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Release of dissolved free amino acids during a bloom of Thalassiosira rotula

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Abstract

Three large plastic enclosures (3–4 m^3) were anchored in the outer harbour of Helgoland (German Bight) and filled with natural seawater which was filtered free of algae. The enclosed water bodies were enriched with inorganic nutrients and inoculated with the diatom *Thalassiosira rotula*. During the growth of the algae diurnal changes in concentration of dissolved free amino acids (DFAA) occurred. The periodic concentration changes of individual amino acids with a low carbon to nitrogen ratio showed significant interrelationships with the partly synchronous devisions of the diatoms. From the exponential to the stationary phase the carbon to nitrogen ratio of DFAA shifted to higher values pointing at an adaptation of the organisms to the decreasing inorganic nitrogen source. During the bloom amino acids relatively rich in nitrogen were mainly excreted but by the end of the growth amino acids with a higher carbon content predominated. At phases of high photosynthetic activity the organisms probably reduced the high osmotic pressure by exudation of DFAA.

Introduction

In marine plankton ecosystems the instable dissolved organic compounds play an important role in biochemical processes (WILLIAMS 1975). These substances are in a steady state between release and uptake by the various members of a plankton community. Two of the most important release mechanisms are: 1. Loss due to the decay of marine plankton (SMITH et al. 1977, SOROKIN 1977), 2. Release from living organisms (FOGG 1977, LARSSON and HAGSTRÖM 1979). The release of organic matter by healthy phytoplankton is still a matter of discussion (SHARP 1977).

Three simultaneous experiments (F, G, H) with enclosed cultures of the diatom *Thalassiosira rotula* were conducted in the outer harbour of Helgoland. Long flexible plastic bags with a diameter of 1 m and a depth of 4–5 m were filled with natural seawater which had been previously filtered to remove phyto- and zooplankton. After nutrient enrichment and inoculation with *T. rotula* cells a partly synchronous development of diatoms occurred under natural nutrient and cell concentrations (BROCKMANN et al. 1977). During the exponential algae growth a significant increase in concentration of dissolved free amino acids (DFAA) was observed in all three experiments (HAMMER and EBERLEIN 1981). The question whether these substances are released by living diatoms is to be discussed in this report.

Material and methods

Experimental procedures as well as sample preparation and amino acid analysis have been described elsewhere (BROCKMANN et al. 1977,HAMMER and EBERLEIN 1981).

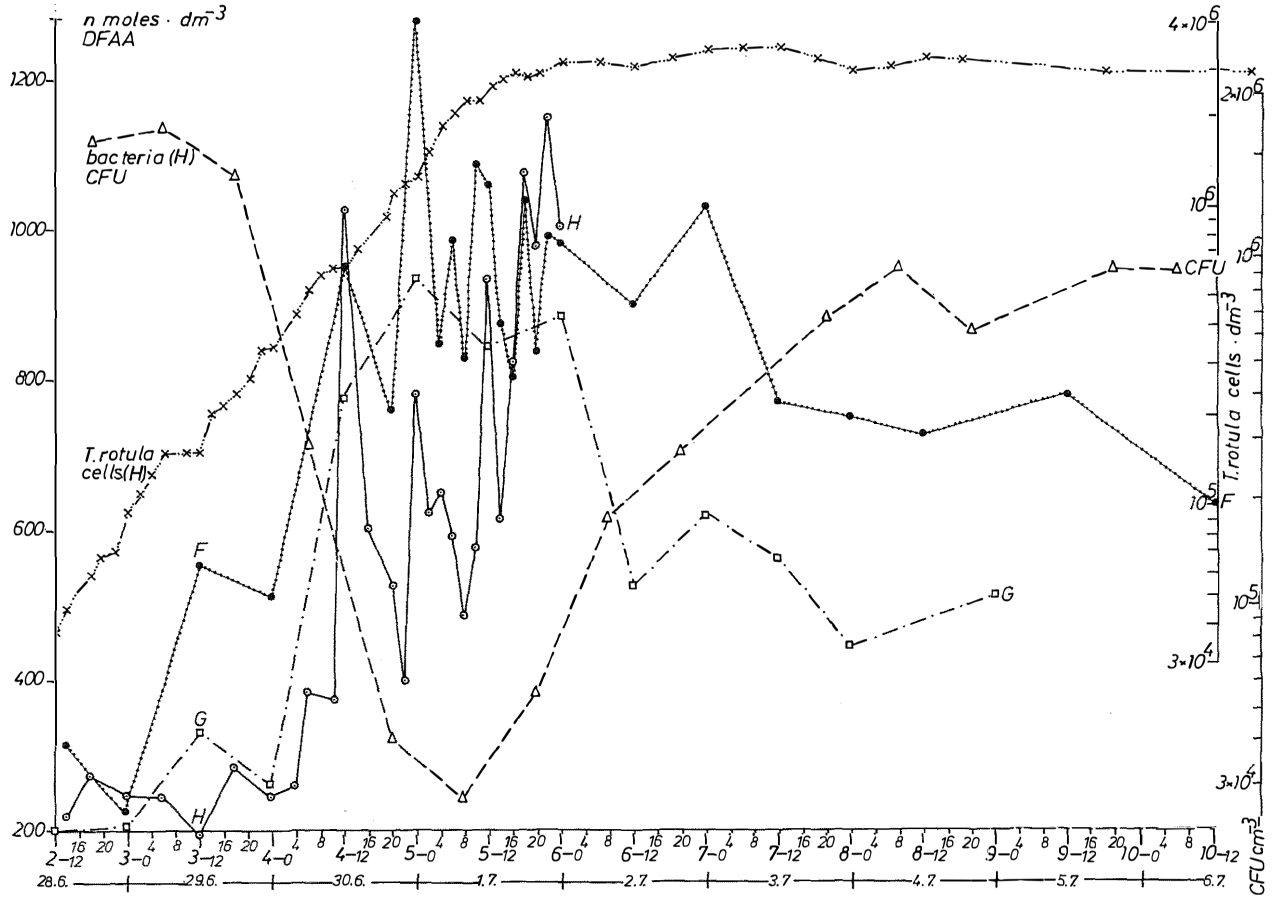

Figure 1

Development of cell-counts of *Thalassiosira rotula* and the heterotrophic colony forming bacteria units (CFU) in culture H (logarithmic representation). F,G,H: Concentration of total dissolved free amino acids (DFAA)

Short-term variability of cell-counts and DFAA concentration were extracted from the data according to the following method. To calculate the trend the concentrations of DFAA and the logarithmic values of cell-counts were smoothed by the running average method:

$$\bar{X}(t_i) = (X(t_{i-2}) + X(t_{i-1}) + X(t_i) + X(t_{i+1}) + X(t_{i+2}))/5$$

where t_{i-2}, t_{i-1}, t_i, t_{i+1}, t_{i+2} are 5 successive sampling times.

Short-term variations were calculated by the difference between temporary original data and corresponding trend values. These variations were related to the respective trend values (= 100 %) in percent.

Results and discussion

On the fourth and fifth day during the exponential growth phase of *Thalassiosira rotula* the DFAA concentration increased by nearly 400 n moles · dm^{-3} · day^{-1} from 200 n moles · dm^{-3} to about 1000 n moles · dm^{-3} (Fig. 1). At this time the cell development reached a stationary phase and the concentration of amino acids began to decrease. The reason for this may be an increase of heterotrophic bacteria (Fig. 1), or – considering reduced nitrate/nitrite concentrations – limited liberation by the algae. Since phytoplankton can use amino acids as a source of nitrogen (ANTIA et al. 1975, WHEELER et al. 1974, 1977), *T. rotula* competed with the bacteria for the remaining amino acids, because the possible uptake rates at these diatom concentrations were in the same range as those reported for bacterial uptake (SEPERS 1977).

During field studies (Fladen Ground Experiment 1976) HAMMER et al. (1980) made similar observations on natural phytoplankton populations: During the first spring phytoplankton bloom a similar strong liberation of DFAA also occurred for two days during the exponential growth phase, and also here the release period was followed by a rapid decrease in amino acid concentration.

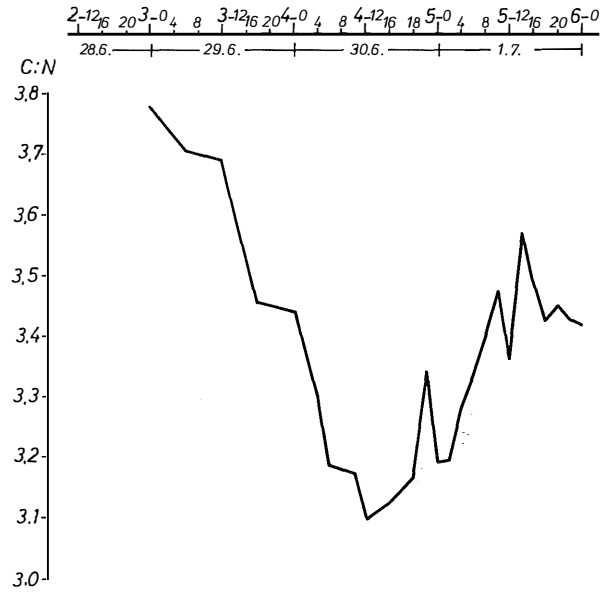

Figure 2

Atomic carbon to nitrogen ratio (C:N) of dissolved free amino acids in culture H

Because of the high frequency of sampling (in 2 hours intervals) culture H was chosen for a detailed study of the liberation mechanisms occurring during the exponential growth phase. At the beginning of the bloom the C:N ratio (atomic weight) of total DFAA fell from 3.8 to 3.1, but rose again to 3.5 towards the end of the growth phase (Fig. 2). The high initial ratio was probably the result of a postbloom situation because the bags were filled at the end of May. Also during the first phytoplankton bloom of the Fladen Ground Experiment (HAMMER et al. 1980) the bulk of DFAA was released with a C:N ratio of 3.1. At this point of time in the enclosed monoculture as well as in the open sea with natural mixed plankton, amino acids rich in nitrogen were excreted increasingly, for example glycine and serine. Both these amino acids are synthesized in short metabolic pathways from the Calvin cycle during photo-synthetic activity, or via glycolate from photo-respiration processes (TOLBERT 1977). On account of an enrichment in the intracellular pool they can be released as bypass reaction products, as they were exuded at times of strong photosynthetic activity (for example serine, Fig. 3).

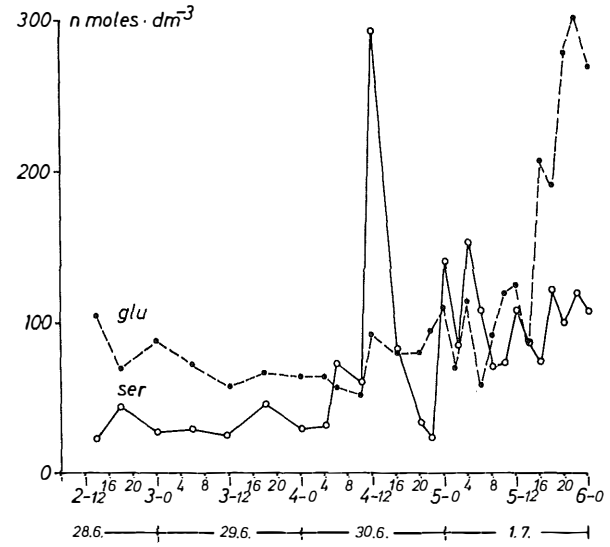

Figure 3

Development of serine and glutamic acid during the exponential growth phase of *T. rotula* in culture H

In contrast glutamic acid, for example, showed only small concentration changes during the growth phase (Fig. 3); an increase only occurred at the beginning of the stationary phase. BOHLING (1972) found glutamic acid in the combined amino acid fraction in 10 times higher concentrations than in the monomeric fraction. Therefore the increase during the stationary phase could be explained by an accelerated proteolytic activity. Another source possibly is a high intracellular glutamic acid pool from the transaminase and glutamine synthetase pathways. The lack of inorganic nutrients reduced the rates in biosynthesis (BROCKMANN et al. 1977), and with the beginning decay of cells this amino acid was liberated into the medium during the late growth phase. An increase in glutamic acid concentration during the stationary phase was also observed in the open sea (Fladen Ground Experiment) when the

phytoplankton spring bloom stopped. At the same time the release of other DFAA with a relatively high carbon-content also increased as can be seen by the augmenting C:N ratio of the DFAA as well in the culture bags as during the Fladen Ground Experiment.

The high dynamics in DFAA concentration changes as observed in culture H could be separated into a trend development and a short-term variation (Fig. 4). The DFAA trend showed a significant increase in concentration only during daylight. This main diurnal liberation coincided in time with the uptake of nitrate/nitrite (HAMMER and EBERLEIN 1981) and is, together with the short-term variation (discussion see below), a strong argument for a connection between excretion and biosynthetic processes of the diatoms.

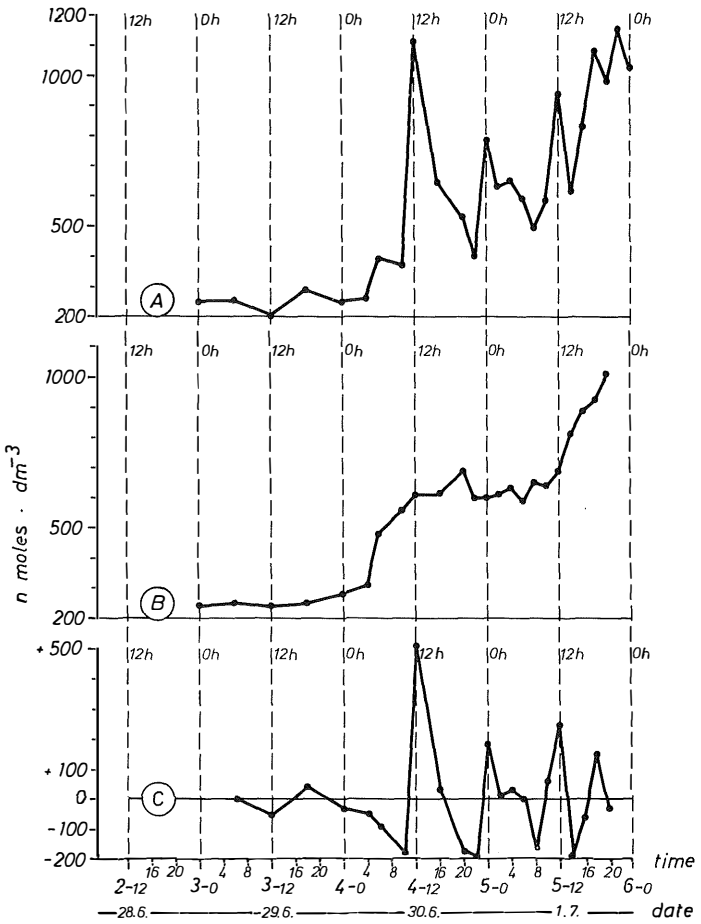

Figure 4

Concentration of DFAA during exponential growth of *Thalassiosira rotula* (A), the trend separated from short-term changes (B), and the short-term variability (calculated from the difference A–B) in culture H (C)

During the exponential growth phase of phytoplankton the release of organic compounds by the decay of cell material should be negligible. Considerable short-time changes in biological parameters at this stage can disturb the establishment of a stable steady state of release and uptake, so that interrelationships could become recognizable. If the excretion of organic substances depends upon the physiological state of organisms, then, in the event of a synchronization of these states, the relationships must become even clearer. During the growth phase the development of cell-counts in culture H showed periodic changes (Fig. 1), and gave an indication of partial synchronization of cell division activity. In Fig. 5, these periodic changes in cell density as well as in lysine concentration are displayed as percentage deviation from the trend. At midday and midnight reduced cell division activity was observed. At these times more lysine was liberated. During division photosynthesis can fall to 1/6 (FOGG 1975). This phenomenon may be one reason for the low lysine concentrations during

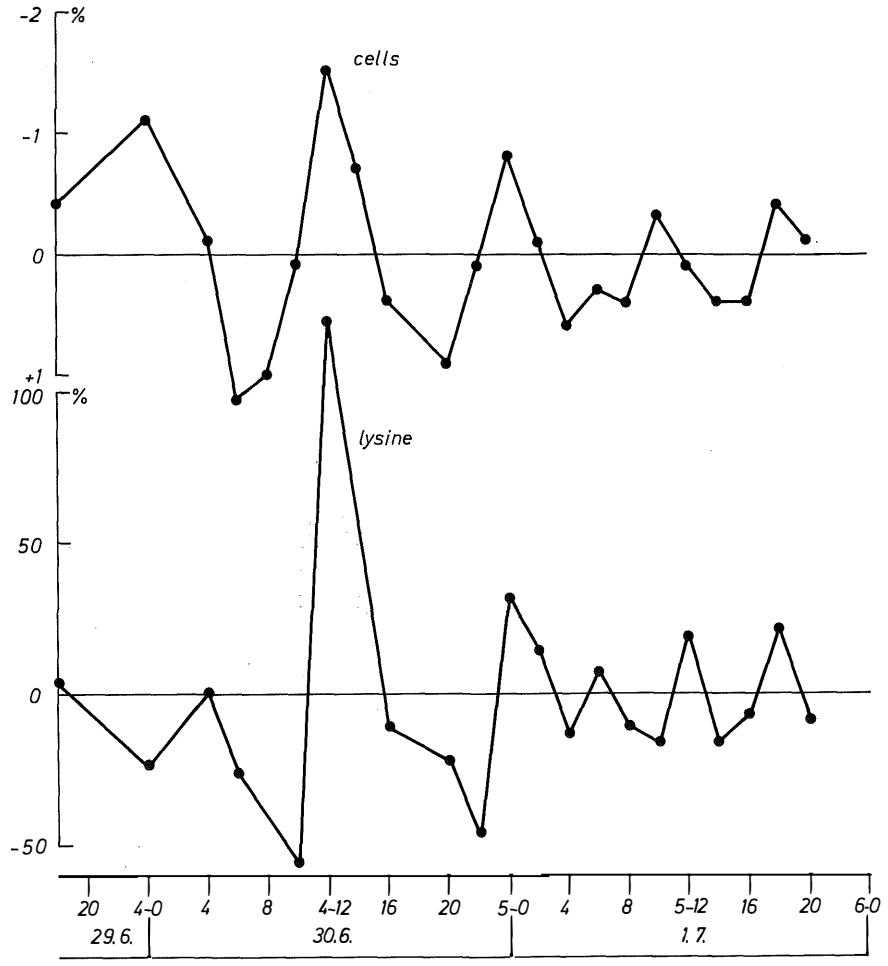

Figure 5

Periodic changes in concentration of cells and lysine as deviation from the trend in percent (changes in cell concentration presented inversely)

high division activity. Similar observations were made by GIMMLER (1969, cited by FOGG 1975). In synchronized cultures he found an increasing excretion of glycolate during photosynthesis and a concentration decrease during cell division. The observed concentration changes could have been caused by a relatively constant uptake of amino acids by bacteria. When there was only low de novo synthesis of dissolved free amino acids during the division phase of diatoms, the uptake by bacteria would dominate, resulting in a decrease of amino acid concentrations.

Besides lysine, periodic changes of six other amino acids were significantly negatively correlated with cell number cycles (Table 1). It is obvious that these amino acids were relatively rich in nitrogen. These findings support the thesis that the decrease in the C:N ratio for total DFAA (Fig. 2) is the result of an intensified excretion of nitrogen rich amino acids during phases of high biosynthetic activity. Amino acids with a lower nitrogen content showed no significant correlations. The boundary lies at a C:N ratio of 4.0. The proportion of amino acids with a greater C:N ratio to the total DFAA only amounts to 20–30 %. For their liberation other mechanisms possibly exist, as has been described for glutamic acid.

Table 1

Linear and rank correlations between periodic changes in concentrations of individual amino acids and cells

amino acid	C : N ratio	n	linear correlation		rank correlation	
			coefficient	significance	coefficient	significance
ala	3	20	− 0.4806	95%	− 0.4523	97.5%
β-ala	3	14	− 0.6207	98%	− 0.4659	95.0%
asp	4	20	− 0.5964	99%	− 0.4959	97.5%
glu	5	20	− 0.3993	–	− 0.3500	–
gly	2	20	− 0.4452	95%	− 0.2613	–
ile	6	20	− 0.3688	–	− 0.2229	–
leu	6	20	− 0.3603	–	− 0.2177	–
lys	3	20	− 0.6088	99%	− 0.4643	97.5%
orn	2,5	20	− 0.4899	95%	− 0.4741	97,5%
phe	9	12	− 0.1760	–	− 0.2395	–
ser	3	20	− 0.6056	99%	− 0.3861	95.0%
thr	4	16	− 0.4873	–	− 0.2882	–
tyr	9	17	− 0.2713	–	− 0.3395	–
val	5	20	− 0.3473	–	− 0.2214	–
total	–	20	− 0.6253	99%	− 0.4653	97.5%

The data presented clearly show a linkage of the DFAA concentration with physiological states of *T. rotula*. It was shown that under nearly natural conditions the greater part of the dissolved free amino acids was liberated by physiologically ,,healthy'' cells. During photosynthesis the intracellular amino acid pool is probably so strongly augmented that, to balance the osmotic pressure of the cell, excretion into the surroundings became probable. If the amino acids had not been released, their additional concentration within the cells can be calculated to about 70 m moles · dm^{-3}

for a maximum increase. This is within the range where osmotic compensation becomes noteworthy, as the cells of *T. rotula* are very sensitive to higher osmotic pressure relative to the medium (SCHÖNE 1974).

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