Biosynthesis of Photosynthates and Taxonomy of Algae

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The representatives of the major algal divisions including Chlorophyta, Rhodophyta and Heterokontophyta usually show specific patterns of accumulated photoassimilatory products. Among these compounds, the accumulated low-molecular weight carbohydrates such as monosaccharides, disaccharides, alditols (*i.e.* polyhydroxy alcohols) and heterosides are of particular interest. Comparative analyses based on photosynthetic ^{14}C-labelling provided sufficient evidence that occurrence and distribution of such compounds are in general indicative for particular algal taxa and in some cases useful biochemical parameters for a classiciation of lower taxa even at the ordinal or the generic levels.

Introduction

Photosynthesis is certainly one of the most intensely investigated metabolic processes of green plants. The reaction sequence from atmospheric CO_2 up to level of the reduced product has long been known as the reductive pentose phosphate cycle (= RPP-cycle). This cycle has mainly been established by findings from coccalean green algae [1], but has subsequently been found in all photosynthesizing organisms which have specifically been investigated. The RPP pathway of carbon reduction is obviously not restricted to eukaryotic plants, but is additionally found in the O_2-evolving blue-green algae (cyanobacteria) as well as in most photosynthetic bacteria which do not evolve O_2 [2]. Even the representatives of a third division of prokaryotic organisms, the O_2-evolving prochlorophytes (= chloroxybacteria), operate photosynthetic carbon assimilation exclusively via RPP cycle [3, 4].

C_4 photosynthesis and CAM metabolism do not occur in marine or freshwater algae. This also applies to marine brown algae which were found to exhibit a considerable potential for β-carboxylation of phosphoenolpyruvate. Particularly brown seaweeds such as *Laminaria*, *Macrocystis*, and *Nereocystis* show certain indications of C_4 metabolism. However, the underlying reactions and conversions are basically different from C_4 photosynthesis and must not be confused or homologized with that particular pathway restricted to ecologically specialized vascular plants [5, 6].

Marine and freshwater algae are thus typical C_3 plants and, with respect to the initial steps of photosynthetic carbon reduction, do not differ from the vast majority of other photosynthesizing organisms. However, characteristic modifications and differences are seen when the accumulated end products of photosynthesis are regarded. These compounds have hitherto hardly been used for the characterization of algae from a comparative point of view. The present contribution therefore attempts to evaluate the taxonomic significance of algal photoassimilate patterns as well as assimilate accumulation and related processes.

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Materials and Methods

Organisms

The algal species listed in Table 2 were obtained from various sources. Field material was collected near Helgoland, North Sea (Nos. 2, 8–10, 16, 21–23). Santa Barbara, California (Nos. 4 + 5), Vancouver Island, British Columbia (Nos. 1, 3–6), Port Jackson, Sydney, Australia (Nos. 11, 12, 18) and Köln, West Germany (Nos. 13–15, 24, 26 + 27). Cultures have been obtained from the culture collections in Cambridge (Nos. 19–25, 30), Innsbruck (Nos. 25, 28, 30 + 31), and Göttingen (Nos. 23, 24, 31, 33). Nos. 37–39 were kindly supplied by Prof. Wehrmeyer (Marburg).

Incubation

Material collected in the field (about 1 g fresh weight) and cell suspensions (equivalent to about 1 mg chlorophyll *a* ml^{-1}) were incubated in small vessels (plexiglass chambers, Erlenmeyer flasks, centrifuge tubes) and allowed to photoassimilate radiocarbon from $H^{14}CO_3^-$ supplied as $NaH^{14}CO_3$ (5–10 μCi 10 ml^{-1}; specific activity 50 μCi $mmol^{-1}$; seawater: pH 7.8; freshwater: pH 7.1) over a range of different incubation periods. Photosynthesis was performed under saturating light conditions and 10–15 °C. Pulse labelling of photosynthetic accumulation products was achieved by incubating thallus samples or cell suspensions for 10–30 min as described above and by replacing the radioactive incubation medium by a non-radioactive seawater or freshwater. The algae were then allowed to photosynthesize for further 60–120 min. After the appropriate exposure time the algae were harvested, brievely rinsed with membrane-filtered water and immediately fixed in liquid N_2, in a mixture of chloroform-methanol-formic acid (6 N) = 12:5:2, or in boiling ethanol (80%).

Analytical

Algal material was repeatedly extracted in the ethanol-chloroform mixture and ethanol (80, 50, 30%). The supernatants of each extraction step were combined and regarded as the crude fraction of soluble intermediates. For further analyses, the neutral fraction of assimilates was prepared by subsequent passing aliquots of the aqueous phase of the crude extracts through microcolumns (polyethylene tubing, 50 × 3 mm) supplied with Dowex 50 W × 8 (H^+-form) and Dowex 1 × 10 ($HCOO^-$-form). Aliquots of the crude or prefractionated extracts were further separated by one- or two dimensional thin-layer chromatography on cellulose or silicagel plates (20 × 20 cm) using diverse solvent systems which have already been detailed earlier [25, 37, 41, 43]. Such solvents have also been used to confirm the specific identy of individual compounds by repeated cochromatography. Compounds which had previously been ^{14}C-labelled during photosynthesis were localized on the chromatograms by contact autoradiography on X-ray film as well as by one-dimensional scanning with a Geiger-Müller flow counter.

Results and Discussion

Chemotaxonomy and primary metabolism

Physiological and biochemical research does not only aim to evaluate metabolic pathways which occur in the organisms in a similar or basically identical form. Even the physiological differences are of considerable interest, since it has become evident that special metabolic characters are often surprisingly consistent with the taxonomy (based on other characters) of a particular category. Thus, biochemical research provides additional criteria apart from the classic (mainly morphological and anatomical) characters. A well-known example is the delimitation of several algal classes on the basis of pigmentation which paralls morphological peculiarities and features and is hence used to distinguish Chlorophyceae, Phaeophyceae, and Rhodophyceae. Distinct differences in biosynthetic pathways and distribution of peculiar compounds are seen with these plants which thus allow the higher categories to be reliably characterized even on a biochemical background. The search for such differences between plants belonging to different phyletic lines as well as a critical evaluation of possible molecular differences is the particular subject of chemotaxonomy.

Hitherto, the main compounds used in chemotaxonomy have been the typical secondary plant substances. It is generally believed that just these compounds provide a reliable basis for chemotaxonomic considerations. There are a number of examples which convincingly show a close parallel between the occurrence of specialized secondary plant substances and the taxonomic concepts as based on morphological and development findings [7].

With respect to the chemical taxonomy and systematics of lower plants such as algae, compounds of primary metabolism are to be selected rather than secondary plant substances, since the latter compounds are frequently lacking in these organisms. As will be shown below, the discussion of chemotaxonomically relevant distribution patterns is mainly focussed here on the occurrence of low-molecular weight photosynthates.

Accumulated photosynthetic products

As in all chemotaxonomic discussions, the accumulated compounds are of special interest. For

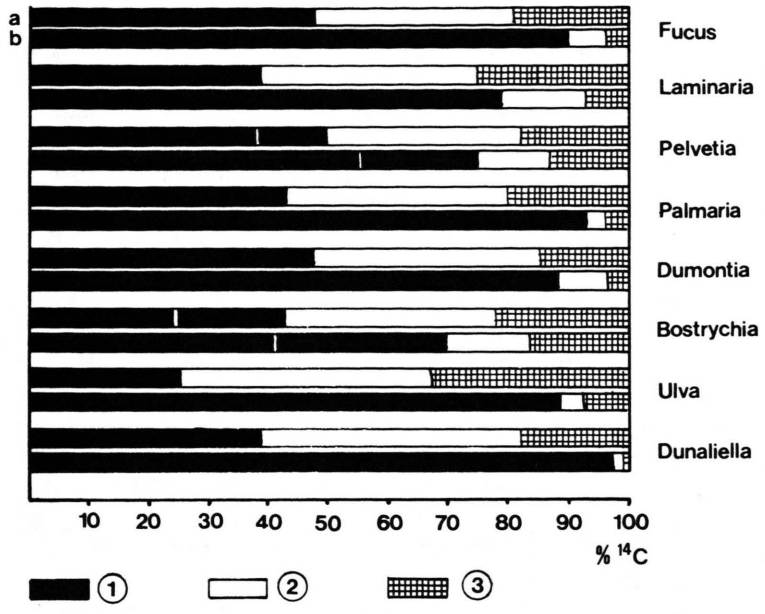

Fig. 1. Percentage ^{14}C-labelling of low-molecular weight compounds after 30 min photosynthesis (a) and 30 min photosynthesis (feeding pulse) followed by 120 min chase period (b). 1 = neutral, 2 = cationic, 3 = anionic fractions.

assaying differential distribution patterns of low-molecular weight photosynthates among various taxonomic entities the qualitative record of any minor compound *per se* does not thus far provide as an useful tool for further consideration. In any case, a critical evaluation necessitates quantitative analyses for deciding if a certain assimilate is accumulated within the cells. This is achieved by a measurement of the appropriate pool sizes or the rates of metabolic turnover. Another reliable guide, due to the bulk of individual records and observations, is the photosynthetic incorporation of ^{14}C from $^{14}CO_2$ or $H^{14}CO_3^-$. By this technique, the participation of individual assimilates in the total ^{14}C-labelling of the diverse fractions of photosynthates is easily observed. On the other hand, the time courses of ^{14}C-labelling allow for distinguishing between relatively short-lived intermediary compounds and typical end products, which attract the major proportion of the photosynthetically reduced carbon. Fig. 1 (Columns arranged under 'a') shows that relatively more radiocarbon is confined to low-molecular weight carbohydrates after 30 min photosynthesis than is recovered from other compounds of the soluble fraction of assimilates. If continuous photosynthetic feeding of radiocarbon is slightly modified and replaced by the pulse/chase incubation technique (feeding pulse 30 min, chase period up to 120 min), different ^{14}C-labelling kinetics are obtained: even a few minutes after a feeding pulse the major proportion of soluble ^{14}C is recovered from the carbohydrate photosynthates, whereas percentage ^{14}C-labelling of further soluble assimilates such as the phosphorylated compounds, amino acids and tricarboxylic acid cycle intermediates steadily decreases. The relative amount of radiocarbon found in these metabolites after 120 min chase period are negligible. Pulse-chase tracer kinetic studies thus help to discriminate between i) intermediates being strongly ^{14}C-labelled during continuous feeding of $^{14}CO_2$, but undergoing rapid turnover, and ii) accumulated assimilates which obviously are deposited in the cells to form a rapidly available energy reserve (Fig. 1, columns arranged under 'b').

Typology of assimilate patterns

In addition to chlorococcaleans *Chlorella* and *Scenedesmus* on which the pioneering work was done, some hundred algal species were analyzed by the pulse/chase incubation technique providing a more detailed picture of the diversity of photosynthates than had originally been expected. A survey of the photosynthetic products accumulated in algae and their chemical nature reveals several interesting and significant features. It is obvious that almost exclusively carbohydrates are accumulated. No further metabolites whose biosynthesis appears

Table I. Types of assimilate patterns in algae.

Pattern type	Composition of assimilate pattern	
	Major compound	Accompanying or minor compound
I	monosaccharide(s)	
II	disaccharide	monosaccharide
IIIa	heteroside(s)	
IIIb	heteroside	disaccharide
IVa	alditol(s)	
IVb	alditol(s)	monosaccharide

to be closely related to the carbohydrates are accumulated or form intermediate pools to a comparable extent except for the poly-/macropeptides and polysaccharides. Moreover, it is interesting to note that generally a few, usually only two or three different carbohydrates, occur as accumulated photosynthates. It is just this group of individual compounds by which the diverse algal classes (taxa) are considerably differing. While unicellular and macrophytic algae as well as the bulk of vascular plants show a rather uniform pattern of primary events in photosynthetic carbon reduction, rather diverging pathways have evolved for the biosynthesis of the respective end products.

On the whole, four types of assimilate patterns consisting of low-molecular weight carbohydrates can be distinguished (Table I). The first type comprises the free monosaccharides glucose and fructose; other hexoses or pentoses are much less frequently found. The second category comprises certain dissacharides such as sucrose or trehalose. Mono- and disaccharides are most often encountered among algal species, whereas oligosaccharides are not found. Another category accomodates the variety of species accumulating particular alditols (= polyols, polyhydroxy alcohols) as photosynthates. Finally, certain heterosides occur among the photosynthetic products of algae. They consist of a monosaccharide and a nonsugar compound (aglycone) (Table I).

It is a special feature of algal photosynthate patterns that the monosaccharides, although occurring in natural compounds in different forms and modifications, are obviously restricted as the free compounds to glucose and fructose. However, almost all of the naturally occurring polyols ranging from C_3 to C_7 compounds are represented. There are certain algae in which glycerol, erythritol, ribitol, arabitol, mannitol, sorbitol (glucitol), dulcitol (galactitol) and/or volemitol are found except their anhydrous homologues. The group of dissacharides encompasses almost exclusively sucrose, less frequently trehalose. Typical heterosides isolated from algae are floridoside (= 2-O-D-glycerol-α-D-galactopyranoside), isofloridoside (= 1-O-D-glycerol-α-D-galactopyranoside) and digeneaside (= 2-D-glycerate-α-D-mannopyranoside) [8–10].

The occurrence and distribution of these compounds is not randomly or irregularly correlated. A closer inspection suggests that such algae which accumulate one or more polyols (alditol) do not simultaneously contain a disaccharide as an accumulated end product of photosynthesis. Species containing heterosides do not photosynthesize free monosaccharides at as high a rate as the heteroside. Heterosides and alditols are mutually exclusive. More often, however, it is observed that an algal species accumulates several isomeric or homologous alditols. Such peculiar features have not yet been reported from higher vascular plants. On the basis of these comparisons one may derive the patterns of accumulated photosynthates which are compiled in Table I. Some examples of photoassimilate patterns are shown in Fig. 2.

Distribution of different assimilate patterns

If the different patterns of accumulated photosynthates as contained in Table I are considered with respect to their occurrence and distribution among different algal taxa, it becomes obvious that a particular pattern appears to be restricted to a particular taxonomic category. Some examples are included in Table II. On the basis of these data one may conclude that the accumulated photosynthates exhibit taxonomic significance for categories of different rank. The peculiar distribution is thus not only a character for the delimitation of higher taxonomic categories, but also permits clear distinction between certain lower taxa.

An example is that a multicellular alga exclusively accumulating mannitol must be a member of the Phaeophyceae. Accumulation of the alditol mannitol thus proves to be a character which is typical for the entire algal class. Simultaneous biosynthesis and accumulation of two homologous alditols, mannitol and volemitol, has hitherto only been observed with a single brown algal species, *Pelvetia canaliculata* [11].

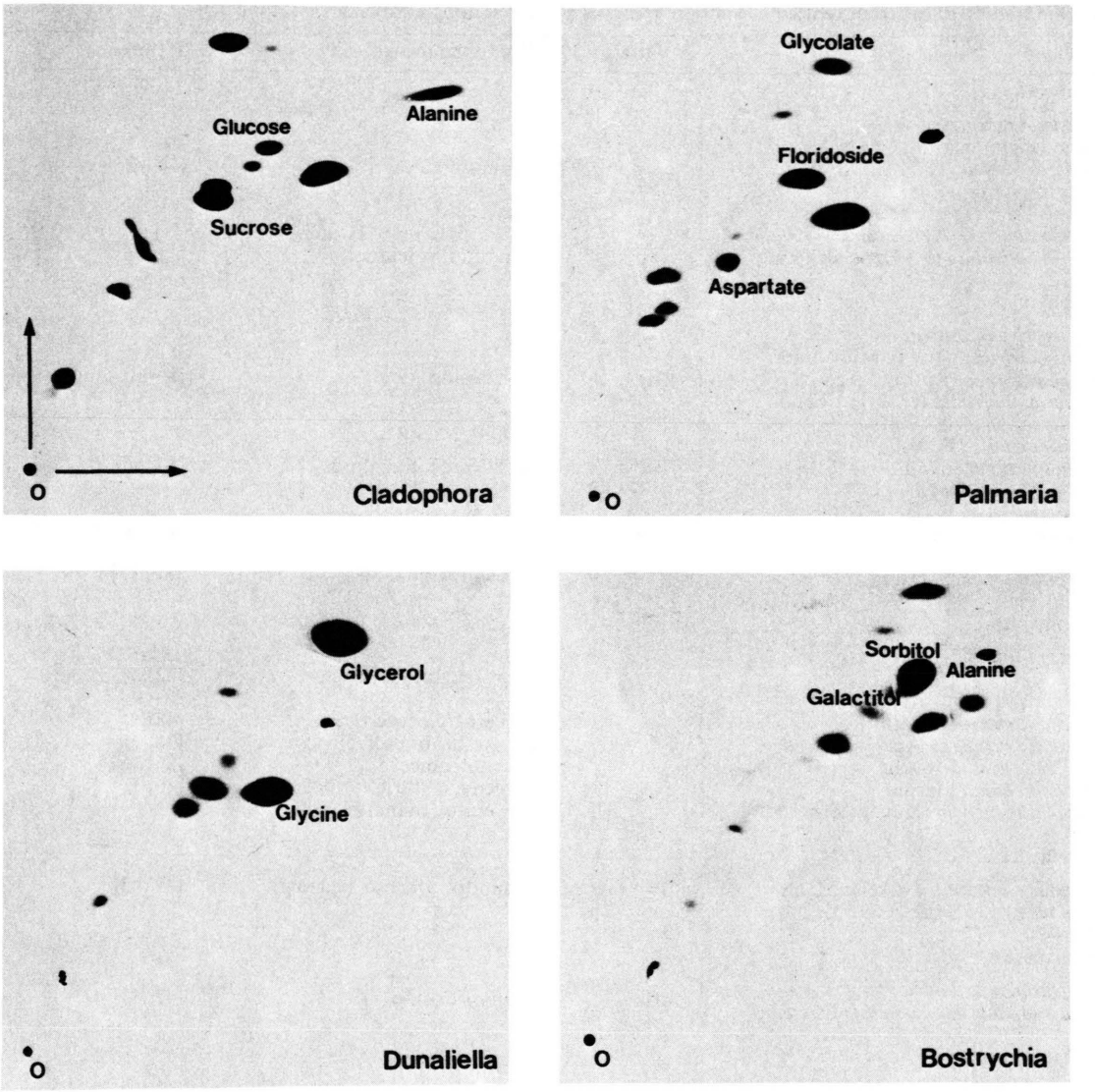

Fig. 2. Autoradiographs showing the patterns of photosynthetically ^{14}C-labelled assimilates as demonstrated after two-dimensional TLC. Species included are (1) *Cladophora rupestris* (Chlorophyceae), (2) *Palmaria palmata* (Rhodophyceae), (3) *Dunaliella tertiolecta* (Chlorophyceae), and (4) *Bostrychia scorpioides* (Rhodophyceae) representing pattern types II (1); IIIa (2); IVb (3), and IVa (4).

The biosynthesis of floridoside is encountered with most of the marine and freshwater red algae. This assimilate, too, is typical for an entire class which does not occur in algae not belonging to the Rhodophyceae. In freshwater rhodophytes, the floridoside is usually accompanied by trehalose [12, 13] which is very rarely found as a carbohydrate constituent of a plant species. The occurrence of digeneaside instead of floridoside is obviously restricted to the order Ceramiales within the Rhodophyceae – it thus turns out to be a chemotaxonomic character at the ordinal level [10, 38]. Red algae generally do not synthesize any polyhydroxy alcohols. One notable exception among all red algal species hitherto investigated are the representatives of the genus *Bostrychia* [49]. These species accumulate two isomeric alditols, galactitol (dulcitol) along with glucitol (sorbitol), but do not synthesize

Table II. Distribution of different types of assimilate patterns among various algal taxa.

Taxon	Pattern[a]	Compounds involved	References
Phaeophyceae			
1 *Pelvetiopsis limitata* Gardn.	IVa	mannitol	[34–36]
2 *Laminaria digitata* (Huds.) Lam.			
3 *Cymathere triplicata* (P. & R.) J. Ag.			
4 *Macrocystis pyrifera* Bory			
5 *Egregia menziesii* (Turn.) Aresch.			
6 *Costaria costata* (C. Ag.) Saund			
7 *Pelvetia canaliculata* (L.) Dcne. & Thui	IVb	mannitol, volemitol	[11, 34]
Rhodophyceae			
8 *Chondrus crispus* Stackh.	IIIa	floridoside	[8–10, 20, 38, 41]
9 *Dumontia incrassata* (O. F. Müll) Lam.			
10 *Porphyra purpurea* (Roth) C. Ag.			
11 *Lomentaria umbellata* (H. & H.) Tendo			
12 *Corallina officinalis* L.	IIIb	floridoside, trehalose	[13, 36]
13 *Lemanea fluviatilis* C. Ag.			
14 *Batrachospermum moniliforme* Roth			
15 *Compsopogon hookeri* Mont.			
16 *Membranoptera alata* (Huds.) Stackh.	IIIa	digeneaside	[10, 14, 15, 38, 41]
17 *Polysiphonia lanosa* (L.) Tandy			
18 *Bostrychia montagnei* Harv.	IVa	galactitol, glucitol	[36, 37]
20 *Bostrychia scorpioides* (Herds.) Mont.			
Chlorophyceae			
21 *Cladophora rupestris* (L.) Kütz.	II	sucrose, glucose, fructose	[22, 24, 39, 40]
22 *Codium fragile* (Sur. Har.)			
23 *Ulva lactuca* L.			
25 *Dunaliella tertiolecta* Butch.	IVb	glycerol, glucose	[48]
26 *Pleurococcus viridis* C. Ag.		ribitol, arabinitol, glucose	[36]
27 *Stichococcus bacillaris* Näg.		glucitol, glucose	[36]
28 *Trentepohlia aurea* Marius	IVa	glycerol, erythritol, ribol	[16]
29 *Trentepohlia umbrina* (Kütz.) Born.		arabinitol, mannitol, volemitol	
Prasinophyceae			
30 *Tetraselmis convolutae* Parke & Manton	IVb	mannitol, glucose, fructose	[42, 43]
31 *Tetraselmis tetrathele* G. S. West			
Crysophyceae			
32 *Poteriochromonas danica* Pringsh.	IIIa	iso-floridoside	[42, 43]
33 *Poteriochromonas malhamensis* Pringsh.			
Bacillariophyceae			
34 *Nitzschia palea* Kütz.	–	no accomulation of free low-molecular weight carbohydrates	[44, 45]
35 *Cyclotella meneghiniana* Kütz.			
36 *Phaeodactylum tricornutum* Bohl			
Cryptophyceae			
37 *Chroomonas* sp.	I	glucose, fructose	[54]
38 *Cryptomonas* sp.			
39 *Hemiselmis rufescens* Parke			

[a] *cf.* Table I.

heterosides. In this case, the accumulation of glucitol and galactitol is a differentiating character at the generic level and certainly of infrageneric uniformity.

Among green pigmented algae (Chlorophyceae s. l.) the type II of accumulated photosynthates is by far the most frequently encountered pattern. Moreover, a very similar type of assimilate patterns is characteristic for the majority of vascular plants. All modifications of this type (see Tables I and II) are interesting exceptions. The type IVb (alditol/monosaccharide) is a combination of mostly only one

definite monosaccharide with any alditol partner. The species of *Trentepohlia* demonstrate the hitherto widest spectrum of accumulated alditols traced in any algal group [16]. This multiple biosynthesis of alditols is thus a character of significance at the generic level. The reasons why *Trentepohlia* spp. and a variety of further green algae (see Table II) do not perform the biosynthesis of sucrose as is typical for this class, but accumulate (several) alditols instead, are not yet fully understood. However, it should be emphasized that *Trentepohlia* spp. as well as the brown *Pelvetia canaliculata* or the red *Bostrychia* spp. are typical aerophytes. All these species occupy the ecologically peculiar boundary fringe between aquatic and terrestrial habitats.

Pathway of biosynthesis

Marked differences in carbohydrate metabolism of algae are seen with respect to the conversion of fructose-6-phosphate, a central intermediate immediately derived from the RPP cycle. Its special relationships with the diverging biosynthetic pathways of accumulated photosynthates as detailed above are shown in Fig. 3. In brown algae, the biosynthesis of mannitol-1-phosphate is catalyzed by mannitol-1-phosphate dehydrogenase (EC 1.1.1.17). This compound is subsequently dephosphorylated to free mannitol by a substrate specific phosphatase. Both enzymes have been demonstrated to operate in a wide variety of brown seaweeds [17]. The biosynthesis of volemitol, which additionally occurs in *Pelvetia canaliculata*, starts with seduheptulose-7-phosphate, another intermediary compound derived from the RPP cycle. The necessary enzyme has been demonstrated (EC number not yet assigned) [18].

Quantitative analyses of ^{14}C-labelled photosynthates from red algal species suggest that in these plants the biosynthesis of floridoside is initiated by a condensation of glycerol-3-phosphate and uridine diphospho galactose. This assumption was proved by a detailed analysis of the individual steps leading to iso-floridoside, the main accumulated photosynthate of certain chrysophytes [19]. Analogous investigations of marine rhodophytes confirm that UDP-galactose (furnished *via* UDP-glucose) and glycerol-3-phosphate are condensed to floridoside phosphate, which releases free floridoside upon cleavage of the

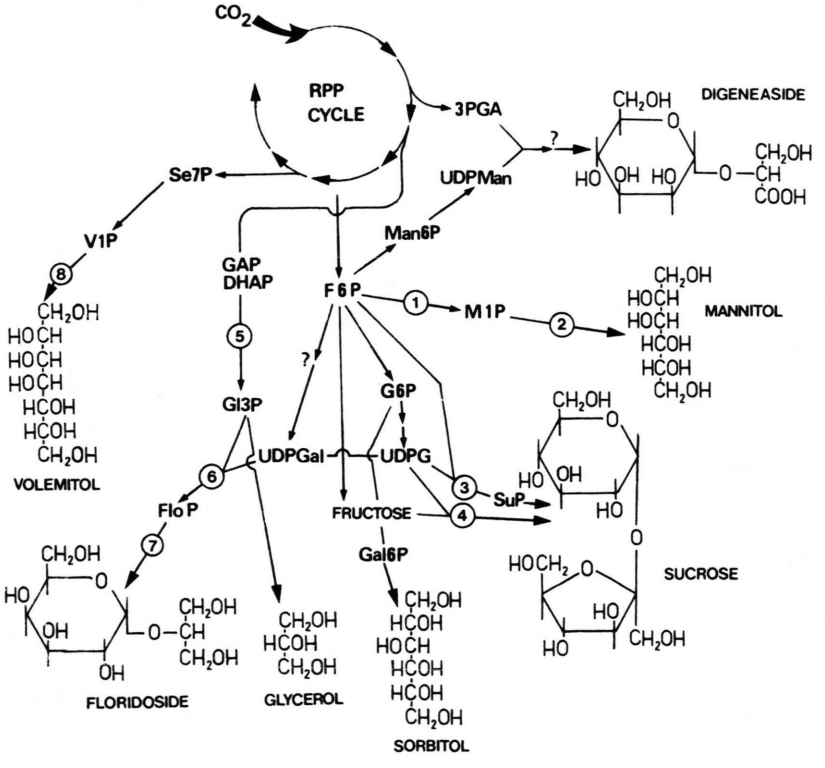

Fig. 3. Schematic representation of pathways and compounds involved in the biosynthesis of algal photoassimilates. Abbreviations: F fructose, Flo floridoside, Gal galactose, Gl glycerol, M mannitol, Man mannose, P phosphate, Se seduheptulose, Su sucrose, UDP uridine diphosphate, V volemitol. Enzymes involved: 1 mannitol-1-phosphate dehydrogenase; 2 mannitol phosphatase; 3 sucrose phosphate synthase; 4 sucrose synthase; 5 glycerol-3-phosphate dehydrogenase; 6 floridoside phosphate synthetase; 7 floridoside phosphatase; 8 volemitol-1-phosphate dehydrogenase.

phosphate ester bound. Several enzymes participate in this multiple step reaction sequence. They are rather unstable in cell free test systems. Despite this, the action of a glycerolphosphate dehydrogenase as well as of a floridoside phosphate has been demonstrated in homogenates of several species [20].

The formation of digeneaside in representatives of the Ceramiales has been much less extensively investigated. The pathway as given in the schematic representation (Fig. 3) is based on analogy to the findings derived from ^{14}C-labelled assimilates. Similarly, the biosynthesis of galactitol and glucitol in *Bostrychia* spp. has not yet fully been elucidated, though it is to be expected that the reaction starts with hexose phosphates.

More details have been evaluated concerning the biosynthesis of sucrose in members of the Chlorophyceae. The biosynthetic way is essentially similar to that characterized from higher plants. A sucrose phosphate synthase has been demonstrated in cell free extracts from several unicellular and thallose green algae which use fructose-6-phosphate and UDP-glucose as specific substrates and performs their condensation to sucrosephosphate [21, 22]. A second enzyme involved in sucrose metabolism has been found in algal extracts: Sucrose synthase requires free fructose besides nucleoside diphospho glucose (ADP-glucose) as specific substrates. This enzyme performs an important function in sucrose cleavage in higher plants and the closely related metabolism of starch.

Compartmentation of biosynthetic pathways

The problem of intracellular localization of the respective pathways involved in biosynthesis of the specific accumulation products is not yet resolved. Labelling experiments with isolated chloroplasts obtained from the giant celled green alga *Acetabularia* suggest an intraplastidary biosynthesis of sucrose [23]. However, such findings must very carefully be considered, since it is not always quite clear that the preparation was absolutely free of cytoplasmic impurities. In this context, observations from green algal chloroplasts occurring as intracellular symbionts in certain marine molluses are rather interesting. Chloroplasts of *Codium tomentosum* contained in the digestive gland of the slug *Elysia viridis* are structurally and functionally fully intact, but do not show any trace of sucrose biosynthesis [24, 25]. Chloroplasts of the ceramialean *Griffithsia flosculosa*, endosymbiotically associated with the slug *Hermaea bifida*, do not form digeneaside, though the photosynthetic performance of these organelles is only reduced to a small extent. Recent observations from isolated brown algal chloroplasts suggest that the biosynthesis of mannitol is not localized within the photosynthesizing organelles [27]. Thus, one may conclude that the final steps of biosynthesis leading to the accumulated photosynthates such as mannitol, floridoside, sucrose and further compounds (see Fig. 3) are prevailingly localized within the cytoplasm. The photosynthesizing chloroplasts initiate these reaction sequences by supplying precursors and intermediary products from the RPP cycle.

Physiological function of accumulated assimilates

Low-molecular weight photosynthates provide several physiological functions. Primarily, these compounds are rapidly available substrates for dark respiratory degradation. Moreover, the accumulated photosynthates have an important role in osmotic adaption by algae. The scheme presented in Fig. 4

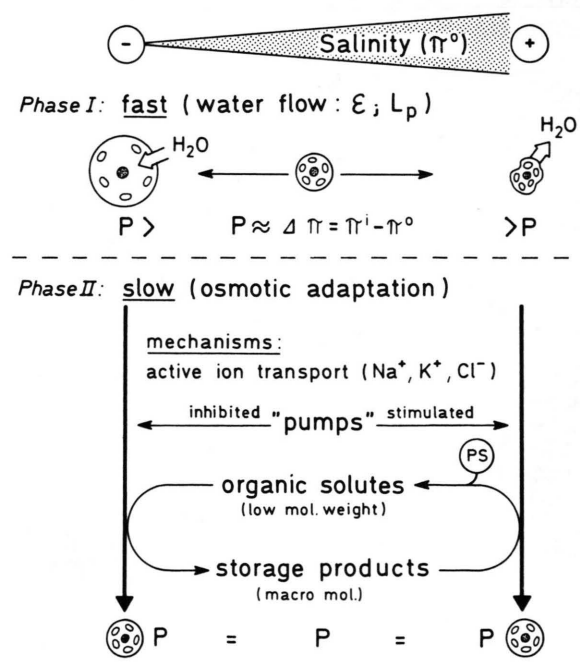

Fig. 4. Reactions involved in osmotic adaption leading to a new steady-state after osmotic shock. π^o = external, π^i = internal osmotic potential; = turgor pressure (equals $\Delta\pi$ in steady-state); ε = elastic modulus and L_p = hydraulic conductivity are physico-chemical properties of the cell wall including plasmalemma; PS = photosynthesis.

summarizes the reactions involved in osmotic adaptation. Two different phases can be distinguished. Phase I is characterized by rapid water fluxes following the osmotic gradient into (hypo-osmotic) or out of the cell (hyper-osmotic conditions) as indicated in the scheme. The water fluxes cause rapid changes in turgor pressure of cell pressing a proper cell wall or in cell-volume in the case of naked cells, *e.g.* the flagellate *Dunalliella* [49] of *Platymonas* (*Tetraselmis*) [50]. The water flow is not under metabolic control and depends only on physicochemial properties of the plasmalemma and the cell wall, *i.e.* the elastic modules and the hydraulic conductivity. While phase I proceeds within seconds, phase II is a slow process, a metabolically controlled adaption resulting in a new steady state after 60–90 min in unicellular microalgae or 48 h in giant algal cells. In the new steady state, the algal cells have regained their turgor pressure and cell volume. Ions as well as low molecular weight organic compounds are involved in osmotic adaption. Ions play a major role, *e.g.* in macroalgae turgor pressure regulation in mainly achieved by internal changes in Na^+, K^+ and Cl^- concentrations. The ion concentrations contribute 90–98% to internal osmotic pressure [29]. Organic solutes are in a rather low concentration compared to ion concentrations. However, in all cases investigated the concentration of the main photosynthetic product is closely correlated with external osmotic pressure, *i.e.* to salinity of the medium [51] indicating that these compounds are involved in osmotic adaption. With respect to the kind of organic compounds there are some exceptions: The diatoms accumulate the amino acid proline under hyper-osmotic stress, *e.g.* *Phaeodactylum* [45] and some species change the concentrations of the compounds, *e.g.* in *Dunalliella* glycerol and sucrose concentrations are shifted with salinity [52].

The organic solutes reported to be accumulated under hyperosmotic stress are combined in a taxonomic scheme (Fig. 5). Although there are no direct relations to taxonomic classes, some interesting cross-connections with chemically related compounds can be observed especially in blue green prokaryotic and eukaryotic red algae [53].

The Table III might present a clue to the role of organic solutes in osmotic adaption: In microalgae with no large vacuole exhibiting a high ratio of cytoplasm to vacuole the organic solute balances about

Table III. Contribution of ions and organic compounds to internal osmotic potential in algae of different cell sizes.

Algal type (Cell volume)	Cytoplasm (in % of cell volume)	Internal osmotic potential	
		% Ions	% Organic compounds
Microalgae ($10^{-6}-10^{-5}$ µl)	25–80	30–70	25–60
Macroalgae (thalloid) ($10^{-5}-10^{-2}$ µl)	5–20	90	3–8 (10)
Macroalgae ('giant cells', coenocytic) (10 µl–10 ml)	<1–2	100	0–2

30–60% of the internal osmotic presence. With increasing vacuolisation of the cells the fraction of the organic compounds decreases and finally vanishes in the extreme example of the giant algal cells. However, calculated on a cytoplasmic volume basis the concentrations of the organic solutes are high enough to account for 40% and more of the internal osmotic prescure. At present the actual concentrations in the cytoplasm cannot be measured. Other observations, however, like the protective effect of some of these compounds (glycerol, proline, polyols), on enzymes in presence of high ionic strength "compatible solutes" [53] are consistent with the hypothesis that different mechanisms for osmotic adjustment occur in the main compartments of the cell. In the vacuole internal osmotic pressure is maintained by ion concentration while in the cytoplasm in addition low-molecular organic compounds are involved.

Predictive value of algal photosynthate patterns

A more detailed analysis of assimilate patterns and accumulated photosynthates is particularly helpful in those cases in which the taxonomic relationships of an algal species is not clear or questionable. A recent example for an immediate application of such comparative biochemical investigation of algae are the red pigmented endosymbionts of certain marine foraminiferans such as *Peneroplis pertusus*. Based on structural investigation, the endosymbiotic algae of this species have first been associated with the Cryptophyceae. Since the endosymbionts accumulate floridoside as a photosynthate, they are very clearly characterized as repre-

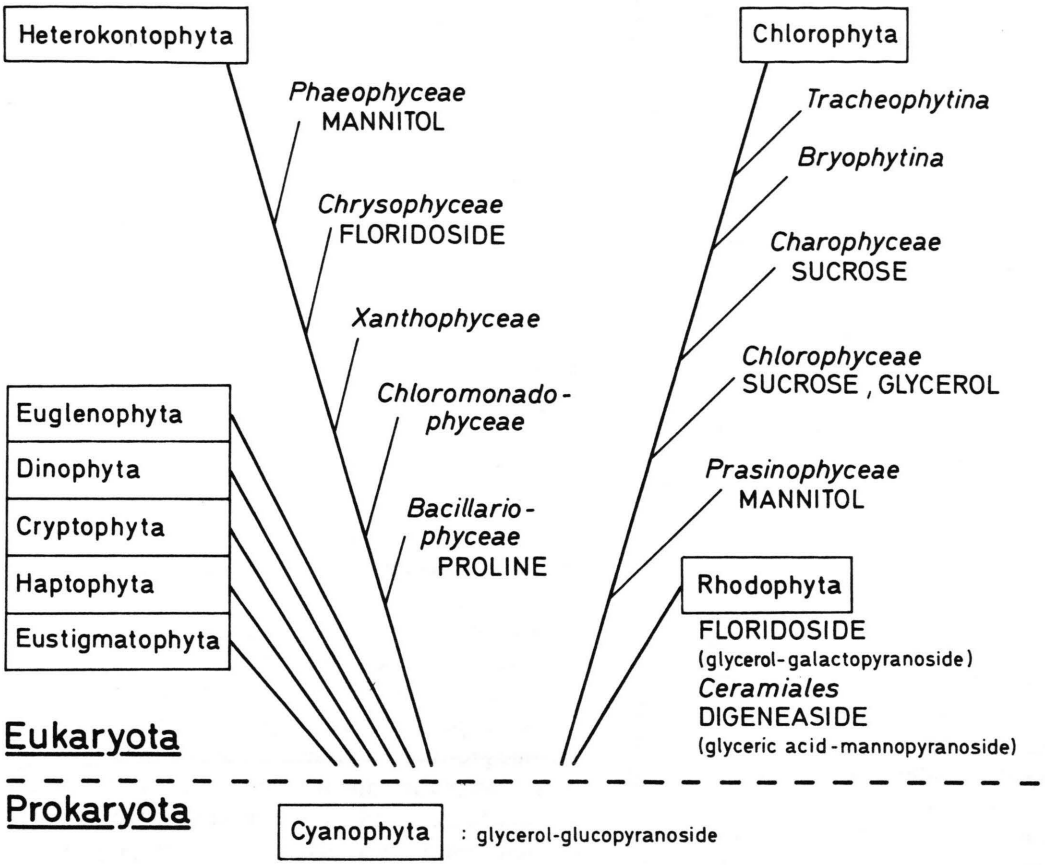

Fig. 5. Algal classification and organic compounds involved in osmotic adaptation. Major taxa arranged according to ref. [55].

sentatives of the Rhodophyceae. Biosynthesis of such heterosides has hitherto not yet been observed with cryptophycean species [31].

There is another enigmatic organism with uncertain taxonomic affiliation. In this case, too, the analysis of the accumulated photosynthates provided a broad basis for further consideration: *Cyanidium caldarium* is an acidothermophilic alga with striking blue green algal characters, but undoubted eukaryotic cellular organization. Proposals for the taxonomic affiliation of this organism included the Cyanophyceae, Chlorophyceae, Cryptophyceae as well as the Rhodophyceae [32]. A comparative analysis of accumulated low molecular weight compounds along with the particular ultrastructural features suggests an alternative interpretation: *Cyanidium* may thus be understood as a colourless, apoplastidal, eukaryotic alga, which contains endosymbionts of blue-green algal ancestry. *Cyanidium* thus shows exactly that mode of cellular organization which has previously evaluated for a variety of associations generally termed cyanomes [32, 33].

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