

Denaturation

Denaturation - "any non covalent change in the structure of a protein molecule"

If changes are reversible, thermodynamic information can be obtained.

If not reversible, can obtain kinetic data.

Bovine Trypsin Inhibitor



$$K = [D]/[N]$$

$$H = 57.3 \text{ Kcal/Mole}$$

$$S = 180 \text{ cal/deg-mole}$$

Determine G at 30, 45 and 60 °C

Calculations

At 30

$$G = 57,300 - 303(180)$$

$$G = +2,760 \quad K_{eq} \approx 0.01$$

At 45

$$G = 57,300 - 318(180)$$

$$G = +60 \text{ cal/mole} \quad K_{eq} \approx 1$$

At 60

$$G = 57,300 - 333(180)$$

$$G = -2540 \text{ cal/mole} \quad K_{eq} \approx 100$$

Measures of Denaturation

Loss of solubility

Increased proteolysis

Loss of biological activity

Increased tritium - hydrogen exchange

Spectroscopic procedures

Ultraviolet

Fluorescence

ORD/CD

Implications of Denaturation

Loss of enzymatic activity

Loss of solubility

Change in water holding capacity

Change in ability to bind or emulsify fat

Change in ability to form foams

Reversibility

Denaturation will usually become irreversible when too many hydrophobic groups have been exposed.

This leads to aggregation.

What is the relative free energy of the native versus the irreversibly denaturated state?

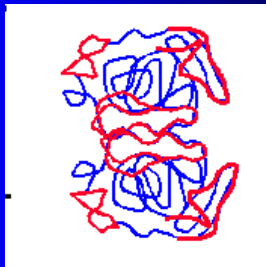
Native



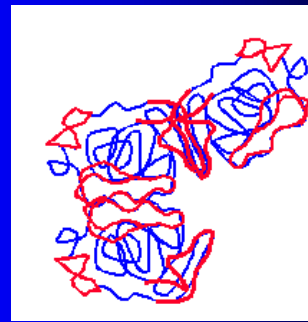
Denatured



Aggregation



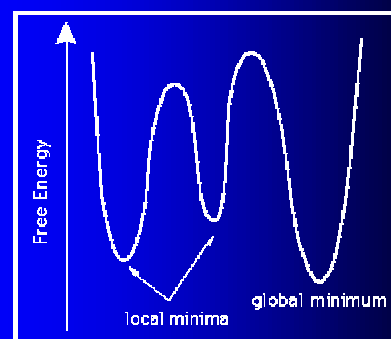
Precipitation



Causes

- pH extremes (isoelectric)
- Organic solvents
- High salt concentrations
- Freezing
- Stirring
- Heating
- Drying
- Interfaces

Energy



Kuru

South Fore on New Guinea (8000 total population)

Total of 1000 died in the 1960's

Women 8 times as likely

Mortuary cannibalism



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Tissue Infectivity

Category I: High Infectivity
Brain, spinal cord, (eye)

Category II: Medium Infectivity
Spleen, tonsil, lymph nodes, ileum, proximal colon, cerebrospinal fluid, pituitary gland, adrenal gland,

Category III: Low Infectivity
Peripheral nerves, nasal mucosa, thymus, bone marrow, liver, lung, pancreas

Category IV: No Detectable Infectivity
Skeletal muscle, heart, mammary gland, serum, feces, kidney, thyroid, salivary gland, saliva, ovary, uterus, testis, seminal testis, (colostrum, bile, bone, cartilaginous tissue, connective tissue, hair, skin, urine).

Scrapie

First detected in sheep in Great Britain 250 years ago

2 to 5 years for infection to show

From ewe via placenta

Cross species spread via inoculation in brain

Creutzfeldt-Jakob Disease

Usually between ages of 50 and 70

Inherited - 5% of all cases

Sporadic - unknown

Infection -rare

Average = 63 one case per million

vCJD - Average age= 28 (151 as of 12/6/2004)



Protein Folding

