HYALURONAN

Volume 1 – Chemical, Biochemical and Biological Aspects

Editors:

JOHN F. KENNEDY BSc, PhD, DSc, EurChem CChem FRSC, CBiol FIBiol, FCIWEM, FCMI, FIFST
Director of Birmingham Carbohydrate and Protein Technology Group,
School of Chemical Sciences, The University of Birmingham, Birmingham B15 2TT, England, UK,
Director of Chembiotech Ltd,
University of Birmingham Research Park, Birmingham B15 2SQ, England, UK,
Director of Inovamed Ltd,
Chembiotech Laboratories, University of Birmingham Research Park, Vincent Drive, Birmingham B15 2SQ, England, UK, and
Professor of Applied Chemistry,
The North East Wales Institute of Higher Education, Plas Coch, Mold Road, Wrexham, Clwyd, LL11 2AW, Wales, UK

GLYN O. PHILLIPS BSc, PhD, DSc, HonDSc, HonLIB, CChem FRSC
Chairman of Research Transfer Ltd,
Newtech Innovation Centre,
Professorial Fellow,
The North East Wales Institute of Higher Education, Plas Coch, Mold Road, Wrexham, Clwyd, LL11 2AW, Wales, UK, and
Professor of Chemistry,
The University of Salford, England, UK

PETER A. WILLIAMS BSc, PhD, CChem FRSC
Director of the Centre for Water Soluble Polymers,
The North East Wales Institute of Higher Education, Plas Coch, Mold Road, Wrexham, Clwyd, LL11 2AW, Wales, UK,
Director of the Centre for Advanced and Renewable Materials at the North East Wales Institute and University of Wales, Bangor,
The North East Wales Institute of Higher Education, Plas Coch, Mold Road, Wrexham, Clwyd, LL11 2AW, Wales, UK
Professor of Polymer and Colloid Chemistry,
The North East Wales Institute of Higher Education, Plas Coch, Mold Road, Wrexham, Clwyd, LL11 2AW, Wales, UK

Guest Editor:

VINCE C. HASCALL PhD
Co-Director of the Orthopaedic Surgery Musculoskeletal Research Center,
Department of Biomedical Engineering ND-20, Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, Ohio 44195, USA
Adjunct Professor
Department of Biochemistry, Case Western Reserve University, Cleveland, Ohio 44106, USA
Adjunct Professor
Department of Biochemistry, Rush Presbyterian St. Lukes Medical Center, Chicago, Illinois, 60612 USA

WOODHEAD PUBLISHING LIMITED

Published by Woodhead Publishing Ltd, Abington Hall, Abington,
Cambridge CB1 6AH, England
www.woodhead-publishing.com

First published 2002

British Library Cataloguing in Publication Data
A catalogue record for this book is available from the British Library.

ISBN 1 85573 570 9 (2 volume set)

Printed in Great Britain by MFK Group Ltd

BIOLOGICAL PROPERTIES OF HYALURONAN ARE CONTROLLED AND SEQUESTERED BY TERTIARY STRUCTURES

John E. Scott[1] and Frank Heatley[2]

[1]Chemical Morphology, Manchester University Medical School, Oxford Rd, Manchester M13 9PT
[2]Chemistry Dept. Manchester University, Manchester M13 9PL

ABSTRACT

Hyaluronan (HA) is the characteristic polysaccharide component of vitreous humor (from which it was first purified[1]), Wharton's jelly and synovial fluid. The unusual mechanical properties of these gels and viscoeleastic fluids were attributed non-specifically to interactions between HA molecules, which are stiff in solution partly because they prefer to take up 2-fold helical secondary structures, stabilised by H-bonds[2] (Fig 1A). HA has since been found in many tissues from many species[3]. Probably all animals produce it. Unexpectedly, a new class of specific and potent biological activities shown by HA fragments (in angiogenesis, inflammation etc) emerged[4-6]. Highly polymerised HA does not share these properties. HA is vitally important during development[7]. It is a pluripotent material with a simple strucure (Fig1A).

Is there a unifying concept behind this diversity? We suggest that physiological properties of HA are controlled and sequestered by reversible tertiary structures[8]. We introduce an NMR approach which can monitor their formation and behaviour. Biological properties may thereby be linked to specific chemical aspects of HA and HA supramolecular organisation.

KEYWORDS

β Sheets, twofold helices, angiogenesis, hyaluronidase, erythrocyte lysis.

INTRODUCTION

^{13}C NMR studies on HA in aqueous solution proved that acetamido and carboxylate groups were involved in NH→COO^- H-bonds, probably in stacked antiparallel agregates formally similar to β sheets as seen in proteins[8] (Fig 1B), but uncommon if not unique in the polysaccharide field. The ^{13}C acetamido C=O signal is much broadened (unlike all the other ^{13}C resonances) because rotation of the amide group is restricted by participation in an H-bond. This broadening can be used as a reporter of H-bond formation, the first specific spectrometric test so far in this field.

DISCUSSION

It is a necessary consequence of the tertiary structure model that the acetamido NH is oriented *trans*-to the C2H of the glucosamine ring, permitting the NH→COO H-

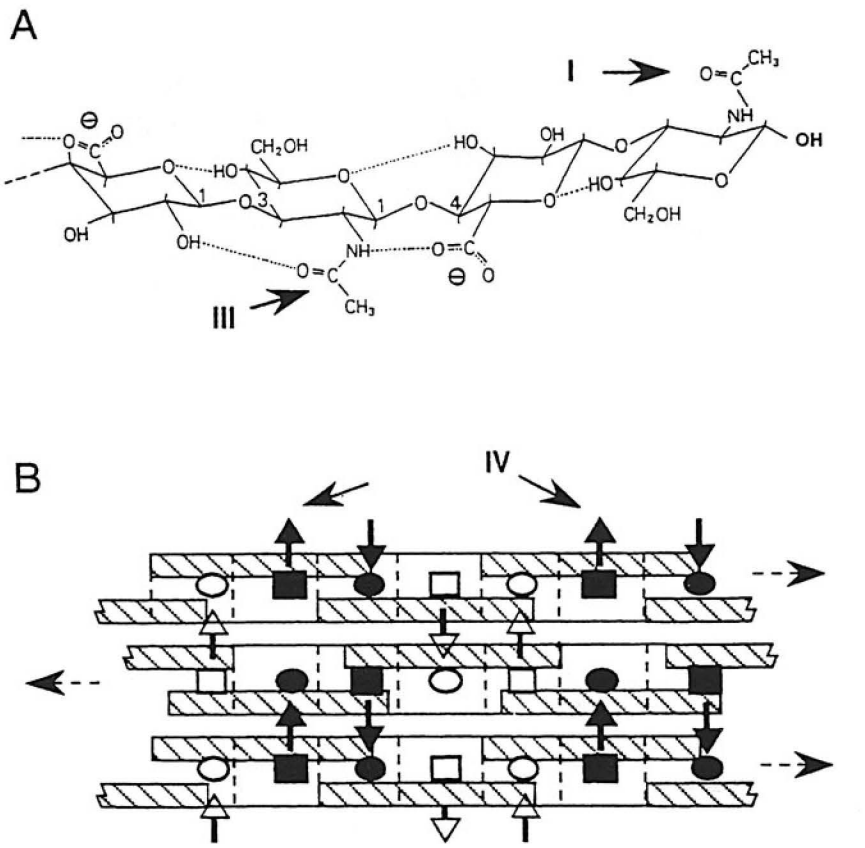

Figure 1 (A) secondary structure of HA (a tetrasaccharide fragment) as a two-fold helix with hydrogen bonds (dotted lines).
(B) tertiary structure of HA viewed from the side, showing three HA molecules in antiparallel array stacked above each other. Arrows to the left and right point to the reducing ends; cross hatched areas are the hydrophobic patches; vertical dotted lines delineate sugar units. Square symbols (□) denote acetamido groups, circles (○) carboxylate groups. Filled symbols are on the distal side and open symbols on the proximal side. Vertical arrows indicating H-bonds from donor NH to acceptor -COO^- are in pairs alternately pointing up and down, linking each HA molecule with both its neighbours in a β sheet-like structure[8]. Overlapping hydrophobic patches provide additional stabilisation by hydrophobic bonding.
Four different environments in which the amide group can exist in HA are labelled I, II, III and IV. I and II are not H-bonded to carboxylates, I because there is no suitably placed carboxylate, and II because the relevant carboxylate has been converted into a poor receptor for H-bonding e.g.as a methyl ester[8]. III and IV are H-bonded either in secondary (twofold helices) or tertiary (β sheets) structures. Characteristic NMR signatures are associated with each environment (see refs 8 and 12).

bonds to form between stacked antiparallel polysaccharide chains (Fig.1B). It was known, using ^{1}H NMR to measure $^{3}J_{CH\text{-}NH}$ coupling, that the *trans*- arrangement was present in monomers and oligomers[2] of up to 27 disaccharides[12] which, however, do not aggregate efficiently to tertiary structures[8]. We tried to extend these data to high mol. mass HA, but all signals were broadened, as is usual in high polymers, and although the NH signal was broader than other peaks in the spectrum (Fig. 2), as expected if it was intrinsically two peaks with a large value of $^{3}J_{CH\text{-}NH}$ coupling, a precise value of the coupling constant could not be obtained. The broadness of this peak is probably also due to lack of rotation of the amide group, as already shown by ^{13}C NMR[8]. If the sharpening of the ^{13}C acetamido C=O resonance on warming is secondary to simple rupture of NH→COO H-bonds, the *trans*- CH-NH orientation should persist at high temperatures and this is compatible with the persistent broadening of the NH signal up to ~80 °C (Fig 2).

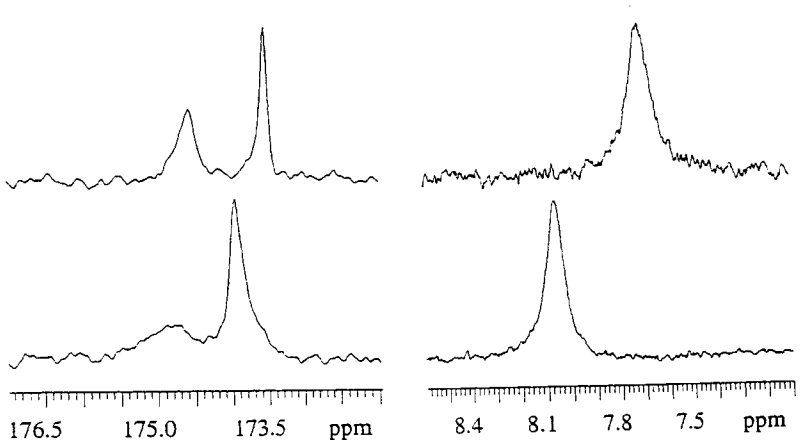

Figure 2. Left, ^{13}C NMR resonances of high mol. mass HA acetamido C=O (174.5 - 175 ppm, amide IV) at 23 °C (lower trace) and 80 °C (upper trace). The sharp signal at 173.5 -174ppm is the carboxylate C=O resonance[8]. HA concentration 10mg/ml in D_2O containing 0.14M NaCl. Note the marked sharpening of the acetamido C=O resonance on warming.
Right, ^{1}H NMR resonance of high mol. mass HA NH proton. HA concentration 2mg/ml in 4:1 H_2O/D_2O solution containing 0.15M NaCl. The broad resonance is of similar width at both 23 °C (lower trace) and 80 °C (upper trace).

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.