شبكة المعلومات الجامعية

بسم الله الرحمن الرحيم

شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

شبكة المعلومات الجامعية

جامعة عين شمس

التوثيق الالكتروني والميكروفيلم

قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها
علي هذه الأفلام قد أعدت دون أية تغيرات

يجب أن

تحفظ هذه الأفلام بعيدا عن الغبار

في درجة حرارة من ١٥-٢٥ مئوية ورطوبة نسبية من ٢٠-٤٠%

To be Kept away from Dust in Dry Cool place of 15-25- c and relative humidity 20-40%

بعض الوثائـــــــق الاصليـــــة تالفـــة

بالرسالة صفحات لم ترد بالاصل

Biophysical Study of X-ray Scattering in Biological Samples

Presented by

Ebtsam Abd Elghany Mohammad Elbeshbishy

A Thesis Submitted
to
Faculty of Science

In Partial Fulfillment of the
Requirements for
the Degree of
M.Sc. of Biophysics
(Medical)

Biophysics Department
Faculty of Science
Cairo University

(2009)

APROVAL SHEET FOR SUBMISSION

Thesis Title: Biophysical Study of X-ray Scattering in Biological Samples.

Name of candidate: Ebtsam Abd Elghany Mohammad Elbeshbishy

This thesis has been approved for submission by the supervisors:

1- Prof. Dr. Anwar Abd-Elazim Elsayed

Signature: Anwar A. Elsayed
6/12/2009

2- Dr. Wael M. Elshemey

Signature: Wael Elshemey

Prof. Dr. Osiris Wanis Guirguis

Chairman of Biophysics Department
Faculty of Science- Cairo University

Osiris W. Guirguis
22/12/2009

Acknowledgment

First, I thank God for helping me to accomplish this work.

I am grateful to prof. Dr. Anwar A. Elsayed for his deep insights and illuminating comments that made this work possible. His supervision for this thesis is a real proud for me.

I am indebted to Dr. Wael M. Elshemey for his magnificent effort in the supervision of this work. His faithful guidance, smart remarks and technical comments played a pivotal role in the development of this thesis.

I should also appreciate Dr. Mosad El ghatuory for his help and guidance. Dr. Mosad helped me to obtain insulin material from VACSERA.

Deep thanks to Dr. Ussry from Chemistry department for his help and donating me uranyl acetate.

Finally, I would like to thank my colleagues, in Biophysics Department for their sincere feelings and enormous support.

To
My Father and
Mother,

To
My Brothers,

To
My Sisters

Table of contents

Abstract

Abstract

Wide angle x-ray scattering (WAXS) from protein in solution and dry (lyophilized) proteins is characterized by the presence of two weak broad scattering peaks. The first peak corresponds to a d-spacing around 10 Å and is attributed to inter-helix packing. The second peak is at about 4.5 Å and is attributed to α-helix backbone. For a protein with β-sheets as the main secondary structure, these peaks correspond to inter-sheet packing and hydrogen bonding distance between β-strands, respectively.

Since the scattering from water dominates over the scattering from protein in solution, one have to use a highly intense x-ray beam (probably from synchrotron) in order to be able to observe the scattering from protein. Otherwise, it would still be possible to unveil the protein scattering peaks through the lyophilization of protein solution into a powder form and obtaining the WAXS profile using a conventional x-ray diffractometer. The latter method is adopted in this study.

Since it has been reported that wide-angle x-ray scattering would provide a means to identify induced changes in the secondary, tertiary and quaternary structure of protein, this work aims to evaluate the potential of WAXS as a probe of induced conformational changes in insulin. For such purpose, native (control) insulin is forced to unfold and breakdown either using thermal denaturation alone or in the presence of thiol (cysteine) catalysts via disulfide scrambling. Denatured products are acid-trapped and monitored using WAXS in addition to FTIR, gel

filtration chromatography and Transmission Electron Microscopy (TEM) as supportive techniques.

Results show that the WAXS peak at 10 Å is sensitive towards the α-helix content of insulin. A reduction in the intensity of such peak (due to thermal denaturation or denaturation using thiol catalysts) is proven to be directly linked to the reduction of native insulin having normal α-helix content. It has been shown that the decrease in α-helix content of insulin is accompanied by the appearance of β-sheet structures that in turn rearrange into a fibrous form (amyloid fibers).

Chapter 1

Introduction

1. Introduction:

Since the discovery of x-rays by Roentgen in 1895, wide range of applications in the biological and medical fields had been developed. Among these applications are x-ray radiography, x-ray crystallography, x-ray florescence analysis, x-ray computed radiography, Dual Energy X-ray Absorptiometery (DEXA) and possibly many other useful developments to come in the future.

Among the recent interesting applications of x-ray in the biological and medical fields are the x-ray scattering techniques. These techniques make use of photons scattered from biological entities to gain characteristic information about the scattering tissue or molecule.

While Compton scattering has shown remarkable sensitivity toward the density of the scattering medium, coherent (elastic or Rayleigh) scattering has shown a potential sensitivity toward the molecular structure of biomolecules. This is a major interest in the present study.

Kosanetzky et al (1987) presented x-ray diffraction patterns of some plastics and several biological samples. Their scattering profiles showed that the investigated biological samples had characteristic scattering distribution with one or more forward scattering peaks. These peaks are attributed to the interference of photons coherently scattered from molecules of the medium (molecular interference effects) and considered as a fingerprint of the investigated sample.