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Metabolic conversion of exogenous ^{14}C-aspartate and ^{14}C-glutamate in the dark by *Fucus serratus* L.

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Abstract

The rates of uptake of exogenous L[U-^{14}C] aspartate and glutamate into tissues of vegetative growing tips of *Fucus serratus* and their metabolism were studied in the dark. In these non-photosynthetic conditions, aspartate was fixed and metabolically converted more rapidly than glutamate. Radioactivity from ^{14}C-aspartate was principally transferred into glutamate. On the other hand, metabolism of absorbed ^{14}C-glutamate was very slow and its rate did not increase during incubation time, but produced more diversified soluble radioactive compounds. Thus in *F. serratus*, glutamate principally seems to be in the dark more a temporary $^{14}CO_2$ storage product coming from β-carboxylation than a rapidly turned over intermediate.

Introduction

Non-autotrophic (light-independent) CO_2 fixation *via* the β-carboxylation of phosphoenolpyruvate with phosphoenolpyruvate carboxykinase (PEPCK) as the carboxylation enzyme, occurs in numerous brown seaweeds (Akagawa *et al.* 1972a, Kremer 1979, Johnston and Raven 1986). In most algae, the first stable products of short-term $^{14}CO_2$ fixation in the dark usually were the four carbon dicarboxylic acids, malate and aspartate, with more $^{14}CO_2$ incorporated in aspartate than in malate (Craigie 1963, Akagawa *et al.* 1972b).

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Abbreviations: PAR - photosynthetic active radiations; PEPCK - phosphoenol-pyruvate carboxykinase; OAA - oxaloacetic acid; 3-MPA - 3-mercaptopicolinic acid; fm - fresh mass.
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Nevertheless, we had shown, beside malate and aspartate, a rapid and high level of labelled glutamate in *Cystoseira* species (Coudret *et al.* 1987 and unpublished results) and of labelled glycerate and later glutamate in *Fucus* species (Coudret *et al.* 1989). Glutamate, whose the total pool did not increase significantly during the pulse period (Coudret *et al.* 1987, 1989), seemed to play, in all these species, a predominant role as a source of carbon, in the dark as well as in the light (Coudret *et al.* 1992).

In order to state their metabolic relationships, the rates of uptake and metabolism in darkness of [U-^{14}C] aspartate and [U-^{14}C] glutamate and the respective labelling patterns were determined as a function of incubation time. It was expected to obtain some informations about the metabolic channels taken by β-carboxylation products and their derivatives.

Material and methods

Uniform thalli of *Fucus serratus* L. were harvested in April, near Arromanches (Calvados, France). The plants were grown for two weeks in continuously aerated seawater (taken from harvesting zone) at 15 °C, under a photon flux density (*Quantum Meter, LI-185B Li-Cor*) of 100 µmol m^{-2} s^{-1} PAR (*Cool White General Electric* fluorescent lamps) supplied in 15 /9 h (light/darkness) cycles.

Before experiments, fragments of vegetative growing tips were randomly selected, weighed (300 to 800 mg fresh mass) and maintained for 2 h under a photosynthesis saturating irradiance (800 µmol m^{-2} s^{-1}, previously it was verified that this high irradiance did not cause photoinhibition) and afterwards left for 1 h in the dark in filtered seawater at 15 °C. The fragments were then transferred in the dark into filtered seawater containing (the initial concentration was 0.5 mM in each case) one of the two following labelled substrates: L-[U-^{14}C]aspartic acid (8.44 GBq mmol^{-1}) and L-[U-^{14}C] glutamic acid (10.7 GBq mmol^{-1}) for 1, 2, 5, 10, or 30 min. After incubation, samples were quickly rinsed with distilled water and metabolism stopped by plunging into liquid nitrogen. The metabolites were extracted and separated as described earlier (Coudret *et al.* 1992), incorporated radioactivity was measured by liquid scintillation counting (*Betamatic I, Kontron*) after identification by auto-radiography. The results are given in radioactivity (dps) incorporated in 1 mg of fresh matter as a function of the incorporation time or as percentage of the total radioactivity of the soluble organic matter. Each experiment was repeated twice and the results given are the means of the two replicates.

Results

The uptake of exogenously supplied ^{14}C-aspartate and ^{14}C-glutamate into tissues cut off from the growing zone of *Fucus serratus* was intense during the first minutes of incubation (Fig.1A). After a longer incubation time (5 min), a fairly constant labelling pattern occurred in the two cases, indicating that metabolic balance was

established for exogenously supplied metabolites within 30 min of incubation, but, the level of aspartate incorporated was significantly more important than the level of glutamate.

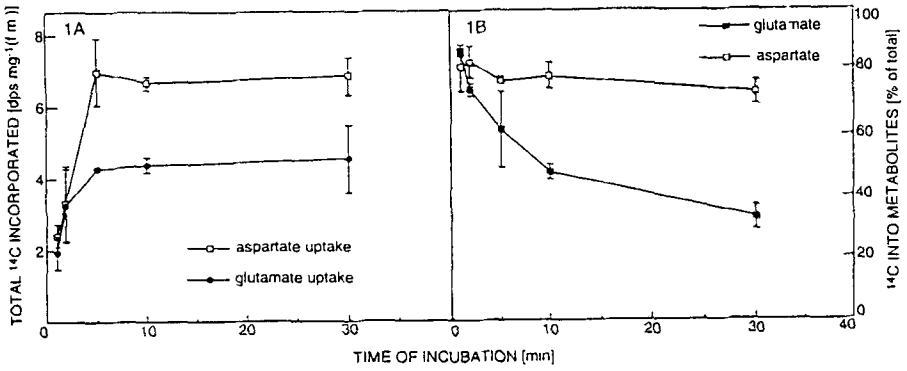

Fig. 1A. Time-course of uptake of L[U-^{14}C] aspartate (*open squares*) and L[U-^{14}C] glutamate (*closed squares*) by *Fucus serratus* in the dark.
Fig. 1B. Changes with time of the amount of non-metabolized L[U-^{14}C] aspartate and L[U-^{14}C] glutamate in the dark (in percent of total radioactivity incorporated in soluble organic matter) into *Fucus serratus*. Vertical bars = ± S.E.

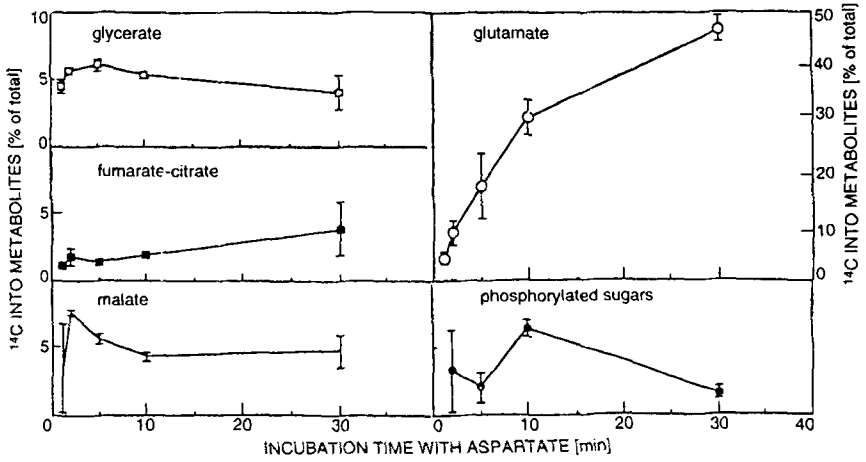

Fig. 2. Patterns of metabolic conversion of incorporated L[U-^{14}C] aspartate into *Fucus serratus* (in percent of total radioactivity incorporated into soluble organic matter) in relation with the incubation time. Vertical bars = ± S.E.

In these non-photosynthetic conditions, aspartate was metabolized much more rapidly and intensively than glutamate (Fig. 1B). From the first minute of incubation, about 20 % of glutamate were metabolized, but then this percentage remained steady. On the other hand, aspartate was continuously metabolized during the incubation period and more than 60% of ^{14}C incorporated from labelled aspartate appeared in other metabolites after 30 min.

Radioactivity derived from uniformly labelled aspartate was principally transferred and continuously accumulated into glutamate (Fig. 2). In the other significantly labelled identified metabolites (malate, citrate/fumarate, glycerate and phosphorylated sugars), fixed ^{14}C did not fluctuate significantly and pools seemed nearly saturated from the first minute of incubation.

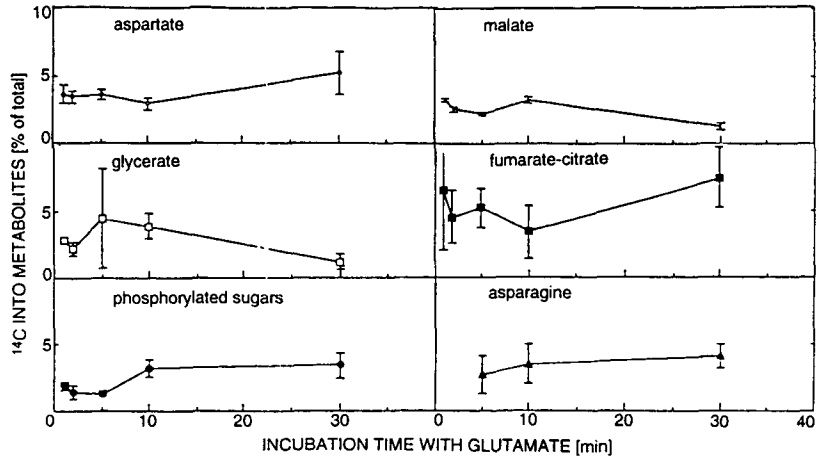

Fig. 3. Patterns of metabolic conversion of incorporated L[U-^{14}C]glutamate into *Fucus serratus* (in percent of total radioactivity incorporated into soluble organic matter) in relation with the incubation time (after 30 min of incubation a low part, <5% of ^{14}C, is incorporated in alanine: this value is omitted on the figure). Vertical bars = ± S.E.

When glutamate was supplied (Fig. 3), only a little part (nearly constant during the incubation time) of the radioactivity was steadily distributed among more diversified metabolites: aspartate, malate, glycerate, fumarate/citrate, phosphorylated sugars, asparagine and alanine. A slight accumulation of ^{14}C occurred after 10 min into phosphorylated sugars, asparagine and fumarate/citrate at the expense of glycerate and malate. Radioactivity in asparagine only appeared after 5 min of incubation and was not detected when aspartate was supplied.

Moreover, after 10 min of incubation with ^{14}C-aspartate or ^{14}C-glutamate, small spots of labelled sucrose appeared on the radiochromatograms but the level of incorporated radioactivity is too low to be measured.

Discussion

Among the two amino acids provided in the dark to *Fucus serratus*, aspartic acid (C4 compound) was more intensively fixed and metabolized than glutamic acid (C5 compound), this reflects the well known fact, previously observed (Coudret *et al.* 1989, 1992), that aspartate (among the first stable products of β-carboxylation) is a rapidly turned over intermediate. The much lower rate of metabolic conversion of glutamate, quickly stopped, (no significant variation of the pool radioactivity after the first minute of incubation) proves, as results previously obtained in pulse-chase experiments (Coudret *et al.* 1992), that glutamate seems to be in the dark, first a CO_2 storage product issued from β-carboxylation and then, for a low part in *Fucus*, a source of carbon for diversified metabolites.

However, the most original and significant result of these experiments was the intense and continuous conversion, in the dark, of aspartate into glutamate by *Fucus serratus* thalli. In other brown seaweeds and in similar non-photosynthetic conditions, only some TCA-cycle intermediates (fumarate and malate) were labelled after the metabolic conversion of ^{14}C-aspartate by tissue disks of *Laminaria hyperborea* (Weidner and Küppers 1982). In *Fucus serratus*, it is possible that aspartate quickly condensed with acetyl Co-A to give citrate may be the substrate of glutamate transaminase to form glutamate.

The distribution of ^{14}C among other metabolites was essentially similar in both experiments, with low levels of radioactivity in TCA-cycle intermediates. Biosynthesis of some phosphorylated sugars, process analogous to gluconeogenesis might be operative in brown algae. Moreover, when glutamate was supplied, some nitrogen compounds (asparagine and alanine) were synthetized.

In both experiments, a little part (5%) of glycerate was labelled during the first minutes of incubation, to decrease afterward particularly when ^{14}C-glutamate was supplied. In *Fucus* thalli as in other plants (Coudret *et al.* 1985, Penot *et al.* 1985, Mortain-Bertrand 1987), glycerate is a significantly labelled derivative from β-carboxylation in the dark. Moreover, glycerate plays in *Fucus* a predominant role as a source of carbon in the dark (Coudret *et al.* 1992). As for aspartate and glutamate, metabolic conversion in the dark of [1-^{14}C]glycerate was studied, but only a low level of incorporation and an insignificant metabolization were observed (unpublished results). Thus, metabolic pathways of glycerate formation and metabolization in non-photosynthetic conditions in brown algae as in other plants still remain unexplained.

As Weidner and Küppers (1982) described, it is possible in brown algae, that under certain conditions (*e.g.* excessively loaded dicarboxylic acid pools) PEPCK can be triggered to operate in the decarboxylating direction. This hypothesis of decarboxylation role in algae was suggested by Reiskind and Bowes (1991). In our experiments, aspartate pool seemed saturated after 5 min of aspartate supply (Fig. 1B), then PEPCK could be switched over to β-decarboxylation of OAA and thus from an anaplerotic reaction to an initial step of various intermediates as glycerate and as anabolic pathway to phosphorylated sugars and sucrose. To confirm the role of PEPCK in these conditions, similar experiments (incubation with ^{14}C aspartate)

were realized in the presence of 3-mercaptopicolinic acid, a specific inhibitor of PEPCK in plants (Ray and Black 1976) and seaweeds (Kerby and Evans 1983, Reskind and Bowes 1991). In these conditions, uptake and metabolic conversion of ^{14}C-aspartate (Tremblin and Coudret, unpublished data) were very low (14 % of the reference). Thus PEPCK might play an essential role in this brown seaweed dark carbon metabolism. Further experiments with or without 3-MPA are under way to specify the possible flexibility (in the dark as in the light) of this enzyme in *Fucus serratus*. Similar experiments should be realized in *Cystoseira* species to precise if the intense and continuous conversion of aspartate to glutamate observed in the dark in *Fucus* occurs similarly in *Cystoseira* species where glutamate is the main labelled product of $^{14}CO_2$ dark fixation (Coudret *et al.* 1987).

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