Features and Nutritional Significance of Photosynthates Produced by Unicellular Algae Symbiotic with Larger Foraminifera

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ABSTRACT: Associations of larger Foraminifera with photoautotrophic partners – including representatives of the Bacillariophyceae, Dinophyceae, and Rhodophyceae – have been investigated for their photoassimilatory compounds using ^{14}C-tracer techniques. Assimilate patterns are compared with appropriate features of free-living relatives. In the foraminifer-rhodophyte-association free galactose and glycerol are found which are usually not present in non-symbiotic Rhodophyceae. In the foraminifer-dinoflagellate-association most of the radiocarbon is recovered from lipid compounds, whereas relatively little ^{14}C is found in the soluble carbohydrates. In the foraminifer-diatom-associations the amount of glucose and glycerol is relatively higher than in free-living diatoms. These differences may be interpreted as effects of the symbiotic condition. From the pattern of photoassimilatory products it is concluded that the animal cells receive organic carbon by release of algal assimilates rather than by digestion of their algal symbionts. The endosymbionts of *Spirolina arietina* were shown to be Rhodophytes.

INTRODUCTION

Endosymbiotic microalgae associated with aquatic invertebrates represent fascinating examples of highly integrative interspecific relationships mutually benefitting both partners involved. In the case of photosynthesizing symbiotic algae it might generally be expected that these autotrophic organisms provide their animal partners with a wide range of accumulated photosynthates. An effective carbon transfer which might even be stimulated by animal tissue, has been demonstrated for a variety of symbiotic associations (Trench, 1979).

While the vast majority of marine coelenterates, for instance, contain dinoflagellate algae (mostly *Gymnodinium microadriaticum*) as endosymbionts, representatives of the Foraminifera exhibit a remarkable diversity in the choice of their photoautotrophic partners. The yellow-brown endosymbionts (zooxanthellae) of *Heterostegina depressa* and *Amphistegina lessonii* have been characterized by fine-structure analysis as diatoms (Berthold, 1978; Schmaljohann and Röttger, 1978). Recently, the diatoms from *H. depressa* and from *A. lessonii* have been isolated in axenic culture (Lee et al., 1979a; Lee et al., 1980a). By means of their

frustules they could be identified as *Nitzschia valdestriata* Aleem and Hustedt and *Nitzschia laevis* Hustedt (*H. depressa* and *A. lessonii* from Hawaii) and as *Nitzschia panduriformis* and *Fragilaria* sp. (*H. depressa* and *A. lessonii* from Elat). Species of the same algal class are found in *Amphistegina lobifera* (Lee et al., 1979a). The zooxanthellae of *Amphisorus hemprichii* belong to the Dinophyceae (Leutenegger, 1977a). The algae associated with *Peneroplis planatus* and *P. pertusus* are unicellular Rhodophyceae (Leutenegger, 1977b), representing the only hitherto known examples of endosymbiotic red algae. The zoochlorellae of other species of larger Foraminifera have been shown to be *Chlamydomonas* spp. (Lee et al., 1974, 1979b). The symbionts of *Spirolina arietina* have not yet been identified, but their red colouration as well as our findings (see below) enable us to classify them as Rhodophytes. Recently, some benthic Foraminifera have even been reported to be associated with algal chloroplasts (Lopez, 1979).

The notable range of algal endosymbionts from different classes require further evaluation with particular regard to the metabolism of photoassimilatory products. Results on the physiology of several species of larger Foraminifera are in press (Lee et al., 1980b). No

further biochemical or physiological characterization of these associations has so far been reported.

MATERIAL AND METHODS

Some 5–30 individuals of *Heterostegina depressa* d'Orbigny, *Amphistegina lessonii* d'Orbigny, *Amphistegina lobifera* Larsen, *Amphisorus hemprichii* Ehrenberg, *Peneroplis pertusus* Forskål and *Spirolina arietina* Batsch were used for the experiments. *P. pertusus*, *S. arietina*, *Aphisorus hemprichii* and *Amphistegina lobifera* were collected in rockpools at Makapuu Point, Oahu, Hawaii. *Amphistegina lessonii* derives from clone cultures whose stock material is also from the Makapuu pools. *Heterostegina depressa* cultivated in Kiel are part of a clone originating from a microspheric parent individual collected off Maui, Hawaii (for culture methods see Röttger, 1972).

The test individuals were incubated in light (5000 lx) in a $H^{14}CO_3^-$-seawater medium (100 μCi ml^{-1}) and were then analyzed for distribution of ^{14}C in the major photosynthates by means of chromatographic techniques. Cultures of *Prorocentrum micans* Ehrenberg and *Scrippsiella faeroense* (Paulsen) Balech and Soares, kindly supplied by Dr. E. Hagmeier (Biologische Anstalt Helgoland), were treated in the same way. All analytical procedures used have been detailed in a recent review (Kremer, 1978a).

RESULTS AND DISCUSSION

All foraminiferans investigated incorporated appreciable amounts of ^{14}C in the light, due to photosynthetic carbon fixation by their endosymbiotic algae. Assimilation rates for the endosymbiotic algae of *Heterostegina depressa* account for 130 nmol CO_2 μg^{-1} Chl *a* h^{-1}. Similar rates have been observed in the other

associations investigated. Radiocarbon was recovered from various compounds soluble in a mixture of methanol-chloroform-formic acid (12 : 5 : 2) and ethanol (50 %) as well as from insoluble (polymeric) compounds. Some of the major assimilates of the neutral fraction and percentages of ^{14}C-labelling are listed in Table 1.

Since the typical patterns of accumulated photoassimilatory products provide a useful biochemical marker for taxonomic purposes (Kremer, 1978b, c), the results obtained are first to be interpreted from this point of view. In the foraminiferan harboring unicellular red algae, the strongest ^{14}C-labelled low-molecular weight carbohydrate has been identified as floridoside (2-0-D-glycerol-α-D-galactopyranoside). This finding is consistent with earlier observations on a wide variety of marine red algae (Kremer, 1978b) and on free-living unicellular species (Kremer and Feige, 1979). The taxonomic identity of the endosymbionts of *Spirolina arietina* was hitherto unknown. However, the pattern of their photoassimilatory products (which is very similar to that of *Peneroplis pertusus*) allows to identify them as Rhodophytes.

The assimilate pattern of the dinophycean endosymbionts of *Amphisorus hemprichii* compares well with the appropriate features of planktonic dinoflagellates, such as *Prorocentrum micans* and *Scrippsiella faeroense*, where glucose and glycerol are usually the predominantly ^{14}C-labelled carbohydrate compounds (Table 2). As in *A. hemprichii*, the assimilates provided by symbiotic diatoms of *Heterostegina depressa* and *Amphistegina* spp. comprise glucose along with glycerol and thus resemble qualitatively those of other species of Bacillariophyceae in the free-living state (Table 2, cf. Kremer and Berks, 1978). Symbiotic unicellular algae, therefore, appear to show the same pattern of accumulated compounds as their free-living relatives.

However, the quantitative and qualitative analysis of assimilate patterns from symbiotic associations im-

Table 1. Distribution of photosynthetically assimilated ^{14}C (3h) among neutral constituents of symbiotic associations (% of ^{14}C-labelling). – not traced

Foraminifera species: Class of symbionts:	Amphistegina lessonii	Amphistegina lobifera	Heterostegina depressa	Amphisorus hemprichii	Peneroplis pertusus	Spirolina arietina
		Bacillariophyceae		Dinophyceae	Rhodophyceae	
Soluble photosynthates:	69.6	61.4	89.5	81.6	79.1	71.2
Insoluble photosynthates:	30.4	38.5	10.5	18.4	20.9	28.3
Soluble compounds = 100 %						
Glycerol	4.7	6.7	10.9	3.5	7.8	5.1
Galactose	–	–	–	–	5.6	4.9
Floridoside	–	–	–	–	18.1	14.4
Glucose	11.5	6.3	4.0	5.2	–	–
Polyglucan	44.6	17.8	42.7	1.4	11.9	34.4
Lipids	31.0	51.0	32.8	73.7	19.4	5.7

Table 2. Distribution of photosynthetically assimilated ^{14}C (3h) among neutral assimilates of free-living relatives of symbiotic algae (% of ^{14}C-labelling): – not traced

	Bacillariophyceae[1]			Dinophyceae		Rhodophyceae[2]	
	Navicula pelliculosa	Nitzschia palea	Phaeodactylum tricornutum	Prorocentrum micans	Scrippsiella faeroense	Porphyridium cruentum	Rhodella violacea
Soluble photosynthates:	79.1	67.7	68.0	88.7	82.3	85.2	78.2
Insoluble photosynthates:	21.9	32.3	32.0	11.3	17.6	14.8	22.8
Soluble compounds = 100%							
Glycerol	1.8	1.0	2.4	8.2	9.5	–	–
Galactose	–	–	–	–	–	–	–
Floridoside	–	–	–	–	–	52.1	45.3
Glucose	1.0	1.0	1.4	22.9	35.7	–	–
Polyglucan	22.9	30.4	26.5	10.2	13.1	5.7	9.2
Lipids	68.4	54.9	52.8	18.4	14.5	8.5	13.3

[1] Data (recalculated) from Kremer and Berks (1978).
[2] Data from Kremer and Feige (1979).

plies further aspects, when compared to the appropriate features of nonsymbiotic algae. It is noteworthy that in extracts prepared from *Peneroplis pertusus* and *Spirolina arietina* appreciable amounts of ^{14}C-galactose and ^{14}C-glycerol are found in addition to the heteroside composed of both compounds. Free galactose and glycerol are usually not encountered in nonsymbiotic Rhodophyceae (Kremer, 1978b, c; Kremer and Feige, 1980). In a first approach, the occurrence of these compounds may be interpreted as an effect of the symbiotic condition. While floridoside is clearly stored inside red-algal endosymbionts and has hitherto never been observed as extracellular product, galactose and glycerol are most probably constituents of the animal cell and hence reflect to some extent the metabolization of floridoside derived from the plant cell. At present it is not possible to decide whether floridoside is released from the algal endosymbionts (which might be impeded by the molecular size of this compound) or whether galactose and glycerol are transferred immediately.

A similar situation is observed in Foraminifera containing symbiotic diatoms: The amount of ^{14}C-labelled glycerol (exceeding 4 %) in these associations is far more than that observed in a variety of free-living species (Table 2). The same is true for ^{14}C-glucose, in *Navicula* spp. and *Nitzschia* spp. for instance, is in the range of detection limits after long periods of incubation in light. Distinct ^{14}C-labelling of these compounds may, therefore, be indicative of metabolic participation of the animal partner. The symbiotic dinoflagellates of *Amphisorus hemprichii* incorporate relatively less ^{14}C into glucose and glycerol than do *Prorocentrum micans* and *Scrippsiella faeroense*.

In all associations, except with *Amphisorus hemprichii*, a remarkable amount of ^{14}C is confined to soluble, but chromatographically immobile, material yielding ^{14}C-glucose upon hydrolysis, and is hence regarded as a glucan-type polysaccharide. This as well as the fraction of insoluble assimilates is usually more intensely ^{14}C-labelled than in free-living algae incubated under comparable conditions. Most of the photosynthetically fixed radiocarbon is recovered from lipid compounds in *A. hemprichii*, whereas relatively little ^{14}C is found in the soluble carbohydrates.

There are two principal ways for the animal partner to obtain assimilates from its endosymbiotic primary producers. The surplus production of assimilates is (i) released from the algae and thus flows from plant to animals cells, or (ii) endosymbionts are digested. Enzymatic degradation of algal storage assimilates in the course of digestion would thus yield ^{14}C-glycerol (from the lipid fraction; Table 1) and ^{14}C-glucose (from the glucan-type polysaccharides). However, ^{14}C-glucose does not occur in *Peneroplis pertusus* and *Spirolina arietina*, though a strongly ^{14}C-labelled glucan-fraction is available. On the other hand, the amount of ^{14}C-glucose found in *Amphisorus hemprichii* does not correspond to the ^{14}C-labelling of glucans in this association. Moreover, in the symbiotic algae of *P. pertusus* and *A. hemprichii*, for instance, percentage labelling of usually accumulated compounds (such as floridoside and glucose) is to some extent lower than in nonsymbiotic relatives.

The features of distribution of radiocarbon among soluble and polymeric material as detailed above reflect the metabolic participation of the animal host and may thus include different rates of turnover in different associations. Nonetheless, it is reasonable to assume that the foraminiferans receive algal photoassimilatory products by carbon transfer involving at least accumulated compounds or their biosynthetic precursors (^{14}C-glycerol in case of diatoms).

However, in *Heterostegina depressa* and probably

also in other foraminiferans, digestion of endosymbiotic algae is possible. Continuous observations show that they often release appreciable amounts of brown-green waste material. While in some species this might originate from the digestion of external food, in *H. depressa* it clearly represents the remains of digested endosymbiotic algae. This has been shown by electron microscopy and demonstration of acid phosphatase within the digestion vacuoles (Schmaljohann, unpublished).

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