From mechanism to medicine
a look at proteins and drug design

Chem 342

Wednesday, May 6, 2009
Drug Design - an Iterative Approach

- Structural Analysis of Receptor
- Computational Analysis
- Ligand Design
- Ligand Synthesis
- Structural Analysis of Ligand-Receptor Complex
- Biological Evaluation of Ligand
- Drug Lead

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Bringing a Drug to the Market

Drug Lead → Toxicity Studies → Phase I Trials

10,000 compounds 1-2 year

1,000 compounds 3-4 years

10 compounds 2-3 years

Phase II Trials → Phase III Trials → Marketable Drug

1-5 compounds 2-3 years

1-2 compounds 2-3 years

1 compound av 15 years $900M to $1.5B

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Amino Acids

- Highly polar zwitterions
Amino Acids

There are 20 common amino acids - 15 Neutral

- Glycine
- Alanine
- Valine
- Leucine
- Isoleucine
- Proline
- Serine
- Threonine
- Cysteine
- Methionine
- Asparagine
- Glutamine
- Phenylalanine
- Tyrosine
- Tryptophan
Amino Acids

- Acidic and Basic

- Apartic acid

- Glutamic acid

- Lysine

- Histidine

- Arginine
Peptides - Proteins

- Polymers of Amino Acids

\[ \text{alanine} \quad + \quad \text{valine} \quad \rightarrow \quad \text{ala-val dipeptide} \]

\[ \text{valine} \quad + \quad \text{alanine} \quad \rightarrow \quad \text{val-ala dipeptide} \]
Peptide Bonds

- Amide is the main bond - but can have disulfide bonds

\[
\text{HO} \quad \text{NH}_2 \quad + \quad \text{H}_2\text{N} \quad \text{O} \quad \text{OH} \quad \xrightarrow{\text{reaction}} \quad \text{HO} \quad \text{NH}_2 \quad \text{H}_2\text{N} \quad \text{O} \quad \text{OH}
\]
Disulfide in Vasopressin

- Vasopressin - antidiuretic hormone from pituitary
Peptide - Protein Structure

- Primary Structure - amino acid sequence
- Secondary Structure - orientation of segments alpha-helix, beta sheets, loops
- Tertiary Structure - overall shape of the molecule
- Quaternary Structure - overall structure of protein aggregates
Alpha - Helix

- A helical secondary structure from keratin
Beta Sheets

- Beta sheet secondary structure from Silk fibroin
Hemoglobin

- Globular protein with 574 amino acid residues
# Types of Proteins

## Table 26.2 Some Common Fibrous and Globular Proteins

<table>
<thead>
<tr>
<th>Name</th>
<th>Occurrence and use</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fibrous proteins (insoluble)</strong></td>
<td></td>
</tr>
<tr>
<td>Collagens</td>
<td>Animal hide, tendons, connective tissues</td>
</tr>
<tr>
<td>Elastins</td>
<td>Blood vessels, ligaments</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>Necessary for blood clotting</td>
</tr>
<tr>
<td>Keratins</td>
<td>Skin, wool, feathers, hooves, silk, fingernails</td>
</tr>
<tr>
<td>Myosins</td>
<td>Muscle tissue</td>
</tr>
<tr>
<td><strong>Globular proteins (soluble)</strong></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>Involved in oxygen transport</td>
</tr>
<tr>
<td>Immunoglobulins</td>
<td>Involved in immune response</td>
</tr>
<tr>
<td>Insulin</td>
<td>Hormone for controlling glucose metabolism</td>
</tr>
<tr>
<td>Ribonuclease</td>
<td>Enzyme for controlling RNA synthesis</td>
</tr>
</tbody>
</table>

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Enzymes

- Proteins which act as catalysts for chemical reactions.

<table>
<thead>
<tr>
<th>Main class</th>
<th>Some subclasses</th>
<th>Type of reaction catalyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrolases</td>
<td>Lipases</td>
<td>Hydrolysis of an ester group</td>
</tr>
<tr>
<td></td>
<td>Nucleases</td>
<td>Hydrolysis of a phosphate group</td>
</tr>
<tr>
<td></td>
<td>Proteases</td>
<td>Hydrolysis of an amide group</td>
</tr>
<tr>
<td>Isomerases</td>
<td>Epimerases</td>
<td>Isomerization of a chirality center</td>
</tr>
<tr>
<td>Ligases</td>
<td>Carboxylases</td>
<td>Addition of CO₂</td>
</tr>
<tr>
<td></td>
<td>Synthetases</td>
<td>Formation of new bond</td>
</tr>
<tr>
<td>Lyases</td>
<td>Decarboxylases</td>
<td>Loss of CO₂</td>
</tr>
<tr>
<td></td>
<td>Dehydrases</td>
<td>Loss of H₂O</td>
</tr>
<tr>
<td>Oxidoreductases</td>
<td>Dehydrogenases</td>
<td>Introduction of double bond by removal of H₂</td>
</tr>
<tr>
<td></td>
<td>Oxidases</td>
<td>Oxidation</td>
</tr>
<tr>
<td></td>
<td>Reductases</td>
<td>Reduction</td>
</tr>
<tr>
<td>Transferases</td>
<td>Kinases</td>
<td>Transfer of a phosphate group</td>
</tr>
<tr>
<td></td>
<td>Transaminases</td>
<td>Transfer of an amino group</td>
</tr>
</tbody>
</table>

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Citrate Synthase

- A dimeric protein that catalyzes an Aldol Reaction

\[ \begin{align*}
\text{Citrate} & \quad + \\
\text{Synthase} & \\
\text{citrate} & \quad + \\
\end{align*} \]
Citrate Synthase Mechanism
Matrix Metalloproteinases

- Family of Zn-dependent enzymes
- 26 known MMP’s
- Responsible for maintaining extracellular matrix
- MMP’s out of balance in many diseases
- **CANCER**, Arthritis, Multiple Sclerosis, Stroke Damage, and many more . . .
Catalytic Site of MMP-1 with natural collagen substrate
The Cook Group
@ NDSU

Hydroxamic acid sar

\[ \text{H} \text{O} \text{N} \text{H} \]

\[ \begin{align*} 
& \text{O} \quad \text{R}_1 \quad \text{N} \quad \text{O} \\
& \text{R}_\alpha \quad \text{R}_1 \quad \text{H} \\
& \text{R}_2 \quad \text{R}_2 \quad \text{R}_3 \\
& \text{Zn binding group} \\
\end{align*} \]

**R\text{1}**: P1' group - critical for activity and selectivity. Small groups selective for MMP-1, larger groups will increase selectivity of other MMP's over MMP-1 and MMP-7.

**R\text{2}**: P2' group - wide range of substitution tolerated with aromatic groups preferred. More bulky groups increase oral bioavailability by decreasing amide bond scission.

**R\text{3}**: P3' group - functionality widely tolerated. Aromatic groups increase MMP-3 selectivity.

**R\text{\alpha}**: P1 group - not necessary for activity, but substitution can lead to increased activity of some MMP's.
Hydroxamate Bound to MMP-3

- MMP-3 (Stromelysin 1)
Sulfonamide Bound to MMP-3

- MMP-3 (Stromelysin 1)
2006 Nobel Prize in Chemistry

"for his studies of the molecular basis of eukaryotic transcription"

Prof. Roger Kornberg
Dept. of Structural Biology
Stanford University
School of Medicine

Wednesday, May 6, 2009
DNA Structure

- 1953 - Watson and Crick discovered DNA was made up of two strands running in opposite directions

- The strands are held together by hydrogen bonding from the bases

- Specific bases bind to each other like lock and key

- The strands are Complementary
Heterocycles in DNA and RNA

Adenine

Guanine

Cytosine

Thymine

Uracil
Nucleotides

- The DNA/RNA bases are attached to phosphosugars
The nucleotides are connected together by the phosphates on the sugars
DNA Double Helix

- Two grooves are formed
- Sugar phosphate runs along the outside
- The major groove is slightly bigger and deeper than the minor groove
DNA is a code for the synthesis of proteins. Every 3 base pair sequence is directly correlated with a specific amino acid.
Transcription

- DNA unwinds and codes an RNA strand
- RNA Polymerase
Translation

- RNA encodes the amino acid sequence for protein synthesis.
DNA Wraps Around Histone Proteins
Nucleosomes Coil Up

30 nm

Octameric histone core

10 nm

DNA

H1 histone

Histone octamer

DNA

H1 histone

30 nm
Wrapped Up Tight
Histone Deacetylase

Transcription Repression

DNA Binds Tightly

HAT
HDAC

DNA Unwinds

Transcription Activation and Gene Expression

Tumor Cell

HDAC Inhibitor

Hyperacetylated Histones

Transcription Activation of Preprogrammed Genes

Cell Growth Inhibited, Apoptosis, and/or Differentiation

Wednesday, May 6, 2009
Effectiveness in Clinical Trials

- HDAC Inhibitors show marked effect in the treatment of cutaneous T-cell lymphoma
- Phase II clinical trials of FK228
HDAC Structure

- HDAC Like Homolog
- 35.2% sequence homology with human HDAC1
- Active site homology much higher

HDAC Inhibitor Design

ZBG → linker → surface binding group

Typical HDAC Inhibitors
HDAC Inhibitors

Carboxylates
phenylbutyrate $mM$

Cyclic Peptides
CHAP50
depsipeptide $nM$

Benzamides
MS-275 $\mu M$

Hydroxamate
TSA

Electrophilic Ketones
SAHA $nM$
TSA and SAHA - Hydroxamic Acids

Trichostatin A (TSA)
IC<sub>50</sub> 12 nm

Suberoylanilide Hydroxamic Acid (SAHA)
IC<sub>50</sub> 165 nm
Hydroxamic Acid Binding
SAHA and HDAC Homolog
Conclusions

- Proteins are the stuff of life.
- DNA encodes amino acid sequence of proteins.
- Enzymes are proteins that catalyze chemical reactions.
- Disease can often be targeted by identifying specific enzymes that is important for the disease state.
- Synthetic chemistry can provide solutions for drug development.