Frond Development and CO₂-Fixation in *Laminaria hyperborea*

Johannes Willenbrink, Monika Rangoni-Kübbeler, and Bärbel Tersky

Botanisches Institut der Universität Köln
Gyrhofstr. 15, D-5 Köln 41, Federal Republic of Germany

Received 26 March; accepted 20 May 1975

Summary. Discs punched out of different zones of the frond of *Laminaria hyperborea* (Gunn.) Fossl. were exposed to H¹⁴CO₃⁻ in the light and in the dark for various lengths of time. Photosynthetic rates in young and old parts were in the same range, 12–39 μmol CO₂ dm⁻² h⁻¹ at 4°C, whereas dark fixation was remarkably higher in the growing zone than in the old frond: 13–28% of the corresponding light fixation in the young phylloid and only 2–6% in the old one. In all parts of the frond the reductive pentose phosphate cycle is the main system of carboxylation, and is accompanied by a notable primary synthesis of amino acids. In the growing zone heavy and primary labelling of malate and aspartate parallel to 3-PGA is due to the undiminished activity of the dark fixation system (PEP-carboxykinase) in the light. Mannitol synthesis seems to be enhanced with increasing age of the phylloid, but rapid and primary ¹⁴C-incorporation into amino acids was found in all parts of the frond.

The data presented here suggest a possible significance of the high dark fixation rates for the growth of the sublittoral *Laminaria* hyperborea. Translocation of mobilized storage material from the old phylloid might supply the young tissue with reduced material during the growth season when the light intensity is low in the habitat of this alga.

Introduction

Previous investigations on translocation in *Laminaria hyperborea* and *L. saccharina* have shown rates of velocity, composition of translocate (Schmitz et al., 1972), and its ecological significance for the growth rates of these sublittoral algae (Lüning et al., 1974), as suggested earlier by Kain (1963). At the same time the operation of the reductive pentose phosphate cycle as formulated by Calvin et al. (1951) was reported for CO₂-fixation in *L. saccharina* (Kremer und Willenbrink, 1972). With regard to CO₂-fixation in the dark which might contribute significantly to the metabolism of *Laminaria* plants in their habitat during winter, Akagawa et al. (1972a,b) presented data on different rates in some species of Pacific brown algae and identified PEP-carboxykinase (EC 4.1.1.32) as being the responsible enzyme (1972c). High PEP-carboxykinase activities have also been localized in *L. hyperborea*, mainly in the growing frond (Weidner and Küppers, 1973). This paper is concerned with a comparative study of CO₂-fixation in this alga and reports on qualitative shifts occurring in the primary carboxylation products in relation to the ontogenetic stage of the frond.

Abbreviations used: PEP = Phosphoenolpyruvate; RuDP = Ribulosediphosphate; PGA = Phosphoglycerate; SMP = Sugar Monophosphates; SDP = Sugar Diphosphates; MLWS = mean low water of spring tides
Fig. 1. Schematic drawing of sampling in a specimen of *Laminaria hyperborea*. The reference numbers mark the samples punched out of: "1" the meristematic zone of the growing frond (= basal), "2" the zone in which the main enlargement of the new frond occurs—predominant elongation processes—(= apical part), "3" the old frond from the preceding year.

**Material and Methods**

*Plant Material:* *Laminaria hyperborea* (Gunn.) Fosl., collected by SCUBA diving at 2 m below MLWS in the sublittoral zone of Helgoland. During the period from February to June the new frond is initiated and develops to full size. Until incorporation experiments were performed the plants were kept in tanks supplied with flowing sea water, where the temperature corresponded to *in situ* temperature of the sea.

Samples of the different parts of the specimens were punched out as illustrated in Fig. 1, and were immediately subjected to pre-illumination or pre-darkening. Parallel samples were usually taken for determination of chlorophyll a (Arnon, 1949); protein-nitrogen (by nesslerization of the TCA-precipitate by the Kjeldahl-method); and dry-weight.

**Experiments.** For 14C02-studies, an incorporation apparatus was used as described earlier (Kremer and Willenbrink, 1972). Illuminance at the surface of the samples was about 4000 lux. After a pre-incubation period in a sea water medium (carbonate content 2.5 mM, salinity 31‰, pH 8.0, after Provasoli, 1966) the discs were transferred into the incorporation chamber which contained the 14C-medium: as above, after Provasoli, plus 2 mCi H14CO3/10 ml medium.

Results of a 14CO2-fixation series at illuminances in the range of 600 lux up to 40 klux showed that 4000 lux at 8°C is a saturating illuminance for this species. At higher temperatures, such illuminance does not saturate the photosynthetic capacity of *L. hyperborea* (Lüning, 1971). Since in these first series no additions were to be introduced other than temperature adaptation, the illuminance of 4000 lux was also kept constant in June (at 11°C).

**Extraction, Separation and Identification of Fixation products.** After the appropriate incubation time, the discs were immersed rapidly in vials containing 80% ethanol cooled by dry ice (in order to avoid evaporation of volatile 14C-products). The samples were kept frozen until being ground in a mortar with quartz sand. After repeated extraction with 50% and 80% ethanol at 20°C the sediment was extracted with 50% ethanol at 50°C. All extractions of each sample were pooled. Because of the high content of mucilaginous polysaccharides in these algae any water extraction had to be avoided. In order to test exhaustive extraction of water-soluble substances, some samples were further extracted with 0.2 N HCl. Chromatography of these acidic extracts did not show any results of significance in this study. The residues were carefully dried, and total 14C was measured after dry combustion (Oxidizer Packard 305) in a liquid scintillation counter. Aliquots of acidified extracts were also measured by scintillation counting. 14C-labelled compounds were separated by: a) descending two-dimensional paper chromatography (paper Schleicher/Schüll 2043 b Mgl, pre-washed), using the solvents of Tyskiewics (1962), and b) ascending two-dimensional thin layer chromatography.