Utilisation of leucine by several phytoplankton species

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Abstract

Since amino acids represent an important component of dissolved organic carbon in lakes, we investigated the uptake and consumption of leucine by several phytoplankton species. Firstly, we measured the leucine uptake of 28 phytoplankton species (several cyanobacteria and chlorophytes, one diatom, and one euglenophyte) and the uptake kinetics by a chlorophyte (\textit{Ankistrodesmus gracilis}) compared to that of heterotrophic bacteria. Furthermore, we tested whether the algae can decrease the concentration of leucine in the light to lower levels than in darkness (hypothesis 1), and whether algae with high minimum substrate requirements exhibit higher consumption rates at plentiful concentrations compared to algae with high substrate reduction capability but low maximum consumption rate (hypothesis 2). Thirteen species of cyanobacteria and chlorophytes showed leucine uptake. Specific uptake rates by \textit{A. gracilis} were lower in the light than in the dark and much lower than that of heterotrophic bacteria. In the consumption experiments, several algae consumed leucine with higher rates and to lower residual concentrations in the dark than in the light, but with lower rates and not to lower concentrations than heterotrophic bacteria. Residual concentrations and consumption rates were not related to algal cell volume and chlorophyll content. Consumption rates were negatively related to residual concentrations, i.e. algae with higher consumption rates also depleted leucine to lower concentrations. Although the hypotheses were not supported, several algae were capable of removing leucine to equally low concentrations as bacteria so that algal uptake of amino acids is potentially important in natural waters.

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\textbf{Keywords:} Algae; Amino acids; Bacteria; Competition; Cyanobacteria; DOC; Leucine; Mixotrophy

Introduction

Mixotrophy, i.e. the combination of autotrophy and heterotrophy by using inorganic and organic carbon sources, is widespread in many algae. Whereas several algae ingest particles, e.g. bacteria (phagotrophy), others utilise dissolved substances (osmotrophy). This osmotrophic capability is known in many algal groups such as cyanobacteria, chrysophytes, chlorophytes, dinoflagellates, diatoms, and xanthophytes (e.g., Droop, 1974). It is regarded to supplement autotrophic nutrition, particularly in deep water strata with low light intensities and in waters with high allochthonous inputs (Droop, 1974; Bouarab et al., 2004). However, heterotrophic bacteria are considered to be the more efficient users of dissolved organic carbon (DOC) and the superior competitors compared to larger organisms due to their small size and the high surface to volume ratio (Wright and Hobbie, 1966).
In most studies, glucose has been used as a model substance to investigate DOC utilisation (Wright and Hobbie, 1966; Bennett and Hobbie, 1972; Pengerud et al., 1987; Feuillade and Feuillade, 1989; Gervais, 1997). However, other organic compounds such as amino acids are also important components of natural DOC. Amino acids may dominate the exudates released by phytoplankton during photosynthesis (Søndergaard et al., 1988) and can cover the main part of the carbon demand of heterotrophic bacteria (Simon, 1994). The uptake of the amino acid leucine was believed to be specific for heterotrophic bacteria and was used as a measure to determine bacterial production (Kirchman et al., 1985; Simon and Azam, 1989). No uptake was found for eukaryotic algae (Kirchman et al., 1985), and utilisation of amino acids by other microorganisms besides heterotrophic bacteria was regarded negligible up to concentrations of 100 nM (Riemann and Azam, 1992). Also for cyanobacteria, no leucine uptake was detected for coccolid forms (at 0.5 nM; Kirchman et al., 1985) and Synechococcus (at 24 nM; Torreton and Dufour, 1996). However, some early papers showed the uptake of several amino acids for a diatom and for chlorophytes using radiolabelled substrates (North and Stephens, 1972; Kirk and Kirk, 1978), and other studies proved leucine incorporation by Antarctic diatoms (Rivkin and Putt, 1987), and by the cyanobacteria Microcystis aeruginosa (Kamjunke and Jähnichen, 2000; Hietanen et al., 2002) and Nodularia spp. (Hietanen et al., 2002).

Mixotrophic organisms ingesting prey items (e.g., bacterivorous Ochromonas) are known to reduce prey concentrations to lower values in the light than in the dark so that they generate lower food concentrations than a heterotrophic specialist in the light whereas the specialist outcompetes the mixotroph in the dark (Rothhaupt, 1996; Tittel et al., 2003). We tested whether these mechanisms can also apply to osmotrophic algae utilising DOC. In the dark, algae grow only heterotrophically and are expected to need a high substrate concentration, whereas in the light they grow mainly phototrophically and are capable of growing at lower DOC concentration. As a consequence, algae are predicted to decrease DOC concentrations to lower levels in the light than in darkness (hypothesis 1; Fig. 1a). Furthermore, we tested whether algae with a low ability to compete at low substrate concentrations exhibit higher consumption rates at high substrate availabilities (r-strategist) compared to organisms with high substrate reduction capability and low maximum consumption rate (k-strategist; hypothesis 2, Fig. 1b) as it is known for mineral nutrients (Tilman, 1982).

In the present study, we firstly tested the leucine uptake of 28 cultured phytoplankton species (several cyanobacteria and chlorophytes, one diatom, and one euglenophyte). Additionally, the uptake kinetics of leucine by a chlorophyte (Ankistrodesmus gracilis) was measured in the dark and in the light and compared to the uptake kinetics of heterotrophic bacteria. Secondly, we measured the decrease of concentrations of leucine over time, in the presence of several cyanobacteria and chlorophytes in the light and in the dark. We measured residual concentrations as an estimate for zero net growth (hypothesis 1) and measured substrate decrease rates as an estimate for uptake capacities (hypothesis 2). Furthermore, we related these parameters to allometric traits (cell volume) and photosynthetic potential (chlorophyll content) to test whether small algae decrease leucine concentrations to lower levels than larger phytoplankton and if less pigmented algae are stronger competitors for organic carbon.

**Material and methods**

**Organisms**

Phytoplankton species were obtained from the German Culture Collection of Algae, Göttingen (SAG) or from the Humboldt University, Berlin (HUB), and cultured in basal salts medium (Sanders et al., 1990) not containing leucine. Algae were cultured at a temperature of 20 °C and shaken by hand. The irradiance of 130 μmol photons m⁻² s⁻¹ (warm white fluorescent tubes, Lumilux 830, Osram; light:dark cycle 16:8 h) was measured as photosynthetically active radiation using a spherical light sensor (QSL 101, Biospherical). A mixed assemblage of heterotrophic bacteria was sampled from mesotrophic Lake Schlachensee near Berlin (see Gervais, 1997) and cultured in basal salts medium with added glucose (5 mg C l⁻¹).

![Fig. 1.](image-url)
Azam (1989). Chlorophyll was measured after ethanolic extraction for 24 h using a fluorometer (TD-700, Turner Designs; Tittel et al., 2005). Chlorophyll a and b were measured after extraction for 24 h using a fluorometer (TD-700, Turner Designs; Tittel et al., 2005).

Uptake experiments

A total of 28 phytoplankton species which are known for their osmrotrophic activity (Droop, 1974) were screened for their leucine uptake (Table 1). Duplicate 3 ml aliquots and one formaldehyde-killed control (3.7%, final concentration) were spiked with 1.66 µl 14C-leucine (12.2 GBq µmol−1, NEN, 12 µg C l−1 final concentration). Incubation in the light (100 µmol photons m−2 s−1, 20°C) was stopped after 10 min by adding formaldehyde. Samples were filtered onto 0.2 µm Nuclepore filters (Whatman). Filters were rinsed with 1ml culture medium and dissolved in a 0.5 ml tissue solubiliser (Soluene, Packard). After adding 2.5 ml scintillation cocktail (Hionic Fluor, Packard) to each scintillation vial, radioactivity was measured using a liquid scintillation analyzer (2300 TR, Packard). The external standard ratio method was used for quenching.

Table 1. Test of the uptake of leucine in 28 phytoplankton species (several cyanobacteria and chlorophytes, one diatom, and one euglenophyte; listed in alphabetical order)

<table>
<thead>
<tr>
<th>Species and strain number</th>
<th>+</th>
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<tbody>
<tr>
<td>Ankistrodesmus gracilis SAG 278-2</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Chlamydomonas applanata SAG 11-9</td>
<td>–</td>
<td>–</td>
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<td>Chlamydomonas noctigama SAG 36.72</td>
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<td>–</td>
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<td>Chlamydomonas pseudococcum SAG 12.79</td>
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<td>Chlamydomonas pseudogloeogama SAG 15.73</td>
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<td>Chlamydomonas reinhardtii SAG 11b-32</td>
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<td>–</td>
</tr>
<tr>
<td>Chlamydomonas segnis SAG 11-13</td>
<td>–</td>
<td>–</td>
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<td>Chlorella kessleri SAG 211-11c</td>
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<td>Chlorella kessleri SAG 211-11h</td>
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<td>Chlorella protothecoides SAG 211-7b</td>
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<td>Chlorella vulgaris SAG 211-11b</td>
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<tr>
<td>Chlorothece purpurea SAG 13.99</td>
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<td>+</td>
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<tr>
<td>Euglena gracilis SAG 1224-5/3</td>
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<td>–</td>
</tr>
<tr>
<td>Haematococcus pluvialis SAG 34-1a</td>
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<td>Microcystis spec. HUB 5-2-4</td>
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<td>+</td>
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<tr>
<td>Microcystis spec. HUB 5-3</td>
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<td>Nanochloris spec. SAG 251-2</td>
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<td>Scenedesmus acuminatus SAG 38.81</td>
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<td>Scenedesmus ellipticus SAG 64.81</td>
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<td>Scenedesmus obtusus SAG 276-3a</td>
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</tr>
<tr>
<td>Tetraselmsis cordiformis SAG 26.82</td>
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<td>+</td>
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</table>

Positive (+) or negative (−) results are indicated.

Uptake kinetics

To investigate leucine uptake in more detail, we measured the concentration-dependent uptake kinetics of A. gracilis in the light and in the dark and compared it with the uptake by heterotrophic bacteria. Organisms were precultured in basal salts medium at 20°C for 1 week with daily substrate addition of 5 µg C l−1 leucine (final concentration) in the light (130 µmol photons m−2 s−1, light:dark cycle 16:8h) or in the dark. Afterwards, they were centrifuged to remove remaining leucine (10–60 min, 389–694 g; Megafuge 1.0 R, Heraeus Instruments) and resuspended in medium without leucine to a biomass concentration of 3.1 mg C l−1 (algae, dark treatment), 3.4 mg C l−1 (algae, light treatment), and 2.0 mg C l−1 (bacteria, dark). Aliquots of 3 ml (triplicates and three formaldehyde-killed controls, 3.7% final concentration) were spiked with 14C-leucine, resulting in 5 concentrations from 0.15 to 20 µg C l−1 (final concentrations), and incubated for 10 min. After adding formaldehyde, samples were filtered onto 0.2 µm Nuclepore filters (Whatman) and filters were treated as described above.

Consumption experiments

Algae that were tested positively in leucine uptake experiments were precultured for 1 week and resuspended in medium to a biomass concentration of 11.6 ± 6.6 mg C l−1 (mean ± SD) as described above. Three light and three dark aliquots of 15 ml volume were then spiked with 3.46 µl 14C-leucine (5 µg C l−1, final concentration) and incubated in the light (100 µmol photons m−2 s−1) or in the dark. All flasks were gently aerated continuously to remove 14CO2 from respiration. Subsamples of 1 ml were taken mostly every 20 min during the first hour and later every hour and filtered through 0.2 µm Nuclepore filters at low vacuum pressure (50 mbar). After acidifying 0.5 ml of the filtrate with 5 µl 1 M HCl and repeated aeration using a pipette tip to remove 14CO2, 2.5 ml Hionic Fluor was added and the activity was measured. Experiments were performed until the activity in the filtrate, i.e. the substrate concentration, remained constant. As the algal cultures were not axenic, the potential influence of bacterial
contaminants was tested. Separate experiments were performed with bacterial contaminants from algal cultures in equal low concentrations as in experiments with algae, and residual substrate concentrations were measured after 6h. For comparison with phytoplankton, the consumption rate of leucine by heterotrophic bacteria from Lake Schlachtensee was measured. Consumption experiments were performed in a similar way to the algae in the dark (bacterial biomasses 2.0 mg C l⁻¹). Residual concentrations were calculated as means of the triplicates of the last three samplings, and consumption rates from slopes of linear regressions.

Results

In the uptake experiments with 28 phytoplankton species (several cyanobacteria and chlorophytes, one diatom, and one euglenophyte), 13 cyanobacteria and chlorophytes showed leucine uptake but 15 other species did not (Table 1, values of living cells at least 50% above control values). Regarding leucine uptake kinetics, the specific uptake rates by A. gracilis were always lower in the light than in the dark (Fig. 2a). The slope of uptake rates was steeper at low concentrations and more obliterated at higher concentrations. Specific rates at a leucine concentration of 4.5 μg C l⁻¹ amounted to 0.078 μg C mg C⁻¹ h⁻¹ in the light and 0.12 μg C mg C⁻¹ h⁻¹ in the dark. Bacterial uptake rates were much higher than that of Ankistrodesmus (Fig. 2b); the specific uptake at 4.5 μg C l⁻¹ leucine was 5.27 μg C mg C⁻¹ h⁻¹.

Regarding the 13 phytoplankton species tested positively for leucine uptake, Chlorogloea purpurea, Oocystis parva and Scenedesmus ellipticus did not consume leucine starting with 5 μg C l⁻¹, but needed higher concentrations to decrease the substrate concentration (data not shown). Most of the 10 remaining algae decreased leucine to 0.4–0.7 μg C l⁻¹ in the dark, whereas several algae left relatively high concentrations in the light (Fig. 3, Table 2). Out of the 10 species, 3 algae did not differ in dark and light residual concentrations, and 7 species reduced concentrations to lower values in the dark than in the light. No alga decreased the leucine concentration to lower levels in the light. Bacterial contaminants from algal cultures reduced the leucine concentration always much less than in experiments with algae. Furthermore, the residual substrate concentrations in the algal experiments (Table 2) were not related to the abundance of bacterial contaminants, indicating a negligible influence of the bacteria in the algal cultures. Efficient algae reduced the leucine concentration to values as low as bacteria, but no alga achieved lower concentrations than bacteria. Regarding leucine consumption rates, two algae showed equal values in the light and in the dark, seven species had higher rates in the dark than in the light, and only Microcystis spec. HUB 5-2-4 showed a faster consumption in the light than in the dark. Algal consumption rates were always lower than bacterial consumption.

Algae with a rapid consumption achieved also lower residual concentrations (Fig. 3, Table 2). The logarithmised values of residual leucine concentrations in light and dark were negatively related to that of consumption rates (p = 0.043). There were no significant relationships between the logarithmised values of the residual concentrations of leucine in dark or light and the algal cell volume or the algal biomass-specific chlorophyll content (p = 0.38–0.97). Also, the logarithmised values of the consumption rates in dark or light were related neither to cell volume nor to chlorophyll content (p = 0.09–0.29).

Discussion

In the consumption experiments, many cyanobacteria and chlorophytes steeply decreased the concentrations of leucine. Earlier investigations have shown that M. aeruginosa and Nodularia spp. took up leucine (Kamjunke and Jähnichen, 2000; Hietanen et al., 2002), and Synechococcus spp. used methionine and probably leucine (Zubkov and Tarran, 2005). The consumption rates of A. gracilis in the light and of heterotrophic bacteria.

Fig. 2. Specific uptake of leucine by Ankistrodesmus gracilis in the light and in the dark (a), and by heterotrophic bacteria (b). Note the different y-axis (error bars: standard deviation of triplicates).
bacteria agreed well with the specific uptake rates at this leucine concentration, whereas the dark consumption by the alga was higher than the measured uptake. The uptake and consumption rates of leucine in our experiments were higher than uptake rates of other amino acids measured by other authors (max. 0.25 μmol cm$^{-3}$ h$^{-1}$; Zotina et al., 2003). Leucine uptake of *A. gracilis* showed indications of saturation at...
0.28 μM concentration. Other algae have also been shown to exhibit Monod-type uptake kinetics (three species) or exhibited linear uptake kinetics (seven species) between 2.5 nM and 2.5 mM leucine (Kirk and Kirk, 1978). The half saturation constants of A. gracilis in the light and in the dark were much lower than those of A. braunii (16 μM; Kirk and Kirk, 1978). In the present study, residual substrate concentrations or consumption rates were not related to algal cell sizes or to chlorophyll contents, whereas Nielsen (2006) found an allometric relationship between cell size and maximum phototrophic growth rate for unicellular cyanobacteria and green algae.

Cyanobacteria and chlorophytes decreased leucine concentrations in the dark to equal or lower values than in the light, and the uptake by A. gracilis was higher in the dark than in the light. Earlier studies revealed that the uptake of amino acids by Planktothrix rubescens (Zotina et al., 2003) and of leucine by Antarctic diatoms and M. aeruginosa (Rivkin and Putt, 1987; Kamjunke and Jähnichen, 2000) was higher in the light than in the dark. In the experiments presented here, no alga generated lower substrate levels in the light than in the dark, so that hypothesis (1) was not supported. This was also found in consumption experiments with glucose (Kamjunke et al., 2008). Furthermore, cyanobacteria and chlorophytes in our consumption experiments did not decrease substrate concentrations to lower levels than the heterotrophic bacteria. Hypothesis (2) was not supported, too, since algae with higher consumption rates achieved also lower residual substrate concentrations, and residual concentrations were negatively related to consumption rates. Obviously, the mechanisms of competition for DOC differ from those for particulate prey (Rothhaupt, 1996; Tittel et al., 2003) and for mineral nutrients (Tilman, 1982).

Uptake velocities by algae may vary for different amino acids (North and Stephens, 1972), and leucine uptake is regarded to be lower than that of other amino acids (Kirk and Kirk, 1978). Although the leucine uptake rates of algae were always lower than those of heterotrophic bacteria, some phytoplankton species exhibited equally low residual leucine concentrations as the heterotrophic bacteria assemblage. Therefore, they potentially compete with bacteria in natural waters. The uptake of amino acids is supposed to contribute to algal carbon uptake, particularly under conditions where phototrophic growth is limited, e.g. in deeper water strata (Zotina et al., 2003). Since nitrogen addition decreased leucine uptake (Kamjunke and Jähnichen, 2000) and maximum amino acid uptake was negatively related to cellular nitrogen content and nitrogen concentration in the culture (North and Stephens, 1972), we expect a greater importance of leucine uptake by phytoplankton in more oligotrophic waters.

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