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Utilization of Glutamate and Glucose for Heterotrophic Growth by the Marine Pennate Diatom *Nitzschia laevis**

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Abstract

Nitzschia laevis Hustedt grew in the dark in the presence of either glutamate or glucose as substrate. Complex mixtures of yeast extract or tryptone plus lactate also supported good heterotrophic growth, while tryptone alone only supported very slow growth in the dark. The observed growth rates of *N. laevis* in the dark at different concentrations of glutamate or glucose could be accounted for by the measured uptake rates of these compounds. The affinity of the uptake systems for glutamate and glucose (K_S = 0.03 mM for each) was quite high, and similar for dark- and light-grown cells. The lack of a lag-phase when cells were transferred from photoautotrophic to heterotrophic growth conditions can be explained by the presence of uptake systems for glutamate and glucose in light-grown cells, as well as in dark-grown cells. However, the uptake capacity was generally higher in the latter than the former. *N. laevis* also took up alanine and lactate according to Michaelis-Menten kinetics, with a K_S for alanine of 0.02 mM and for lactate of 0.4 mM. Malate and glycerol were not taken up to a significant extent by the cells. Cells grown in continuous light had a doubling time of 18 h. The shortest doubling time observed in the dark on glutamate was 48 h and on glucose 24 h. Glutamate was used for heterotrophic growth with an efficiency of 43% and glucose with an efficiency of 48%.

Introduction

A number of studies have dealt with the active transport of amino acids into diatom cells, both the uptake systems involved and rates of uptake depending on external substrate concentration (Hellebust and Guillard, 1967; Hellebust, 1970; North and Stephens, 1972; Liu and Hellebust, 1974a, b; Wheeler *et al.*, 1974). These studies have shown that amino acids taken up in the light may serve as nitrogen sources under conditions of nitrogen limitation or as nitrogen and carbon sources at reduced light intensity.

Our recent work has had a different emphasis in that we have been concerned with the capability of diatom species to utilize amino acids as substrates for growth in complete darkness, in addition to their more usual autotrophic mode of nutrition. In previous investigations, we have worked with two marine pennate diatom species, each capable of utilizing the amino acid glutamate as a substrate for dark growth. One species, *Navicula pavillardi*, utilized only glutamate as a substrate (Lewin and Hellebust, 1975), while *Nitzschia angularis* utilized glutamate slowly but grew at an increased rate when glucose was present in addition to glutamate (Lewin and Hellebust, 1976). Neither of these two species was capable of growing in the dark on glucose as the only available substrate.

The present paper deals with the heterotrophic capabilities and uptake systems of a third pennate diatom species, *Nitzschia laevis*, which differs from the others in that it can utilize either glutamate or glucose as a single substrate for dark growth, thus affording the opportunity of making comparisons between amino acid uptake and utilization and sugar uptake and utilization in one and the same species.

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Materials and Methods

Organism

The strain of *Nitzschia laevis* employed came originally from a seawater tank in Woods Hole, Massachusetts, USA. It was isolated into bacteria-free culture by streaking material on an agar surface, and was designated as Strain No. 72-M (Lewin and Lewin, 1960).

Culture Medium

The cells were grown in seawater enriched with nutrients. The culture medium employed was essentially medium "f" as specified by Guillard and Ryther (1962), with addition of $Na_2SiO_3 \cdot 9H_2O$ to give a Si concentration of 10 mg l^{-1}.

Dark-Growth Experiments

The growth experiments were carried out using test-tube cultures; the culture medium with added organic compounds was prepared as described in a previous paper (Lewin and Hellebust, 1976).

Inoculum was taken from log-phase cultures growing in the light in the enriched seawater medium described above. Concentrated cell material was introduced into a previously sterilized Potter-Elvejhem tissue homogenizer. With gentle to-and-fro motion of the Teflon pestle, cell clumps were broken up to form a uniform cell suspension. This treatment has been shown to cause no harm to individual cells. Aliquots of the uniform cell suspension were then inoculated into culture medium in test tubes to give an initial cell concentration of 4×10^3 cells ml^{-1} at the beginning of the growth experiments. The cultures were grown at 20°C.

Many replicate tubes were set up for each concentration of an organic compound to be tested. Growth rates were measured by making microscopic cell counts, using a haemacytometer. Duplicate tubes of each series were removed from the culture room (every day at the beginning of an experiment and less frequently later on), and the contents of each tube were well dispersed (again using a tissue homogenizer to gently separate the cells from clumps). Cell numbers from duplicate cultures were averaged and plotted on semi-log graph paper; the slopes (growth rates) were determined from the plots.

Uptake of ^{14}C (Uniformly)-Labeled Substances

Axenic cultures and aseptic experimental techniques were used. The cells were incubated for 30 min in the dark at 20°C with about 0.1 µCi of ^{14}C-uniformly-labeled compound, with sufficient unlabeled compound added to give the final concentration desired. Detailed descriptions of methods have been presented earlier (Hellebust and Guillard, 1967; Lewin and Hellebust, 1975, 1976). All the results of uptake experiments are expressed as averages of duplicate determinations. Only the radioactive carbon in the cells was determined at the end of the 30-min incubation period. Losses due to respired ^{14}C-substrate are, therefore, not included in the estimates of rates of uptakes of the various substrates. However, experiments with ^{14}C-glucose uptake have shown that only about 10% of the ^{14}C-glucose is lost due to respiration during the 30-min uptake period (own unpublished data). The V_{max} data presented in Table 3 for the uptake of glutamate and glucose are, therefore, underestimates of the rates of total uptake, but probably not by more than about 10%.

Cell-Carbon Determinations

The carbon content of cells incubated in the dark with glutamate as a substrate for 4 days was determined by first washing the cells with fresh medium, resuspending them in distilled water, and then homogenizing them by 2-min sonication with a Biosonic (Bronwill) sonicator. Subsamples of the resulting suspension were injected into a Beckman Model 915 Total Carbon Analyzer. Corrections were made for the presence of small amounts of inorganic carbon.

Results

Substrates Capable of Supporting Growth of Nitzschia laevis in the Dark

The various compounds tested, the ranges of concentrations used in the experiments, and the resulting growth both in the light and in the dark are summarized in Table 1. Cultures in the light were run as controls for the dark cultures to determine whether or not the concentrations used were inhibitory.

Among the single compounds tested, both glutamate and glucose could serve as substrates for growth in the dark; lactate, acetate, succinate, and glycerol were not utilized. Yeast extract was capable of supporting growth in the dark, but tryptone was much less effective. Tryptone and lactate together gave better growth than tryptone by itself (Table 1).

Table 1. Organic compounds tested as possible substrates supporting heterotrophic growth of *Nitzschia laevis*. (+++: dense growth; ++: fair growth; +: trace of growth; -: no growth

Compound	Concentration	Light	Dark
Tryptone	1.0 (%)	+++	-
	0.5, 0.25 (%)	+++	+
	0.125 (%)	++	+
Yeast extract	1.0 (%)	++	+++
	0.5 (%)	+++	+++
	0.25, 0.125 (%)	+++	++
Na lactate	44, 22, 11, 5.5 (mM)	+++	-
Tryptone (0.1%)	44 (mM)	++	++
plus Na lactate	22, 11, 5.5 (mM)	+++	+++
Glucose	28 (mM)	+++	+++
	14 (mM)	+++	++
	7, 3.5 (mM)	++	++
Tryptone (0.1%)	28, 14, 7 (mM)	+++	++
plus glucose	3.5 (mM)	++	+
Na glutamate	60, 30, 15, 7.5 (mM)	+++	+++
Na acetate	120, 60, 30, 15, 7.5 (mM)	+++	-
Na succinate	30, 15, 7.5, 3.8 (mM)	+++	-
Glycerol	108, 54 (mM)	+++	+
	27 (mM)	+++	-
	13.5, 6.8 (mM)	++	-

Growth Rate in the Light

Cells kept in continuous light at an intensity of 4,000 lux had a doubling time of 18 h.

Growth Rates in the Dark with Varying Concentrations of Na Glutamate and Glucose

A positive response to glutamate could be demonstrated at a substrate concentration as low as 0.016 mM (Fig. 1). The doubling time appeared to be approximately the same, about 48 h, for the entire range of glutamate concentrations tested: 0.16 to 2 mM.

The most rapid growth rate obtained with cells growing in the dark was in the presence of 1.2 mM glucose: doubling time 24 h (Fig. 2). A positive response to glucose could be demonstrated at a substrate concentration as low as 0.0375 mM. No effect of glucose concentration on growth rate was evident over the range from 0.0375 to 0.6 mM. The doubling time for this range of concentrations was 36 h.

Absence of a Lag-Phase

As can be seen from Figs. 1 and 2, there was no apparent lag-phase when cells were taken from an exponential-phase culture grown in continuous light and used as an inoculum for cultures set up in the dark. This was true when either glu-

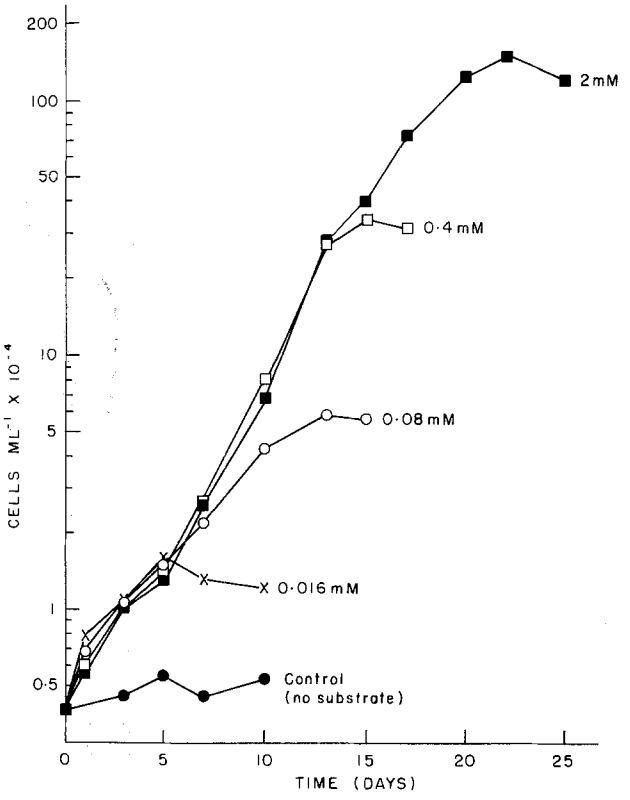

Fig. 1. *Nitzschia laevis*. Growth in the dark in the presence of different concentrations of glutamate

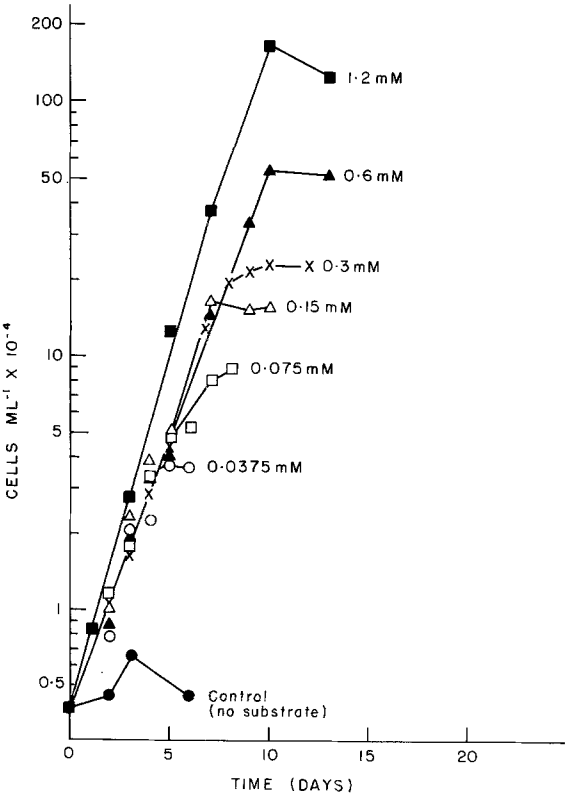

Fig. 2. *Nitzschia laevis*. Growth in the dark in the presence of different concentrations of glucose

tamate or glucose was employed as a substrate.

Efficiency of Conversion of Glutamate Carbon and Glucose Carbon to Cell Carbon

From cell yields obtained with 0.016, 0.08, 0.40, and 2.0 mM glutamate, according to Fig. 1, it could be calculated that 85, 89, 75, and 86 pg glutamate carbon were required at each of these concentrations, respectively, to produce 1 cell, resulting in an average glutamate carbon utilization of 84 pg to produce 1 cell. Analytical determinations of the actual carbon content of cells grown for several generations on glutamate in the dark gave an average value of 36 pg C $cell^{-1}$. From these two values, it can be calculated that the efficiency for converting glutamate carbon to cell carbon must have been 43% (assimilation efficiency = carbon in cell x 100/carbon taken up by cells).

Similar calculations were made from the results shown for glucose (Fig. 2). The average carbon utilized to produce 1 cell was 80 pg and the mean carbon content of cells grown on glucose in the dark was 38 pg C $cell^{-1}$, giving an assimilation efficiency of 48%.

Calculation of Uptake Rate of Glutamate Required for Observed Doubling Time of 48 h

It is possible to calculate the rate of glutamate uptake $\left(\frac{dx}{dt}\right)$ required for the observed doubling time (t_d) of approximately 48 h for cells growing in the dark on glutamate from the glutamate carbon requirement of 84 pg to produce 1 cell (x_0). The following relationships derived from the equation for exponential cell growth were used in calculation of the uptake rate:

$$\frac{dx}{dt} = x_0 \mu, \qquad (1)$$

where μ is the specific growth constant; and

$$\mu = \frac{0.693}{t_d} \qquad (2)$$

(the relation between specific growth constant and doubling time). By introducing the known values for x_0 and t_d into the above equations, it was found that $\frac{dx}{dt}$ = 2.0 x 10^{-2} pg C $cell^{-1}$ min^{-1}, or 3.3 x 10^{-10} µmole glutamate $cell^{-1}$ min^{-1}.

Calculation of Uptake Rate of Glucose Required for Observed Doubling Times of 24 and 36 h

Similarly, it is possible to calculate the rates of glucose uptake needed to produce the observed doubling times of 24 and 36 h at high (1.2 mM) and low (0.0375 to 0.6 mM) glucose concentrations. The glucose carbon requirement of 80 pg to produce 1 cell was used in both calculations. By using this value for x_0 and introducing the observed doubling times into the equations, it was found that a glucose uptake rate of 5.3 x 10^{-10} µmole $cell^{-1}$ min^{-1} was required for a doubling time of 24 h, and a rate of 3.5 x 10^{-10} µmole $cell^{-1}$ min^{-1} for a doubling time of 36 h.

Rates of Uptake of Radioactively Labeled Compounds

Cells grown in continuous light or in the dark in the presence or absence of organic compounds were in all cases able to take up amino acids, glucose, and lactate (Table 2). The rates of dark-incubated cells, however, were generally somewhat higher than rates of light-grown cells. Malate was not taken up to a significant extent by cells grown under any of these conditions, and glycerol was not taken up by light-grown cells. Glutamate and glucose were taken up at significantly higher rates than were the other compounds tested for cells grown in the light or dark, except in the case of glucose-grown cells, where the capacity for glucose uptake was reduced – probably due to excess glucose available during dark-incubation prior to the uptake experiment. Glucose-grown cells also exhibited a very high capacity for aspartate uptake.

Table 2. *Nitzschia laevis*. Rates of uptake (µmole $cell^{-1}$ min^{-1} x 10^{10}) of different substrates by cells previously grown in continuous light, or in continuous light followed by 48 h in the dark alone or in the presence of 1 mM glutamate, glucose, or lactate. -: not determined

Substrate (0.1 mM)	Continuous light	After 48 h in dark	48 h in dark with glutamate	48 h in dark with glucose	48 h in dark with lactate
Alanine	0.75	1.21	0.82	0.70	1.32
Aspartate	1.42	1.80	1.62	3.82	2.56
Glutamate	1.85	4.12	3.10	3.00	4.20
Glutamine	0.96	2.15	1.00	2.12	2.02
Isoleucine	0.16	0.21	0.30	0.48	0.36
Glucose	2.26	4.21	4.80	1.60	4.44
Lactate	0.43	0.86	0.67	0.30	1.28
Malate	0.03	0.02	0.05	0.08	0.08
Glycerol	0.00	-	-	-	-

Kinetic Constants for Uptake of Organic Compounds

Determinations of kinetic constants for uptake of glucose, glutamate, alanine, and lactate at different substrate concentrations by cells incubated in the dark for 48 h in the absence of organic substrates were made from the plots shown in Fig. 3 with the following results: (a) for glucose uptake, $K_S = 0.03$ mM and $V_{max} = 5.3 \times 10^{-10}$ µmole cell^{-1} min^{-1}; (b) for glutamate uptake, $K_S = 0.03$ mM and $V_{max} = 3.7 \times 10^{-10}$ µmole cell^{-1} min^{-1}; (c) for alanine uptake, $K_S = 0.02$ mM and $V_{max} = 1.6 \times 10^{-10}$ µmole cell^{-1} min^{-1}; (d) for lactate uptake, $K_S = 0.4$ mM and $V_{max} = 1.8 \times 10^{-10}$ µmole cell^{-1} min^{-1}. Similar kinetic studies with light-grown cells yielded essentially the same K_S values as for dark-incubated cells, but lower maximum uptake values.

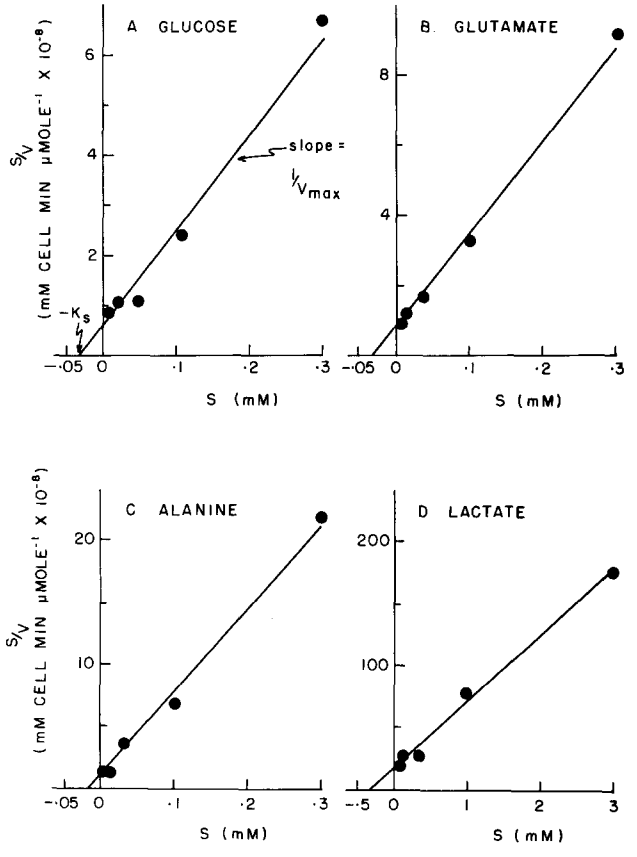

Fig. 3. *Nitzschia laevis*. Rate of uptake (V) of substrate measured at different substrate concentrations (S), and S/V plotted against S (Dowd and Riggs, 1965). Cells were incubated in the dark without organic substrates for 2 days prior to uptake experiment. K_S: half-saturation constant

Discussion

The heterotrophic capabilities of *Nitzschia laevis* with respect to utilization of glutamate and glucose can now be compared with data obtained with the two other marine pennate diatom species studied previously (Lewin and Hellebust, 1975, 1976). (See Table 3 for comparison of all three species.) The results show that each of the three species was able to utilize glutamate for heterotrophic growth and that the doubling time in the dark was always longer than for cells growing autotrophically. The efficiency of conversion of glutamate carbon to cell carbon was essentially the same for each of the three species (41 to 43%). The three species differed from one another quite dramatically, however, in their heterotrophic capabilities with respect to glucose utilization: *Navicula pavillardi* was incapable of utilizing glucose; *Nitzschia angularis* only took up glucose and utilized it as a substrate when it was supplied in the presence of glutamate (or when in the presence of tryptone, alanine, aspartate, or asparagine); *Nitzschia laevis* could grow when in the presence of glucose as a single substrate. Glucose carbon was converted to cell carbon more efficiently than was glutamate carbon.

For all three of these species, there was no observed lag phase before growth took place after transferring cells from photoautotrophic conditions to heterotrophic conditons, indicating that no induction period was required for synthesis of necessary transport systems. The finding that cells of *Nitzschia laevis* grown in the light were capable of taking up glutamate and glucose confirms the presence of transport systems in light-grown cells and explains the reason why no lag phase was observed. Other diatom species (*Cyclotella cryptica* and *Cylindrotheca fusiformis*) studied earlier by us behaved differently, in that there was a lag phase of about 2 days in the dark while the cells developed the transport systems that were required for substrate uptake (Lewin, 1963; Lewin and Hellebust, 1970; Hellebust, 1971; Hellebust and Lewin, 1972, 1977). These major differences between species confirm our previous statements about the considerable variability and high degree of specificity shown by the diatom species that we have investigated thus far (see Hellebust and Lewin, 1977, for discussion). It is not obvious why *N. laevis* did not grow well on tryptone alone in the dark, since this commercial preparation consists mainly of an enzymatic hydrolysate of casein and, therefore, also contains glutamate. The stimulation of growth on tryptone in the

Table 3. Comparison of glutamate and glucose utilization between 3 species of marine pennate diatoms

	Navicula pavillardi Hustedt[a]	*Nitzschia angularis* (Grun.) Perag.[b]	*Nitzschia laevis* Hustedt
Doubling time in light (4000 lux)	24 h	10 h	18 h
Doubling time in dark with glutamate as substrate	70 h	78 h	48 h
Efficiency of conversion of glutamate C to cell C	41%	43%	43%
Kinetic parameters for glutamate uptake			
K_S	0.018 mM	0.02 mM	0.03 mM
V_{max}	7.0×10^{-10} µmole cell^{-1} min^{-1}	3.0×10^{-10} µmole cell^{-1} min^{-1}	3.7×10^{-10} µmole cell^{-1} min^{-1}
Doubling time in dark in presence of glucose alone	Not utilized	Not utilized	24 h (1.2 mM) 36 h (0.0375-0.6 mM)
Doubling time in dark in presence of glucose + glutamate	70 h (as with glutamate alone)	42 h	Not determined
Doubling time in dark in presence of glucose + tryptone	66 h (as with tryptone alone)	16 h	Not determined
Efficiency of conversion of glucose C to cell C	-	50%	48%
Kinetic parameters for glucose uptake			
K_S	-	0.03 mM	0.03 mM
V_{max}	-	2.2×10^{-10} µmole cell^{-1} min^{-1}	5.3×10^{-10} µmole cell^{-1} min^{-1}

[a] Data from Lewin and Hellebust (1975).

[b] Data from Lewin and Hellebust (1976).

dark by the simultaneous presence of lactate is in agreement with the finding that *N. laevis* has a saturable uptake system for lactate, however, the affinity of this uptake system is fairly low (K_S = 0.4 mM).

The rate of growth on glutamate (doubling time about 48 h), corresponding to a required rate of glutamate uptake of 3.3×10^{-10} µmole cell^{-1} min^{-1}, can be accounted for by the observed maximum rates of glutamate uptake of 1.85 to 4.20×10^{-10} µmole cell^{-1} min^{-1} (see earlier and Table 2). Similarly, the rates of growth on glucose (doubling times 24 to 36 h), corresponding to required rates of glucose uptake of 3.5 to 5.3×10^{-10} µmole cell^{-1} min^{-1}, can be accounted for by the observed maximum rates of glucose uptake of 1.60 to 4.8 $\times 10^{-10}$ µmole cell^{-1} min^{-1} (see earlier and Table 2).

The relatively high affinities of the uptake systems for glutamate (K_S = 0.03 mM) and glucose (K_S = 0.03 mM) explain why the observed growth rates in the dark on these substrates were independent of substrate concentration down to 0.02 mM glutamate (Fig. 1) or 0.04 mM glucose (Fig. 2). The only exception was an increased growth rate at the highest glucose concentration (1.2 mM; Fig. 2), which may possibly be due to a significant diffusion component to glucose uptake at this high substrate concentration.

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