

The uptake of amino acids by the cyanobacterium *Planktothrix rubescens* is stimulated by light at low irradiances

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Abstract

The rates of uptake of five amino acids – alanine, glutamate, glycine, leucine and serine – by axenic cultures of the cyanobacterium *Planktothrix rubescens* were measured over a range of irradiances using the ¹⁴C-labelled amino acids at the nanomolar concentrations observed in Lake Zürich. The rates in the light exceeded the dark rates by as much as two- to ninefold. The light-affinity constants for stimulation were similar, indicating a similar process for each of the five amino acids. The E_k (light saturation irradiance) for light stimulation was only $1 \mu\text{mol m}^{-2} \text{s}^{-1}$, less than the compensation point for photosynthesis and autotrophic growth, and much lower than the E_k for either process. The E_k for amino acid uptake was also less than the irradiance at which filaments obtain neutral buoyancy, which determines the depth at which they stratify and the irradiance they receive. This indicates that stimulation of amino acid uptake by light of low irradiances provides a mechanism for supplementing growth of filaments stratifying deep in the metalimnion, which, while able to grow at low irradiances, are often left with insufficient light to sustain them. Acetate uptake was also stimulated by light, but the kinetics differed.

Introduction

The photoautotrophic cyanobacterium *Planktothrix rubescens* flourishes in deep layers of stratified lakes where the irradiances are very low. The occurrence of organisms depending solely on photoautotrophy is restricted to euphotic zones receiving sufficient light to provide a positive energy balance. Cyanobacteria are widely distributed and coexist with many other photoautotrophs, but they may be the sole inhabitants at the margins of photoautotrophic zones, where the conditions form fluctuating gradients. Such habitats include soils, stones, the benthic zones of lakes, and the lower fringes of the pelagic zone, which receive very little light over the year. Where additional chemical energy sources are available, however, these sources will compensate for the lack of light energy.

We have found that an isolate of *P. rubescens* from Lake Zürich, Switzerland, shows a light-stimulated uptake of amino acids at extremely low concentrations through a mechanism that is saturated at irradiances close to the photosynthetic compensation point. This mechanism is

clearly distinct from photoheterotrophy, which increases with irradiance.

Although cyanobacteria are primarily photoautotrophic organisms, some species are able to grow in the dark on sugars and other compounds (Harder, 1917; Allison *et al.*, 1937; Allen, 1952; Kiyohara *et al.*, 1960; Fay, 1965), and many other cyanobacteria can photoassimilate organic substances, including sugars (Fay, 1965), organic acids (Hoare *et al.*, 1967) and amino acids (Smith *et al.*, 1967). Kiyohara *et al.* (1960) showed that *Tolypothrix tenuis* assimilated eight different amino acids when grown in the dark in the presence of glucose, and Chen *et al.* (1991) demonstrated a circadian rhythm in the uptake of several different amino acids. The assimilation of organic compounds, such as acetate, can make an appreciable contribution to the growth of cyanobacteria at low irradiances (Pearce & Carr, 1966). The ability to take up and metabolise organic compounds will be particularly important in cyanobacteria that grow at low fluctuating irradiances, where the organisms often find themselves below the photosynthetic compensation point (Fogg *et al.*, 1973).

The red-pigmented cyanobacterium *P. rubescens* forms a population maximum in the metalimnion of stratified lakes, where the irradiance is low (Thomas & Märki, 1949; Zimmermann, 1969); in cultures, the organism will grow at photon irradiances as low as $0.6 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Davis & Walsby, 2002). A strain of *P. rubescens* (BC 9303) isolated from Lake Zürich, Switzerland, exhibited growth at photon irradiances down to $2 \mu\text{mol m}^{-2} \text{s}^{-1}$ in the light phase of a 12:12 h light–dark cycle, equivalent to a compensation point in continuous light of $1.6 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Bright & Walsby, 2000). From hourly measurements of the irradiance at the lake surface and the vertical light attenuation down the water column it was calculated that photoautotrophic growth would account for the major increases in biomass concentration at different periods of the year in Lake Zürich (Bright & Walsby, 2000; Walsby & Schanz, 2002). In these calculations, however, it is assumed that growth higher in the water column compensates for losses at greater depths, as filaments circulate through the metalimnion by constantly adjusting their buoyancy in response to the changing light field (Walsby *et al.*, 2004). Nevertheless, filaments at the lower limits of the depth distribution are often below the compensation point for photosynthesis and growth, and even those higher up encounter light limitations on days when the daily insolation at the water surface is decreased by cloud or when the depth of the thermocline is depressed by wind-driven seiches (Walsby *et al.*, 2001). Moreover, photoautotrophy ceases at night.

Köster (2002) found measurable amounts of proteino-genic amino acids in the water column of Lake Zürich; the concentrations of several of these substances increased after the introduction of daphnids and other zooplankton to the samples. Zotina *et al.* (2003) demonstrated that axenic cultures of *P. rubescens* strain BC 9307 took up little of the sugars and urea but did take up acetate and the amino acids glycine, serine, glutamate, aspartate and arginine. For the first four of these amino acids, the rate of uptake was stimulated by light at a low irradiance, $3.5 \mu\text{mol m}^{-2} \text{s}^{-1}$, which is only slightly above the photosynthetic compensation point (Bright & Walsby, 2000). It was suggested that the uptake of these organic compounds might make an important contribution to the maintenance or even growth of these organisms, which evidently specialize in these low-irradiance habitats.

There has been little investigation of the stimulation of amino acid uptake by light. Leekaden & Simonis (1982) showed stimulation at moderate irradiances in *Anacystis nidulans*. Church *et al.* (2004) have recently demonstrated the light-enhanced uptake of leucine in natural marine populations of picoplankton that include the cyanobacterial *Prochlorococcus* spp.; the maximum uptake occurred at photon irradiances of 24 to $60 \mu\text{mol m}^{-2} \text{s}^{-1}$, and the uptake/irradiance response curve resembled that expected

for a photosynthesis/irradiance curve. We have now investigated the effect of a range of irradiances on the enhancement of amino acid uptake by *P. rubescens*: the investigation reveals stimulation and light saturation at remarkably low irradiances, considerably lower than those saturating photosynthesis. We also included in our investigation the uptake of acetate, which is taken up at higher rates than the amino acids and which may occur at appreciable concentrations in lake water (Zotina *et al.*, 2003). Our analysis permits a quantitative evaluation of the maximum potential contribution of exogenous amino acids to the growth of *P. rubescens* under conditions it would encounter at different depths in the lake.

Methods and calculations

Cultures of *Planktothrix*

The strain of *P. rubescens* used was the axenic isolate derived from BC Pla 9307, in the Bristol Cyanobacteria Collection obtained from Lake Zürich in 1993 (Walsby *et al.*, 1998); in morphology and genotype analysis (Beard *et al.*, 1999) it resembles strain BC Pla 9303, used in studies on growth and photosynthesis (Bright & Walsby, 2000). The further purification of strain BC Pla 9307 is described by Blom *et al.* (2001); a previous investigation of the uptake of organic compounds by this strain is described by Zotina *et al.* (2003).

Cultures were grown at 20 °C in 300 mL Erlenmeyer flasks in the mineral medium described by Blom *et al.* (2001) and in continuous light of low irradiance, $7 \mu\text{mol m}^{-2} \text{s}^{-1}$. Before each experiment, the evenly suspended culture was diluted with fresh medium to give a low filament volume concentration, of between 7 and $60 \text{ cm}^3 \text{ m}^{-3}$, and the culture was kept at the ambient temperature, about 20 °C, in an irradiance of $< 1 \mu\text{mol m}^{-2} \text{s}^{-1}$. A sample (of 2 mL or 8 mL) was mixed with 100 mL of medium and filtered with a cellulose nitrate membrane filter of 50 mm diameter and pore size 8 μm (AE 99, Schleicher & Schuell, Dassel, Germany), which retained the filaments; the total filament length was determined by epifluorescence microscopy and image analysis (Walsby & Avery, 1996). The mean filament width (5.2 μm) was determined by microscopy and image analysis, and the filament volume in the sample was determined from the calculated cross-sectional area of the cylindrical filaments ($21.0 \mu\text{m}^2$) and the total filament length.

Incubation of *Planktothrix* in ^{14}C -labelled compounds in the light

Concentrations and amounts of uniformly ^{14}C -labelled amino acids (Amersham) taken up were calculated as illustrated by the example of ^{14}C -glycine. From the initial

specific radioactivity (3740 MBq mmol⁻¹) and concentration of radioactivity (1.85 MBq mL⁻¹), the initial chemical concentration was calculated (495 nmol mL⁻¹). Adding 7.5 µL of this solution to 15 mL of medium gave a carrier-free stock solution of 925 Bq mL⁻¹ and 0.247 nmol mL⁻¹; 1 mL of this stock was added to 10 mL of diluted *Planktothrix* culture in a 24 mL capacity scintillation vial, giving a final concentration of 22.5 nmol L⁻¹. Lower concentrations were used for other amino acids (Table 1), and higher concentrations for acetate.

The sample in the vial was immediately mixed, covered with an opaque, truncated black plastic bottle, and placed on the light-exposure bench for 30 min, where it was illuminated from below with a single fluorescent tube through a light filter formed by a partially exposed photographic negative (Fig. 1). The irradiance was measured with a Li-Cor Quantum meter LI-185B fitted with a flat Q 12749 quantum sensor: calibration details are given in the Appendix of Walsby (2001). Comparison of the readings given at the illuminated base (E_0) and above the suspension (E_b) gave the mean irradiance in the suspension: $E_m = (E_0 - E_b) / \ln(E_0/E_b)$ (Walsby *et al.*, 2006).

After 30 min the *Planktothrix* suspension was filtered under vacuum through a 8 µm pore-size cellulose nitrate filter of 30 mm diameter, which retained the filaments. The vial and the filter were washed twice with 1.5 mL of culture medium. While still under slight vacuum, the membrane filter was removed and dissolved in 1 mL of solubilizer (Soluene-350, Packard). This procedure was conducted under very low irradiance (<1 µmol m⁻² s⁻¹). After 17 h, 10 mL of scintillation cocktail (Hionic-Fluor, Perkin Elmer) was added. Samples were counted in a Quanta Smart Liquid

Scintillation Analyser (Packard). The amount (in nmol) of ¹⁴C-labelled amino (or organic) acid retained in each sample was calculated from measurements of the total radioactivity (in disintegrations per s) added to each sample, which in each case corresponded closely to the total activity (in Bq) added. The contributions from background radiation and activity retained on membrane filters from samples without *Planktothrix* (mostly <0.1%) were subtracted from the values with *Planktothrix*.

Results and calculations

Uptake rates of ¹⁴C-labelled amino acids

The concentrations of ¹⁴C-labelled compounds used were adjusted to correspond approximately to the range of concentrations found in water samples from Lake Zürich by Köster (Köster, 2002); acetate concentrations (1.4–4.6 µM) in lake water are known only for the eutrophic zone of Lake Kizaki (Hama & Handa, 1981). The concentrations of amino (or organic) acids used were: 23 nM for glycine, alanine and serine, 9 nM for leucine and glutamate, and 325 nM for acetate; the actual concentrations of the added compounds are given in Table 1.

In preliminary experiments, the concentrations of the *Planktothrix* were adjusted so that at least 70% of the ¹⁴C activity remained available at the end of the incubation. For four of the experiments, the filament biovolume concentration used was in the range 7 to 12 cm³ m⁻³, which is similar to the concentration of *Planktothrix* found in the metalimnion in late summer (Walsby & Schanz, 2002). For leucine and glutamate, in which the rate of uptake was lower than

Table 1. The measured values and calculated coefficients used in equations (1) and (2) describing the light-dependent uptake of the amino acids

Amino acid	Alanine	Glutamate	Glycine	Leucine	Serine
Total filament biovolume/mm ⁻³	0.076	0.606	0.125	0.606	0.038
<i>Amino acid concentrations*</i>					
Supplied in medium/nmol cm ⁻³	0.0224	0.0089	0.0225	0.0074	0.0223
In filament, after 0.5 h/nmol cm ⁻³	235	66.5	628	0.61	357
Ratio, inside/outside	11000	7400	28000	83	16000
<i>Measured uptake rates</i>					
U_D /nmol cm ⁻³ h ⁻¹	117	15.2	150	0.574	87.0
U_L /nmol cm ⁻³ h ⁻¹	471	133	1256	1.23	714
U_L/U_D	4.0	8.8	8.4	2.1	8.2
<i>Calculated coefficients</i>					
U_{max} /nmol cm ⁻³ h ⁻¹	261	113	887	0.530	489
a /(µmol m ⁻² s ⁻¹) ⁻¹	1.75	0.969	1.01	1.59	1.43
b /(µmol m ⁻² s ⁻¹) ⁻¹	2.24	0.110	5.24	0.00317	3.30
<i>Uptake-specific coefficients</i>					
α /nmol cm ⁻³ h ⁻¹ (µmol m ⁻² s ⁻¹) ⁻¹	457	110	896	0.842	699
β /nmol cm ⁻³ h ⁻¹ (µmol m ⁻² s ⁻¹) ⁻¹	583	12.5	4645	0.00168	1613
$E_L/\mu\text{mol m}^{-2} \text{s}^{-1}$	0.57	1.03	0.99	0.63	0.70

*0.001 nmol cm⁻³ = 1 nM. U_D , uptake rate in dark; U_L , uptake rate in light; U_{max} , nominal maximum uptake rate.

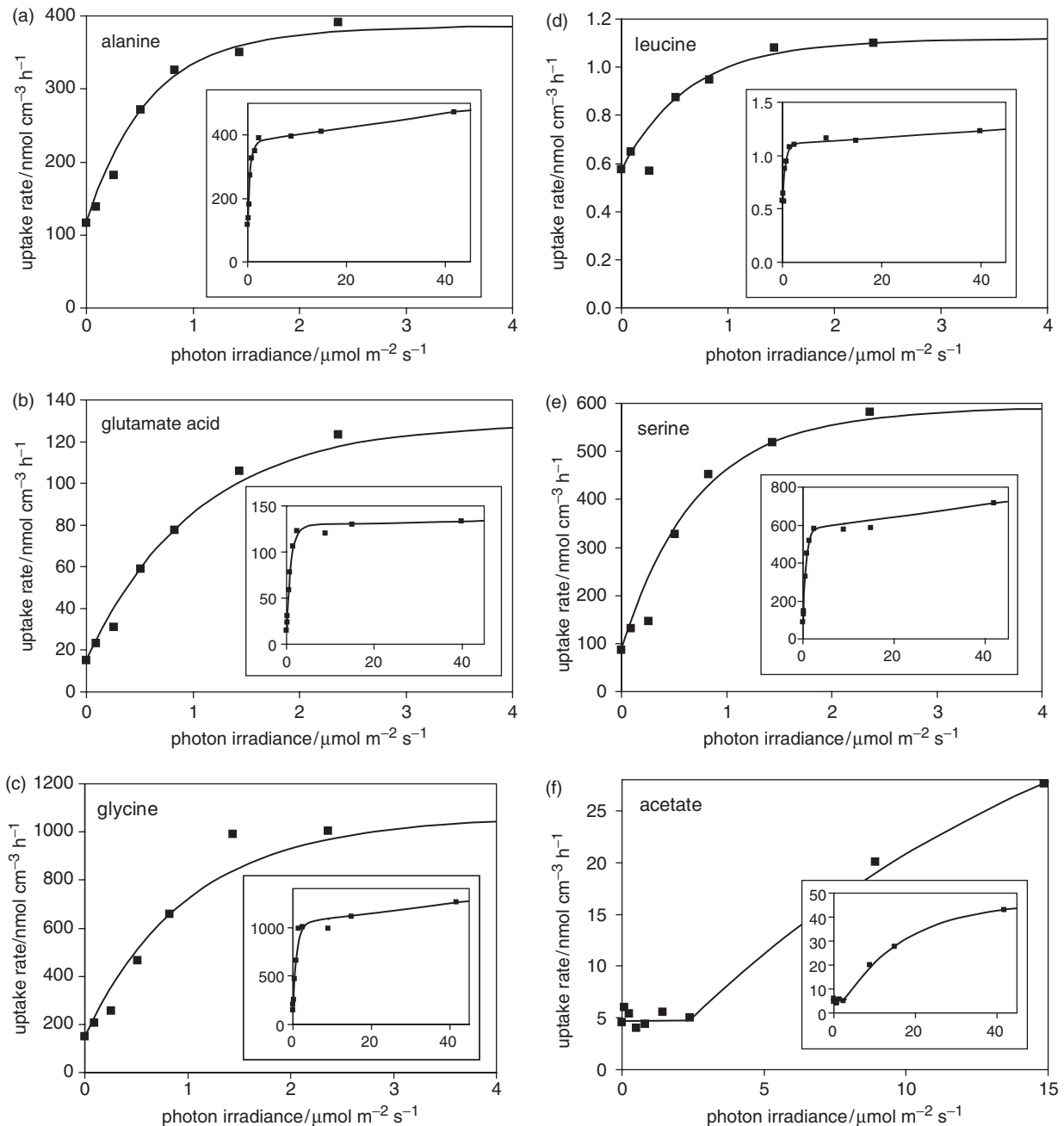


Fig. 1. The rate of uptake of amino (or organic) acids with increasing irradiance; the markers indicate the measured values and the lines the uptake – irradiance curves calculated by the least-squares method, with the coefficients listed in Table 1: (a) alanine; (b) glutamate; (c) glycine; (d) leucine; (e) serine; (f) acetate. Insets show the uptake over the full range of irradiances investigated.

for the other amino acids, the filament concentration was increased to $55 \text{ cm}^3 \text{ m}^{-3}$. Time-course experiments for glycine indicated that the rate of uptake at the highest irradiance remained linear for 40 min, as found previously (Zotina *et al.*, 2003): subsequent incubation periods were limited to 30 min for all of the amino acids.

The rate of amino acid uptake in the dark varied widely, from $0.6 \text{ nmol cm}^{-3} \text{ h}^{-1}$ for leucine to $150 \text{ nmol cm}^{-3} \text{ h}^{-1}$ for glycine (Table 1). All of the uptake rates increased markedly in the light, however, from 2-fold (leucine) to over 8-fold (glycine, serine and glutamate). In each case (Fig. 1), most of the increase was achieved at a very low

irradiance; in the case of glycine, for example, the rate increased to $1000 \text{ nmol cm}^{-3} \text{ h}^{-1}$ at a photon irradiance of only $1.5 \mu\text{mol m}^{-2} \text{ s}^{-1}$, and then only slightly higher, to $1260 \text{ nmol cm}^{-3} \text{ h}^{-1}$, at $42 \mu\text{mol m}^{-2} \text{ s}^{-1}$.

Uptake – irradiance curves

A quantitative description of these trends – the stimulation by light, the steep increase at low irradiance, and further small linear increase at higher irradiances – was provided by analysis of the rate of uptake, U , at an irradiance E , with the equation

$$U = U_m[1 - \exp(-aE)] + bE + U_D \quad (1)$$

in which U_m is the nominal maximum uptake rate, U_D is the rate in the dark, a is the (exponential) light-affinity coefficient for the lower irradiance phase, and b is the (linear) light-affinity coefficient for the higher irradiance phase: a and b have units of $(\mu\text{mol m}^{-2} \text{ s}^{-1})^{-1}$; a is identical to α/U_m , and b to β/U_m , where α and β are the rate-specific coefficients [with units of $\text{nmol cm}^{-3} \text{ h}^{-1} (\mu\text{mol m}^{-2} \text{ s}^{-1})^{-1}$] used in similar equations for photosynthesis/irradiance curves. At higher irradiances, the additional increase (bE) varies linearly, rather than exponentially, with E .

The rate coefficients for the five amino acids are listed in Table 1. As also demonstrated by Zotina *et al.* (2003), there were large differences between the highest uptake rates (from $1.2 \text{ nmol cm}^{-3} \text{ h}^{-1}$ for leucine to $1256 \text{ nmol cm}^{-3} \text{ h}^{-1}$ for glycine); in each case, however, the light-affinity coefficient, a , was of the same magnitude, $1.35 \pm 0.35 (\mu\text{mol m}^{-2} \text{ s}^{-1})^{-1}$, which suggests that the process of light stimulation was similar.

The reciprocal of a is equivalent to E_k (light saturation irradiance), which in photosynthesis denotes the onset of light saturation (Talling, 1957): $E_k = P_m/\alpha = P_m/aP_m = 1/a$. The mean value of E_k for the five amino acids (Table 1) is $0.78 \mu\text{mol m}^{-2} \text{ s}^{-1}$ (Table 1), which is less than the compensation point for growth in the light by *P. rubescens* strain 9303 of $1.6 \mu\text{mol m}^{-2} \text{ s}^{-1}$ (Bright & Walsby, 2000).

Concentration of amino acids by the cells

Substances can be taken up into cells by diffusion or by substance-specific affinity uptake systems (such as the ubiquitous ABC-transporters). We have not investigated the uptake mechanisms, but a comparison is made of the overall nominal concentration of amino acid (i.e. not that of the free amino acid) accumulated over the 30 min exposure with the concentration at which it was supplied in the medium. For example, from a suspension containing 7.4 nM leucine (i.e. $0.0074 \text{ nmol cm}^{-3}$), filaments of total volume 0.606 mm^3 accumulated 0.00037 nmol leucine, equivalent to $0.615 \text{ nmol cm}^{-3}$, only 83 times higher than the outside concentration (Table 1). This accumulation

might be accounted for by a low-affinity system, or by passive diffusion into the cell followed by assimilation into proteins or other compounds. In contrast, in a suspension containing 22.5 nM glycine (i.e. $0.0225 \text{ nmol cm}^{-3}$), filaments of total volume 0.125 mm^3 accumulated 0.79 nmol glycine, equivalent to 628 nmol cm^{-3} , more than 10^4 times higher than the outside concentration. Accumulation to a similar order of magnitude was also observed with alanine, glutamate and serine (Table 1), and strongly suggests active uptake in each case. In making these comparisons we are not making any assumptions about the form in which the compounds accumulate inside the cell – doubtless some of the amino acid will be converted into protein during the exposure.

Uptake rate in relation to the growth rate: for carbon

From the rate of amino acid uptake we calculate the potential contribution to the growth rate of carbon and nitrogen in the *Planktothrix* cells, and we compare this with previous measurements of photoautotrophic growth rates at various irradiances.

The molar ratio of carbon: amino acid, A_C , is for glycine 2, alanine and serine 3, glutamate 5, and leucine 6. The ratio of carbon: biovolume, B_C , is calculated from the ratio of dry mass (M) to cell volume (V) in *Planktothrix*, $M/V = 0.201 \text{ g cm}^{-3}$ in strain BC Pla 9316 (Vaughan *et al.*, 2001) and 0.18 kg m^{-3} in the strain used (Zotina *et al.*, 2003); the M/V ratio used here was 0.20 g cm^{-3} . The ratio of the mass of carbon (M_C) to dry mass in the axenic strain of *P. rubescens* strain 9307 is $M_C/M = 0.452$ (Zotina *et al.*, 2003); the molar mass, for carbon, is $C = 12 \text{ g mol}^{-1}$, and hence $B_C = (M/V)(M_C/M)/C = 0.20 \text{ g cm}^{-3} \times 0.452/12 \text{ g mol}^{-1} = 0.007533 \text{ mol cm}^{-3}$, or $7533 \mu\text{mol cm}^{-3}$.

The maximum rate of increase in cell carbon supported by amino acid uptake, when there is no expenditure of carbon in the assimilation of the amino acid, is calculated as follows. If the initial biovolume is $V_0 = 1 \text{ cm}^3$, the amount of cell carbon at time 0 is $C_0 = B_C V_0 = 7533 \mu\text{mol}$. During the incubation of time t , the cells take up the amino acid at the cell-volume specific amino acid uptake rate U (in $\mu\text{mol cm}^{-3} \text{ h}^{-1}$); the amount of carbon taken up is therefore $\Delta C = U A_C t V_0$ (in μmol). The amount of cell carbon at time 0 is $C_0 = B_C V_0$, and after time t it is $C_t = C_0 + \Delta C$. Since $C_t = C_0 \exp(kt)$, the rate of growth of cell carbon is described by

$$k_C = \ln[C_t/C_0]/t = \ln[(B_C V_0 + U A_C t V_0)/B_C V_0]/t = \ln[(B_C + U A_C t)/B_C]/t \quad (2)$$

The calculation is illustrated by the example of glycine uptake at the highest photon irradiance ($42 \mu\text{mol m}^{-2} \text{ s}^{-1}$). During the incubation of $t = 0.5 \text{ h}$, the cell-volume-specific glycine uptake rate in the light was $U_L = 1256 \text{ nmol cm}^{-3} \text{ h}^{-1} =$

Table 2. Maximum growth rates of cell carbon and cell nitrogen that could be supported by uptake of the various amino acids at the highest photon irradiance investigated ($42 \mu\text{mol m}^{-2} \text{s}^{-1}$) and the neutral buoyancy irradiance ($6.5 \mu\text{mol m}^{-2} \text{s}^{-1}$). Values in parentheses are the percentage of the measured cell growth rates, 0.2395 day^{-1} at $42 \mu\text{mol m}^{-2} \text{s}^{-1}$ and 0.1437 at $6.5 \mu\text{mol m}^{-2} \text{s}^{-1}$

Amino acid	Alanine	Glutamate	Glycine	Leucine	Serine
Atoms of C mol^{-1}	3	5	2	6	3
Atoms of N mol^{-1}	1	1	1	1	1
<i>Growth rates at $42 \mu\text{mol m}^{-2} \text{s}^{-1}$</i>					
Carbon growth rate/day $^{-1}$	0.0045	0.0021	0.0080	0.000024	0.0068
(% of measured growth rate)	(1.9)	(0.89)	(3.3)	(0.010)	(2.8)
Nitrogen growth rate/day $^{-1}$	0.0073	0.0021	0.020	0.000019	0.011
(% of measured growth rate)	(3.1)	(0.87)	(8.2)	(0.008)	(4.6)
<i>Growth rates at $6.5 \mu\text{mol m}^{-2} \text{s}^{-1}$</i>					
Carbon growth rate/day $^{-1}$	0.0037	0.0021	0.0068	0.000022	0.0057
(% of measured growth rate)	(2.6)	(1.43)	(4.7)	(0.015)	(4.0)
Nitrogen growth rate/day $^{-1}$	0.0061	0.0020	0.017	0.000017	0.0093
(% of measured growth rate)	(4.2)	(1.40)	(11.6)	(0.012)	(6.5)

$1.26 \mu\text{mol cm}^{-3} \text{h}^{-1}$ (Table 1); the amount of carbon taken up by cells of volume of $V_0 = 1 \text{ cm}^3$ was therefore $\Delta C = UA_C t V_0 = 1.26 \mu\text{mol}$, and the growth rate of cell carbon was $k_C = \ln[(7533.33 + 1.26) \mu\text{mol} / 7533.33 \mu\text{mol}] / 0.5 \text{ h} = 0.00033 \text{ h}^{-1}$ or 0.00800 day^{-1} (Table 2). This value can be compared with the photoautotrophic growth rate at the same irradiance, using the algorithm based on the growth rate in culture of *P. rubescens* strain 9303 (Bright & Walsby, 2000): at a photon irradiance of $42 \mu\text{mol m}^{-2} \text{s}^{-1}$, the equivalent growth in the light period of a light–dark culture is 0.24 day^{-1} ; the glycine-uptake value is equivalent to 3.3% of this. The equivalent value is somewhat higher at lower irradiances, for example 4.7% at $6.5 \mu\text{mol m}^{-2} \text{s}^{-1}$, the ‘neutral buoyancy irradiance’ experienced by *Planktothrix* filaments stratifying in Lake Zürich (Walsby *et al.*, 2004). The rates calculated for this and the other amino acids are shown in Table 2. For the other four amino acids, the maximum percentage values are lower than for glycine.

From the rate of glycine uptake in the dark, $\Delta C = 0.075 \mu\text{mol}$ in $t = 0.50 \text{ h}$, the growth rate supported in the dark would be $k_C = 0.00096 \text{ day}^{-1}$. The overall growth rate over one day on a 12:12 h light–dark cycle would be $(0.00800 \text{ day}^{-1} + 0.00096 \text{ day}^{-1}) / 2 = 0.0045 \text{ day}^{-1}$. This is the maximum growth rate of cell carbon that could be supported by uptake of glycine (but see Discussion below), and represents 4.1% of the overall growth rate of this strain on a 12:12 h light–dark cycle (Bright & Walsby, 2000).

At other irradiances, the maximum carbon growth rate supported by uptake of the amino acids can be generated in a two-stage calculation: the rate of amino acid uptake, U (in $\text{nmol cm}^{-3} \text{h}^{-1}$), at each irradiance is first calculated from equation (1) with the coefficients listed in Table 1; the carbon growth rate is then calculated by substituting the value of U in equation (2). The mean carbon growth rates supported by amino acid uptake over one day on a 12:12 h light–dark cycle are shown in Fig. 2. In each case, the principal increase occurs below the compensation point for

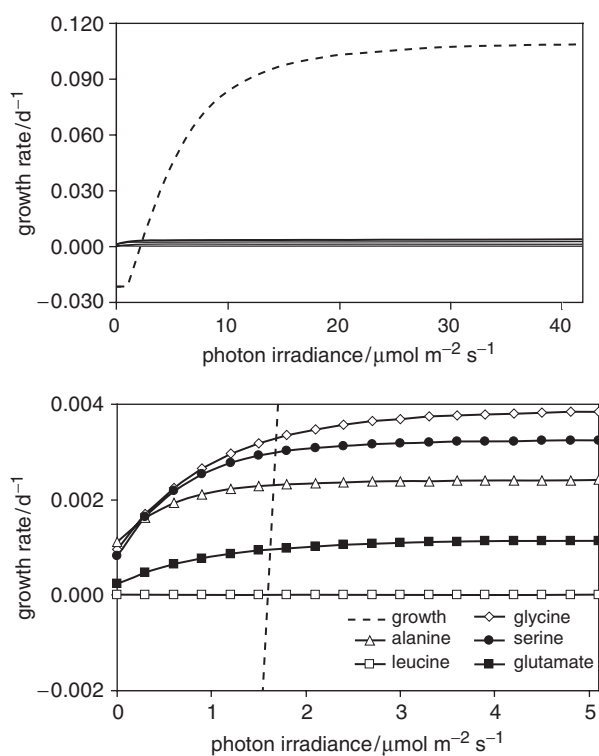


Fig. 2. The maximum growth rate of cell carbon that could be supported by the uptake of the various amino acids (solid lines), compared with the observed growth rate (broken line). The rates are the overall mean values over one day, with 12 h in the dark and 12 h in the light at the irradiance shown. The curve marked ‘growth’ indicates the photoautotrophic growth rates calculated from the algorithm based on measured growth rates in cultures of *Planktothrix rubescens* strain BC 9303 (Bright & Walsby, 2000): (a) graph over the full range for growth rate; (b) graph over a smaller range, showing details of rate curves for the various amino acids.

photoautotrophic growth of *P. rubescens* strain 9303 (Bright & Walsby, 2000), as indicated by the calculation of E_k , described above.

Uptake rate in relation to the growth rate: for nitrogen

The same procedure can be used in calculating the growth rate of cell nitrogen, given by $k_N = \ln[(B_N + UA_N t)/B_N]/t$: the ratio A_N is 1 for each of the five amino acids; the ratio of the mass of nitrogen (M_N) to dry mass is $M_N/M = 0.108$ (Zotina *et al.*, 2003); and the ratio of nitrogen: biovolume is $B_N = (M/V)(M_N/M)/C = 0.20 \text{ g cm}^{-3} \times 0.108/14 \text{ g mol}^{-1} = 0.001542 \text{ mol cm}^{-3}$, or $1542 \mu\text{mol cm}^{-3}$. The results for the five amino acids are shown in Table 2: the cell-nitrogen based rates differ from the cell-carbon rates owing to the different C:N ratios, and for four of the amino acids range from 0.9% to 8.2% of the overall growth rate.

Uptake of acetate

In the dark, the rate of biovolume-specific uptake of acetate was only $5 \text{ nmol cm}^{-3} \text{ h}^{-1}$, lower than that of all the amino acids studied except leucine. The rate of uptake increased strongly in the light, by 9.5-fold at the highest irradiance investigated, $42 \mu\text{mol m}^{-2} \text{ s}^{-1}$, although no significant stimulation was found below $2.4 \mu\text{mol m}^{-2} \text{ s}^{-1}$. The form of the uptake enhancement by light appears to be different from that of the amino acids, but it needs to be further characterized in experiments with irradiances between 3 and $9 \mu\text{mol m}^{-2} \text{ s}^{-1}$, to determine where the onset of enhancement begins, and with irradiances above $40 \mu\text{mol m}^{-2} \text{ s}^{-1}$, to determine the saturation point.

Discussion

Uptake at very low irradiances

The discovery of the enhancement of amino acid uptake by light of low irradiance raises the following questions. What is the physiological mechanism of the enhancement? Does it involve photophosphorylation, to provide ATP for amino acid transporters? Can the light requirement for enhancement be replaced by pretreating the cells at high irradiance, providing reserves for substrate phosphorylation? How does the light-enhanced uptake vary with amino acid concentration? Is the uptake rate of each amino acid independent of the presence of other amino acids? How is amino acid uptake and its enhancement by light affected by temperature? Does the enhancement of uptake by light effectively decrease the compensation point for growth of *P. rubescens*? All of these questions require further experimental investigations. We confine our comments here to the discussion of the role of light-enhanced uptake in the ecology of *Planktothrix*.

The low irradiance at which the enhancement is saturated can be related to the low irradiance at the depth at which *Planktothrix* stratifies. The irradiance there is suboptimal for its growth, but it allows *Planktothrix* to outcompete other

phytoplankton: the selection of these depths therefore provides an evolutionary stable strategy for *Planktothrix* (Walsby *et al.*, 2004). In adapting to such low irradiances, the organism may have modified its individual photochemical responses collectively (in which case the response-irradiance curves should be similar) or separately (in which case they may differ). Although the photosynthesis-irradiance curve of *Planktothrix* indicates adaptation to lower irradiances than other phototrophs, it saturates (Bright & Walsby, 2000) at a higher irradiance than does the amino acid response curve, which suggests an independent photochemical system.

Another correlation may be sought with the buoyancy-regulating mechanism involved in the stratification of *P. rubescens*. In laboratory culture, filaments of another strain of *P. rubescens* become buoyant at low irradiances and lose buoyancy at higher irradiances: they are neutrally buoyant (50% floating) on average in cultures grown on a 12:12 h light-dark cycle receiving an irradiance of $6.5 \mu\text{mol m}^{-2} \text{ s}^{-1}$ in the light phase (the neutral buoyancy irradiance, Table 2), equivalent to a daily insolation, Q_n , of 0.28 mol m^{-2} . During the summer in Lake Zürich, *P. rubescens*, by regulating its buoyancy, oscillates about the neutral buoyancy depth, z_n , where the daily insolation is equal to Q_n (Walsby *et al.*, 2004), but a consequence is that a considerable proportion of the population will always occur below z_n and will therefore receive a daily insolation of less than Q_n . There are obvious benefits, therefore, in having an E_k for enhancement of amino acid uptake (of mean $0.78 \mu\text{mol m}^{-2} \text{ s}^{-1}$) that is less than the irradiance supporting neutral buoyancy ($6.5 \mu\text{mol m}^{-2} \text{ s}^{-1}$). The mean E_k is closer to, but still less than, the compensation point for growth, namely $1.6 \mu\text{mol m}^{-2} \text{ s}^{-1}$ for *P. rubescens* strain 9303 (Fig. 2). This suggests, then, that the enhancement is relevant to the survival of those filaments left below the compensation depth.

Although the highest uptake rate, of glycine, would support only 4% of the maximum growth rate of *P. rubescens*, its contribution would be more important at lower irradiances approaching the photosynthetic compensation point. There are, however, several unknowns in quantifying this. First, it is seen that the highest rate of uptake of glycine may not by itself compensate for respiratory losses at the lowest irradiances (Fig. 2), although there are uncertainties in interpreting the negative growth rates at low irradiances (Bright & Walsby, 2000). Secondly, for organisms growing heterotrophically on amino acids, there is a cost in assimilation of the amino acid: if the minimum requirement is one ATP to drive the ABC transporter and two ATPs for assimilation into protein, this will represent about one-third of the ATP that might be generated by respiration of the deaminated amino acid product. In photoheterotrophy, photophosphorylation may provide this requirement, but only partly at irradiances approaching

zero. Thirdly, an organism with access to a mixture of amino acids may derive a higher overall growth rate from the sum of compounds obtained, but only as long as the enhancement provided does not have to be shared amongst them all. Although the light-stimulated uptake rates from mixtures of amino acids have not been investigated, it is unlikely that they would be much different: competitive interference is unlikely because the concentrations used (~ 10 nM) are about 10^3 times lower than the K_m for amino acid uptake, namely 2–40 μ M for basic and neutral amino acids and > 100 μ M for acidic amino acids (Montesinos *et al.*, 1995). Fourthly, the enhancement depends on the availability of the various amino acids in the suspending lake water. All of these factors require measurements before firm conclusions can be drawn on the quantitative importance of light-enhanced uptake of amino acids. Nevertheless, these findings support the conclusions of Zotina *et al.* (2003), that uptake of amino acids must make a significant contribution to the carbon and nitrogen economy of *Planktothrix* populations growing at very low irradiances and especially at levels slightly below the compensation point for photoautotrophy. The contribution of light-stimulated uptake to the nitrogen economy of the cell may be of particular importance as the costs of uptake of amino acids are much less than the costs of their synthesis from the products of photosynthesis.

In theory, light-stimulated uptake at very low irradiances may supplement photoheterotrophy or photoautotrophy. The strain of *P. rubescens* investigated here may, however, depend on photoautotrophy, because, while glucose and fructose are more abundant than other sugars in pelagic waters, this strain is not able to incorporate them in significant amounts (Zotina *et al.*, 2003). Moreover, it is unable to perform gluconeogenesis, and so, in the long term, the uptake of exogenous amino acids and acetate would not replace the light-driven Calvin cycle, whose products are needed for various biosynthetic pathways.

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