Visualizing Insulin Structure Under Shear Stress

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Insulin is a hormone that regulates glucose intake by cells.

Patients with diabetes are insulin-deficient.

Diabetes is treated by injection of engineered insulin analogues.
INSULIN THERAPEUTICS

OBJECTIVE

Long-acting insulin drug for once-daily dosing

Hexamers

Rod-like Structures

Monohexamers

Dihexamers

Rods

Dimers

Monohexamers

Dissociation

Insulin injection

Subcutaneous Tissue

Monomers

Insulin in Bloodstream

Capillary Membrane
Next Step: Does Shear Change Insulin Structure?

Subcutaneous Injection
Shear rates on order of $10^5$-$10^6$ s$^{-1}$

Blood Vessels
Shear stresses as high as 9.8 N/m$^2$
Possible Outcomes

Rod Lengthening/Breaking

Alignment with Applied Shear

Interactions

Repulsion

Attraction
Project Overview

**Objective:** Determine if shear affects insulin self-assembly

- **Insulin samples prepared:**
  - Insulin concentrations (mM): 0.6 | 1.2 | 1.8
  - NaCl concentrations (mM): 30 | 150 | 450

- Samples sheared at different shear rates to probe their viscosity

- **Small-angle neutron scattering** applied during shear for structural data
Viewing Nanostructure With Small Angle Neutron Scattering

Scattering vector: $\mathbf{q} = \frac{4\pi \sin(\theta)}{\lambda}$

Diagram showing the paths of the incident, transmitted, and scattered beams with labels for the source, sample, detector, and beamstop. The scattering intensity is plotted against $\mathbf{q}$ (in units of $\text{Å}^{-1}$) on a graph.
Why SANS?

Probes appropriate length scale to view:

- Structure
- Orientation of structures

![Diagram showing isotropic orientation and alignment in flow](http://cns.che.udel.edu/)
Rheology Measurements

**Instrument:** Anton Paar MCR 502 Rheometer

**Shear Rates:** 0-3500 s$^{-1}$

Images:
- Cup and Bob: ter-mcr-102-302-502/
SANS Measurement

Source \( k_i \) \( \rightarrow \) Incident beam

Sample under shear

Transmitted beam

Scattered beam \( k_s \)

Beamstop

Detector

Radial Measurements

Tangential Measurements

Sample under shear

2\( \theta \)
Sample Preparation

**NaCl Concentrations**

- 30 mM
- 150 mM
- 450 mM

**Insulin Concentrations**

- 0.6 mM
- 1.2 mM
- 1.8 mM

**Components**

- Insulin
- NaCl
- Zinc
- D₂O
- Phenol
- Phosphate Buffer
Rheology: Viscosity vs. Shear Stress

<table>
<thead>
<tr>
<th>Insulin Concentration</th>
<th>Volume Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.6mM</td>
<td>0.44%</td>
</tr>
<tr>
<td>1.2mM</td>
<td>0.88%</td>
</tr>
<tr>
<td>1.8mM</td>
<td>1.32%</td>
</tr>
</tbody>
</table>

\[ \sigma^* = 1.3 \text{ Pa} \] for 30mM NaCl

\[ \sigma^* = 0.22 \text{ Pa} \] for 150mM NaCl

\[ \sigma^* = 0.19 \text{ Pa} \] for 450mM NaCl
SANS Data: Increasing Salt Concentration

0.6mM Insulin

<table>
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<tr>
<th>mM NaCl</th>
<th>30</th>
<th>150</th>
<th>450</th>
</tr>
</thead>
</table>

-1 slope

Radial Configuration

Tangential Configuration

Isotropic Orientation
Higher Order Structures: SAXS Comparison

450mM NaCl

[SAXS Data] [SANS Data]

Quadratic ordering
Layering

I(Q) (1/cm)
Q (1/A)

1.00E-01 1.00E-02 1.00E-03 1.00E-04 1.00E-05

1.00E-01 1.00E-02 1.00E-03 1.00E-04 1.00E-05
Shear Thickening Dependence on Shape

- 30mM NaCl
- 150mM NaCl
- 450mM NaCl

0.6mM Insulin

- $|q| (1/\text{cm})$
- $q (1/\text{A})$

- $-1$ slope

35% change

Intracellular Stress Measure

100
10
1

30mM NaCl

150mM NaCl

450mM NaCl
SANS Data: Increasing Salt Concentration

**Shear-induced reversible structural change at 150mM NaCl, 3500s⁻¹**

- **0.6mM Insulin**
- **1.2mM Insulin**
- **1.8mM Insulin**

Fitted models

With structure factor

Without structure factor
Electrostatic Interactions?

30mM NaCl
- $\sigma^*=1.3$ Pa

150mM NaCl
- $\sigma^*=0.22$ Pa

450mM NaCl
- $\sigma^*=0.19$ Pa

Shear stress overcomes repulsion at $\sigma^*$

Electrostatic Repulsion

SHEAR THICKENING
Electrostatic Interactions?

30mM NaCl

$\sigma^* = 1.3 \text{ Pa}$

150mM NaCl

$\sigma^* = 0.22 \text{ Pa}$

450mM NaCl

$\sigma^* = 0.19 \text{ Pa}$

With NaCl Screening

Weaker Repulsion

Easier to overcome repulsion

Higher NaCl = Lower $\sigma^*$
Summary of Findings

Structure
Rods **form, lengthen, and order** with increasing NaCl and insulin concentrations
Very little shear dependence

Rheology
Shear-thinning → Shear-thickening
Critical shear stress (electrostatic barrier?)

Future Outlook
Tests with higher shear rates, lower q
More rheological studies
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