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Conformation and Intermolecular Interactions of Carbohydrate Chains

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For consideration of their conformations and interactions, carbohydrate chains can conveniently be divided into 3 classes on the basis of their covalent structure; namely periodic (a), interrupted periodic (b), and aperiodic (c) types. In aqueous solution carbohydrate chains often exist as highly disordered random coils. Under appropriate conditions, however, polysaccharides of types (a) and (b) can adopt a variety of ordered conformations. Physical methods, and in particular optical rotation, circular dichroism, and nuclear magnetic resonance, provide sensitive probes for the study of the mechanism and specificity of these disorder-order transitions in aqueous solution.

Intermolecular interactions between such polysaccharide chains arise from cooperative associations of long structurally regular regions which adopt the ordered conformations. For acidic polysaccharides these cooperative associations may involve alignment of extended ribbons with cations sandwiched between them. In other systems the interactions involve double helices which may then aggregate further, and geometric "matching" of different polysaccharide chains can also occur. These ordered, associated regions are generally terminated by deviations from structural regularity or by "kinks" which prevent complete aggregation of the molecules.

The complex carbohydrate chains which occur at the periphery of animal cells have very different, aperiodic structures and although their conformations are as yet poorly understood, preliminary indications are considered.

Key words: conformational analysis, polysaccharides, cooperative interactions, synergistic interactions, cooperative cation binding, spectroscopic techniques, circular dichroism, nuclear magnetic resonance, optical rotation

INTRODUCTION

Carbohydrate chains are ubiquitous components of the molecular assemblies which characterize the extracellular organization of biological tissues and we have argued (1–4), as have others, that a detailed understanding of their shapes and of their potential for intermolecular interactions is essential to the illumination of their biological roles. Recent interest has centred on complex macromolecules in which carbohydrate chains are complexed to proteins or lipids but "all carbohydrate" polymers remain important, not only in their own right but also as models on which approaches and techniques can be sharpened for application to more elaborate systems. In this discussion we shall concentrate pre-

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dominantly on solution structures, since these are generally considered more relevant to biological situations, although ultimately they must be compared with the wide variety of 3-dimensional conformations defined in the solid state by x-ray diffraction (for review see Refs. 3 and 5).

In aqueous solution the favored conformations of these highly hydrated chains cannot automatically be assumed to be ordered since the conformational entropy (3, 6) arising from the continuous fluctuation of the polymers about a large number of internal linkages provides a strong drive to disordered states. Under particular circumstances, however, favorable nonbonded energy terms (hydrogen bonding, dipolar and ionic interactions, and solvent terms) can act cooperatively (3, 4, 6) to fix the macromolecules in ordered shapes. For the examples characterized to date such disorder-order transitions are almost invariably stabilized by intermolecular interactions involving alignment and cooperative association of long structurally-regular sequences of carbohydrate chains. Physical methods, and in particular circular dichroism (CD), optical rotation (OR), and nuclear magnetic resonance (NMR), provide sensitive probes of the mechanism and specificity of the interactions and we have used "simple," interacting plant, bacterial, and animal systems to characterize the subtypes of ordered structures.

For consideration of their conformations carbohydrate chains can conveniently be divided (4) into 3 classes on the basis of their covalent structure, namely periodic (a), interrupted periodic (b), and aperiodic (c) types. Naturally each class will have shared properties with other types in addition to the distinctive features illustrated here.

1. PERIODIC TYPE

These carbohydrate chains are composed of "repeating units" of sugar residues linked through identical positions and glycosidic configurations.

1. i. Homopolysaccharide Periodic Structure

For chains containing only 1 type of residue, conformational analysis confirms (7) that simple periodic sequences can generate ordered conformations which will correspond to 1 of the subclasses shown in Fig. 1. Such ordered conformations can be considered as helices and defined by their symmetry (n) and projected residue height (h).

a. Extended ribbons. These structures have $2 \leqslant n \leqslant 4$ and h close to the maximum for sugar residues (4.1–4.5 Å). Archetypes are the β-1,4 glucans: cellulose, the major skeletal component of plant cell walls, and chitin, the analogous component in fungi, insect cuticles, and crustacean shells. In ordered structures these particular examples of extended ribbons most probably adopt the "bent chain" conformation (5, 8) with alternate residues related by a 2-fold symmetry axis and hydrogen bonded between O(3) and O(5). Their skeletal function arises from their supreme ability to pack together like "planks in a timber yard," in dense, strong, microcrystalline arrays with efficient hydrogen bonding within and between the organized layers.

b. Coiled springs. These structures resembled coiled springs in various states of extension; n has a much wider variation ($2 \leqslant n \leqslant 10$) and, more significantly, h can be very small. Amylose, the α-1,4 glucan, is the supreme example. Its best-known ordered states have the spring very compressed with its geometry varying between 6, 7, and 8 residues per helical turn. Such structures do not occur in solution, where the chains have the hydrodynamic properties of a random coil (9), but for amylose they can be prepared as crystalline derivatives by addition of suitable nonpolar complexing agents. Apparently

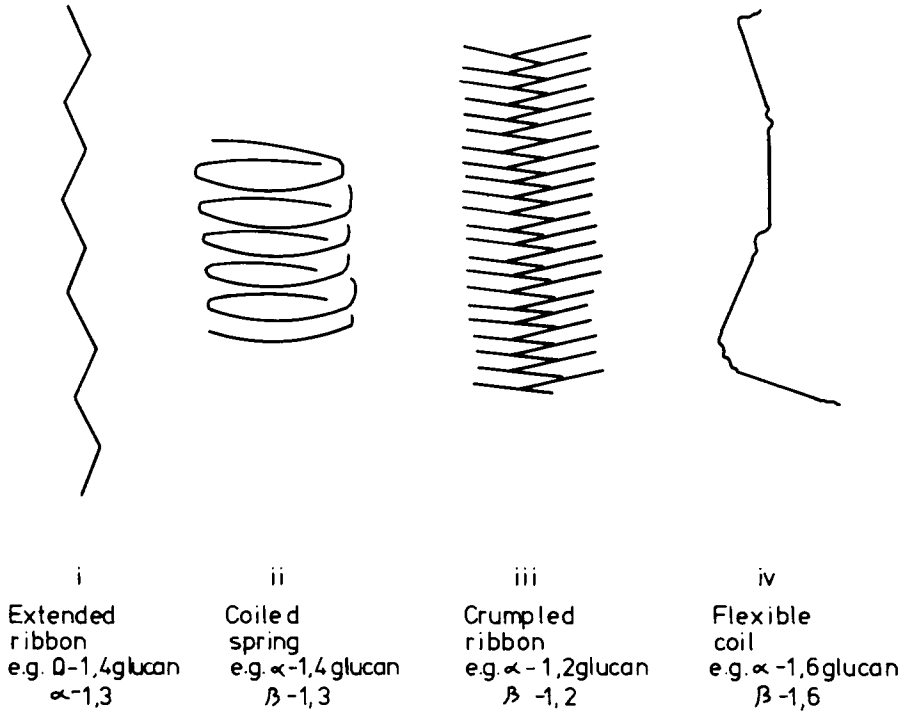


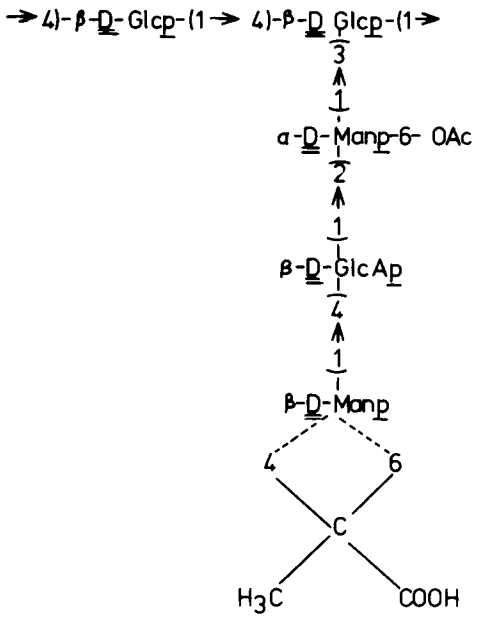
Fig. 1. Schematic representation of the subclasses of ordered conformations predicted by conformational analysis for periodic structures containing only 1 type of residue: left to right, extended ribbon, coiled spring, crumpled ribbon, and flexible coil, respectively. Such structures can be illustrated by the listed polymers of glucose.

"guest" molecules are bound in the core of the helix which can adjust to accommodate a wide variety of included molecules. The best characterized conformation, the 6-fold "V-form," is probably left-handed (10) and hydrogen bonded between O(2)–O(3) of adjacent residues and O(2)–O(6) of residues opposed on the helical surface but 6 apart in the primary sequence. Alternatively, ultimate extension is found in a potassium bromide complex considered to have n = 4 and h = 4.03 (11).

c. Crumpled ribbons and flexible coils. No ordered conformations of these structural types have yet been established. For crumpled ribbons extended sequences would result in multiple steric clashes, and this complex shape is seldom found in nature, although limited sequences resembling this type characterize aperiodic chains. Flexible coil sequences are characterized by an extra bond between residues, by virtue of the 1,6 linkages, which greatly enhances their conformational entropy and minimizes opportunities for ordered conformations.

1. ii. Complex Periodic Structures — Xanthan

Periodic chains can also be considerably more complex, e.g., lipopolysaccharide O-antigen chains and capsular polysaccharides of gram-negative bacteria which, in general, may contain up to 6 residues in the repeating sequence (12). A particular example is xanthan, the extracellular polysaccharide from Xanthomonas campestris, which has highly



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branched "repeating units" with complex trisaccharide side chains extending from alternate residues of a "cellulose" backbone (I). In the condensed state the molecule probably exists (13) as a 5-fold helix with a repeat of 47 Å: side chains align closely with the backbone and most probably stabilize the structure by favorable nonbonded interactions.

In aqueous solution, as shown in Fig. 2, viscosity, NMR, and OR all show sigmoidal temperature-dependent changes which follow very similar temperature courses (14, 15); such changes are characteristic of cooperative processes. Our interpretation is that on cooling individual chains undergo a cooperative transition (Fig. 2) from a random coil to an ordered helical conformation which may be very similar to that in the solid state. Supporting evidence is the confirmation from CD studies (14) that O-acetate chromophores on the side chains experience a more dissymmetric environment in the ordered state, as expected if side chains and backbone align. Moreover, the overall change in the observed n-π^* ellipticity is negative and hence its contribution to OR must be negative. Since the observed OR transition is positive this indicates large contributions from the inaccessible CD transitions which dominate monochromatic OR. As we shall show, such effects are entirely consistent with the "locking" of glycosidic angles which accompanies such coil → helix transitions. The concentration independence of the temperature of the OR transition (15) provides supporting evidence that the change is unimolecular.

Confirming evidence for a temperature-dependent conformational restriction is provided by NMR (14). High resolution linewidths are inversely proportional to the relaxation time of the nuclei, and molecular flexibility interferes with this. For polysaccharides [^{1}H] NMR signals are normally broad and overlapping due to the restricted segmental motion of the chains. Above 85°C, however, the O-acetate and pyruvate substituents in xanthan give sharp, distinct signals. On cooling to 60°C the entire spectrum collapses confirming a transition to a rigid conformation with short relaxation times and hence signals that are

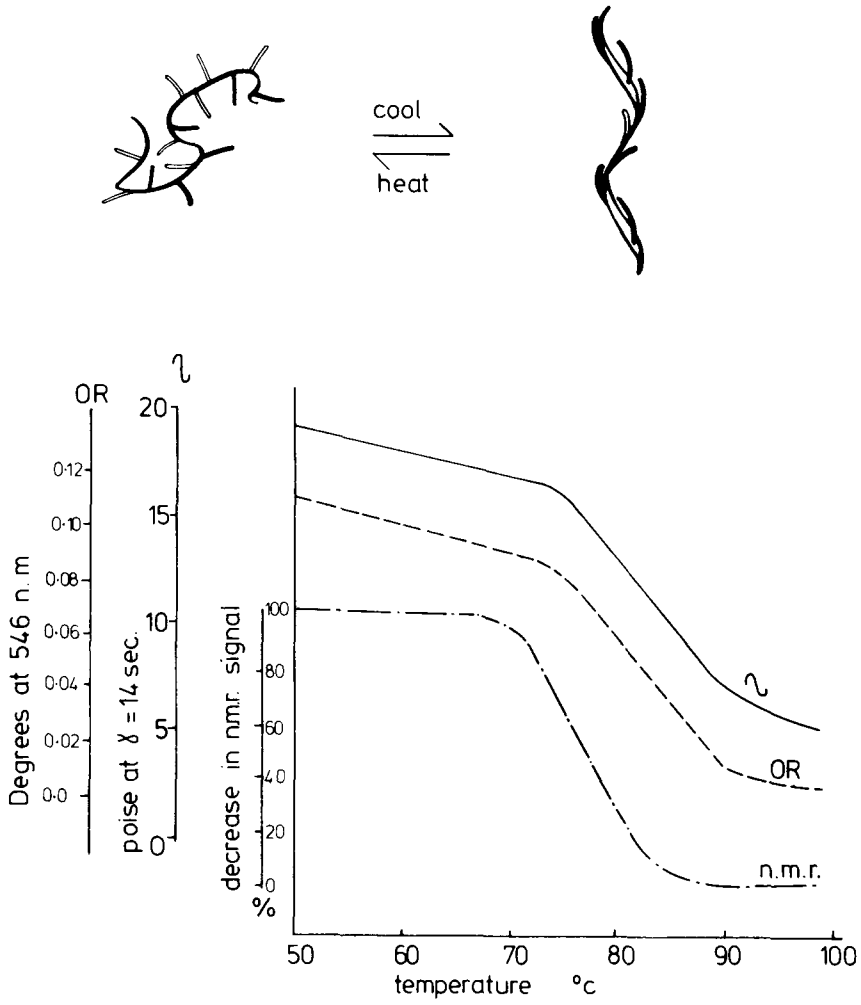


Fig. 2. Schematic representation of the suggested coil → helix transition for xanthan. The temperature dependence of the low shear viscosity (∿), OR, and high resolution NMR peak areas for 1% (wt/vol) aqueous solutions of this bacterial polysaccharide is also shown.

too broad to be observed. At intermediate temperatures no significant broadening or shifting accompanies the sigmoidal decrease in peak area confirming a "two-state all or none" transition.

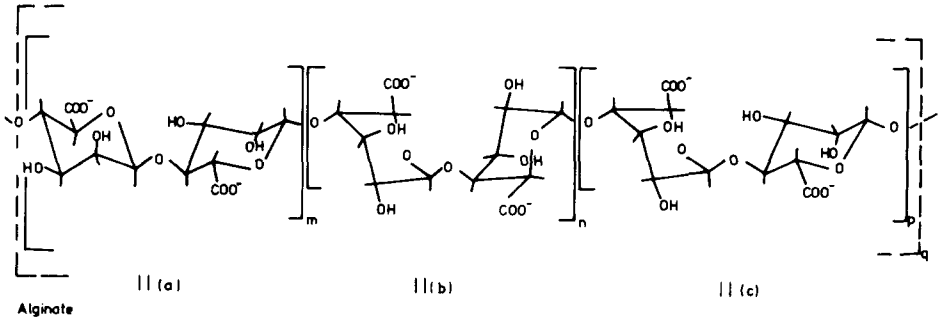
2. INTERRUPTED PERIODIC TYPE

These chains also contain periodic sequences capable of adopting ordered conformations, but these are separated or "interrupted" by deviations from regularity which force chains to leave ordered associations and combine with more than 1 partner. The resultant balance of ordered and "soluble" regions leads to the highly hydrated gel state which is so

important to nature and industrialists. The microcrystalline "junction zones" (2) which characterize the gel network may be 1 of several types.

2. i. Interrupted Ribbons – Alginates and Pectins

A group of acidic polysaccharides which gel by cooperative binding of divalent cations are the alginates and pectins, derived from brown algae and the cell walls and soft tissues of higher plants, respectively. For alginates (11) homopolymeric poly α-L-guluronate sequences (IIb) play the most important role in junction formation. The sequences (IIc)



which approach, albeit irregularly, alternating sequences (16) may play a minor role in gelation but probably also function with poly β-D-mannuronate sequences (IIa) as interrupting "soluble" sequences. Moreover, the relative proportion of sequence types varies with the tissue, the state of maturation, and the geographical origin of the seaweed. The available biosynthetic evidence (17) indicates that the polymer is produced as poly β-D-mannuronate and subsequently "tailored" for its biological function by incorporation of guluronate-containing sequences by an enzymatic epimerization at C(5). Conformational analysis predicts (3) that ordered conformations of both homopolymers will be extended ribbons, and this is confirmed by fiber diffraction studies (18) on such "idealized" extremes in the condensed state.

Evidence for the mechanism of gelation can be obtained from the cation binding behavior of isolated block types (19). Both homopolymeric sequences (IIa and b) show the expected statistical cation binding, but polyguluronate sequences also show a sigmoidal increase in binding affinity above a chain length of 18–20 residues. This strongly implies a cooperative binding process, confirmed by competitive ion-binding studies (20) for polyguluronate but not for other types of sequence.

Circular dichroism studies provide spectacular support for specific site-binding by polyguluronate sequences. Gelation of alginate solutions by controlled addition of divalent cations is accompanied by dramatic changes in CD (Fig. 3). The negative trough arising predominantly from contributions from polyguluronate sequences (21) is swamped by a positive transition. Moreover, the magnitide of the observed difference spectra (Fig. 2) can be correlated with the proportion of such sequences, increasing polyguluronate content giving increased difference spectra. The cation binding behavior of isolated block types confirms this view. Large CD changes are observed (22) for polyguluronate sequences with much smaller changes for alternating and insignificant changes for polymannuronate sequences. These changes in the n-π^* spectral region are consistent with the n-orbitals of polyguluronate carboxylate chromophores being specifically involved in coordination of the divalent cation.

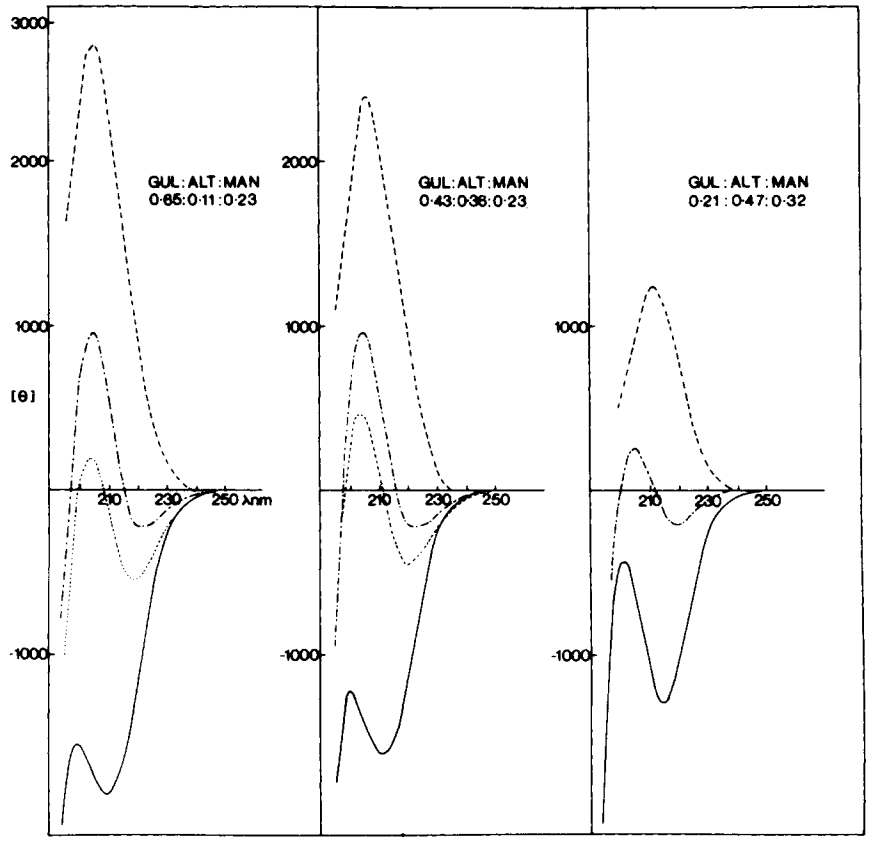


Fig. 3. Changes in CD spectra with diffusion of Ca^{++} to a final concentration of 6 mM into solutions of sodium alginates (0.1%) having the sequence compositions shown in the diagram. Spectra are shown for solutions (—) and for gels at intermediate (· · · ·) and final (· – · – ·) stages of gelation. Difference spectra (- - -) are obtained by subtraction of solution spectra from final gel spectra: $[\phi]$ = molecular ellipticity (degree – cm^2 per decimole) and λ = wavelength (nm).

The principal mechanism of alginate gelation can thus be considered in terms of an "egg-box" model (23) involving cooperative binding of cations between associated polyguluronate "ribbons" (Fig. 4). Competitive inhibition of gelation by isolated blocks and the stoichiometry of cation binding (22) strongly indicate that the predominant mechanism of association is dimerization. Physical and mathematical model building suggests an ordered structure very similar to that observed in the condensed state. Buckled ribbons pack together with cations tightly coordinated in oxygen-lined "nests" between the chains like "eggs" in an "egg-box." For individual chains (Fig. 4) each hydrophilic coordination site involves O(6) and O(5) with the glycosidic oxygen and O(2) and O(3) on the next residue in the "nonreducing" direction. Termination of polyguluronate sequences terminates particular junction zones and establishes the gel network.

Pectin, like alginate, forms firm gels with divalent cations and the "junction forming" sequences are homopolymeric, 1,4 diaxially-linked poly α-D-galacturonate sequences (IIIa; R = Na) which are geometrically very similar to polyguluronate sequences. Partial

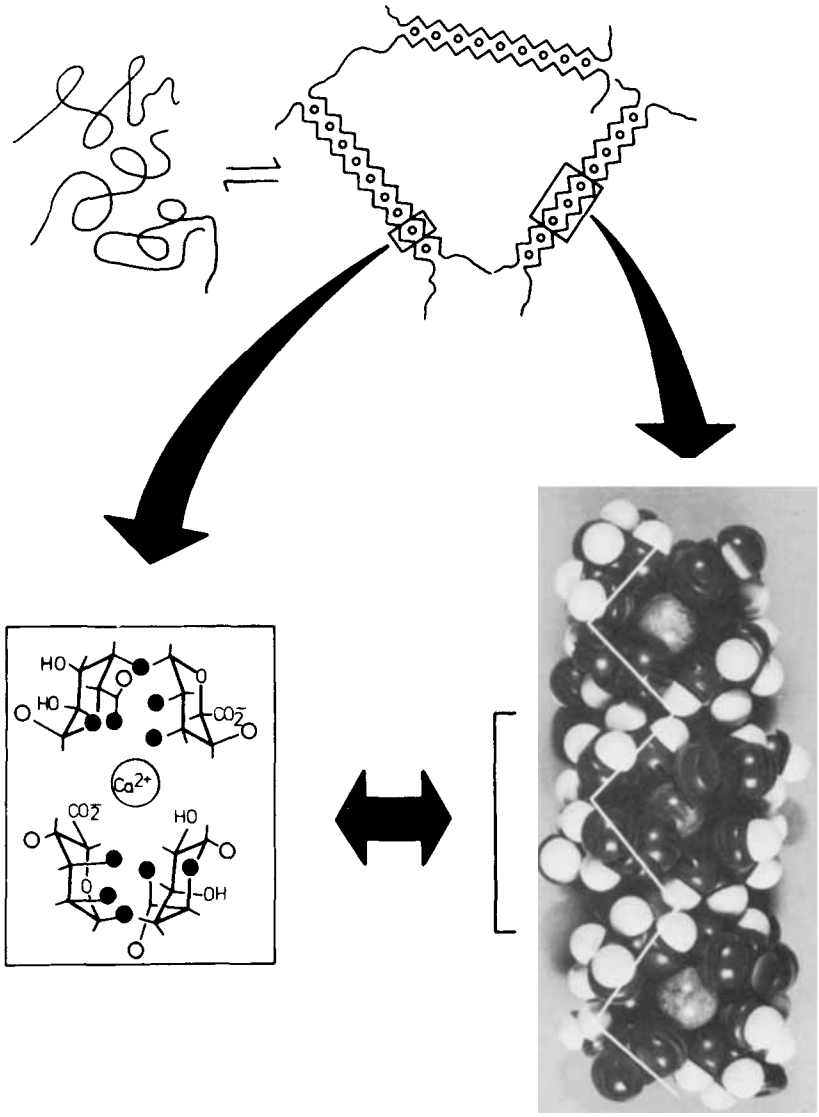
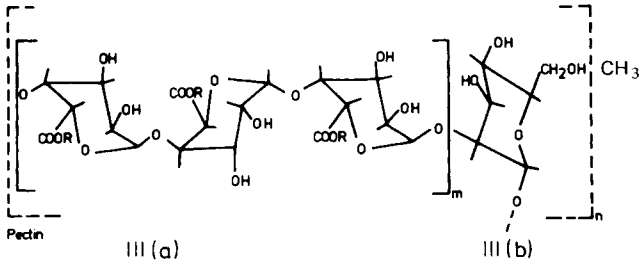


Fig. 4. Schematic representation of the "egg-box" model for the sol → gel transition in alginates. The array of calcium binding sites is shown in the space-filling model and the contour of 1 chain is traced to illustrate its buckled character. The disposition of groups coordinating the divalent cation is also shown schematically and for clarity chains have been moved apart.

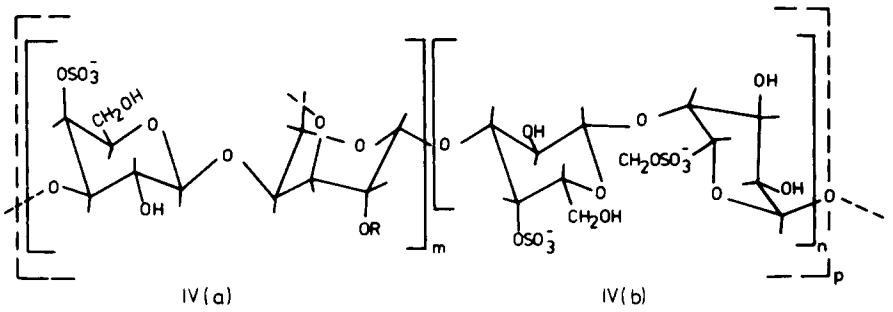
methyl esterification (IIIa; R = CH_3) provides "soluble" regions and further complications are additional structural interruptions of severe α-1,2 linked L-rhamnose "kinks" (IIIb) and neutral sugar side chains (1, 24). The cation binding behavior of deesterified polygalacturonate sequences and low methoxy pectins are very similar to those of polyguluronate. Thus on gelation large CD charges are observed (23) in the n → π* region suggestive of an "egg-box mechanism" and polygalacturonate sequences show (20) cooperative cation binding above a chain length of 14–16 residues. As expected, highly methyl-esterified pectins show little affinity for cations and correspondingly small CD changes. They can,

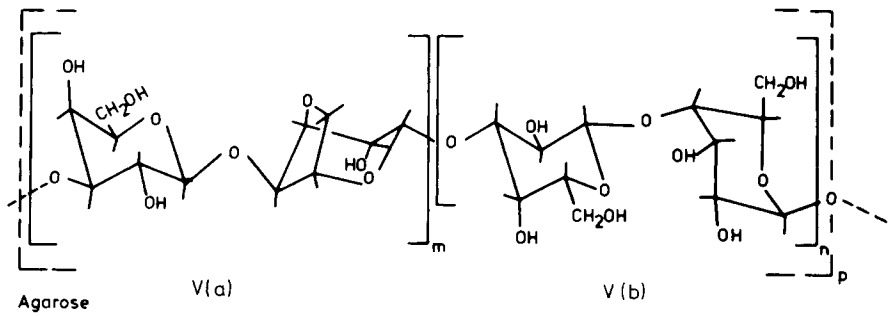


however, gel under conditions of low pH and low water activity. The gelation predominantly involves association of extended esterified sequences (1, 23) and is accompanied by increases in the CD spectrum. Pectin can thus form ordered associations by 2 very different mechanisms, and our interpretation is that both involve stacking of extended ribbons in arrays broadly similar to those of alginate. Significant differences include termination of junction zones by a diverse and complex range of structural interruptions.

2. ii. Interrupted Helices – Carrageenans and Agarose

Algal carbohydrate chains where the gel framework involves temperature-dependent, cooperative associations in double-helical arrays are the carrageenans (κ and ι) and agarose. These are alternating copolymers, containing variable amounts of sulphate ester, and "junction forming" sequences contain 1,3-linked β-D-galactose and either 1,4-linked 3,6-anhydro-α-D- or -L-galactose residues for carrageenans (IVa) and agarose (Va) respectively (25, 26). Associated helices are terminated by "soluble kinks" (IVb and Vb) of sequences involving deviations from the regular 3,6-anhydro residues. These interruptions can be removed by chemical modification (27) to create shorter blocks or "segments" which retain the ability to adopt ordered structures but cannot gel.





For the highly sulphated ι-carrageenan the double helices have been thoroughly characterized both in solution and the solid state (28). The reversible coil → helix transition (Fig. 5) of ι-segments shows a sigmoidal increase in OR which is concentration-dependent (at constant temperature and ionic strength), corresponds to chain dimerization, and is accompanied by an exact doubling of number average and weight average molecular weights (29). The sign and magnitude of this OR shift correspond closely to that predicted (30) from the double-helix geometry in the condensed state, using semiempirical calculations of optical activity from the glycosidic angles of the polysaccharide backbone (31). Thermodynamic measurements are also consistent with double-helix formation by a "two-state all or none" mechanism and low angle x-ray scattering shows ordered rods corresponding to the expected double helical dimensions (32).

Corroborative evidence for conformational restriction is provided by [$^{-13}$C] and [$^{-1}$H] NMR measurements (33). Because of their larger chemical shifts and diminished dipolar broadening compared to [$^{-1}$H] nuclei, [$^{-13}$C] spectra provide (Fig. 5) sharp, well-resolved peaks above 80°C, i.e., for the random coil. All these peaks collapse on conversion to the rigid double helical form and concomitantly a dramatic decrease in the relaxation time (T_2) for the carbon nuclei was observed in the pulse NMR spectrometer. As for xanthan, the absence of broadening or shifting of peaks when the transition was partially completed suggested no time averaging of resonances between disordered and ordered states and hence supported the "two-state all or none" model.

For $\underline{\kappa}$-carrageenan segments OR measurements again suggest (34) that double helices exist in solution, but the lower charge density due to the decrease in sulphate ester substituents leads to markedly different properties. Thus the cooperative, concentration-dependent OR transition shows distinct hysteresis in its "melting" and "setting" behavior which can be correlated with aggregation of the ordered helices. In the nonsulphated chains of agarose this aggregation is so marked that double helix formation cannot be monitored by OR, and the chains precipitate (34) from solution at the onset of the disorder → order transition. Substituted agarose segments do, however, show OR behavior indicative of double helix formation (35) and with the expected distinct hysteresis loop similar to $\underline{\kappa}$-carrageenan.

In the complete carbohydrate chains the "soluble kinks" establish the 3-dimensional gel network. These gels show OR behavior which is very similar to that of the isolated segments, and their setting and melting temperatures are close to the midpoints of the observed transitions (34). Detailed calorimetric and OR analyses (32, 33) indicate that helix formation achieves 70–90% of the theoretical maximum and that each chain can evidently overcome topological constraints and take part in at least 6 independent helices. Competitive inhibition studies provide further substantiation of gelation mechanisms (36) by distinguishing between associations involving dimerization and those involving larger aggregates. As shown on Fig. 6, incorporation of isolated segments into carrageenan gels markedly decreases gel strength for ι-carrageenan whereas that of $\underline{\kappa}$-carrageenan actually increases. This is the predicted result since the substitution of short segments into the double-helical junctions of ι-carrageenan must prevent the essential cross-linking whereas for $\underline{\kappa}$-carrageenan gels segments should no longer do this and actually seem to produce more effective incoporation of polymer chains into aggregating juction zones and hence a stronger gel.

2. iii. Complex Interrupted Structures – Association Between Unlike Chains

Examples of this type of ordered structure are the interactions of xanthan, carrageenans,

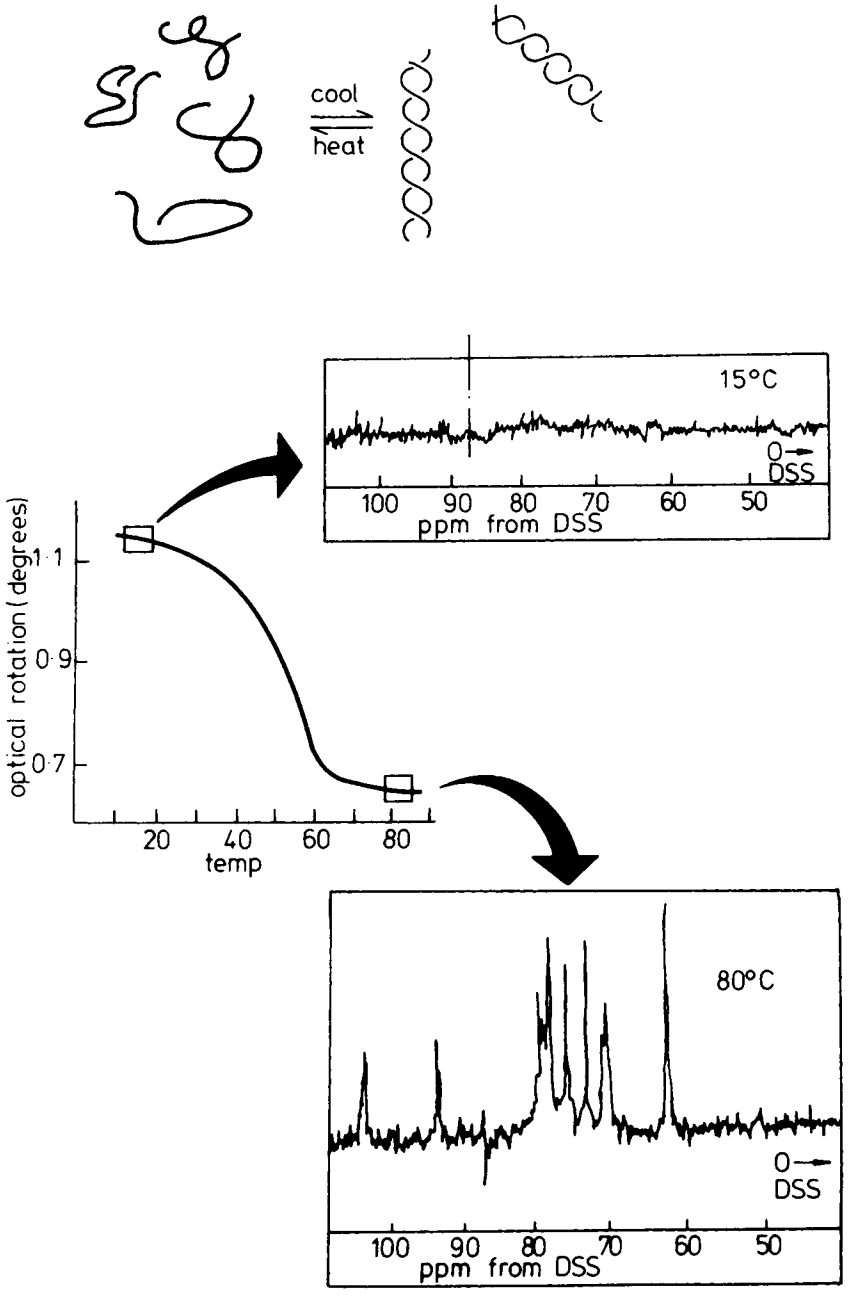


Fig. 5. Schematic representation of the coil → double helix transition of ι-carrageenan "segments." The temperature-dependent sigmoidal increase in OR and comparison of [$^{-13}$C] NMR spectra at 80°C and 15°C are also shown. The concentration was 6% (wt/vol) and 0.1 M sodium chloride.

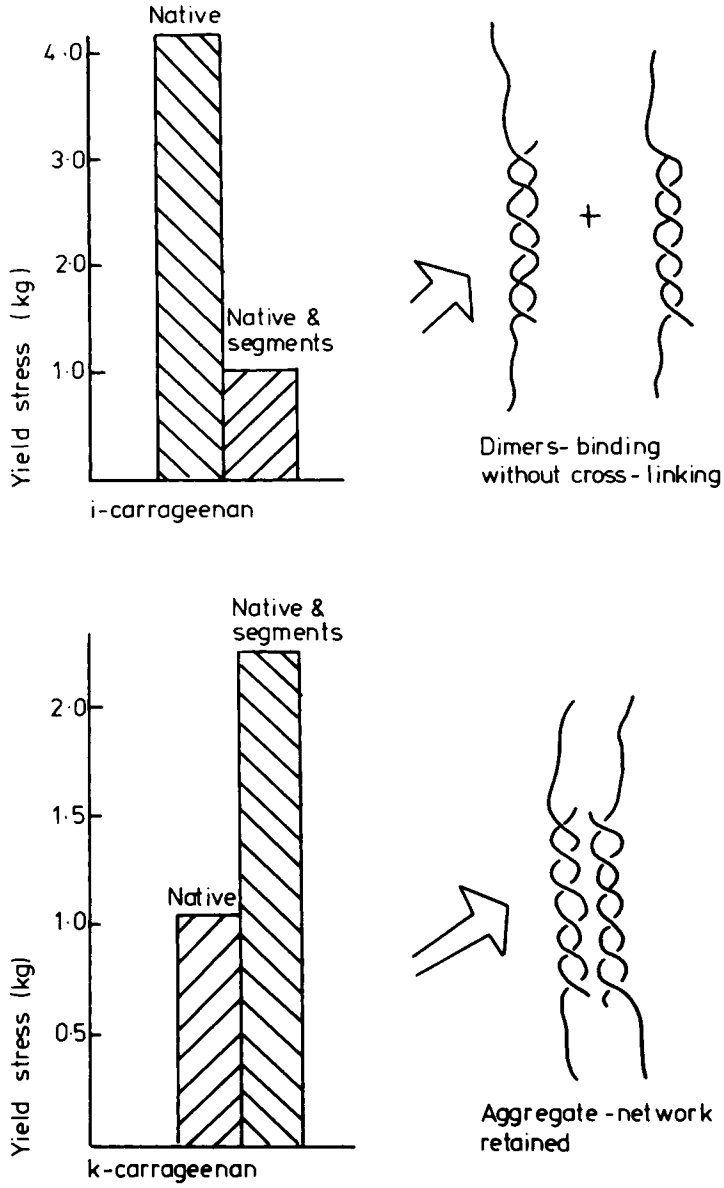


Fig. 6. Comparison of the measured gel strength for native carrageenan gels and gels formed in the presence of equal concentrations of isolated "segments": concentrations were 2.5% and 1% (wt/vol) for ι- and κ-carrageenan respectively. Proposed molecular interpretations of the experimental observations are also shown (see text).

and agar with certain plant β-1,4-linked galactomannans. Concentrations of these polymers which are incapable of gelation can be made to gel by addition of the galactomannans and isolated, nongelling "segments" show similar behavior (15, 34). Such gels show physical properties indicative of cooperative associations and are evidently cross-linked by quaternary interactions between unlike carbohydrate chains. Comparison of the gelling ability and

secondary structure of different galactomannans (37) indicates that in this case junction zones involve (Fig. 7) associations between ordered helices and extended "ribbons" of "smooth" unsubstituted mannose backbone, with "hairy" galactose-substituted sequences providing complementing "soluble" sequences.

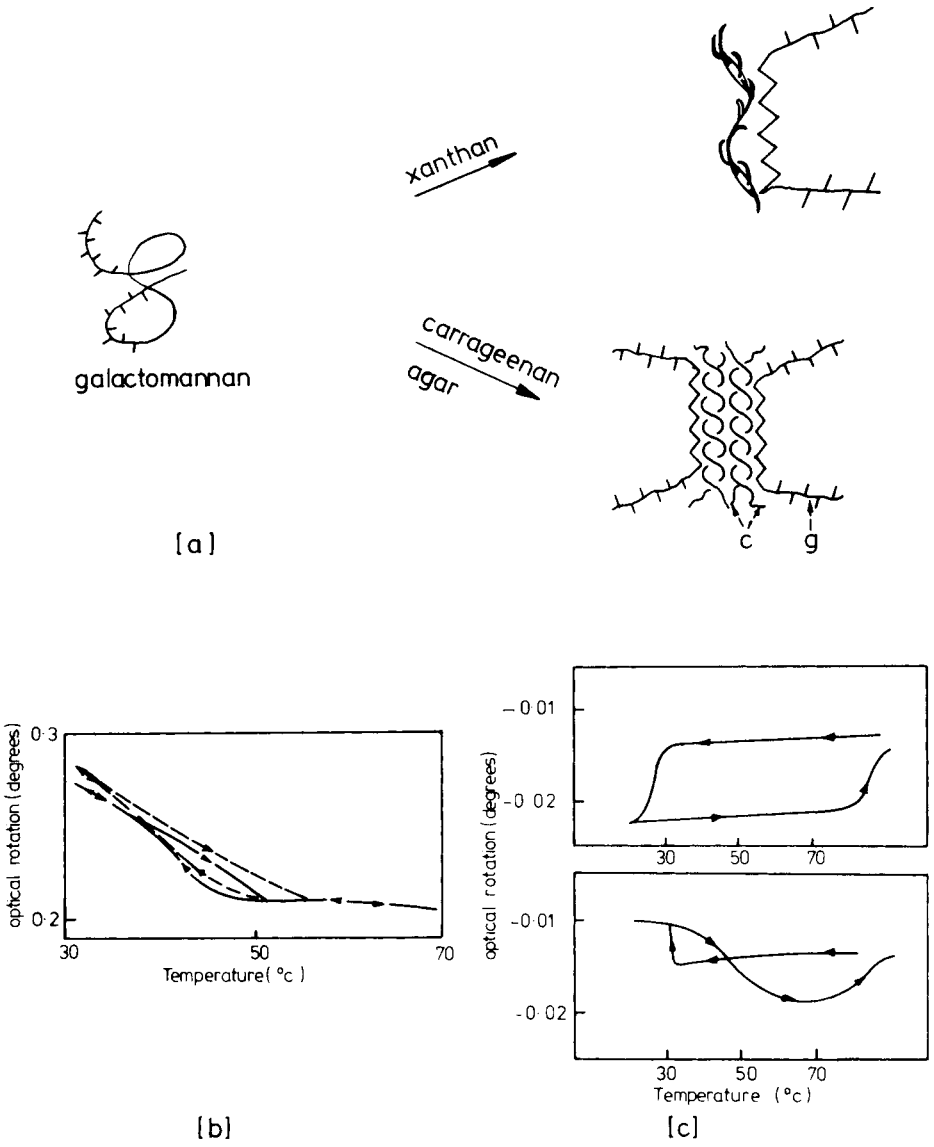


Fig. 7. a) Schematic representation of the quaternary associations between unlike carbohydrate chains: the stoichiometry of such interactions is, as yet, undetermined. Comparisons are also shown of OR variations with temperature for b) κ-carrageenan "segments" 4% (wt/vol) alone (—) and in the presence of 1% (wt/vol) galactomannan (– – –) and for c) nongelling agarose concentrations (0.05%) alone (upper graph) and with added galactomannan (0.1%; lower graph). To facilitate comparisons galactomannan-containing curves have been adjusted by subtracting expected contributions from the galactomannan alone.

On cooling, the OR transition for κ-carrageenan segments is shifted (Fig. 7) to higher temperatures, suggesting an involvement of galactomannan in the nucleation event, and likewise on heating the observed change is consistent with some stabilization of double helices by galactomannan (34). For agarose, comparison of OR transitions in the presence and absence of galactomannan shows more marked perturbations (Fig. 7). Temperature-dependent shifts on cooling again suggest some participation in helix nucleation (34) but, in addition, the transitions are opposite in sign and the "melting transition" shows a complex form when galactomannan is present. Our interpretation is that the galactomannan itself adopts an ordered shape in the junction zone and that this can be "melted out" before the agarose helix itself melts.

As previously described, disorder → order transitions in xanthan are nongelling but in the presence of galactomannans this polymer also forms (14, 15) firm gels with sharp transition points suggestive of a cooperative association between the unlike chains (Fig. 7). Such quaternary interactions may also occur (15) with other 1,4-linked chains including glucomannans, xylans, and cellulose derivatives. For carrageenans and agarose such interactions may be important in maintaining the structural integrity of plant cell walls. In the particular case of xanthan, however, the interactions may be part of a specific pathogenic "recognition process." Xanthomonas campestris is a plant pathogen which invades tissues such as cabbages, beans, and cotton through the vascular system. This intermolecular interaction suggests that the extracellular xanthan chains could serve to "recognize" particular areas of plant cell walls and to bind the bacteria to them in a single layer.

3. APERIODIC CHAINS

These carbohydrate chains, typically found covalently bound to proteins and lipids, occur at the periphery of animal cells and in secretions and have aperiodic sugar sequences. Moreover, although it is increasingly evident that such chains are often involved in cellular recognition phenomena their shapes and potential for intermolecular associations are, as yet, poorly understood. It is apparent, however, that any such ordered conformations must be very different to those previously described. The chains are characterized by short, irregularly linked sequences of up to 6 sugar units. Ordered helices or ribbons are, therefore, impossible and it is tempting to speculate that the frequent occurrence of common "core" regions with "peripheral" side chains extending from dense branch points serve to induce the aperiodic chains to adopt either stable "globular shapes" or "planar arrays" capable of aligning and interacting with ordered "backbone" structures in a manner losely analogous to that of the simpler bacterial systems (12, 13) discussed above.

Excitingly, the first steps into the detailed understanding of the conformations of these chains are now being taken. Conformational analysis (38), using predictive methods for determining protein secondary structure from primary sequence, indicates a very high probability that aperiodic chains will attach to reverse β-turns of the amino acid backbone; all 9 O-glycosidic and 19 out of 28 N-glycosidically-linked chains considered were attached to sequences of this conformational type. In addition the striking revelation of the first crystallographic evidence for spatially distinct aperiodic chains confirms that such chains can themselves adopt ordered conformations under appropriate circumstances. The crystallized polypeptide is the Fc region of an immunoglobulin IgG and, although the shape of the carbohydrate chains is not yet finalized, Huber and his co-workers (39) have shown that they adopt a distinct 3-dimensional geometry and are, indeed, linked to a

β-turn of the protein backbone. As shown on Fig. 8 each carbohydrate chain does "shield" distinct, apolar regions of the polypeptide "sheet" and is also in intimate contact with a number of amino acids in the region functioning as the "hinge" in the complete antibody (Fig. 8). The uncomplexed antibody itself corresponds roughly to a symmetrical "Y" shape with little or no contact between F_{AB} and F_C domains when crystallized and showing complete disorganization of the F_C region below the hinge. Huber et al. have, therefore, proposed that the ordered F_C form corresponds to the antibody structure following complexation to antigen and this is consistent with solution studies suggesting contraction and conformational stabilization when antigen binding occurs.

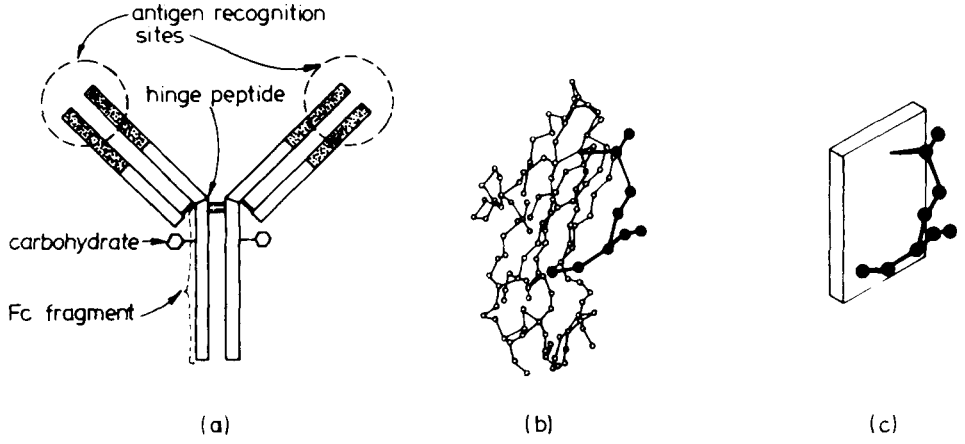


Fig. 8. Representation of antibody (IgG) molecule: a) Schematic to show relative locations of the different parts, b) contour of the carbohydrate chains (heavy lines – circles represent centers of each sugar ring) and neighbouring regions of peptide (thinner lines – circles represent C_α of each peptide unit) as deduced from X-ray diffraction analysis of F_C fragment, c) schematic of b), showing how the carbohydrate chain projects from the peptide sheet to cover 1 face.

In summary antigen binding is considered (39) to convert the antibody from a "flexible Y" to a "rigid T" shape and the function of the aperiodic carbohydrate chains appears to be in "screening" apolar protein segments and "keying in" the final association between ordered protein domains. If such conformations and interactions of these complex, aperiodic carbohydrate chains prove to be of general occurrence then the implications for molecular biology, and in particular for cellular recognition phenomenon involving multi-subunit assemblies of glycoproteins, are enormous.

NOTE ADDED IN PROOF

Additional evidence (40) consistent with the proposed role for aperiodic chains in determining the final shape and organisation of IgG antibodies is that glycoside digestion confirms the requirement of the carbohydrate moieties in recognition events mediated by the F_C region (ie through F_C receptor or complement binding) without significantly affecting their binding ability. Furthermore, differential glycosidase digestion indicated that the key sugar residues (N-acetylglucosamine and mannose) were those that make up the "core" of these complex, aperiodic carbohydrate chains.

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