Pure & Appl. Chem., Vol.55, No.4, pp. 677-694, 1983.
Printed in Great Britain.

0033-4545/83/040677-18$03.00/0
Pergamon Press Ltd.

STRUCTURAL EVOLUTION OF GLYCANS IN ALGAE

Terence J. Painter

Institute of Marine Biochemistry, N-7034 Trondheim-NTH, Norway

Abstract - The compositions and structures of the principal glycans in the eleven recognised algal phyla are reviewed and correlated with the positions of the phyla in a phylogenetic tree proposed by Chapman. By appealing to fossil evidence for the conservative nature of many algal species, tentative inferences are made concerning the probable order of evolution of the glycans. In general, the evolution of morphological complexity has been accompanied by the replacement of irregular glycan structures by more regular ones, containing a smaller number of different sugar residues. There are, however, some notable "missing links" and apparent exceptions among glycans that participate in calcification and silicification processes.

INTRODUCTION

In any attempt to review agal glycans, there is the risk of presenting a catalogue of structures which, however interesting, are so disparate as to defy integration into a single theme. If there is a single theme that unites all research in this area, it is surely the fascination engendered by the primitive morphology of the algae, and their aquatic habitat, which still has its mysteries, even though it covers 70% of the Earth's surface.

That the algae are, to some extent, "living fossils" is palpable even to the casual observer. They are, certainly, among the world's oldest living organisms, and they share this title only with the photosynthetic bacteria, from which they are distinguished by somewhat ill-defined criteria. The oldest fossils of both groups date back about 3 billion years (Ref. 1). Their evolution has also been very slow compared to that of terrestrial plants. For example, whereas the first blue-green alga must have appeared at least 3 billion years ago, the red algae, which are believed to be the second oldest phylum and the first eucaryotes, have not been traced back further than 1.3 billion years (Ref. 1 & 2). It therefore seems to have taken about half the recorded history of life for this first, major development to occur.

Somewhere near the other end of the expanse of time we are considering are the diatoms, whose appearance is fairly easy to "pin-point", because of their non-biodegradable, silica frustules. They are "only" 140 - 190 million years old (Ref. 3), which represents only the last 5% of the recorded history of life. That, however, is still a long time compared to the history of the angiosperms, which comprise the main bulk of the Earth's present terrestrial vegetation. The diatoms are roughly contemporary with the ancient ginkgo tree which is the oldest extant gymnosperm.

The slow evolution of the algae may be attributed, in part, to the absence of evolutionary pressures such as floods, droughts and ice ages, the relative constancy of temperature afforded by the huge volume of the oceans and the high specific heat of water, and the comparatively low level of ionising radiation. Probably the greatest evolutionary pressures have been the steady increase in the salinity of the oceans (Ref. 4), and the grazing of unicellular, planktonic species by filter feeders (zoöplankton).

It is not surprising, therefore, to find a close morphological similarity between many present-day algal species and fossils of great antiquity. Some authorities even state that there has been "little, if any" evolution in an ancient phylum such as the blue-green algae (Ref. 5). Can one, therefore, seriously expect that a study of the glycans of extant algal species would

reveal something about the early history of the evolution of glycan structures? The question seems worth asking, because direct examination of fossils is unlikely ever to provide that fascinating glimpse into the distant past: most of them take the form of empty cavities in sedimentary rocks, or the skeletons of calcareous or siliceous algae (Ref. 1-5). In this review, we propose tentatively to assume the truth of the idea, and then to examine the facts to see whether they lead to internally self-consistent conclusions.

The difficulties of such an undertaking are evident. There is a desperate shortage of fossil evidence, which is confined mainly to calcareous and siliceous species, and the obvious fact that any species is older than its oldest known fossil. Most fossils probably relate more to the heyday of a phylum than its appearance, and in periods when evolution has been rapid, "missing links" must be expected. The author has been greatly assisted by the recent appearance of several valuable textbooks on algal paleontology (Ref. 3, 6-9). The available information about algal glycans is also disappointing. Although more than 25,000 algal species have been described (Ref. 4,5,10), the glycans of only about 150 of these have been investigated, and there are four whole phyla for which no skeletal glycan has been studied structurally. It is therefore hoped that this review will stimulate research in the neglected areas.

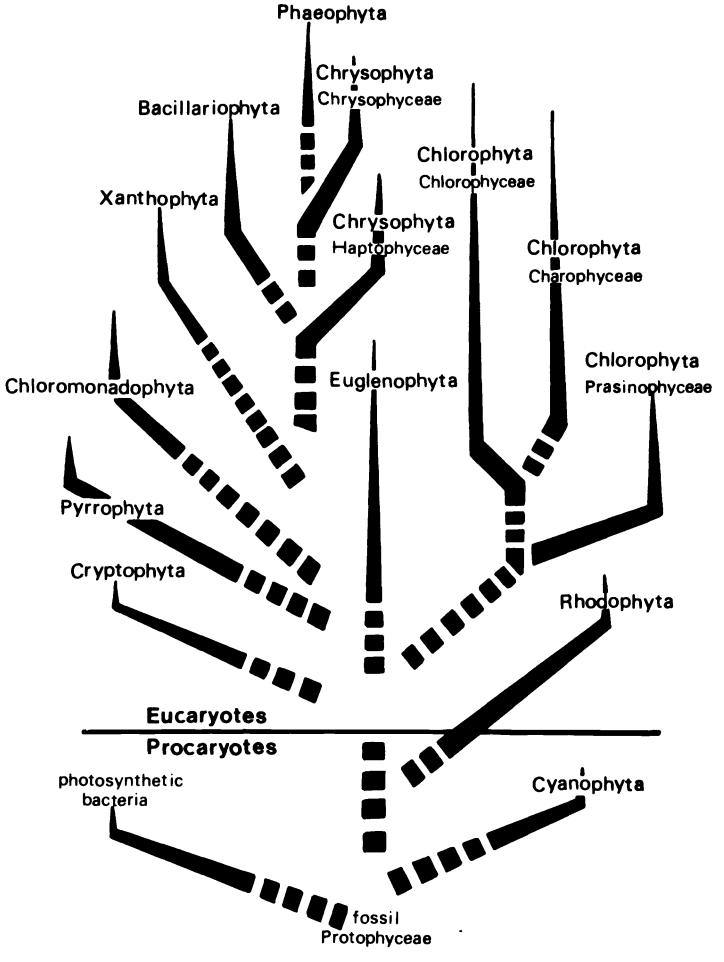


Fig. 1. Phylogenetic tree of algae, according to Chapman (Ref. 10) Reproduced, by permission, from The Encyclopædia Britannica

PHYLOGENY OF ALGAE

The phylogenetic tree shown in Fig. 1 is Chapman's latest (Ref. 10). He has stressed that the picture is still fluid, but most algologists seem to agree about the relative positions of the Cyanophyta, Rhodophyta, Chlorophyta, and Phaeophyta, whose glycans have received most attention. What matters most, perhaps, is that Chapman's conclusions are based upon a combination of fossil and morphological evidence, supplemented by inferences based upon pigments. They do not depend at all upon the compositions or structures of the glycans.

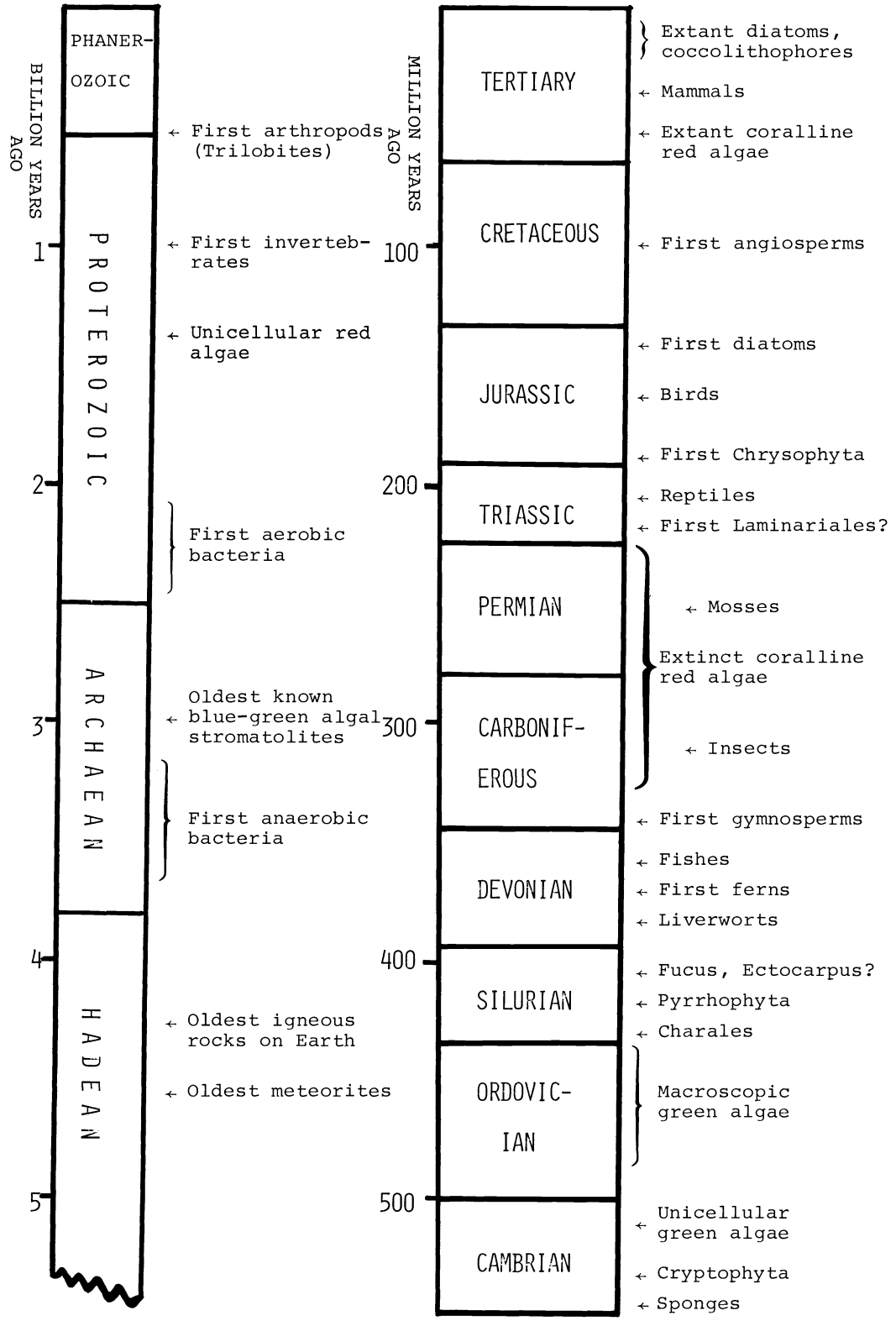
Table 1 gives a summary of information about the eleven algal phyla in the order in which they appear to have evolved, according to Chapman's scheme. There is a danger of over-interpretation here. Evolution has obviously continued within each phylum from the time of its separation, and it is quite possible to find, for example, a red alga that is "younger" than a green one. We shall try as far as possible to take account of this in the discussion.

TABLE 1. Habitat, morphology and glycans of the algal phyla

Phylum	Food reserve	Habitat	Morphology	Other glycans
Cyanophyta (Blue-green)	Glycogen	Mostly FW, wet soil	Unicells, colonies, OCT	Lipopolysaccharides rich in Glc and Man; sulphate-free extracellular slimes
Rhodophyta (red)	Glycogen	Mostly SW, benthic	Mostly OCT, some unicells	True cellulose doubtful. Mannans or glucomannans, heteroxylans, sulphated galactans and complex extracellular mucilages.
Cryptophyta	Starch	FW and SW	Unicells	No information.
Chlorophyta (green)	Starch, inulin, laminaran	FW (90%) SW (10%)	Unicells, colonies, OCT	True cellulose, glucomannans, homoxylans, pectic acid, complex hemicelluloses and sulphated mucilages.
Pyrrhophyta (dinoflagellates)	Starch?	Mostly SW	Unicellular flagellates	Cellulose putative.
Chloromonadophyta	Lipid	Mostly FW	Unicellular flagellates	No information.
Euglenophyta (green, some heterotrophs)	Laminaran	FW	Unicellular flagellates	No information.
Xanthophyta (yellow-green)	Lipid	FW, wet soil	Unicells, colonies, OCT	Cellulose and protein-bound heteroxylans, lichenan and 1,6-β-glucan.
Chrysophyta (golden)	Laminaran?	FW and SW	Unicells, colonies, OCT	Complex, sulphated, Ca^{++} - binding heteroglycans.
Bacillariophyta (Diatoms)	Laminaran	FW and SW, wet soil	Unicells, colonies, OCT	Cellulose putative. Chitin, complex sulphated extracellular slimes, sulphated glucuronomannans.
Phaeophyta (brown)	Laminaran	SW, mostly benthic	OCT, some vascular tissue	True cellulose, lichenan, alginate, fucoidan, complex sulphated heteroglycans.

Abbreviations: FW, fresh water; SW, salt or brackish water; OCT, organised cellular tissue, implying intercellular connection and dependence.

For obvious reasons, the following summary of the fossil record is both tentative and incomplete. Except in cases of mistaken identity, the effect of new fossil discoveries will clearly be to increase the estimated age of a phylum.



It should be noted in passing that terrestrial plants are believed to have evolved from green algae (Chlorophyta). This view, which is based mainly upon the similarity of their pigments (notably, chlorophyll b and carotenoids), will find few dissenters among glycan chemists, who will note the presence also of starch, cellulose, xylans, glucomannans, and pectic acid. The freshwater Chlorophyta of the Class Charophyceae (Fig. 1) are the most likely, immediate antecedents of terrestrial plants, because they are erect and fern-like in appearance (Ref. 4 & 5).

THE NOTION OF COMPLEXITY - A SEMANTIC DIFFICULTY

In view of the primitive morphology of algae, one might expect that the structures of the glycans they elaborate would also be simple. In fact, the reverse seems to be true. Many algal glycans surpass even the gum exudates of terrestrial plants in apparent complexity. It is becoming increasingly clear, however, that much of what used to be described as "complexity" in glycan structures would better be described as "irregularity". The notion of simplicity is, arguably, subjective. For example, isotactic and syndiotactic polystyrene are technologically more "advanced" polymers than the atactic variety, but we may judge their structures to be "simpler", because they are easier to remember. In fact, it can be shown mathematically that their information content is higher.

As another example, one may compare a bacterial lipopolysaccharide with four different types of sugar residue arranged in regular repeating-units with the highly ramified molecules that would result from polymerising the same sugars in the presence of an acidic catalyst. Evidently, both structures are complex, but the former is regular, and the latter is chaotic.

Considerable irregularity might perhaps be expected in the glycans of organisms that are low on the evolutionary scale, because it implies that the glycotransferase enzymes are low in specificity rather than large in number. Irregular glycan structures tend to form slimes and gels rather than fibres, and the evolution of morphological complexity implies the elaboration of ordered macromolecular structures for specialised functions. Moreover, slimes and rubbery, shock-resistant gels have an obvious survival value for a plant in the aquatic environment. For example, the former retard desiccation of seaweeds at low tide, and the latter protect them against violent wave action.

This is not to pre-judge the amount of order that might be found in the structure of an algal glycan, much less a licence not to look for it. On the whole, however, one would expect the evolution of glycan structures to consist in the replacement of irregular structures by more ordered ones (an apparent simplification), followed by an increase in "true" or "ordered" complexity.

STRUCTURAL EVOLUTION OF FOOD-RESERVE GLYCANS

Glycogen, amylopectin and amylose

Glycogen seems unquestionably to be the world's oldest food-reserve glycan (Table 1). In the filamentous, blue-green alga Nostoc muscorum, it exists as minute, roughly cylindrical particles, 65 nm in length and 31 nm in diameter (Ref. 11). The cylinders appear to consist of two spherical sub-units fused together, and are probably single macromolecules, because they do not split up unless treated with α-amylase. The material resembles shellfish glycogen in the λ_{max} of its iodine complex (410 nm), its yield of α-limit dextrin (11 - 14%), and its average chain-length, determined by periodate oxidation (13 units). In the glycogen from Anacystis nidulans, another filamentous Cyanophyte, the chain-length distribution, determined after debranching with bacterial iso-amylase, was similar to that of sweet-corn phytoglycogen (Ref. 12).

The red algae also contain glycogen, with most values for the average chain length falling in the range of 10 - 14 units (Ref. 13), but it is present as starch-like granules, 0.5 - 25 μm in diameter. It differs from typical animal glycogens in giving higher iodine blue-values, and in its susceptibility to partial debranching by R-enzyme (Ref. 14). These facts suggest a different distribution of internal chain-lengths. Red-algal glycogen behaves typically with starch-degrading enzymes, but, in addition to the usual oligosaccharides, it also gives nigerose (3-O-α-D-glucopyranosyl-D-glucose). It must therefore contain a small proportion of =(1→3)-linkages, in contrast to all the glycogens and starches of higher plants and animals (Ref. 14). Unfortunately, blue-green algal glycogen has not been examined for this special feature.

Starch makes its first appearance in the Cryptophyta, a small phylum of mostly flagellated unicells, whose pigments place them fairly clearly between the Rhodophyta and the Chlorophyta. This would place their appearance somewhere in the Cambrian Period, 500 - 550 million years ago. The starch is present as granules, 2 - 5 μm in diameter. The material from Chilomonas paramecium contained 45% of an amylose of low molecular weight, and an amylopectin similar to that of potato starch (Ref. 15). A similar starch from Chroomonas salina was estimated to contain 30% of amylose (Ref. 16).

Chlorophycean starches have been well investigated, with the conclusion that they are true starches (Ref. 13 & 17). The presence of amylose is firmly established in most, but not all, of the cases investigated, and one species, Urospora penicilliformis, is reported to contain pure amylose (ref. 18). The principal peculiarities are the smallness of the granules, and the low molecular weights of both the amylose and amylopectin, compared to their counterparts in cereal and tuber starches. Some green algae contain only a typical amylopectin (Ref. 19 & 20), but that, of course, is also true of some cereal starches.

One wonders whether the reader will share the author's astonishment upon being confronted with the notion that glycogen had existed for 2.5 billion years, before amylose made its first appearance on Earth? The process must have entailed a partial loss and compartmentalisation of branching activity, requiring the evolution of an endoplasmic reticulum. Fredrick, who has done much to promote the notion that enzymic evolution can be validly studied with algae, has studied this question with Oscillatoria princeps, a filamentous blue-green alga (ref. 21). It was shown that an anti-phosphorylase rabbit serum cross-reacted, not only with the two phosphorylase isozymes in the alga, but also with the two glycogen synthetase isozymes, and, more weakly, with the branching isozymes (Q-enzyme). This suggested that glycogen synthetase and Q-enzyme had evolved from a common precursor. By separating the two activities, therefore, evolution had produced two enzymes from one, and increased the specificity of both.

The apparent loss of the ability to synthesise (1→3)-linkages in glycogen may represent the loss of an enzyme, but it could also be due to an increase in the specificity of the glycogen synthetase.

Laminaran

The apparently smooth transition from starch to laminaran as the principal energy-reserve, as evolution progressed, is particularly striking, even though the identification of starch in the Pyrrhophyta and laminaran in the Chrysophyta is tenuous (Table 1). A form of laminaran makes its first appearance in the Chlorophyta. In the unicellular, freshwater Chlorella species, well known for their promise as protein-producers, it is linked, at least in part, to protein (Ref. 22). Small amounts have also been found in the macroscopic, marine species, Caulerpa filiformis (Ref. 23), Cladophora rupestris (Ref. 24) and Caulerpa simpliciuscula (Ref. 25). In the last case, a small proportion of β-(1→6)-linkages was demonstrated in addition to the β-(1→3)-linkages.

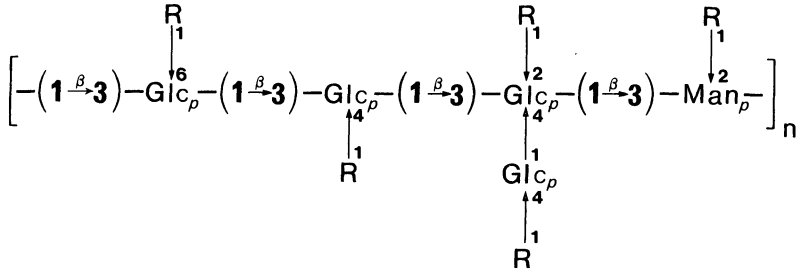
In the Euglenophyta, a small phylum of green algae once considered to be a sub-phylum of the Chlorophyta, laminaran clearly takes over as the main energy-reserve. It exists in the cells as discrete, rod-shaped bodies, and is rapidly broken down when the cells are deprived of light and organic carbon (Ref. 13). The laminaran from Euglena gracilis resembles brown-algal laminaran in containing a high proportion of (1→3)-linkages, but no mannitol is present (Ref. 26).

Laminaran has not been found in the Xanthophyta, but a protein-bound β-D-glucan, linked mainly through positions 1 and 6, has been found in the filamentous species, Tribonema aequale (Ref. 27). It is not clear whether it served as a food-reserve. In both freshwater (Ref. 28) and marine (Ref. 29 & 30) diatoms, there is no doubt that laminaran is the food reserve; when starved of nitrogen, they may accumulate up to 40% of their weight of it, corresponding to 80% of the total dry weight of organic matter. The laminaran of diatoms contains both (1→3)- and (1→6)-linkages, and is generally similar to brown algal laminaran, except for the total absence of mannitol.

The classical, pioneering work on brown-algal laminarans is well known (Ref. 13 & 31). An important, recent development was the clear separation of Laminaria hyperborea laminaran into a fraction in which the chains are terminated by mannitol and a fraction in which they are not (Ref. 32). The mannitol-terminated chains were all unbranched, while the others included both branched and unbranched molecules (Ref. 33). An unusual laminaran from Eisenia bicyclis contained no mannitol, and consisted of linear chains of

(1→3)- and (1→6)-linked β-D-glucopyranose residues in a ratio of 2:1 respectively; at least some of the (1→6)-linkages were contiguous (Ref. 34).

It is hard to perceive any clear evolutionary trend in the structure of laminaran, apart from the replacement of protein by mannitol as an (ultimate) acceptor. There is perhaps some indication of a compartmentalisation of branching activity in brown algae, this being absent in chains terminated by mannitol. We can, however, find an indication of where the genetic information required for the synthesis of contiguous, (1→3) β-D-glucopyranosidic linkages came from. Structure (1) has been reported for a glycan isolated from the envelope and spores of the filamentous blue-green alga Anabaena cylindrica (Ref. 35):



(1)

Inulin

Since it has been found only in macroscopic Chlorophytes, little can be deduced about the evolutionary history of inulin except its minimum age. This is known with some precision, because it occurs in calcareous green algae of the Order Dasycladales. The thalli of these algae are so elaborate and distinctive in architecture that extant species can be identified, with reasonable certainty, with well-preserved fossils from the Ordovician Period, 430 - 500 million years ago. Inulin, apparently identical in every respect with that in dahlia tubers, has been isolated from Acetabularia mediterranea (Ref. 13), Acetabularia crenulata (ref. 36) and Batophora oerstedi (Ref. 13). There is no evidence for the presence of branching through (2→6)-linkages, such as is found in the levans of grasses.

There are no reports of fructans of any kind in the Cyanophyta or Rhodophyta, and one can only speculate as to where the ability to synthesise inulin came from. Stereochemically, the environment around HO-1 of a β-D-fructofuranose residue is very similar to that around HO-6 of a β-D-glucopyranose residue. A minor change in the conformation of the enzyme that synthesises (1→6)-linkages in laminaran, such as might be brought about by a change in one amino-acid, could therefore explain the appearance of inulin.

STRUCTURAL EVOLUTION OF FIBROUS GLYCANS

Cellulose

There is apparently no cellulose in the Cyanophyta, whose cell walls are constructed similarly to those of the heterotrophic Gram-negative bacteria, with a net-like membrane of peptidoglycan, and an outer envelope of lipopolysaccharide (Ref. 35, 37-41). Unfortunately, the outer, glycan part of only one of these lipopolysaccharides has been studied structurally, with the result shown as structure (1) above. A number of quantitative compositions have been reported, however, and these are summarised in Table 2. Residues of both glucose and mannose are prominent, and, if structure (1) is typical, (1→4)-linkages, some of them contiguous, are also present.

It is very hard to decide whether "true" cellulose (in the sense of cotton cellulose) exists in red algae. Exhaustive extractions with acids and alkalis, with or without chlorite bleaching, do yield from 1% to 9% of fibrous residue

TABLE 2. Compositions (moles %) of the outer glycan part of Cyanophycean LPS

Species	Glc	Man	Xyl	Gal	Rha	Ref.
Anabaena cylindrica	65	23	5	7	-	35
Anabaena flos-aquae	17	83	-	tr.	-	40
Anabaena variabilis	44	5	-	3	48*	39
Anacystis nidulans	8	74	-	12	6	38
Agmenellum quadruplicatum	17	16	56	tr.	11	41

*Partly 3-O-methylated

(Ref. 42), but this contains a large proportion of sugar residues other than glucose (mostly mannose and xylose), and one such residue from a Porphyra species contained no glucose residues at all: the "α-cellulose" was a pure mannan! (Ref. 43). The verdict of the objective eye of the X-ray camera is that untreated red-algal tissues do not contain Cellulose-I, but that a low degree of crystallinity of this type may develop in some cases after extraction of encrusting materials (Ref. 44 & 45).

The status of the "cellulose" in the unicellular Chlorophyta is also unclear, but there are quite strong indications that it is covalently linked to other sugars. For example, most of the cell wall of Halicystis osterhoutii is a glycan containing residues of xylose and glucose in a ratio of 1:2 respectively. This ratio does not change as the walls are progressively solubilised by repeated alkaline extractions, a result that would not be expected for a physical mixture of xylan and cellulose. The material also stains with dilute aqueous iodine, thus resembling the "amyloid" of tamarind seeds, which consists of cellulosic chains substituted at position 6 with α-D-xylopyranose residues (Ref. 46). The α-cellulose from Pleurotaenium trabecula is likewise closely associated with galactose residues (Ref. 47), while that from Chlorella pyrenoidosa contains many different sugars (Ref. 48).

Cellulose makes its first undisputed appearance in the green seaweeds, albeit only in some. A beautifully crystalline form of cellulose is present in the globular or club-shaped vesicles of Valonia species. These are actually giant cells, up to 4 cm in diameter. Their walls contain long, regular fibrils, 100 - 350 Å in diameter, arranged in a criss-cross manner. These contain residues of D-glucose only, and give very sharp X-ray diffraction patterns characteristic of Cellulose-I (Ref. 49 & 50). Cellulose has also been identified by chemical methods in the freshwater Charales (Ref. 51).

Within the phylum Xanthophyta, cellulose has been identified by chemical methods in Tribonema aequale (Ref. 27), but in the Pyrrhophyta and Bacillariophyta, it was only tentatively identified by staining reactions. In the Phaeophyta, cellulose has been firmly identified by chemical and X-ray data (Ref. 52).

In conclusion, cellulose has been a slow developer. Since the macroscopic green seaweeds and the Charales have been traced back to the Ordovician Period it is probably no more than 500 million years old. Its functional antecedents were all heteroglycans containing non-glucose sugar residues, notably mannose and xylose.

Mannans and Glucomannans

Although glucomannans, in general, can be traced back to the Cyanophyta, the essentially linear, β-(1→4)-linked mannans and glucomannans are prominent only in the Rhodophyta and Chlorophyta. In some species, they seem to replace cellulose entirely as the principal, fibrous, cell-wall glycan. They are isolated essentially as "α-cellulose", by extraction with acids and alkalis, with or without chlorite bleaching. They are then solubilised, with considerable difficulty, by hot, concentrated alkali, and purified by precipitation as an insoluble copper complex (Ref. 53 & 54). The solubilisation procedure probably entails considerable depolymerisation, and, since the copper complex is formed selectively with fragments rich in mannose, the products may not be represent-

ative of the native fibres.

The red alga, Porphyra umbilicalis, gave a pure mannan with a $\overline{DP}_n$ of only 12, and an $[\alpha]_D$ of -41. The (1→4)-linkage was established by methylation analysis (Ref. 53). The product from the green alga, Codium fragile, contained 5% of D-glucose residues, and the β-(1→4)-linkage was established by the isolation of oligosaccharides, including 4-O-β-D-glucopyranosyl-D-mannose and 4-O-β-D-mannopyranosyl-D-glucose (Ref. 54). The product from Acetabularia crenulata contained only traces of glucose, and had $\overline{DP}_n$ < 60. Methylation analysis established that the linkages were (1→4), with occasional branching through position 2 (Ref. 36). A glucomannan has also been reported in the freshwater, colonial green alga, Hydrodictyon africanum (Ref. 55).

Insofar as the presence of pure, homopolymeric mannans in red and green algae can be considered to have been established, the balance of the evidence is quite strongly in favour of the notion that their functional antecedents, like those of cellulose, were glucomannans. This implies the existence of a fork in the evolutionary pathway:

Xylans

These do not seem to exist as such in blue-green algae, but it is evident from Table 2 that some of their lipopolysaccharides are rich in xylose. The subsequent history of the xylans, however, provides a very clear example of a fork in the evolutionary pathway. The prototype for red algae is rhodymenan, a xylan from the benthic seaweed, Rhodymenia palmata ("dulse"). The material extracted under neutral or acidic conditions consists of linear chains of β-D-xylopyranose residues, joined by (1→4)- and (1→3)-linkages in ratios that vary from about 4:1 to 2:1 respectively (Ref. 56). The presence of both kinds of linkage in the same molecule is firmly established by the isolation of oligosaccharides (Ref. 57-60) and by Smith degradation (Ref. 60), and the yields of the fragments strongly suggest a random arrangement (Ref. 58). Subsequent extraction of the algal residue with 3M-sodium hydroxide then removes a xylan containing 97% of (1→4)-linkages (Ref. 61).

Another red seaweed, Porphyra umbilicalis, gives a xylan closely similar to rhodymenan when extracted under the conditions of chlorite bleaching, but subsequent extraction with alkali then removes a xylan containing 95% of (1→3) linkages (Ref. 61). A summary of these and other data is given in Table 3. Evidently, these red algae contain families of xylan molecules, each with a broad spectrum of linkage compositions, but biased, as it were, in favour of one or another of the two homopolymeric extremes.

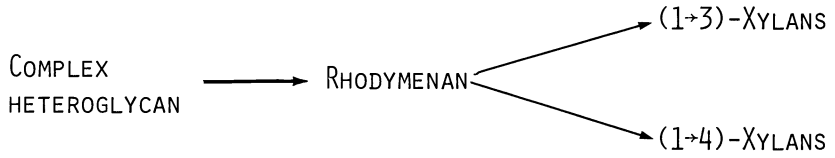
TABLE 3. Extraction conditions and structures of red-algal xylans

Species	Extraction conditions	(1→4)-links (%)	(1→3)-links (%)	Structural method	Ref.
Rhodymenia palmata	H_2O, 100°	80	20	Methylation	56
	3M-NaOH	97	3	Methylation	61
	H_2O, 100°	71	29	Periodate	58
	0.5N-HCl	62	38	Periodate	58
Porphyra umbilicalis	$HClO_2$, 70°	83	17	Methylation	61
	3M-NaOH	0	95	Methylation	61
	Mechanical	0	100	X-ray	62
Chaetangium erinaceum	H_2O, hot	82	18	Methylation	57
Bangia fuscopurpurea	Mechanical	0	100	X-ray	62

Upon proceeding to the Chlorophyta, the remarkable fact emerges that no xylan comparable to rhodymenan has yet been found in them. In some species, notably those that seem to be adapted exclusively to the marine environment, the xylans are uniformly (1→3)-linked. They are fibrous, and in some species, notably those of the Order Siphonales, they take over entirely from Cellulose-I as the principal cell-wall glycan (Ref. 63). These algae are quite delicate in construction, and it may be supposed that cellulosic fibres are too inflexible to withstand the pounding that they receive from the waves. The prototype is the xylan from Caulerpa filiformis, which has about one branching point for every 96 (1→3)-linked β-D-xylopyranose residues (Ref. 63).

Since β-(1→3)-linked xylans are unknown in terrestrial plants, and these are considered to have evolved from green algae, it would be surprising not to find a β-(1→4)-linked xylan in those green algae that are more closely related to the antecedents of terrestrial vegetation. Indeed, one has been found in the morphologically complex, freshwater Chara genus, and moreover, it is associated with minor amounts of arabinose and uronic-acid residues, just like the xylan hemicelluloses of land plants (Ref 64). These algae stand quite erect in placid, freshwater lakes, and it may be supposed that their ability to grow tall is a response to the need for sunlight.

It therefore seems that, in response to the environmental pressures, evolution has selected out these two, homopolymeric extremes of xylan structure from the comparatively disordered structure of rhodymenan:



The "complex heteroglycan" may be a xylose-rich lipopolysaccharide such as that of the blue-green alga, Agmenellum quadruplicatum (Table 2), but one can see a more immediate antecedent in the Porphyridium mucilages that will be discussed in the next section.

Upon passing on to the freshwater Xanthophyta, one finds no evidence for the selection of either kind of homopolymeric xylan. In the one species in which a xylan has so far been found, namely, Tribonema aequale, it was firmly bound to protein, and contained both (1→3)- and (1→4)-linkages (Ref. 27). Upon reflection, one can find no reason why such a selection should have been made: since the species in question floats freely on the surface of stagnant ponds, it does not have to respond to either of the environmental pressures named above. This finding does not conflict with Chapman's phylogenetic tree (Fig. 1), because the implication is simply that the Xanthophyta and Chlorophyta evolved in different ways from a common antecedent.

Chitin

Chitin is found in some diatoms of the Order Centrales, which flourished in the Cretaceous Period, 70 - 100 million years ago. It occurs as spiny or tubular protrubrances, which probably help to keep the diatoms bouyant, by trapping air bubbles. The crystal structure is different from that of arthropod chitin, into which it is irreversibly converted by hot, aqueous lithium thiocyanate (Ref. 65).

Glucosamine is present in the peptidoglycan and inner core of blue-green algal lipopolysaccharides (Ref. 37), and although the former has not been very closely investigated, it may be presumed to resemble the peptidoglycan of bacterial cell walls in containing, as its carbohydrate moiety, 3-O-(1-carboxyethyl) ethers of chitin. If this is true, then chitin, albeit in modified form, must be considered the most primordial of all skeletal glycans.

That so much time should have elapsed before chitin appeared in unmodified form seems astonishing, especially when, in the animal kingdom, arthropod chitin appeared much earlier. The fact is, however, that we so far know nothing about the skeletal glycans of the other, unicellular phyla that appeared in the intervening billions of years, namely, the Cryptophyta, Pyrrhophyta, Chloromonadophyta and Euglenophyta. In the Chrysophyta, glucosamine has been found as a component of a complex, sulphated proteoglycan of a Phaeocystis species (Ref. 66). It is, therefore, too early to complain about "missing links", but not too early to look for them!

It should be noted that diatoms are under intensive evolutionary pressure, due to grazing by filter feeders. It has been estimated that no single, diatom

species has existed for more than half a million years, which is an exceptional rate of evolution for an alga (Ref. 8). This is the most likely explanation for other surprises that will be mentioned later.

STRUCTURAL EVOLUTION OF ALGAL MUCILAGES

This section includes slimes secreted into the growth medium, capsules, and the glycans forming the gelatinous matrix in both the intercellular spaces and the cell walls.

Blue-green algal slimes

Perhaps the most striking feature of blue-green algae is the slimy jelly that develops as a capsule around the cells in unicellular and colonial forms, or as a sheath and intercellular substance in multicellular forms. It is sloughed off, or exuded, into the growth medium. Unfortunately, there have been no structural studies, but some typical sugar compositions are given in Table 4.

TABLE 4. Constituent sugars of blue-green algal slimes (moles %)

Species	Glc	Xyl	GlcA	Gal	Rha	Ara	Other	Ref.
Anabaena cylindrica	31	25	25	6	6	6	-	67
Nostoc sp.	35-X	25	30	X	10	-	GalA?	68
Anabaena flos-aquae	67	30	0.8	-	-	-	Rib	69
Palmella mucosa	52	-	4.8	-	-	14	Fuc	70
Anacystis nidulans	64	-	-	15	-	-	Man	71

These sugars are well worth remembering, because they persist with remarkable tenacity throughout the evolutionary history of algal slimes. It should be noted that none of the slimes contains hemiester sulphate or O-methyl sugars, which appear for the first time in red algae.

Red-algal mucilages

The unicellular *Porphyridia* and the closely related genus, *Rhodella* provide an important link between the Cyanophya and the Rhodophyta, because they contain pigments common to both phyla, and there is still some dispute as to which one they belong to. These primitive algae grow as red or purple films on damp soil or sand. The mucilages, which form gelatinous capsules around the cells, are sulphated proteoglycans. Some reported sugar compositions and the principal linkages, determined by methylation analysis, are shown in Table 5. The remarkable variations in composition reported for the mucilage of *Porphyridium cruentum* seem to be due to differences in growth conditions, and they argue for a low specificity on the part of the biosynthetic enzymes. In turn, this implies considerable irregularity of structure, which also seems to be confirmed by investigation.

TABLE 5. Unicellular freshwater Rhodophyta (molar ratios)

Species	D-Glc	D-Xyl	D-GlcA	D-Gal	L-Gal	2MeGlcA	Ref.
P. cruentum	1.00	2.50	0.28	0.23	2.02	0.22	72
P. cruentum	1.00	2.42	1.22	0.21	1.91	2.61	73-75
P. cruentum	1.00	3.00	0.80	1.90	0.60	0.20	76
P. aerugineum	1.00	1.70	0.50	0.74	0.36	0.00	76
Linked mainly	1,3	1,3:1,4	1,3	1,3	1,4	?	

In addition to these main sugar components, minor proportions of 3- and 4-O-methyl galactose, 2,4-di-O-methyl galactose, an unidentified 2-O-methyl hexose, and 3-O-methyl xylose were found in the mucilages.

The presence of both D- and L-galactose is noteworthy, and the fact that the former is mainly 1,3-linked, while the latter is mainly 1,4-linked, foreshadows the situation in agarose. The presence of a high proportion of D-xylose linked mainly 1,3 and 1,4 likewise portends the subsequent appearance of rhodymenan. The presence of D-glucose, linked mainly 1,3, is reminiscent of the lipopolysaccharide of Anabaena cylindrica (1). The isolation of aldobiouronic acids by partial acid-hydrolysis established that residues of D-glucopyranuronic acid were linked to positions 3 of D-glucose and D-galactose residues, but to position 4 of L-galactose residues (Ref. 72 & 76).

Proceeding up the scale of morphological complexity, we come next to the macroscopic, freshwater genus, Batrachospermum, which grows as bushy, violet - green tufts on sticks and stones in streams and pools, and thrives in peaty water. One species yielded 42% of its weight as a sulphate-free glycan, containing residues of D-glucose, D-xylose, D-glucuronic acid, D-galactose, L-galactose, L-rhamnose, L-arabinose, and D-mannose, together with the 3-O-methyl ethers of D-galactose and L-rhamnose (Ref. 77). It is interesting to compare these results with those in Table 4. Partial acid-hydrolysis led to the isolation of aldobiouronic acids in which residues of D-glucuronic acid were linked glycosidically to galactose, possibly in the same way as in the Porphyridium mucilages.

The salient features of the chemistry of agars and carrageenans, namely, the perfectly alternating sequence of 1,3-linked D-galactose and 1,4-linked D- or L-galactose residues partly converted into 3,6-anhydrides, are well known and have been extensively reviewed (Ref. 31). These structures are so "simple" and regular in comparison with those just discussed, that one may seriously question whether they are related to them at all. There are certainly some "missing links" to be sought after, but these galactans do come from a rather small selection of commercially important red seaweeds, and even they contain a small proportion of xylose residues (Ref. 31). When a broader spectrum of red seaweeds is considered, the notion of a direct evolutionary relationship back to the Porphyridium mucilages and beyond becomes more acceptable. For example, the galactan of Anatheca dentata not only contains 8% of D-xylose, but also D-glucuronic acid. The latter was recovered after partial hydrolysis as 4-O-α-D-glucopyranuronosyl-L-galactose, and was therefore linked to L-galactose in the same way as in the Porphyridium mucilage (Ref. 78). The galactan of Dilsea edulis also contains residues of D-xylose and D-glucuronic acid and moreover, its D-galactose residues are mainly (1→3)-linked, thus deviating from the pattern of perfect alternation (Ref. 79). The galactan of the calcareous red alga, Corallina officinalis, contains 22.7% of D-xylose, and the D-galactose residues are mainly (1→4)-linked (Ref. 80). In Pachymenia carnosa, the ratio of (1→3) to (1→4)-linkages is 2.3:1 (Ref. 81), and in Aeodes ulvoidea, the galactan chains contain some (1→6)-linkages (Ref. 82).

The extent of the "simplification" represented by agars and carrageenans is nonetheless remarkable, and one wonders how long it took. Unfortunately, none of the seaweeds concerned is calcareous, and there have been no fossil discoveries of significance. The fossil record for the coralline red algae is good, however, and fossils similar to extant species have not been traced back further than the early Tertiary Period, 65 million years ago. Even the most ancient, extinct species seem to have appeared in the Jurassic, about 140 - 190 million years ago. Since Corallina officinalis contains a fairly "simple" sulphated xylogalactan, it may be tentatively assumed that the well known agarophytes and carrageenophytes are no older.

Green-algal mucilages

Table 6 shows the principal sugars and linkages found in the sulphated mucilages of benthic green seaweeds. Both the Codiaceae and the Dasycladiaceae include calcareous species which place them firmly in the Ordovician Period, and probably even in the Cambrian. They are therefore probably at least 500 million years old. Since the Cladophorales are morphologically much more complex than the others, they are probably the most recent. Here again, we have a selection of the most prominent sugars found in blue-green algal slimes (Table 4). The principal linkages are also broadly similar to those of the Porphyridium mucilages, and a similar aldobiouronic acid has been isolated. Particularly striking is the elimination of D-glucose and D-mannose with decreasing antiquity, and there has also been a simplification in the way in which the L-arabinose residues are linked. It is established that most of the D-xylose residues in these mucilages are mutually linked.

TABLE 6. Constituent sugars and principal linkages in sulphated mucilages of green seaweeds

Species	D-Xyl	D-GlcA	D-Gal	L-Rha	L-Ara	Other	Ref.
ORDER SIPHONALES							
Codium fragile	1,4	Trace	1,3 1,6	Trace	1,4 1,3	D-Glc D-Man 3,6AnGal	83 84
Caulerpa filiformis							
ORDER DASYCLADALES							
Acetabularia crenulata	1,4 1,3	1,3*	1,3*	1,4 1,2	-	D-Glc 4MeGal	85
ORDER ULVALES							
Ulva lactuca	1,4 1,3	1,3 1,4	1,3 1,6	1,4	-	-	86 87
ORDER CLADOPHORALES							
Cladophora rupestris	1,4	-	1,3 1,6	Trace	1,4	-	24 88 89

*Present as D-GlcA-(1→3)-D-Gal

Extracellular slimes of diatoms

We must now move forward at least 300 million years, because of a lack of information about the intervening phyla. Some diatoms secrete exceedingly viscous slimes into the ambient water. It must be presumed that this energy-consuming activity has some survival value, and it seems most likely that it discourages grazing. To be effective, the slimes must resist bacterial attack, and some unusual sugars and structures might therefore be expected. Some colonial diatoms, including Berkeleya rutilans (Table 7), protect themselves by living inside mucilaginous tubes, called "schizonema". The details in Table 7 do indeed show some radical developments, and the appearance of fucofuranose and 3,6-anhydro-glucose residues is especially noteworthy. In spite of this, most of the sugars and some of the linkages still suggest a genetic link with the more ancient phyla. The slimes are all sulphated.

TABLE 7. Constituent sugars and principal linkages in extracellular slimes of marine diatoms

Chaetoceros affinis	$Gal_p(1,3:1,4:1)$, $Rha_p(1:1,2)$, $Fuc_p(1,3:1,2,3)$	Ref. 90
Chaetoceros curvisetus	$Gal_p(1,2,3)$, $Rha_p(1,2)$, $Fuc_f(1,2:1:1,2,3/5)$	91
Coscinodiscus nobilis	$Xyl_p(1)$, $GlcA_p(?)$, $Rha_p(1,2)$, $Fuc_p(1,3)$, $Man_p(1,6)$	92
Berkeleya rutilans	$Xyl_p(1,2:1,4)$, $ManA_p(1,3:1,2,3)$, $36AnGlc_p(1,4)$	93

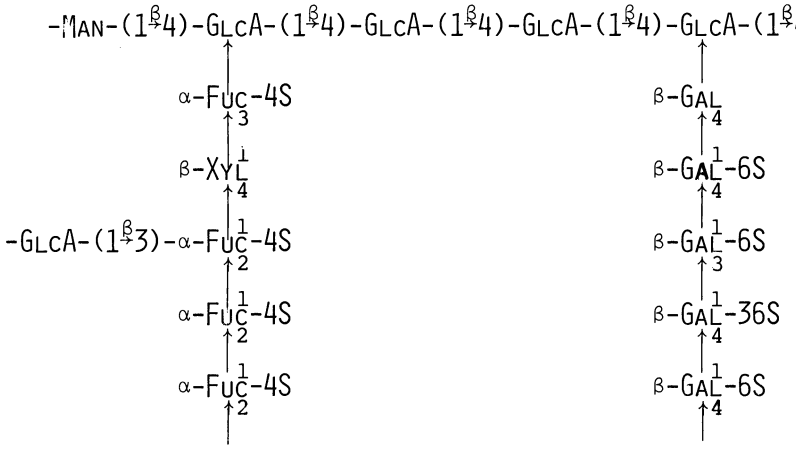
Brown-algal mucilages

Only one (Padina) of the 240 extant genera of brown algae is calcareous, and the fossil record is poor. Fossils of the extinct Order, Nematophytales, have been traced back to the Paleozoic Era (Ref. 5), but the advanced state of evolution of extant species is inferred from their comparatively high degree of cellular specialisation (Ref. 4). We are concerned with the mucilages of only two Orders, namely, the Fucales and the Laminariales, because the others have been neglected. The Laminariales are the more advanced morphologically, and they include the giant kelps, Macrocystis and Nereocystis, which even have a vascular system.

We are concerned, too, with only two kinds of mucilage, namely, fucoidan and alginate, provided that the former term is applied to all fucose-containing glycans in brown algae. Both glycans are present in both Orders, but the amount of fucoidan in the Laminariales is comparatively small.

It is appropriate to begin with the more primitive Fucales, and the most complex form of fucoidan yet described. This was found in Sargassum linifolium, and named "sargassan" (Ref. 94). Its principal structural features may be represented, in simplified form, by the structure (2). Again, there is a selection of the more prominent sugars found in blue-green algal slimes (Table 4),

but, after disappearing from the slimes of diatoms, (Table 7), glucose has not reappeared, and fucose has now entirely supplanted rhamnose as the 6-deoxy-hexose.



(2)

In their native state, it is likely that all fucoidans contain the same sugars as sargassan, but that the proportions vary widely. Fractions extracted with acid may be devoid of glucuronic acid and mannose, but pure fucans are rarely obtained. Acid extraction of another member of the Fucales, Ascophyllum nodosum, has yielded a fucoidan ("ascophyllan") that contains no mannose or galactose, but which is otherwise similar to sargassan (Ref. 95 & 96). Systematic studies of the acid-extraction procedure showed that, in its native state, ascopyllan is part of a much more complex molecule that contains alginate-like chains (Ref. 97), and a fraction similar to ascophyllan was isolated containing residues of D-mannuronic and L-guluronic acids in addition to D-glucuronic acid (Ref. 98). Fucus vesiculosus gave similar results.

The inference that fucoidan and alginate are chemically linked in Fucales is therefore strong. It is not clear how much of the alginate is present in the free state, but it is very difficult to prepare pure alginate from the Fucales without using acid at some stage in the isolation. Unfortunately, similar studies of the Laminariales are lacking, but they are generally considered in the industry to be superior raw materials for the manufacture of alginate.

We are so accustomed to thinking in terms of biosynthesis, that it seems hard to think of alginate as having evolved from a much more complex macromolecule of which it is only one of the building units. Obviously, it would have to be synthesised first, before xylofucan chains could be built on to it. The indications are, however, either that the fucotransferase activity declined, or that alginate stopped acting as the acceptor.

GLYCANS PARTICIPATING IN CALCIFICATION AND SILICIFICATION

Although these glycans are also non-fibrous, they are distinguished from the other mucilages by their specialised functions and apparently highly ordered structures.

Pectic acid

This has been found in the freshwater green alga, Nitella translucens, which belongs to the Order Charales (Family, Characeae), whose close affinity to terrestrial plants has already been noted (Ref. 99). The genus Nitella is not itself calcareous, but most of the Charales are, and fossil species are known from the late Silurian Period, about 400 million years ago. This means that pectic acid is roughly contemporary with true cellulose and the homopolymeric xylans.

The pectic acid was extracted from the alga with hot aqueous ammonium oxalate, and after purification via a copper complex, it had $[\alpha]_D$ +245 and contained 74% of uronic anhydride. The neutral sugar components were galactose, arabinose, xylose and rhamnose, present in the molar ratio 4:6:3:1, and D-galacturonic acid was isolated by hydrolysis with pectinase. The material was therefore remarkably similar to some of the more complex pectins of terrestrial plants, but it contained no methoxyl.

It is evident that the presence of pectic acid does not invariably lead to the

precipitation of calcium carbonate in vegetable matter, but its affinity for calcium ions is well known, and the binding site has been identified (Ref. 100). It is therefore likely that it participates in some way in the accumulation of calcium by the calcareous Charales.

The apparently sudden appearance of pectic acid in the Silurian is hard to explain. A trace of galacturonic acid has been tentatively identified in the extracellular slime of one blue-green alga (Nostoc sp., Table 4), but one would have expected that the ability to synthesise long sequences of contiguous, (1→4)-linked α-D-galacturonic acid residues would have developed more slowly, as we have already seen with the xylans, for example. We are therefore confronted by a particularly puzzling "missing link". It is perhaps possible that investigation of other Charales, not to mention other blue-green algae, would throw some light on this problem. A fraction rich in pectic acid has already been isolated from Chara australis (Ref. 101).

Calcium-binding glycan of Emiliania huxleyi

This alga belongs to the Family Coccolithophoridae of the Class Chrysophyceae (Fig. 1). These are marine, unicellular Chrysophyta, and a popular item in the diet of filterfeeders. Probably for this reason, they are, like the diatoms, still rapidly evolving. It has been estimated that a new species is currently appearing at the rate of one every 30,000 years (Ref. 8). The coccoliths are elegantly fluted discs of crystalline calcium carbonate (calcite). Since their design is different in every species, it must be under close genetic control. Since coccoliths are mineral, the fossil record is excellent. The oldest coccolithophore appeared in the early Jurassic Period, but it has long been extinct. Emiliania huxleyi itself is only about a million years old.

The coccoliths are produced inside the cells, and are then thrusted outwards through the cell envelope. Their geometric design is pre-determined by that of a gelatinous matrix synthesised within the cytoplasm, which then becomes impregnated with calcium carbonate crystals. The matrix is a glycan, extractable with EDTA, and containing residues of D-glucose, 3-O-methyl-D-xylose, D-galacturonic acid and its 3-O-methyl ether, L-galactose, L-rhamnose and its 2,3-di-O-methyl ether, L-arabinose, D- and L-mannose and their 6-O-methyl ethers, and D-ribose (Ref. 102 & 103). It also contained 4% of hemiester sulphate.

From the extremely complex mixture of oligosaccharides obtained upon partial acid-hydrolysis, the acidic components were isolated and separated on an ion-exchange resin, and thirteen of them were fully or partly characterised by methylation analysis and mass spectrometry (Ref. 104). Reference is made to the original paper for full details, but the following sequences represent one possible (simplified) interpretation of the data:

OUTER CHAINS:

GALA-(1→4)-GALA-(1→6)-MAN-(1→3)-MAN-(1→4)-GALA-(1→2)-MAN-

GALA3ME-(1→2)-MAN6ME-(1→4)-GALA-(1→2)-MAN6ME-(1→4)-GALA-

INNER CHAINS:

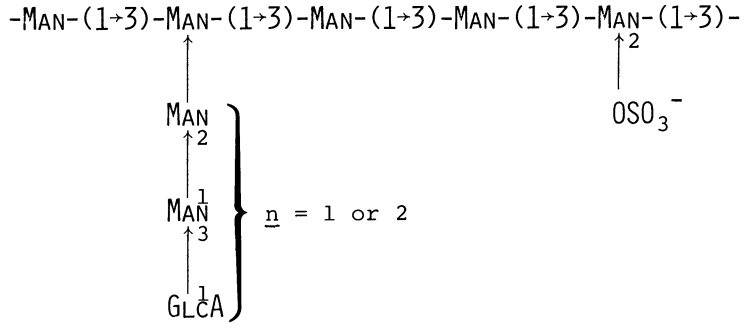
→4)-GALA-(1→2)-RHA-(1→4)-GALA-(1→2)-RHA-(1→4)-GALA-

Glycans involved in the silicification of diatoms

The silica frustules of diatoms are even more elaborate and varied in design than coccoliths. Evidently, these designs, too, are under absolute genetic control. The silica is of the amorphous, hydrated kind, and appears to be laid down over, or within, a mucilaginous capsule around the cell wall. The mechanism of silicification is obscure, but there are strong indications that residues of mannose and glucuronic acid are prominent components of the mucilages. In six different species of diatoms, the proportion of mannose residues was always higher in the logarithmic phase of growth, when silicification would have been most active (Ref. 105). Direct microanalysis of a "skin" of glycan material surrounding the silica frustules in Navicula pellicosa led to the detection of mannose, glucuronic acid, xylose and fucose residues (Ref. 106).

There has been only one structural study, namely, of a sulphated glucuronomannan from Phaeodactylum tricornutum (Ref. 107). Structure (3) gives a

simplified picture of the findings:



(3)

A sulphated, α-(1→3)-linked mannan has been isolated from the red seaweed, Nemalion vermiculare (Ref. 108), and an unsulphated, α-(1→3)-linked mannan has been found in the green seaweed, Urospora penicilliformis (Ref. 18) but neither species is known to accumulate silica in significant amounts.

CONCLUSIONS

There seem to be few exceptions to the general rule that evolution has resulted in a reduction in the number of different sugar residues, the number of different kinds of linkages, and the degree of branching in algal glycans. This process starts earlier, and it carried much further, in the fibrous glycans than in the mucilages, but the same trend is clear in both. New sugars seem to have appeared, but usually at the expense of others. For example, D-fructose in inulin replaces D-glucose in starch and laminaran; D-mannuronic acid replaces D-glucuronic acid in some diatoms and brown algae; and D-galacturonic acid replaces D-glucuronic acid in pectic acid and the calcium-binding mucilage of Emiliania huxleyi.

Running counter to this general impression of simplification, which seems to represent a progressive compartmentalisation of glycotransferase activity, are the introduction of sulphate hemiester and methyl ether groups, very prominent in red algae; the conversion of galactose into its 3,6-anhydride in red algae; the conversion of glucose into its 3,6-anhydride in a diatom; and the conversion of D-mannuronic acid into L-guluronic acid in brown algae. It is, however, either firmly established or very probable that these modifications all occur at the polymer level. They might therefore be considered more as an extension of the process of compartmentalisation than as exceptions. Some support for this idea might be seen in the fossil evidence that red algae were the first eucaryotes (Ref. 1 & 2), which implies that they were the first organisms to develop an endoplasmic reticulum.

Perhaps the only real exception is the formidably complex, calcium-binding glycan of Emiliania huxleyi, but here we can perhaps appeal for an explanation to its very recent evolution, and the very rapid rate of evolution of the phylum to which it belongs. It certainly appears to be a highly ordered molecule, and perhaps we are witnessing a turning-point in evolution here, in which an apparent simplification is giving way to the development of "true" or "ordered" complexity.

REFERENCES

1. J. W. Schopf, Evol. Biol. 7, 1-13 (1974).
2. M. D. Brasier, Chem. Brit. (Roy. Soc. Chem., Lond.) 15, 588-592 (1979).
3. R. Simonsen (Ed.), Fourth Symp. on Recent and Fossil Diatoms, Oslo, Sept. 1976, Beihefte zur Nova Hedwigia, Heft 54, J. Cramer, FL-9490 Vaduz, 1977.
4. G. W. Prescott, The Algae - A Review, Thomas Nelson, London, 1969.
5. V. J. Chapman, The Algae, Macmillan, New York, 1962.
6. E. Flügel (Ed.), Fossil Algae, Springer-Verlag, Berlin, 1977.
7. W. A. S. Sarjeant, Fossil and Living Dinoflagellates, Academic Press, London, 1974.
8. B. U. Haq and A. Boersma (Eds.), Introduction to Marine Micropaleontology, Elsevier, New York, 1978.
9. M. A. Ragan and D. J. Chapman, A Biochemical Phylogeny of the Protists, Academic Press, New York, 1978.
10. V. J. Chapman, in Encyclopædia Britannica, 15th Edn., Vol.1, 487-499.

11. Lee Chao and C. C. Bowen, J. Bact. 105, 331-338.
12. M. Weber and G. Wöber, Carbohydr. Res. 39, 295-302 (1975).
13. B. J. D. Meeuse, in Physiology and Biochemistry of Algae (R. A. Lewin, (Ed.), Academic Press, New York, 1962, pp. 289-291.
14. S. Peat, J. R. Turvey and J. M. Evans, J. Chem. Soc., 3223-3227 (1959).
15. A. R. Archibald, E. L. Hirst, D. J. Manners and J. F. Ryley, J. Chem. Soc. 556-560 (1960).
16. N. J. Antia, J. Y. Cheng, R. A. J. Foyle and E. Percival, J. Phycol. 15, 57-62 (1979).
17. J. Love, W. Mackie, J. P. McKinnel and E. Percival, J. Chem. Soc., 4177-4182 (1963).
18. E. J. Bourne, M. L. Megarry and E. Percival, J. Carbohydr. Nucleosides Nucleotides 1, 235-264 (1974).
19. E. L. Hirst, D. J. Manners and I. R. Pennie, Carbohydr. Res. 22, 5-11 (1972).
20. J. R. Fredrick, Phytochemistry 16, 1489-1490 (1977).
21. J. R. Fredrick, Phytochemistry 16, 55-57 (1977).
22. M. Kojima, M. Dobashi and S. Ino, J. Reticuloendothelial Soc. 14, 192-195 (1973).
23. I. M. Mackie and E. Percival, J. Chem. Soc., 1151-1156 (1959).
24. I. S. Fisher and E. Percival, J. Chem. Soc. 2666-2675 (1957).
25. E. Percival, Brit. Phycol. J. 14, 103-117 (1979).
26. A. E. Clarke and B. A. Stone, Biochim. Biophys. Acta 44, 161-163 (1960).
27. M. Cleare and E. Percival, Brit. Phycol. J. 7, 185-193 (1972).
28. A. Beattie, E. L. Hirst and E. Percival, Biochem. J. 79, 531-537 (1961).
29. C. W. Ford and E. Percival, J. Chem. Soc., 7035-7041 (1965).
30. B. S. Paulsen and S. Myklestad, Carbohydr. Res. 62, 386-388 (1978).
31. E. Percival and R. H. McDowell, Chemistry and Enzymology of Marine Algal Polysaccharides, Academic Press, London, 1967.
32. J. R. Stark, Carbohydr. Res. 47, 176-178 (1976).
33. T. E. Nelson and B. A. Lewis, Carbohydr. Res. 33, 63-74 (1974).
34. N. Handa and K. Nisizawa, Nature (Lond.) 192, 1078-1080 (1961).
35. L. Cardemil and C. P. Wolk, J. Biol. Chem. 251, 2967-2975 (1976).
36. E. J. Bourne, E. Percival and B. Smestad, Carbohydr. Res. 22, 75-82 (1972).
37. G. Drews, in The Biology of Blue-Green Algae (N. G. Carr and B. A. Whitton, Eds.), Blackwell, Oxford, 1973, pp. 99-116.
38. G. Weise, G. Drews, B. Jann and K. Jann, Arch. Mikrobiol. 71, 89-98 (1970).
39. J. Weckesser, A. Katz, G. Drews, H. Mayer and I. Fromme, J. Bact. 120, 672-678 (1974).
40. A. W. Wang and A. Hill, J. Bact. 130, 558-560 (1977).
41. T. M. Buttke and L. O. Ingram, J. Bact. 124, 1566-1573 (1975).
42. A. G. Ross, J. Sci. Fd. Agric. 4, 333-335 (1953).
43. J. Cronshaw, A. Myers and R. D. Preston, Biochim. Biophys. Acta 27, 89-103 (1958).
44. A. Myers and R. D. Preston, Proc. Roy. Soc. B150, 447-459 (1959).
45. E. Frei and R. D. Preston, Nature (Lond.) 192, 939-943 (1961).
46. P. A. Roelofsen, V. Ch. Dalitz and C. F. Wijnman, Biochim. Biophys. Acta 11, 344-352 (1953).
47. D. W. Lorch and A. Weber, Arch. Mikrobiol. 83, 129-140 (1972).
48. D. H. Northcote, K. J. Goulding and R. W. Horne, Biochem. J. 70, 391-397 (1958).
49. D. R. Kreger, in ref. 13, pp. 317-323; 328-332.
50. P. B. Green, in ref. 13, pp. 628-629.
51. El S. Amin, J. Chem. Soc., 281-282 (1955).
52. E. G. V. Percival and A. G. Ross, J. Chem. Soc., 3041-3043 (1949).
53. J. K. N. Jones, J. Chem. Soc., 3292-3295 (1950).
54. J. Love and E. Percival, J. Chem. Soc., 3345-3350 (1964).
55. D. H. Northcote, K. J. Goulding and R. W. Horne, Biochem. J. 77, 503-508 (1960).
56. E. G. V. Percival and S. K. Chanda, Nature (Lond.) 166, 787-788 (1950).
57. J. R. Nunn, H. Parolis and I. Russell, Carbohydr. Res. 26, 169-180 (1973).
58. H. Bjørndal, K. E. Eriksson, P. J. Garegg, B. Lindberg and B. Swan, Acta Chem. Scand. 19, 2309-2315 (1965).
59. B. H. Howard, Biochem. J. 67, 643-651 (1957).
60. D. J. Manners and J. P. Mitchell, Biochem. J. 89, 92P (1963).
61. J. R. Turvey and E. L. Williams, Phytochemistry 9, 2383-2388 (1970).
62. E. Frei and R. D. Preston, Proc. Roy. Soc. (Lond.) B160, 314-327 (1964).
63. I. M. Mackie and E. Percival, J. Chem. Soc., 1151-1156 (1959).
64. El S. Amin, J. Chem. Soc., 282-284 (1955).
65. M. Falk, D. G. Smith, J. McLachlan and A. G. McInnes, Can. J. Chem. 44,

2269-2281 (1966).
66. T. J. Painter, unpublished results.
67. C. T. Bishop, G. A. Adams and E. O. Hughes, Can. J. Chem. 32, 999-1004 (1954).
68. L. Hough, J. K. N. Jones and W. H. Wadman, J. Chem. Soc., 3393-3399 (1952).
69. B. G. Moore and R. G. Tischer, Can. J. Microbiol. 11, 877-885 (1965).
70. R. G. Tischer and B. G. Moore, Arch. Mikrobiol. 49, 158-166 (1964).
71. V. K. Sangar and P. R. Dugan, Appl. Microbiol. 24, 732-734 (1972).
72. D. G. Medcalf, J. R. Scott, J. H. Brannon, G. A. Hemerick, R. L. Cunningham, J. H. Chessen and J. Shah, Carbohydr. Res. 44, 87-96 (1975).
73. J. H. Kieras and D. J. Chapman, Carbohydr. Res. 52, 169-177 (1976).
74. J. H. Kieras, F. J. Kieras and D. V. Bowen, Biochem. J. 155, 181-185 (1976).
75. J. H. Kieras, L. Rodén and D. J. Chapman, Biochem. J. 165, 1-9 (1977).
76. E. Percival and R. A. J. Foyle, Carbohydr. Res. 72, 165-176 (1979).
77. J. R. Turvey and L. M. Griffiths, Phytochemistry 12, 2901-2907 (1973).
78. J. R. Nunn, H. Parolis and I. Russell, Carbohydr. Res. 20, 205-215; 29, 281-289 (1971,1973).
79. V. C. Barry and J. E. McCormick, J. Chem. Soc., 2777-2783 (1957).
80. J. R. Turvey and P. R. Simpson, in Proc. Vth Intern. Seaweed Symp. (E. G. Young and J. L. McLachlan, Eds.), Pergamon Press, Oxford, 1966, pp. 323-327.
81. H. Parolis, Carbohydr. Res. 62, 313-320 (1978).
82. A. J. R. Allsobrook, J. R. Nunn and H. Parolis, Carbohydr. Res. 16, 71-78 (1971); 40, 337-344 (1975).
83. J. Love and E. Percival, J. Chem. Soc., 3338-3344 (1964).
84. I. M. Mackie and E. Percival, J. Chem. Soc., 3010-3015 (1961).
85. E. Percival and B. Smestad, Carbohydr. Res. 25, 299-312 (1972).
86. E. Percival and J. K. Wold, J. Chem. Soc., 5459-5468 (1963).
87. J. P. McKinnel and E. Percival, J. Chem. Soc., 2082-2083 (1962).
88. E. J. Bourne, P. G. Johnson and E. Percival, J. Chem. Soc. C, 1561-1569 (1970).
89. E. L. Hirst, W. Mackie and E. Percival, J. Chem. Soc., 2958-2967 (1965).
90. B. Smestad, A. Haug and S. Myklestad, Acta Chem. Scand. B28, 662-666 (1974).
91. B. Smestad, A. Haug and S. Myklestad, Acta Chem. Scand. B29, 337-340 (1975).
92. E. Percival, M. A. Rahman and H. Weigel, Phytochemistry 19, 809-811 (1980).
93. B. S. Paulsen, A. Haug and B. Larsen, Carbohydr. Res. 66, 103-111 (1978).
94. A. F. Abdel-Fattah, M. M. Hussein and H. M. Salem, Carbohydr. Res. 33, 9-17; 19-24; 209-215 (1974).
95. B. Larsen, A. Haug and T. J. Painter, Acta Chem. Scand. 20, 219-230 (1966).
96. E. Percival, Carbohydr. Res. 7, 272-283 (1968); 17, 121-126 (1971).
97. B. Larsen, A. Haug and T. J. Painter, Acta Chem. Scand. 24, 3339-3352 (1970).
98. D. G. Medcalf and B. Larsen, Carbohydr. Res. 59, 531-537; 539-546 (1977).
99. D. M. W. Anderson and N. J. King, J. Chem. Soc., 5333-5338 (1961).
100. H. Grasdalen, T. Anthonsen, B. Larsen and O. Smidsrød, Acta Chem. Scand. B29, 99-108 (1975).
101. D. M. W. Anderson and N. J. King, Biochim. Biophys. Acta 52, 449-454 (1961).
102. E. W. De Jong, L. Bosch and P. Westbroek, Eur. J. Biochem. 70, 611-621 (1976).
103. A. M. J. Fichtinger-Schepman, J. P. Kamerling, J. F. G. Vliegenthart, E. W. De Jong, L. Bosch and P. Westbroek, Carbohydr. Res. 69, 181-189 (1979).
104. A. M. J. Fichtinger-Schepman, J. P. Kamerling, C. Versluis, and J. F. G. Vliegenthart, Carbohydr. Res. 86, 215-225 (1980).
105. A. Haug and S. Myklestad, Marine Biol. 34, 217- (1976).
106. J. Coombs and B. E. Volcani, Planta 80, 264-279 (1968); 82, 280-292 (1968).
107. C. W. Ford and E. Percival, J. Chem. Soc., 7042-7046 (1965).
108. A. I. Usov, K. S. Adamyants, S. V. Yarotsky, A. A. Anoshina and N. K. Kochetkov, Carbohydr. Res. 26, 282-283 (1973).