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Polysaccharide Research at Norwich

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INSTITUTE OF FOOD RESEARCH, NORWICH LABORATORY

The Laboratory at Norwich is one of three sites which together constitute the Agricultural and Food Research Council (AFRC) Institute of Food Research (IFR). A reorganisation of food research in the UK during the 1960s led to the closure of the Low Temperature Station for research in biochemistry and biophysics at Cambridge and the Ditton Laboratory in Kent, and to the opening of the Meat Research Institute at Bristol and the Food Research Institute at Norwich (Fig. 1). A further restructuring in the 1980s resulted in an amalgamation of the two sites (now called laboratories), together with part of the National Institute for Research in Dairying at Reading, into a single Institute, the IFR. The Norwich Laboratory (Fig. 2) is one of several affiliated research establishments situated adjacent to the University of East Anglia. The Laboratory specialises in the study of agricultural and food produce, mainly of plant origin.

CARBOHYDRATE RESEARCH AT NORWICH

Interest in carbohydrates began at Cambridge with Dr F. A. Isherwood who studied biosynthesis and degradation of plant tissue. Prof. R. L. M. Synges' interest in the chromatographic separation of methylated sugars, plus the development of mass spectrometric methods within the Institute by Mr R. Self provided the impetus for detailed structural analysis of polysaccharides, and a cell wall group was set up to investigate the structure of plant cell walls. In the middle 1970s a Nutrition division was formed by Prof. D. A. Southgate, leading to additional

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Fig. 1. Outline of the mainline coast of England showing Norwich.

Fig. 2. The AFRC Institute of Food Research, Norwich Laboratory. The Norwich site contains the University of East Anglia, the British Sugar plc Research Laboratory, and the AFRC Institute for Plant Science Research. The latter, previously known as the John Innes Institute, is to be expanded to include a part of the AFRC Plant Breeding Institute from Cambridge and a new Sainsbury laboratory for studying molecular plant pathology. The Ministry of Agriculture, Fisheries and Food is to build a new Research Laboratory on the Norwich site starting in 1988.

interest in the composition, analysis and physiological effects of dietary fibre. In the late 1970s Dr H. W.-S. Chan set up studies of biopolymer functionality which led to the formation of a starch group and the establishment of new groups concerned with the physical, physical–chemical and rheological characterisation of biopolymers. A substantial part of this work was devoted to studies of polysaccharides. In the mid-1980s, Prof. P. Richmond (now Head of Laboratory) extended these studies to include the physical modification of polysaccharides and the characterisation of modified structures. The laboratory now possesses a wide range of techniques and a breadth of expertise for studying polysaccharides. I will attempt to introduce the people and their expertise, and illustrate the work of the Laboratory through the use of specific examples, and hope that this article will engender new collaborative research which will extend and broaden our present interests in carbohydrate polymers.

MEN, WOMEN, METHODS AND MACHINES

Structural analysis

Analysis and sequencing of polysaccharide structures increasingly involves many disciplines and several research groups at the IFR now contribute to such work. Structural studies are centred on the *cell wall group* run by Dr Robbie Selvendran. For many years Robbie has been studying plant cell walls and the structure of the component polysaccharides, glycoproteins and proteoglycans. The group has developed and improved methods for isolating cell wall material and for the selective and sequential extraction of biopolymers from this material. Recently, with Malcolm O'Neill (now working with Prof. Albersheim's group in the USA), Robbie has extended his interests to include bacterial polysaccharides. This work is now continuing through the efforts of Barry Stevens. Structural analysis involves collaboration with the *mass spectrometry group* (set up by Ron Self and now run by Dr Fred Mellon). A combination of chemical analysis, specific degradations and mass spectrometry is used to determine sugar composition, anomeric configurations, linkages and sugar sequences. Facilities available include gas chromatography–mass spectrometry (GC–MS), high performance liquid chromatography–mass spectrometry (HPLC–MS) and fast atom bombardment (FAB) mass spectrometry. The implementation of LC–FAB offers the potential for sequencing mixtures.

1D and 2D NMR techniques show great promise for structural analysis and may soon rival MS methods. Coupled with selective degradations and separations of oligosaccharides, the methods may be used for sequencing, defining chair conformations, measuring spatial arrangements of sugar rings and locating non-carbohydrate substituents. Expertise in this area is represented by Dr Ian Colquhoun, the head of the *molecular spectroscopy group*. Detailed analysis of starch and starch polysaccharides, using enzymic degradations and HPLC, is the function of the *starch group* run by Dr Steve Ring. The interests of the *nutritional composition group*, led by Richard Faulks, lie in the development of methods of analysis and their application to determine the sugar, starch and dietary fibre content of foods.

Physics, physical chemistry and rheology

These techniques are divided amongst three research groups. Dr Steve Ring and Dr Mary Whittam use dilatometry, surface tension and heat capacity studies to monitor the solution properties of starch polysaccharides, oligosaccharides and their complexation with other molecules. Routine rheological methods are used to monitor starch pastes, gels and retrogradation.

Nuclear magnetic resonance (NMR) and fourier transform infrared spectroscopy (FTIR) are managed by the *molecular spectroscopy group*. Dr Steve Tanner is interested in the use of multinuclear NMR to probe changes in the mobility (line broadening) and chemical environment (chemical shifts) of cations due to binding to carrageenans upon gelation. High-resolution solid-state NMR is being used by Steve Tanner to study the hydration of α-(1→4)-glucans, the crystal structure of model carbohydrates and the crystal structure and polymorphism of cellulose, chitin (for example, see Fig. 3) and chitosan. Dr Brian Hills is interested in the theory of exchange processes in complex heterogeneous systems and its application to probe pore sizes in polysaccharide gels, whilst Kevin Wright has been involved in ^{17}O NMR studies of sugar–water systems. High-resolution 1D and 2D proton and ^{13}C NMR methods are used by Ian Colquhoun for structural analysis. Reg Wilson is responsible for FTIR studies on polysaccharides with areas of interest ranging from molecular studies of cation binding, or conformational transitions, to the development and use of improved sampling methods (attenuated total reflectance (ATR) and photoacoustic spectroscopy (PAS)) for studying complex heterogeneous systems.

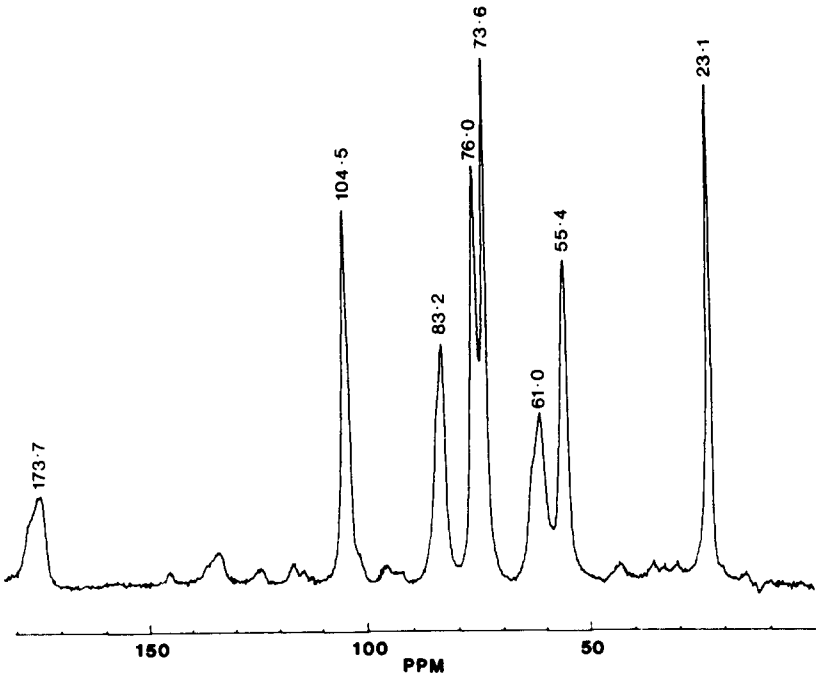

Fig. 3. High-resolution solid-state NMR spectrum of α-chitin.

I (Vic Morris) run the *molecular biophysics group* which has optical, X-ray and rheological methods for studying polysaccharides. Static and dynamic light-scattering, together with electric and elongational flow-birefringence are used to probe the size, shape and stiffness of polysaccharides. Circular dichroism and optical rotation are available to investigate conformational transitions. X-ray methods, managed by Dr Mervyn Miles comprise small-angle scattering, powder diffraction and fibre diffraction, which are used to study the helical structures, interactions and crystallisation of polysaccharides. Use of the SERC Daresbury synchrotron source has permitted kinetic small-angle scattering studies of polysaccharide gelation. Rheological techniques (dynamic mechanical spectroscopy, relaxational shear and elongational methods, and fracture) are managed by Dr Geoff Brownsey. A recent acquisition is a scanning-tunnelling microscope which will be used to assess the potential of this technique for imaging biopolymers. The group has a general interest in plant, animal and bacterial polysaccharides. Jane Harris is the group microbiologist involved in the screening, growth and isolation of useful polysaccharides. Genetic studies of bacterial polysaccharide production

are carried out in the neighbouring AFRC Institute of Plant Science Research, John Innes Laboratory, in connection with studies of bacterial pathogenicity, root nodulation and nitrogen fixation. Facilities for differential scanning calorimetry to probe crystallisation, conformational transitions, molecular complexation or glass transitions are available courtesy of the *protein group* headed by Dr Nigel Lambert.

Physical processing

Physical processing of polysaccharides is carried out by the *solids processing group* headed by Dr Andrew Smith. Single- and twin-screw extruders, with in-line rheometers and a capillary rheometer, are being used to model extrusion processes. Control and monitoring instrumentation is being developed by Eddie Prescott and novel probes are being developed by Dr Sidi Chouikhi. The links between polysaccharide modification, melt rheology and product characteristics are being studied by Dr Roger Parker. Work on solid foam structures is being carried out in collaboration with scientists at the Cavendish Laboratory as part of an AFRC Link Group with the University of Cambridge.

Nutritional studies

The *nutrient availability group* is largely concerned with the factors affecting the absorption and metabolism of carbohydrates. The head of the group, Dr Ian Johnson, is an intestinal physiologist interested in the effects of non-digestible polysaccharides on gastrointestinal function. Dr Geoff Livesey is a biochemist specialising in the determination of the energy values of foods, particularly involving poorly available polysaccharides. Current interests are in the physiological effects of resistant starch and the development of in-vitro methods of predicting in-vivo digestion of starch polysaccharides.

AREAS OF RESEARCH

Dietary fibre is important in nutrition and health. In addition to work on the absorption and metabolism of carbohydrates there is a major programme on the structure of plant cell walls. Extensive studies on the parenchymatous tissues of plants have revealed, and are revealing, details of the cell wall structure. Such studies assist in the development or modification of dietary fibre analysis, and form a basis for studies on ripening, growth, and the cell wall as a source of oligosaccharins.

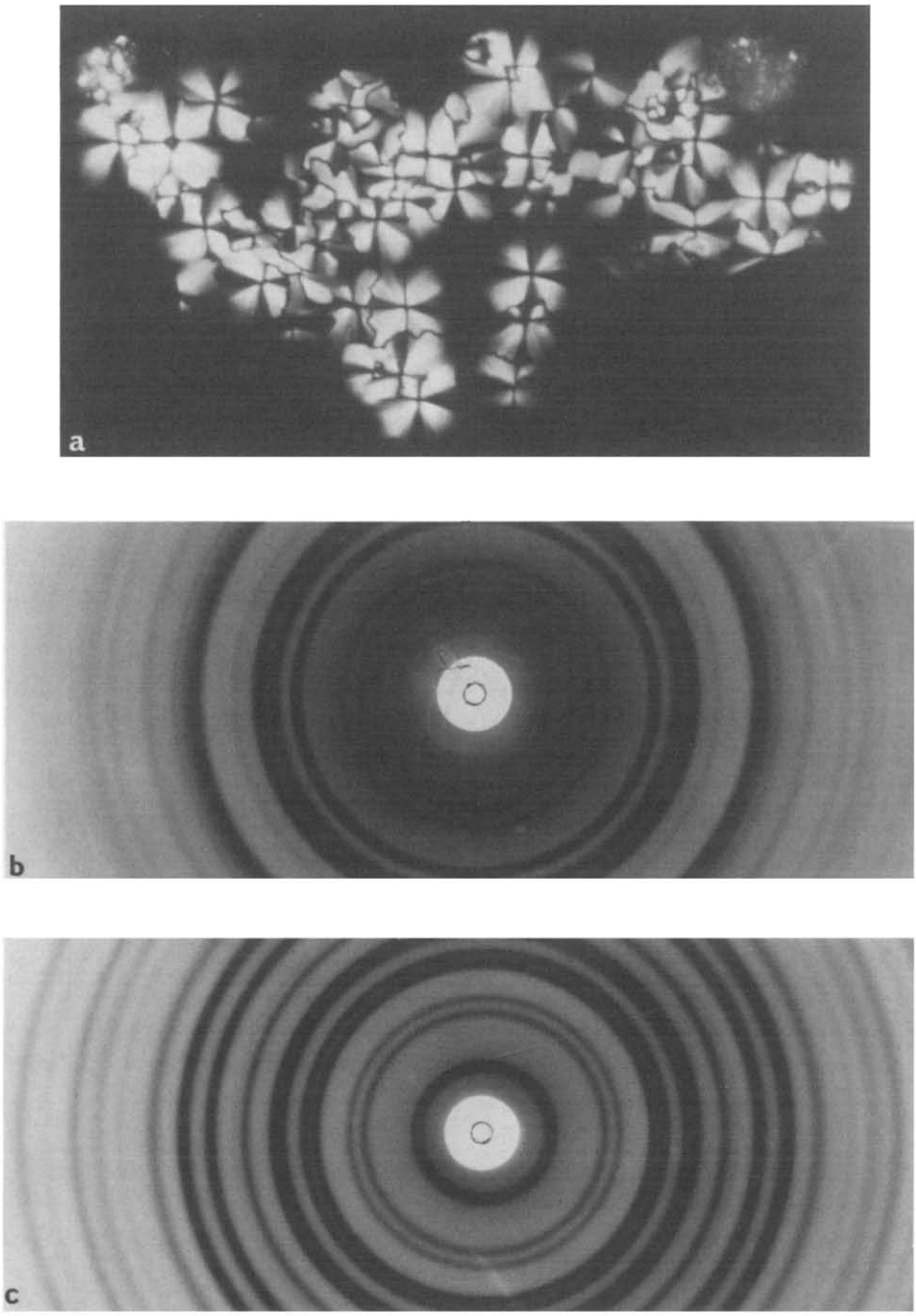

Fig. 4. As part of the study of the crystallisation of starch polysaccharides it was found that (a) spherulites could be formed from the crystallisations of dp22 amylosic chains. Different solvent conditions lead to (b) A-type and (c) B-type crystal modifications. The significance for biosynthesis needs further investigation.

Work on starch has led to a molecular description of the processes occurring upon gelation and storage (see Fig. 4). Such models are being used to investigate effects such as the influence of botanical source, effect of additives on staling behaviour, resistant starch or physical modifications on processing.

The in-vivo nature and industrial use of animal and plant polysaccharides involves mixtures of several biopolymers. To analyse such behaviour the Institute has been studying binary polysaccharide or polysaccharide–protein mixtures. Such studies have led to molecular descriptions of intermolecular binding (Fig. 5), new models for synergistic interactions and new uses such as encapsulation.

There is interest in bacterial polysaccharides both in terms of their in-vivo role and their use as industrial additives. Polysaccharides from a

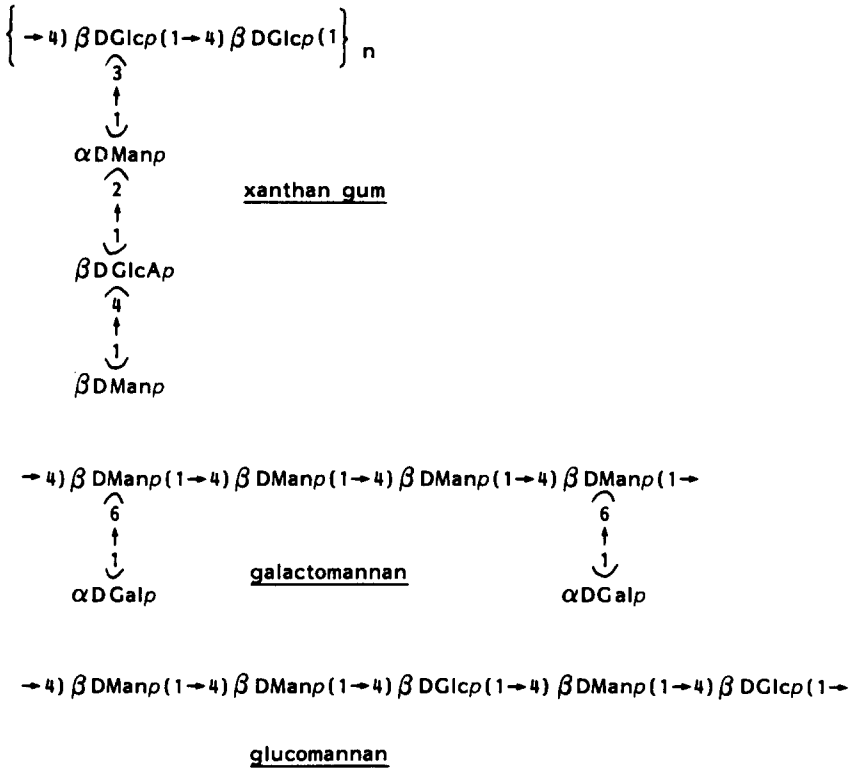

Fig. 5. A molecular model has been developed for intermolecular binding between xanthan and certain galactomannans or glucomannans. An essential step is denaturation of the xanthan helix revealing the cellulosic backbone. Binding results from the stereochemical compatibility of the cellulose structure with the mannan backbone of the galactomannan or mixed backbone of the glucomannan.

wide range of natural and genetically-modified microorganisms are being evaluated and investigated, in addition to studies on established (xanthan) or new (gellan) polysaccharides. We are also comparing polysaccharides from families showing chemically similar structures in order to relate structure and function.

COLLABORATIVE RESEARCH

The Institute already collaborates with many other research institutes, universities and industrial companies within the UK, Europe and abroad. We wish to expand our interests and expertise by further collaboration. Such studies can be supported by visiting workers, research students or industrially-sponsored confidential or semi-confidential research. Initial contact for collaborative or contract research should be with the group leader concerned with a given area of research. A complete list of research projects, research publications and information on all aspects of the Institute activities can be obtained from the Liaison office.