Aggregation of α-Chymotrypsinogen A in Aqueous Solutions

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What is Protein Aggregation?

- It is a broad term used to define a type of protein self assembly
- Classified as native or nonnative proteins
- Can include both soluble and insoluble protein aggregates
- Typically these aggregates are unfavorable products

Figure 1. Schematic diagram of protein aggregation pathways.

Quality control of protein folding in extracellular space. J. J. Yerbury.
Why is it important?

1. Bio-processing operations

2. Pharmaceuticals

3. Debilitating diseases

4. Cytotoxicity
Our study

- α-Chymotrypsinogen A

**Goals:**
1. Aggregation dynamic of α-Chymotrypsinogen A
2. Broaden this understanding to other proteins
3. Apply to real world problems

**Techniques:**
1. Dynamic Light Scattering
2. Circular Dichroism
3. Small-Angle Neutron Scattering

“...30% of the original protein sample aggregated in a period of 2 hours into clusters comprising four or more molecules each.”
Dynamic Light Scattering Results

5 mg/ml pH=7.3
Decay Curve Fitting Results

\[ I_2(t) = A(e^{-2t/\tau}) + B \]

```matlab
clc;
clear;
h = fopen('testdata.txt', 'r');
data = fscanf(h, '%g %g', [4 inf])';
t = data(:,1);
Rexp = data(:,2);
loglog(t, Rexp, 'ro'); hold on

b0 = [.22 40 1];
b = lsqnonlin(@expfunc, b0, [], [], [], data)
Rcal = (b(1)*exp(-2*t/b(2))) + b(3);
plot(t, Rcal, 'b');
k = 2*pi/(782.7e-9/1.33);
q = (sqrt(2)*k);
D = 1/((b(2)*(10^-6))*(q^2));
a = (1.3806503e-23*298.15)/(6*pi*8.94e-4*D)

Hydrodynamic Radius:
a = 2.2293e-009 = 2.23 nm
```

[Graph showing decay curve fitting results]
Dynamic Light Scattering Results

5 mg/ml  pH=7.3

96 h
Dynamic Light Scattering Results

5 mg/ml pH=7.3

96 h

~ 50000
Dynamic Light Scattering Results

1 mg/ml  pH=7

Typical Decay Curves
Decay Curve Fitting Results

\[ a_1 = 2.2110 \times 10^{-9} = 2.21 \text{ nm} \]
\[ a_2 = 9.3650 \times 10^{-8} = 93.7 \text{ nm} \]
\[ a_3 = 8.3982 \times 10^{-7} = 840 \text{ nm} \]

\[ a_1 = 1.9266 \times 10^{-9} = 1.93 \text{ nm} \]
\[ a_2 = 1.9271 \times 10^{-7} = 193 \text{ nm} \]
\[ a_3 = 2.4384 \times 10^{-6} = 2.44 \text{ \(\mu\)m} \]
Circular Dichroism and the Conformational Analysis of Biomolecules. Gerald D. Fasman.

Figure 5. Far-UV CD spectra of chymotrypsinogen and α-chymotrypsin at pH 7. The inset shows the difference spectrum (chymotrypsinogen – chymotrypsin) as the solid curve (right ordinate) and the CD spectrum of N-acetyl-L-tryptophanamide as the dashed curve (left ordinate). The data on chymotrypsinogen are from unpublished work of M. J. Gorbunoff and S. N. Timasheff, and those for AcTrpNH₂ are from Shiraki (1969). (Reprinted with permission from Cantor and Timasheff, 1982, by permission. © 1982, Academic Press, Inc.)
Circular Dichroism

1 mg/ml Chymotrypsinogen pH=7

- Fresh
- 1 Day
- 2 Days
- 5 Days
- 7 Days
- 9 Days
- 2 Weeks
Small-Angle Neutron Scattering (SANS)
Small-Angle Neutron Scattering (SANS)

Chymotrypsinogen 5mg/ml pH=7 fresh

Smeared Sphere Model
Radius = 2.0 nm

X1mg/ml_pH7_7days

FitYw

Smeared Sphere Model
Radius = 2.1 nm

X1mg/ml_pH9_7days

FitYw

NIST
Conclusion

- Aggregate
  - Linear semi-flexible polymer chain
  - Does not precipitate
Future Plans

• Chymotrypsin Testing
• Trypsin Inhibitor
• Vary pH
  ▫ pH = 3
  ▫ pH = 9
  ▫ pH = 11
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Thank You!
¿Questions?
References


Materials

- α-chymotrypsinogen
- deionized water from a Millipore Milli-Q system (solutions for DLS)
- NaCl (used to adjust the electrolyte concentration)
- 10 mM citrate buffer (0.01 mol/L) deionized water (1 L) citric acid (192.14 x 0.01 = 1.9214 g)
- 0.1 M NaOH (0.1 mol/L) deionized water (0.1 L) NaOH (40.01 x 0.01 = 0.4001 g)
- 0.1 M HCl dilute from concentrated HCl solution