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The Centre de Recherches sur les Macromolécules Végétales (CERMAV)

M. Rinaudo

Centre de Recherches sur les Macromolécules Végétales (CNRS), BP 68, 38402, Saint Martin d'Hères Cedex, France*

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1 INTRODUCTION

The Centre de Recherches sur les Macromolécules Végétales 'CERMAV' (Institute of Plant Macromolecules) is an Institute of CNRS (The French National Center for Scientific Research). Created and built in 1966, the Institute was one of the first buildings to be erected on the new University campus of Grenoble, in the suburb Saint Martin d'Hères. On the campus, located by the River Isère, CERMAV lies between the Biology Department, the Chemistry Department and the Paper Engineering School.

As with all CNRS Institutes, the goal at CERMAV is to conduct fundamental research. Initially, efforts were targeted on plant chemistry and biochemistry with a strong emphasis on cellulose science. Later on, these were redefined and extended to the biochemistry, biology, chemistry and physical chemistry of polysaccharide materials, of either plant or bacterial origin.

Today, CERMAV is located in a single three-storey building which has a total floor area of 2600 square meters (Fig. 1). The Institute is manned by 65 research scientists and technicians. Among these are 15 non-teaching scientists, belonging to the CNRS organization, nine University Professors (from the Biology and Chemistry Departments), 21 engineers, technicians and secretaries and 20 non-permanent staff members (post-doctoral research fellows, students, trainees, etc.). In addition, there are in residence one or more visiting scientists from foreign countries, each collaborating with one or more CERMAV scientist. The first director of CERMAV was Professor D. Gagnaire, and

*Affiliated with the Joseph Fourier University of Grenoble, France.

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Fig. 1. Front view of the Centre de Recherche sur les Macromolécules Végétales, early 1988, before the construction of the building extension.

he was succeeded in 1984 by Professor M. Rinaudo, both members of the Chemistry Department of the University Joseph Fourier of Grenoble (the former Scientific, Technical and Medical University of Grenoble).

In 1987, the overall budget of CERMAV was 13·7 M Fr; 9·2 M Fr for salaries and 4·5 M Fr for operations. Except for a few students or temporary staff, the salaries were paid by government ministeries, either through CNRS or University channels. Half of the operating budget was given by the University and CNRS whereas the other half had to be acquired from research contracts funded either by government agencies or private industry.

2 AREAS OF RESEARCH

Research activities at CERMAV are organized in seven areas as follows:

2.1 Preparation, synthesis and modification of mono- and oligosaccharides

The Institute has been traditionally involved with basic sugar chemistry and synthesis. In particular, we have developed the synthesis of cyclic oligosaccharides composed of 2, 3 or 4 β-D-glucopyranosyl residues connected by β-(1–6) linkages. These products have remarkable complexing properties, quite different from those of conventional cycloamyloses. We have also devised the stereoregular syntheses of α- and β-thiooligosaccharides in which the interosidic oxygens have been replaced by sulfur atoms. These compounds are useful non-hydrolyzable substrate analogs for induction, for purification by affinity chromatography or as active site probes for glucan hydrolases.

Another key aspect of the research has been the preparation of purified and well-characterized oligosaccharides from acid hydrolysis of polysaccharides. Over the years, the Center has accumulated a source bank of these products. They have proved of great importance for the understanding of polysaccharide properties. In several homologous series, it has been shown that the basic polysaccharide features (conformation, specific interactions, spectroscopic identification, three-dimensional crystalline structure, etc.) were well established in oligosaccharides of DP as low as 4 to 6.

2.2 New polysaccharide-based materials

To find an alternative route to the old technology of the viscose process, the Center's activity in this field has focused on the study of cellulose dissolution in organic solvents. It has concentrated on the ternary dissolution system; cellulose, water, *N*-methyl-morpholine-*N*-oxide. The Center has established the fundamental aspects of cellulose dissolution, the phase diagram, the properties of the solutions and the coagulation scheme when precipitants (water in particular) are added to the solutions. This has led to the preparation and study of original solvent-spun cellulose fibers, films and cellular materials. An interesting aspect of this work has been the dissolution and spinning of steam-exploded wood. It was shown that exploded wood chips could be fully dissolved, then subsequently spun into continuous wood filaments; the overall conversion from wood chips to a spun bobbin being achieved in only a few minutes.

2.3 Induction, purification and uses of glycan hydrolases

In order to understand the mechanisms of polysaccharide biodegradation, the laboratory has established several techniques for the preparation of glycan hydrolases. In particular, a purification scheme has been established, where insoluble matrices, grafted with thiooligosaccharides, were used for affinity chromatography. Among the hydrolases which have been prepared by this technique, is recent work where 1,4-β-glucan cellobiohydrolases from *Trichoderma reseei* were purified with the ligands *p*-aminothiophenyl 1,4-β-thiocellobioside and 1,4,4′-β-trithiocellotrioside.

Another feature of the Center's work with glycan hydrolases has been the study of their induction in plant cells which have been subjected to the action of specific oligo- and polysaccharides. For this, silica beads were grafted with given oligosaccharides, then co-suspended with plant cells in an appropriate medium. By this technique, in particular, the elicitation of *endo*-laminarinase in cells of *Rubus*, when the immunoadsorbent *H* was selected as elicitor could be shown. The elicitation mechanism involves the activation of oligosaccharide sensitive receptors at the surface of the plant cell wall. Their localization and mode of action is being investigated.

A third aspect of the Center's work on hydrolases has been their use as specific markers for ultrastructural work with electron microscopy. For this, researchers have prepared complexes of colloidal gold particles with specific hydrolases and have observed the adsorption of these complexes on ultra-thin cross-sections of plant tissues. This allows the precise localization of a given polysaccharide within a composite cell wall (Fig. 2). In particular, the Center has successfully used this technique to follow the ultrastructural aspects of the fungal degradation of lignified woody tissue. In another series of experiments, the enzyme–gold

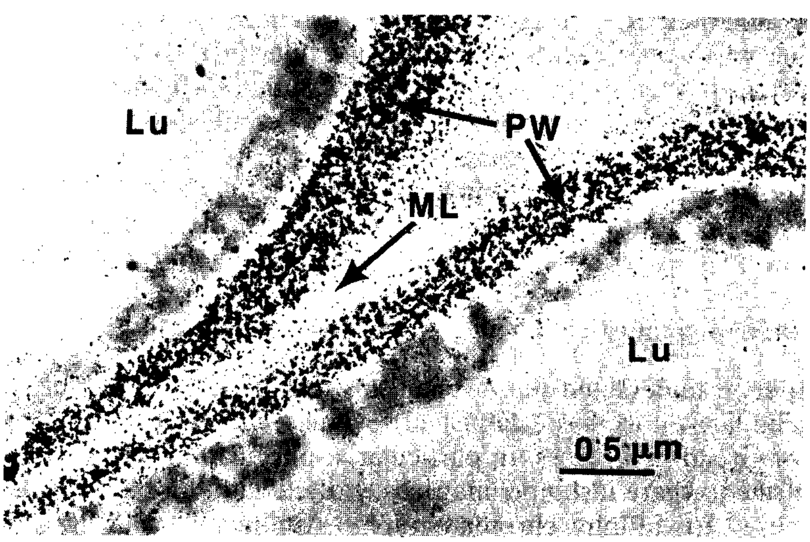

Fig. 2. Use of the colloidal gold xylanase complex to localize glucuronoxylans in the primary wall of a graminea. Lu, Lumen; PW, primary wall; ML, middle lamella.

complexes have been used to observe the mode of adsorption of 1,4-β-glucan cellobiohydrolase-1 on highly crystalline cellulosic substrates such as isolated *Valonia* microfibrils or microcrystals. In this case, it was shown that the adsorption occurred only on two of the four edges of the microfibrils or microcrystals, leaving the other two untouched by the enzymes.

2.4 Structural studies on oligo- and polysaccharides

Since the opening of the laboratory, the Center has been involved in the determination of the various levels of the structure of polysaccharides. Using modern spectroscopic techniques (NMR, mass spectrometry, etc.) as well as chemical and chromatography analysis, the primary structure of a large number of complex polysaccharides and oligosaccharides has been established from bacterial or plant origin.

The Center has also been very active in studying the conformation of polysaccharides. The knowledge of the conformation or conformational changes of polysaccharides when in different states (in solid, gel or in solution) leads to the understanding of their specificity. The Center's laboratory has been involved in developing experimental approaches leading to the determination of the conformation of polysaccharides and their models, the oligosaccharides. It is with the crystalline state that research has been most successful so far. In particular, the three-dimensional structures of a series of oligosaccharides (thiomaltose, panose, laminarabiose octaacetate, etc.) have been crystallized and solved by conventional crystallography techniques. Activity has also been directed toward the crystal structure determination of polysaccharides. For this, researchers have established a new methodology based on the preparation of micron-sized lamellar single crystals and their investigation by electron crystallography techniques, where the diffraction information comes from either electron diffractograms or high-resolution images. This method, when coupled with conformational analysis, has yielded precise descriptions of the structure of crystalline materials such as cellulose triacetate, mannan (exemplified in Fig. 3), dextran, amylose, etc.

For polysaccharides in solution, the exact determination of the conformations is more difficult, since one only has access to average conformations. Furthermore, the interpretation of the experimental data is not as straightforward as it is for crystalline materials. Nevertheless, the Center has undertaken some solution studies, where the conformational analysis was combined successfully with the exploitation of experimental data, obtained either from solution NMR (in particular, coupling constant determinations and NOE experiments) or from optical rotation data.

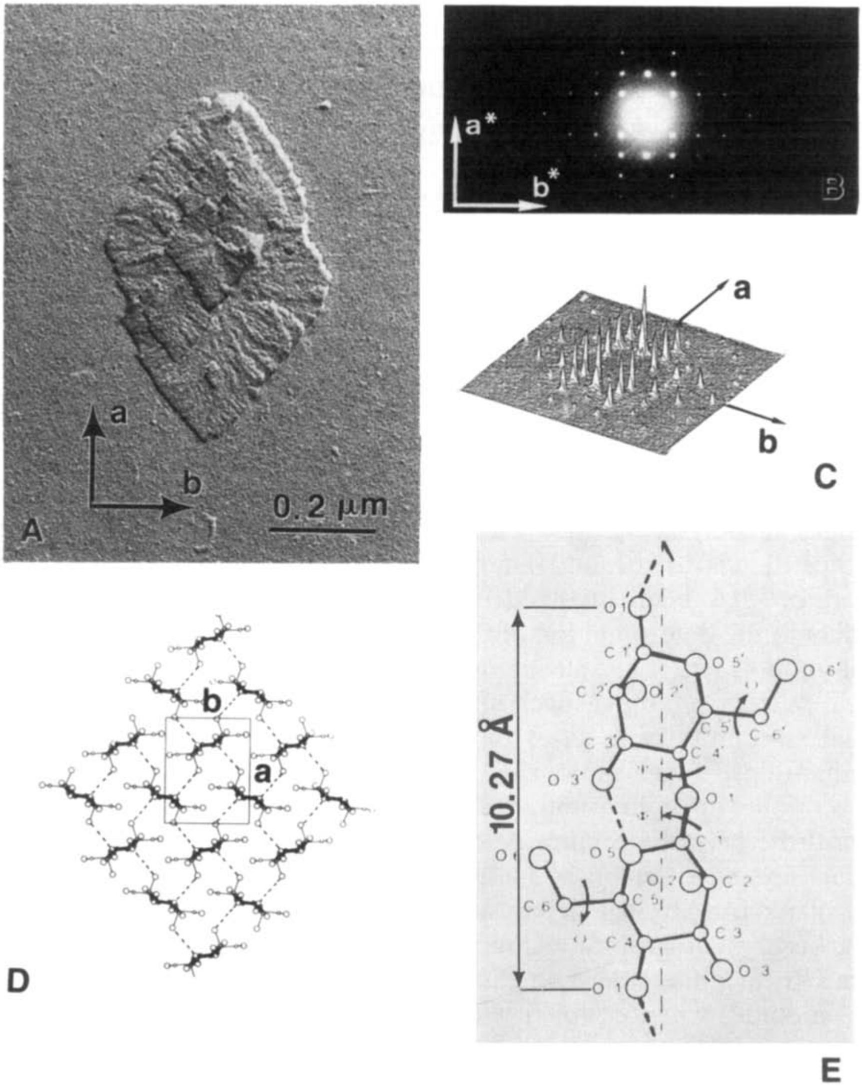

Fig. 3. Investigation of mannan-I by electron crystallography: (A) a single crystal of mannan; (B) its electron diffraction diagram; (C) digitization of (B); (D) a–b projection of the structure of mannan, deduced from the analysis of the patterns as in (B); (E) projection of the structure of mannan along the chain axis, deduced from the analysis of tilted electron diffractograms (not shown here).

2.5 Studies on the properties of polysaccharide solutions and gels

The Center's laboratory has also been involved with fundamental investigations on the properties and characterization of neutral and charged polysaccharides in dilute and semi-dilute solutions. For this, a series of analytical techniques (aqueous gel permeation chromatography (GPC), viscometry, light-scattering measurements, etc.) have been developed. Research has focused on the preparation and study of well-characterized polysaccharide fractions. This has been used to establish the fundamental hydrodynamic parameters of the solutions in different conditions (salt content, conformational changes, temperature changes, polymer concentration, etc.). During the last decade a large part of the Center's activity in this field has been directed toward the study of water-soluble bacterial polysaccharides and in particular, xanthan gum. This important product has quite unusual properties, connected with the conformations that can be adopted by the xanthan molecules, when different conditions of salt and temperature are applied. In particular, it has been shown that xanthan could form cholesteric mesophases even for moderate polymer concentrations. Also the comparison between unprocessed and processed xanthan has been studied in detail. Improved purification schemes have been devised and patented to prepare and clarify its solutions. Finally, an interesting study on the biodegradation of xanthan by cellulase has shown that only xanthan in its disordered conformation could be digested. It was resistant to cellulases, when in the ordered conformation.

The study of gel formation in polysaccharide systems has been another area in which the laboratory has been quite active. Center staff have studied in detail the gels of agarose, pectin, gellan and carrageenan. In this latter case, studies with κ-carrageenan have led to the establishment and description of a new model for its gel formation.

2.6 Structural and ultrastructural studies of the plant cell wall

The study of the plant cell wall was an initial subject of interest and remains an area in which the Center is very active. It is particularly interested in the primary wall composition, constitution and elaboration. Studies are performed either on suspension cultured cells or on cultured calli tissue. The structures of all the polysaccharide constituents are studied, after extraction and purification, using chemical methods, enzymatic degradation, NMR and mass spectrometry. Research in this field has led to the proposal of a model of interaction between cellulose, xyloglucan and polygalacturonans. This model, deduced from biochemical and chemical analysis, is now corroborated at the ultrastructural level by electron microscopy.

At the ultrastructural level, studies have also been directed toward the knowledge of the secondary wall and the exact localization of the various polysaccharides and lignin components. The Center has investigated in great detail, the enzymatic degradation of such walls, when subjected to purified xylanases, ligninases or cellulases. This basic study has led to a description of a model for the biodegradation of the secondary cell wall when subjected to fungal degradation.

2.7 Biomass valorization

Following the current trends in French public laboratories, some of the Center's activities have been devoted to studies with both applied and fundamental interest. In this framework, knowledge of the chemistry of plants and polysaccharides has led the staff to investigate some aspects of the valorization of the vegetal biomass.

In one project, researchers have designed and contracted the construction of a one-liter steam explosion gun (Fig. 4). This unit was designed to evaluate the fundamental aspects of the steam explosion

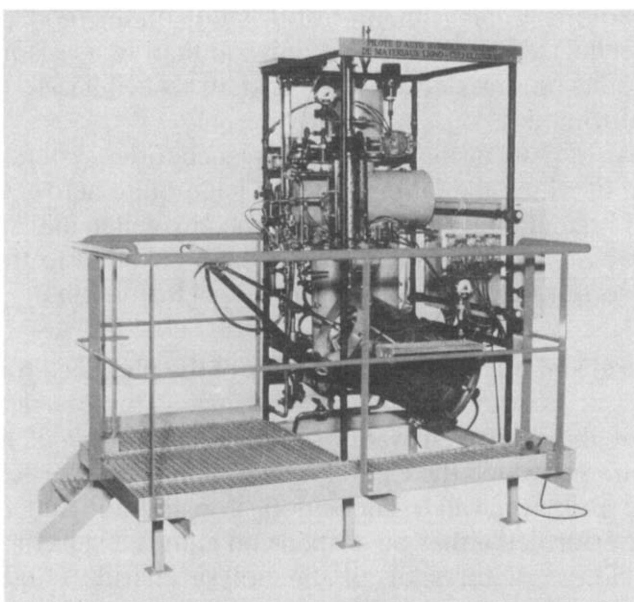

Fig. 4. Photograph of the steam explosion gun designed at CERMAV and built and commercialized by DELTALAB (Voreppe, France). The gun has a capacity of one liter and is fully automated.

process applied to plant biomass. The process leads to an easy separation of lignin, hemicelluloses and cellulose from complex plant materials. A large number of specimens from various origins have been investigated: hardwood and softwood, bagasse, straw, corn stover, nut and almond shells, etc. Work has focused on the fundamental aspects of the chemistry and physics of the process. The release of the acetyl groups that are normally present in the plant biomass and that are responsible for the autohydrolysis of the plant components during the steaming process have been studied in detail. It has been shown that for some components (e.g. lignin or hemicelluloses), in certain specific conditions, the depolymerization could be quite severe; for others (e.g. cellulose), a fairly high degree of polymerization could be maintained. These observations have led to the consideration and study of many applications of the steam explosion process: as an effective pretreatment before biomass biodegradation, in ethanol production; in the preparation and purification of sugars such as xylose or mannose; in the easy preparation of a chemical grade cellulose pulp; in the spinning of exploded wood, etc.

In another project, knowledge of the enzymatic degradation of plant cell wall was applied to some aspects of the paper-making process. In particular, the beneficial action of purified xylanases when interacted with unbleached or bleached paper-making pulp, could be demonstrated. In the first case, studies showed that superior bleaching could be achieved after the enzymatic treatment. With bleached pulp, xylanases were also quite effective in inducing fibrillation of the pulp fibers. This led to a reduced energy consumption when the enzymatically treated pulp was beaten, prior to paper-making.

3 CONCLUSIONS AND PERSPECTIVES

This article illustrates how a 65-person CNRS Institute has been working for 22 years on polysaccharides of plant and bacterial origin. As described above, work has mainly concentrated on the chemical, physico-chemical and biochemical aspects of these biopolymers. There are many areas in which the Center would like to get involved, but so far it has not been able to do so, due to lack of manpower and space. This situation will soon change. In a joint effort, CNRS, the Rhône-Alpes Administration and the city of Grenoble have pooled together to finance a 10 M Fr extension to the laboratory. The construction, due to start at the end of 1988, will lead to an additional 1500 square meters of laboratory and office space. This new development will have a strong influence on the organization of research activity.

With more space available, new research areas will be initiated and new scientists are expected to join the existing staff. They will include biologists, who, in particular, will initiate and develop research on polysaccharide biosynthesis. The Center also expects to attract physicists to study the mechanics of polysaccharide systems either in solution, in gels or in solids. When all these new areas are operating and producing results, the Institute will have achieved its goal as a center of multidisciplinary research in all aspects of polysaccharide science.