BIOLOGICAL STUDIES ON GLYCOSAMINOGLYCANS

Thesis

Submitted for the Degree of M.Sc.

By

Amai Abd El Azeem Mohammed

B.Sc. Zoology Dept. Faculty of Girls Ain Shams University, 1980

Supervised by

Professor

Ekram Z.I. Khafagy

Prof. of Biochemistry and Head of Biochemistry Dept. National Research Centre Dokki - Cairo

Researcher

Dr. Fathya A. Shaheen

Dr. of Biochemistry Biochemistry Dept. National Research Centre Dokki - Cairo

Professor

10 m // // /

Madiha A. Ashry

Prof. of Histochemistry
Zoology Dept.
Faculty of Girls
Ain Shams University

Assit. Prof.

Dr. Roukaya M. Ali

Dr. of Physiology
Zoology Dept.
Faculty of Girls
Ain Shams University

1993

BIOLOGICAL STUDIES ON GLYCOSAMINOGLYCANS

BOARD OF SCIENTIFIC SUPERVISION

Professor Dr.: Ekram Z.I. Khafagy

Professor of Biochemistry and Head of Biochemistry Dept. National Research Centre Dokki - Cairo.

Professor Dr.: Madiha A. Ashry

Professor of Histochemistry Zoology Dept. Faculty of Girls Ain Shams University Cairo - Egypt.

Dr. Roukaya M. Ali Saeid

Doctor of Physiology Zoology Dept. Faculty of Girls Ain Shams University Cairo - Egypt.

Dr. Fathya A. Shaheen

Doctor of Biochemistry Biochemistry Dept. National Research Centre Dokki - Cairo



BIOLOGICAL STUDIES ON GLYCOSAMINOGLYCANS

COURSES

Studied by the Candidate in Partial Fulfilment of the Requirements for the Degree M.Sc.

- 1- Physiology
- 2- Histology
- 3- Histopathology
- 4- Invertebrate
- 5- English
- 6- Statistical

ACKNOWLEDGMENT

The author wishes to acknowledge her thanks and gratitude to late / Professor Dr. Moread Yanni, Professor Dr. Madiha A. Ashry and Assistant Professor Dr. Roukaya M. Ali, Zoology Dept., Faculty of Girls, Ain Shams University for their support.

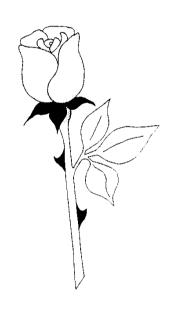
The author is indebted to Professor Dr. Ekram Z.I.

Khafagy, Head of Biochemistry Department, National
Research Centre, for her suggestion, valuable advice, deep
interest, supervision and help. Her patience and thought
fulness have made the work a pleasant task.

She wishes to express her thanks to Dr. Fathia A. Shaheen, Researcher in the Biochemistry Dept. for her help.

The author is greatly indebted to the National Research
Centre for the financial support and facilities that enabled her
to carry out this work.

To My Parents
To My Husband Gouda
And To My Daughters
(Rehap, Eman & Amml)



ABSTRACT

Heparin (which is the most important member of glycosaminoglycan group) was isolated from beef, sheep and camel lungs and then subjected to purification. Chemical analysis and anticoagulant activity tests of the purified heparin samples comply well with the data of the international clinically used heparin.

From the biological studies of glycosaminoglycan it is found that, this group has an important role in cellular regulatory processes through gene regulation at the chromatin level and the enzymes involved in the breakdown of DNA and RNA.

CONTENTS

	Paç	је
Introductio		1
A. Gene	ral introduction	.1
B. Gene	ralized primary structures of glycosaminoglycans	.6
я H	yaluronic acid	. 7
n C	hondroitin	. 7
¤ C]	hondroitin 4-sulfate	. 7
ъ С	hondroitin 6-sulfate	. 7
¤ D	ermatan sulfate	8
	eparin	
n K	eratan sulfate	9
C. Biosy	nthesis of glycosaminoglycans	11
D. Biolo	gical action of glycosaminoglycans	18
E. Tissu	e presentation of glycosaminoglycan disorders	24
Aim of \	Work	30
Material ar	id: Methods	31
l- Hepar	in preparation, purification and chemical analysis	31
¤ Pı	reparation and purification of Heparin	31
¤ C	hemical analysis of heparin samples	34
	Determination of uronic acid by Carbazol method	
	Determination of glucosamine by the Elson-Morgan method Determination of sulfate ions by the Turbidity method	
	iological assay of heparin (anti-coagulant activity)	

Pag
II. Influence of glycosaminoglycans (Heparin samples, chondroitin sulfate and Hyaluronic acid) on calf thymus chromatin
III. Influence of glycosaminoglycans on the Deoxyribonuclease 1 (DNase I) induced degradation of DNA and DNP50
IV. Influence of glycosaminoglycans on the breakdown of ribonucleic acid (RNA) by bovine pancreatic ribonuclease (RNase)
Results 5
Discussion 7
Summary 7
References 8
Arabic Summary

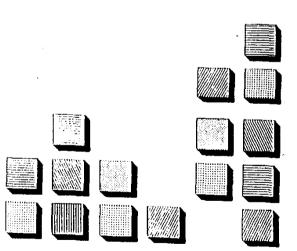
LIST OF TABLES

Table		Page
1	Major constituents of glycosaminoglycans	5
2	Composition and anticoagulant activity of heparin samples	54
3	Influence of heparin samples on the release of DNA from chromatin	56
4	Influence of camel heparin, chondroitin sulfate and hyaluronic acid on the release of DNA from chromatin	57
5	The DNase I. induced degradation of DNA and DNP in the presence of standard heparin	59
6	The DNase I. induced degradation of DNA and DNP in the presence of beef heparin	60
7	The DNase I. induced degradation of DNA and DNP in the presence of sheep heparin	62
8	The DNase I. induced degradation of DNA and DNP in the presence of camel heparin	
9	The DNase I. induced degradation of DNA and DNP in the presence of chondroitin sulfate	64
10	The DNase I. induced degradation of DNA and DNP in the presence of hyaluronic acid	

LIST OF FIGURES

ire		P
	Standard curve of glucuronic acid	
!	Standard curve of glucosamine	
3	Standard curve of sulfate ions	
ŀ	Standard curve of calf thymus DNA	
5	Effect of standard heparin on the activity of bovine pancreatic RNase	
6	Effect of beef heparin on the activity of bovine pancreatic RNase	
,	Effect of sheep heparin on the activity of bovine pancreatic RNase	
3	Effect of camel heparin on the activity of bovine pancreatic RNase	.
•	Effect of chondroitin sulfate on the activity of bovine pancreatic RNase	<i>.</i>
0	Effect of hyaluronic acid on the activity of bovine pancreatic RNase	

INTRODUCTION



INTRODUCTION

A. General Introduction

As the advances of chemistry and biochemistry have permitted some insight into and understanding of our chemical natures and processes, new complexities, and complexities within complexities have been encountered.

One such group of complexities are the proteoglycans and glycosaminoglycans, which together constitute a group of macromolecules that clearly are important and essential, if not major, components of our bodies, but whose structures and functions are complex and far from being understood completely (Kennedy, 1973).

As will be developed later, these macromolecules occur extensively in almost all, if not actually all, mammalian tissues along with collagen fibers whose positions they maintain and with which the tissue structure, type, and rigidity is produced and maintained.

Therefore they are of prime importance in health and disease.

Nomenclature of Glycosaminoglycans

The introduction in (1938) of the term mucopolysaccharide (Meyer, 1938) was to describe collectively 2-amino-2-deoxyhexose containing polysaccharide materials of animal origin occurring either as free polysaccharides or as their protein derivatives.

However, with the various subsequent discoveries of other types of carbohydrate containing macromolecules, the term has come to be used in so many ways that it is now in a sense quite vague.

Since the glycosaminoglycans have always come within the mucopolysaccharide category irrespective of the way in which that the term has been used, they were described widely as acidic mucopolysaccharides on account of their highly cationic nature.

However, this nomenclature arose at a time when it was not realized that the glycosaminoglycans, as we call them today, are attached covalently to protein, and at a time when the polysaccharide was isolated with some amino acid units attached.

Thus, acidic mucopolysaccharide means the glycosaminoglycan of a proteoglycan plus (some times) a few amino acid units, whereas glycosaminoglycans means purely the polysaccharide part of a proteoglycan.

On account of the apparent regularity of the polysaccharide chains in proteoglycans and the early belief that the protein present in preparations of the polysaccharide parts represented impurity, greatest attention has been given to the glycosaminoglycan chains themselves rather than to proteoglycans as a whole.

Thus the glycosaminoglycans have been named individualy, but not so much according to their component monosaccharides and their simplified disaccharide repeating structures (these were often unknown at the times of original isolation), but according to trival reasoning, e.g., by naming after the source.

In all, eight glycosaminoglycans of essentially different chemical structures have been identified.

Through the times, these glycosaminoglycans have been individually named in a number of ways shown in Table 1. Most of these names are used currently. Where the term chondroitin sulfate appears in the more recent literature, this can mean chondroitin 4-sulfate or chondroitin 6-sulfate or a mixture of the two.

The terms keratan sulfate 1 and keratan sulfate II are sometimes used to denote keratan sulfates of corneal and skeletal origin, respectively, there being, some differences between the two.