

# Report: Project Week in the Abel Group: Kinetic Study of the Aggregation of Insulin at an Acidic Condition

Anmol Kumar Ray

## Introduction and Background:

This project week was carried out in the group of Prof. B. Abel under the supervision of Andreas Bögehold. The goal was to study the kinetics of aggregation of bovine insulin in aqueous acidic solution (pH 2.) at 70 °C using mass spectrometry and CD spectroscopy.

The project week involved new technique of mass spectrometry, known as laser induced liquid beam desorption mass spectrometry. In this project, *Analytical laser induced liquid beam desorption mass spectrometry (ALILBD-MS) of biomolecules and their aggregates*, pre-formed ions were desorbed directly from the liquid phase and were analyzed using time of flight analyzer. This method of analysis is very important especially in case of biomolecules which involves rather weak non-covalent interactions. Since my PhD work was involved also in the study of the non-covalent interactions between natural products and biomolecules using Fourier Transform Ion Cyclotron Resonance- Electrospray Ionization (FTICR-ESI) mass spectrometry, this project week appeared to be the alternative of FTICR mass spectrometry.

I basically worked on bovine insulin proteins. Aggregation of this protein leads to fibril formation which is responsible for the Alzheimer's disease. The main purpose of this experiment was to carry out the kinetic study of the aggregation pattern of this protein in an aqueous solution (pH 2) at 70 °C. The CD measurement was done to provide the supplementary informations about the structural changes with time, like from alpha-helical to beta-sheets.

## Results and Discussions:

From the mass spectrometry (ALILBD-MS) as depicted in Fig. 1, the signal for the insulin monomer decreased very rapidly in the beginning (during the first 30 min) followed by the constant and very low signal intensity. It is known that under acidic condition, insulin exists as monomer. Therefore, under this experimental condition (aqueous acidic solution, pH 2), insulin showed the maximum monomer's signal intensity and it remained almost constant at room temperature (25 °C). When this solution was heated at 70 °C, the monomer's concentration decreased rapidly as shown in Fig. 1.

Circular dichroism (CD) is a spectroscopic method which is used for monitoring protein folding and unfolding. Therefore, this technique was used to find the changes in the structure with time under the aforementioned experimental conditions (aqueous acidic solution, pH 2, 70 °C). In Fig. 2, Initially, the CD spectrum (at 0 min) showed three peaks: a negative peak at ~220 nm, a similar negative peak at ~208 nm, and a positive peak at ~200 nm. This spectrum is typical for the  $\alpha$ -helix. After 30 min, the spectrum changed completely to the one having one significant negative peak at ~217 nm which is normally for the random coil or the for the disordered protein. After 60 min, the spectrum again changed completely to the one having one negative peak at ~225 nm and one positive peak at ~200 nm. This spectrum is mostly for the  $\beta$ -sheets of proteins. Also, with time the shape of this  $\beta$ -sheet CD spectra remained almost

same with varying magnitudes of the peaks intensities. These three different types of CD spectra were obtained at 70 °C while at room temperature (25 °C), the CD spectra were having similar shapes (belonging to the  $\alpha$ -helix). This result was also in agreement with that of mass spectrometry (ALILBD-MS).

a) Kinetic study from mass spectrometry (ALILBD-MS)

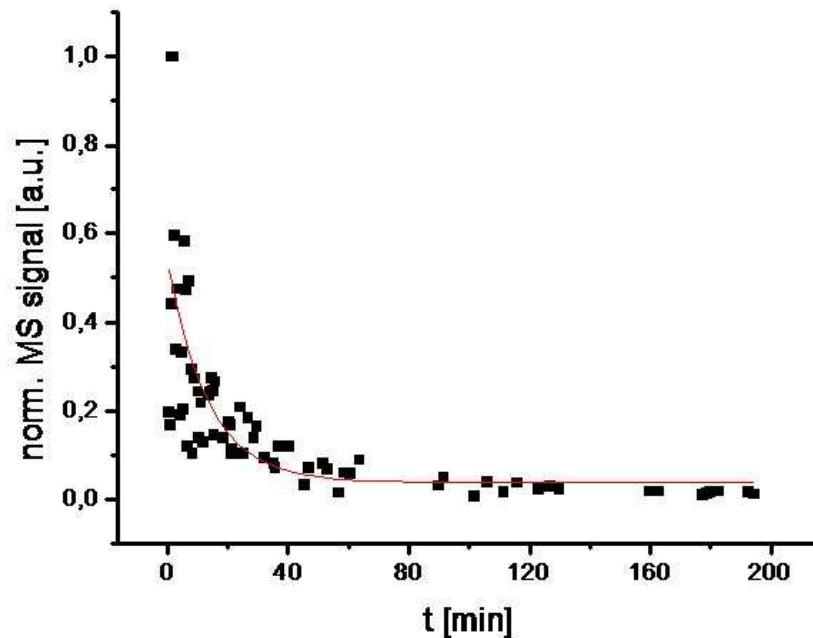


Fig. 1: Kinetic study of Insulin monomer's decrease using mass spectrometry

b) CD spectra

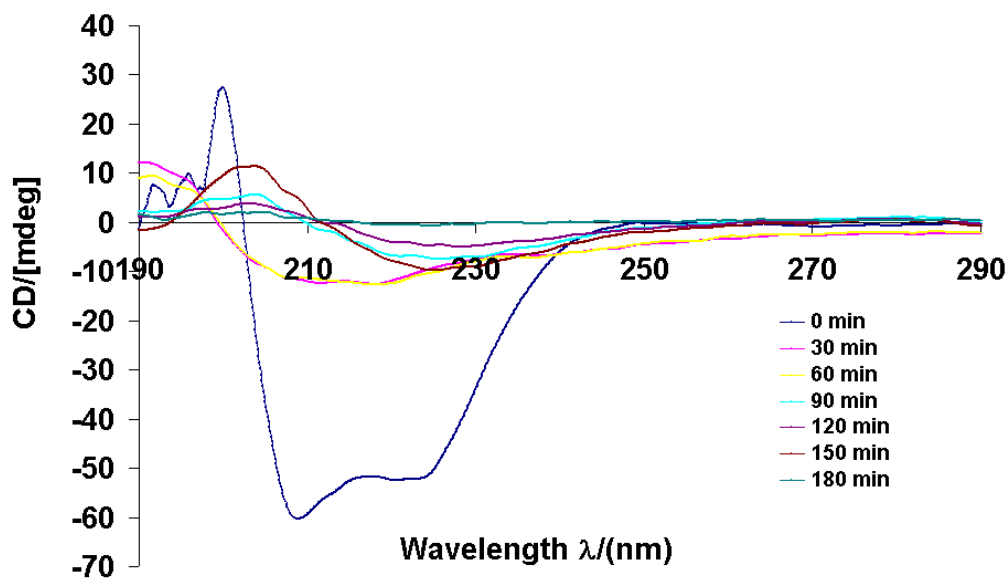


Fig. 2: CD spectra of bovine insulin at 30 min intervals

## **Conclusion**

Higher temperature (70 °C in this case) expedites the structural changes of this insulin protein. Under these experimental conditions, insulin undergoes the structural changes from  $\alpha$ -helix to random coil (or disordered form) followed by  $\beta$ -sheets. Once this  $\beta$ -sheet forms, they might lead to the fibril formation which is responsible for the Alzheimer's disease.

I would like to thank Prof. Bernd Abel and Andreas Bögehold for providing me such opportunity to learn about this interesting project done in the group.

Feb 17 2009