere Release

- **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



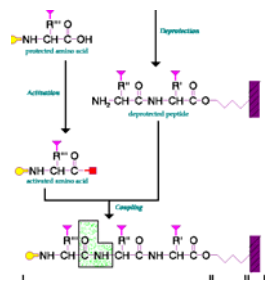
Peptide Micelles



Peptide Micelles

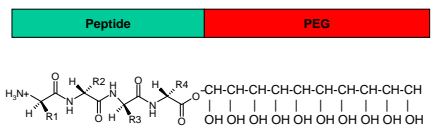
- Small, viral sized (10-50 nm) particles
- Similar to lipid micelles
- Composed of peptide core (hydrophobic part) and PEG shell (hydrophilic part)
- Peptide core composition allows peptide/protein solubilization
- Also good for small molecules

Peptide Synthesis

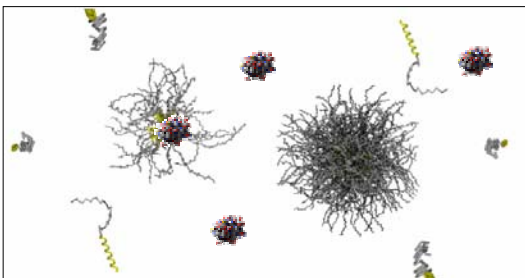


Peptide-PEG monomers

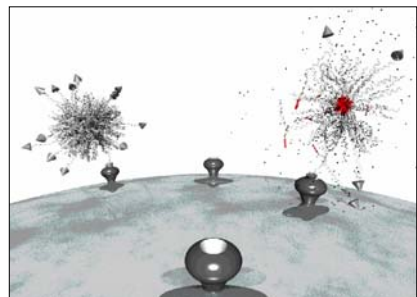
Hydrophobic block Hydrophilic block



Peptide Micelles



Targeted Micelles



Nanoparticles for Vaccine Delivery to Dendritic Cells

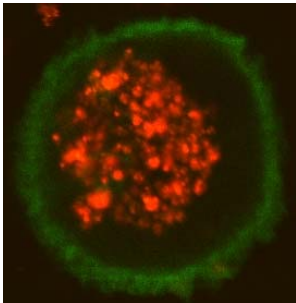
- Dendritic Cells - 'sentries' of the body
- Eat pathogens and present their antigens to T cells
- Secret cytokines to direct immune responses



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)

Polymeric Nanoparticle Uptake by Human DCs: Confocal Image



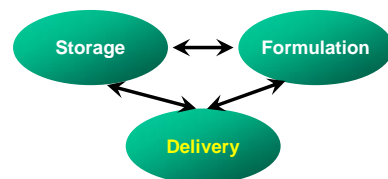
Permeabilizers (Adjuvants)

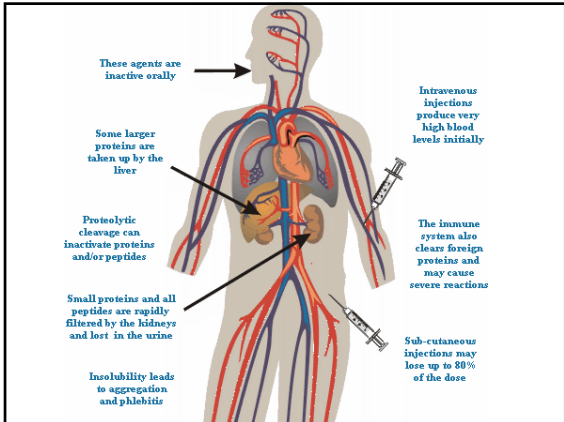
- Salicylates (aspirin)
- Fatty acids
- Metal chelators (EDTA)
- Anything that is known to "punch holes" into the intestine or lumen

Protein Formulation


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

Protein Pharmaceuticals

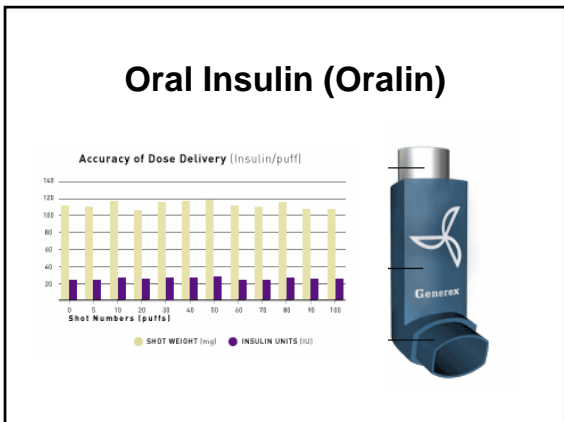




- ## Routes of Delivery
- Parenteral (injection)
 - Oral or nasal delivery
 - Patch or transdermal route
 - Other routes
 - Pulmonary
 - Rectal/Vaginal
 - Ocular

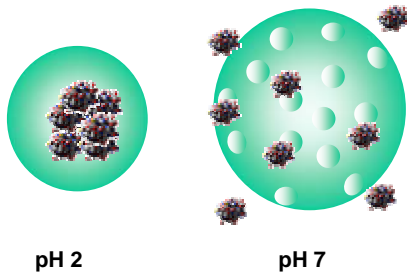
- ## Parenteral Delivery
- 
- Intravenous
 - Intramuscular
 - Subcutaneous
 - Intradermal

- ## 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**



- ## Oral Insulin (Oralin)
- Buccal aerosol delivery system developed by Generex
 - Insulin is absorbed through thin tissue layers in mouth and throat
 - Insulin is formulated with a variety of additives and stabilizers to prevent denaturation on aerosolization and to stabilize aerosol particles

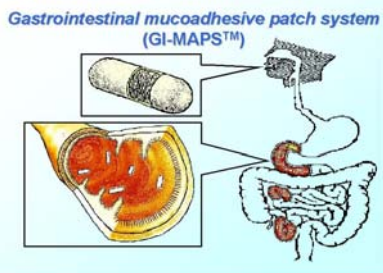
Oral Delivery by Microsphere



pH Sensitive Microspheres

- Gel/Microsphere system with polymethacrylic acid + PEG
- In stomach (pH 2) pores in the polymer shrink and prevent protein release
- In neutral pH (found in small intestine) the pores swell and release protein
- Process of shrinking and swelling is called complexation (smart materials)

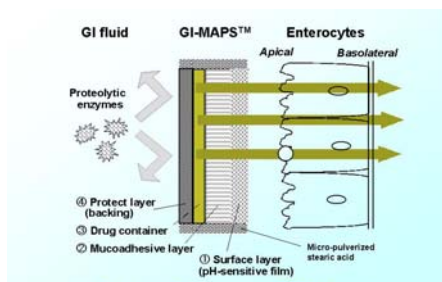
Patch Delivery



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



GI-MAPS Layers

- pH sensitive surface layer determines the adhesive site in the GI tract
- Gel-forming mucoadhesive layer adheres to GI mucosa and permits controlled release - may also contain adjuvants
- Drug containing layer holds powders, dispersions, liquids, gels, microspheres,
- Backing layer prevents attack from proteases and prevents luminal dispersion

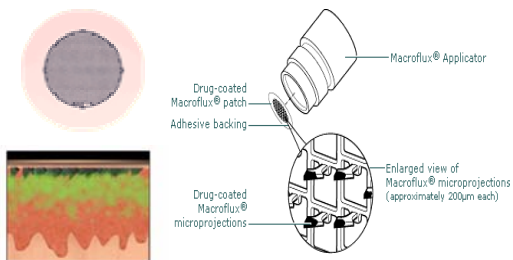
Transdermal Patches



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

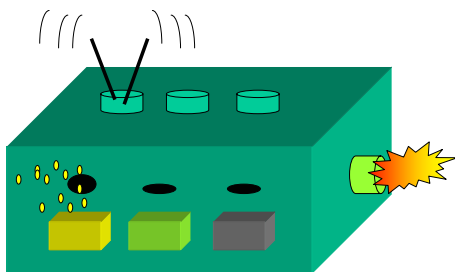
MacroFlux Transdermal Patch



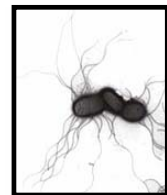
The Future

- Greater use of Nanotechnology in biopharmaceutics (nanopharm)
- Using cells as “Protein Factories” or as targetable “Nanosensors & Nanorobots”
- Artificial or Synthetic Cells as drug delivery agents

Smart Pills



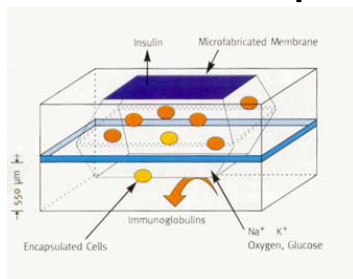
Smart Pills (Nano-Robots)



Unlikely

Likely

Micromachined Biocapsules

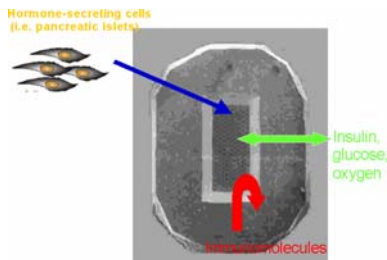


Artificial Islet Cells - Tejal Desai (UI)

Micromachining

- Uses photolithography or electron beam etching to carved small (5 nm) holes into metal (titanium) plates
- Porous plates are placed over small metal boxes containing islet cells
- Insulin (2 nm) leaks out through diffusion, but antibodies are too big (~10 nm) to get in

Biocapsules



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