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Ion Composition of Unicellular Marine and Fresh-Water Algae, with Special Reference to *Platymonas subcordiformis* Cultivated in Media with Different Osmotic Strengths

G.O. Kirst

Institut für Botanik der Technischen Hochschule, Fachbereich Biologie, Schnittspahnstr. 10, D-6100 Darmstadt, Federal Republic of Germany

Summary. Ion compositions (K^+, Na^+, Mg^{2+}, Ca^{2+}, Cl^-, phosphate) of the euryhaline algae, *Platymonas subcordiformis, Chlorella salina,* grown in media with a salinity range from 0.1 to 0.6 M NaCl and of the fresh-water algae, *Ankistrodesmus braunii* and *Scenedesmus obliquus,* were compared. Enhancement of ion concentrations with increasing salinity in *Platymonas* was attributed largely to decreasing cell volume. In both the euryhaline algae, Na^+ and – partially – Cl^- content per cell increased significantly with rising salinity. The contents per cell of the other ions were not affected. Considering the relevance of ions and mannitol (*Platymonas*) and proline (*Chlorella*) as osmotically active particles, it was found that the ions alone maintained osmotic balance with low external salinity. With increasing salinity the organic compounds contributed up to 20–30% of the cellular solute potential. The main cation, K^+, was the main contributor to the osmotic balance; the accumulation of organic compounds as well as of Na^+ and Cl^- contributes further to the ability of the algae to adapt to high salinity. The results confirm the hypothesis of low Cl^- concentrations in nonvacuolate cells in comparison to vacuolate cells.

Introduction

The scale-bearing microalgae of the genus *Platymonas* (Prasinophyceae) are widely distributed in the phytoplankton in coastal waters, estuaries, and tide pools. Organisms living in these environments must be able to adept to changes of salt concentrations.

The mannitol content of *Platymonas subcordiformis* is closely correlated with the external osmotic pressure (Kirst, 1975b). However, the endogenous mannitol concentrations proved to be insufficient to maintain the observed osmotic equilibrium. It is likely that ions also contribute to osmotic homeostasis.

Ochromonas malhamensis, a fresh-water unicellular alga, responds to osmotic stresses by changing the internal isofloridoside content and the K^+ concentra-

tion (Kauss et al., 1975). The relation between osmotic regulation and ion content was more intensively investigated with coenocytic (giant) and with thalloid (multicellular) algal cells grown in marine and fresh-water environments as well (reviews: Hellebust, 1976; Cram, 1976), while investigation of additional participation of endogenous organic solutes in these species was neglected. It is generally accepted that these large, walled cells adjust their cellular osmotic pressure so as to maintain almost constant turgor pressures under various external salinities. Which of the main ions – Na^+, K^+, and Cl^-, – plays a role in regulating the internal osmotic pressure varies with the species concerned and also depends on the natural growth habitat (Kesseler, 1965; Bisson and Gutknecht, 1975).

The role played by ions in maintaining osmotic balance in *Platymonas* was investigated by studying the changes in the ion composition of the algae. External osmotic pressure was varied by changing the NaCl concentration in the culture media, while the concentrations of the other ions were held constant. In contrast to the well-investigated giant and multicellular algae, data on ion content of microalgae are scarce (Gutknecht and Dainty, 1968; Raven, 1976). Thus for comparison it seemed suitable to investigate a further salt-tolerant microalga, *Chlorella salina* (Chlorococcales), and two fresh-water species: *Ankistrodesmus braunii* and *Scenedesmus obliquus* (both Chlorococcales), well known from studies on uptake of metabolically important ions such as phosphate (Simonis and Urbach, 1973).

Materials and Methods

Platymonas subcordiformis Hazen, strain 161-1 a from the culture center at Göttingen, West Germany, and *Chlorella salina* Kufferath, strain LB 211/25 from the culture center at Cambridge, England, were cultivated in a medium containing 1000 ml Knop's solution (Bold, 1942), 1 ml A–Z solution (microelements according to Hoagland), 50 ml soil decoct, and sufficient NaCl to give culture solutions with 0.1, 0.2, 0.3, 0.4, 0.5, or 0.6 M NaCl (*Chlorella* in 0.2 and 0.5 M NaCl only). The concentrations of the main ions in the media were (in mM): K^+ 8.20 ± 0.24; Ca^{2+} 7.20 ± 0.15; Mg^{2+} 1.53 ± 0.02; PO_4^{3-} 3.50 ± 0.03.

The cultures were illuminated with 12 'white' fluorescent lights (14,000 lx) in a light-dark regime of 14:10 h; the temperature of the water bath was 25° C, and the algal suspensions were bubbled with air +2% CO_2.

Ankistrodesmus braunii Brunnth., strain 202-7c, and *Scenedesmus obliquus* Turp., strain 276–3d (both from the culture center at Göttingen, West Germany) were cultivated under conditions (see above) similar to those described by Ullrich (1972) and Findenegg (1974). Ion concentrations in the media were (in mM): *Ankistrodesmus:* K^+8; Na^+13; Ca^{2+}0.1; Mg^{2+}1; Cl^- O; PO_4^{3-}4. *Scenedesmus:* K^+ 5.3; Na^+O; Ca^{2+} 4.2; Mg^{2+} 1; Cl^-0; PO_4^{3-}1.8.

The cells were harvested in the logarithmic phase of growth usually one day (*Ankistrodesmus, Scenedesmus*) and 4 days (*Platymonas, Chlorella*) after inoculation. They were washed twice by centrifugation and resuspended in fresh culture solutions. To avoid contamination of the cell walls of the Chlorococcales and of the theca of *Platymonas* with ions, the following washing procedure was usually applied: the cell suspension was spun down, the supernatant discarded, and the pellet resuspended and washed for 10 min in the same volume of ice-cold isotonic sucrose solution containing HCl (0.05 N). Two to three additional washes with ice-cold isotonic sucrose solution without HCl were sufficient to remove HCl and to give reproducible results. Because of possible precipitation of calcium phosphate (hydroxylapatite) in cell walls and thecae (Frank, 1962), three modifications of the first step of the washing procedure were tested: (1) sucrose solution with 0.1 N HCl; (2)

sucrose solution with 0.05 N HCl, followed by a washing with sucrose solution containing 40 mM EDTA-Na_2; (3) sucrose solution with 40 mM EDTA-Na_2.

The data presented in this paper were obtained with algae subjected to the first washing procedure. The other washes tested did not significantly alter the results. When EDTA-Na_2 was used, the Na^+ content was overestimated due to the EDTA-Na_2 salt, which could not readily be removed with successive washes in sucrose solution. In particular the values of the Ca^{2+} estimations remained almost constant after washings with HCl (0.05–0.1 N) containing solutions. However, if the algae (all species tested) were washed without HCl or solutions with EDTA-Na_2 only, the Ca^{2+} concentrations proved to be unusually high, indicating precipitations of calcium carbonate or calcium phosphate in the cell wall and the theca, respectively (Frank, 1962). The algal pellets were resuspended in known volumes of bidestilled water (usually 10 ml). Aliquots (0.5 ml) were used for mannitol estimation (*Platymonas;* Kirst, 1975b). Aliquots (3 ml) were extracted for 30 min in a water bath at 100°C. After centrifugation, the supernatant of the extract was diluted and used for determination of ion concentrations: Na^+ and K^+ with the flame photometer (Eppendorf, Hamburg, West Germany); Ca^{2+} and Mg^{2+} with the atomic absorption spectrophotometer 448 (Beckman, Munich, West Germany); Cl^- with the chloride titrator (American Instruments Co. Inc., Silver Spring, Maryland, USA).

Total phosphate content of the cells was determined after digestion of samples (0.5) in a mixture of equal parts of concentrated H_2SO_4 and 70% $HClO_4$, neutralization with conc. NH_3, and dilution to a known volume (10 ml). Inorganic phosphate and polyphosphate (together with organic phosphate compounds) were fractionated from algal cells by the following simplified extraction: 2 ml samples were treated for 15 min with ice-cold TCA (5% final concentration). After centrifugation the supernatant contained orthophosphate and acid-soluble stable organic phosphates (according to Kuhl, 1974). The pellet was hydrolyzed with 1 ml 1 N HCl, 100°C, for 7 min, yielding orthophosphate from polyphosphates and organic phosphates. The extracts (total phosphate, TCA and HCl extracts) were analyzed for phosphate according to the method of Gerlach and Deuticke (1963).

Cell counts were made in a hemocytometer; cell volume was determined as described by Kirst (1977). All measurements have been corrected for extracellular volume ('inulin-space'). The nonosmotic volume was not considered.

Preparations of thecae in *Platymonas* cultures for analysis were made according to Lewin (1958). The suspensions of thecae were subjected to the washing and the extraction procedures described previously for algal cells. The results were calculated on a dry weight basis.

Results

Ion Concentration of Platymonas

The ion concentrations of *Platymonas* changed depending on the external NaCl concentration (Figs. 1 and 2).

As in many other algae and in higher plants, K^+ was the cation with the highest internal concentration. Compared with its external concentration (8.2 mM), it was accumulated about 14-fold, i.e., a concentration of ca. 110–120 mM in algae cells grown in media with lower salinities (0.1–0.3 M NaCl). With increasing external NaCl concentrations (0.4–0.6 M NaCl), the K^+ concentration of the cells rose to 210 mM (Fig. 1), i.e., an accumulation of about 25-fold. The Na^+ concentrations were always less than the external concentration but there was a significant increase from 10 mM to 100 mM as external NaCl was increased from 0.1 to 0.6 M (Fig. 1). Ca^{2+} and Mg^{2+} concentrations, like that of K^+, remained almost constant at low external salinities, showing a slight increase from ca. 50 mM to 75 mM (Ca^{2+}) and 12 mM to 26 mM (Mg^{2+}) with increasing salinity (Fig. 1).

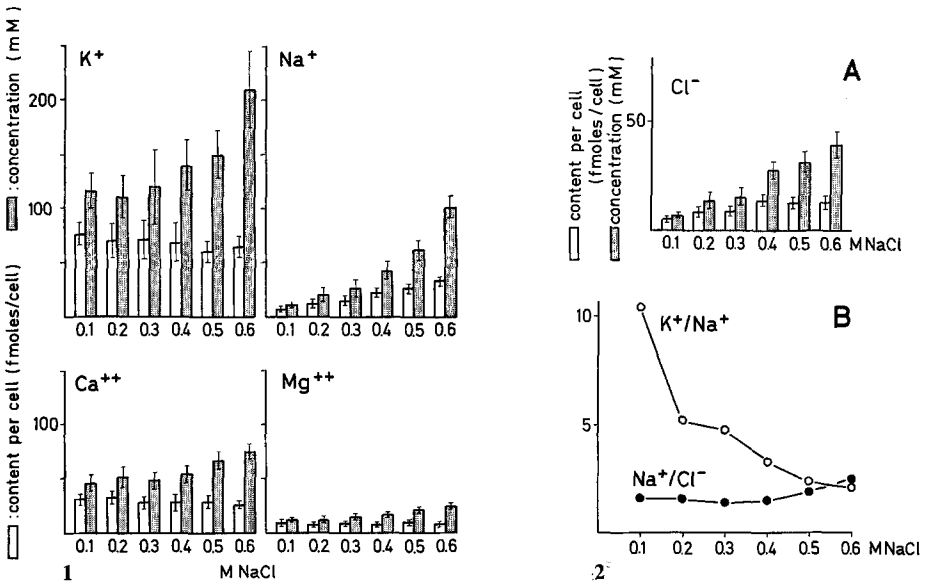

Fig. 1. Concentration and content of cations in cells of *Platymonas subcordiformis* cultivated in media with various NaCl concentrations. Means of data from 12 to 20 cultures for each NaCl concentration, and SDs are indicated. Concentrations of the ions in the media: K^+ 8.2 ± 0.24 mM; Ca^{2+} 7.2 ± 0.15 mM; Mg^{2+} 1.53 ± 0.02 mM

Fig. 2. A Concentration and content of Cl^- in cells of *Platymonas subcordiformis*. For further details see Figure 1. **B** Ratios of ion concentrations in *Platymonas*, dependent on the external NaCl concentration

Considering the absolute ion content per cell (Fig. 1, content in fmol/cell), the data indicate clearly that K^+, Ca^{2+} and Mg^{2+} contents were not influenced by the NaCl concentration of the media, which is in contrast to the Na^+ content. Thus the increased concentrations of these cations in relation to increasing external NaCl concentration were due to the decreasing cell volume, the dependence of which on external osmotic pressure was recently investigated (Kirst, 1977). However, the increased Na^+ concentration could not be attributed solely to the decreased cell volume; there was also an increase in the absolute content. Hence the increase in the concentration of Na^+ with increasing salinity was greater than for other cations, especially K^+. The ratio of K^+/Na^+ dropped from about 10 at 0.1 M NaCl in the medium to ca. 2 at 0.6 NaCl (Fig. 2B).

Although Cl^- was the main anion in the media, its concentration in *Platymonas* was fairly low, on the average less than one-tenth of the external concentration (Fig. 2A). Unlike the cations, the Cl^- content (fmol/cell) increased with increasing NaCl concentration at the lower salinities (0.1–0.4 M NaCl) and remained constant with further rise of external NaCl (0.4–0.6 M). Therefore the increase in concentration of Cl^- in *Platymonas* in media of higher salinities was largely an effect of volume shrinkage. In cultures grown in low salinities (0.1–0.4 M NaCl) the increase of Cl^- concentration paralleled that of Na^+;

Table 1. Concentration of ions (μmol/mg dry weight) in the thecae of *Platymonas* grown in 0.2 and 0.5 M NaCl medium. External ion concentrations, see Figure 1. Means of measurements from 3 preparations of each salinity

M NaCl of medium	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	P_{total}
0.2	0.082	traces	0.583	0.046	traces	0.03
0.5	0.077	traces	0.528	0.037	traces	0.018

the Na^+/Cl^- ratio was constant at 1.5, which is close to a 1:1 stoichiometry (Fig. 2B). In higher salinities (0.5 and 0.6 M NaCl), however, the Na^+/Cl^- ratio changed to at least 2.5, which is significantly different from a 1:1 stoichiometry.

The effect of the salinity on content and concentration of phosphate in *Platymonas* was not clear due to the high variability between cells from separate cultures. The mean values of the content decreased slightly with increasing NaCl concentration, thus compensating for the effect of volume shrinkage: phosphate concentration remained constant.

The data on cultures from 0.2 and 0.5 M NaCl media are presented in Table 3. The results for algae in the other salinities were of the same order. The concentration of total phosphate from 100 to 120 mM was distributed approximately equally between the TCA fraction (about 60 mM, most of the orthophosphate), and the hydrolysate with HCl (about 50 mM, most of the polyphosphate and the stable organic phosphate; see "Materials and Methods"). This distribution was independent of the external salinity.

Ion Content of the Thecae

The theca, the cell 'envelope' of *Platymonas*, consists of galactose, arabinose, and galacturonic acid (Lewin, 1958; Gooday, 1971). The counterion of galacturonic acid is Ca^{2+} (Manton et al., 1973). To ascertain the contributions of Ca^{2+} and other ions to the composition of the theca, preparations of thecae were tested, and the results are summarized in Table 1. The Ca^{2+} content of the theca was 2–3% (w/w). This is in good agreement with the value of 9.5% (w/w), which was based on the simplifying assumption that the theca consists of galacturonic acid only. Na^+, Mg^{2+}, and phosphate were also detected in measurable amounts: the Na^+ and Mg^{2+} contents were less then 0.2% and the phosphate content was about 0.3% (w/w) of the theca. The concentrations of the other ions, K^+ and Cl^-, were too low to be estimated with the methods used here.

Chlorella salina

As a further example of a euryhaline unicellular alga, *Chlorella salina* was also investigated. It was cultivated under conditions identical to those used

Table 2. Concentration (mM) and content (fmol/cell) of ions in *Chlorella salina* cells, grown in 0.2 and 0.5 M NaCl medium and in two fresh-water species, *Ankistrodesmus braunii* and *Scenedesmus obliquus*. External concentrations of the ions are in parentheses and given with values of the internal concentrations

Species	Na^+		K^+		Ca^{2+}		Mg^{2+}		Cl^-	
	fmol/cell	mM	fmol/cell	mM	fmol/cell	mM	fmol/cell	mM	fmol/cell	mM
Chlorella salina	2.6 ±0.6	6.5 ±1.3	23.7 ±3.8	69.2 ±6	12.2 ±1.4	28.6 ±4.2	4.9 ±1.3	9.4 ±2.5	4.5 ±1	9.3 ±2.5
(0.2 M NaCl)		(200)		(8.2)		(7.5)		(1.53)		(200)
Chlorella salina	5.0 ±0.6	12.4 ±2.9	18.1 ±2	51 ±9	7.8 ±1.8	23.3 ±5.3	4.9 ±1.7	8.0 ±0.8	4.4 ±0.6	13 ±3
(0.5 M NaCl)		(500)		(8.2)		(7.5)		(1.53)		(500)
Ankistrodesmus braunii fresh-water	0.9 ±0.08	5.1 ±0.4 (13)	6.9 ±1.1	36.1 ±6.8 (8)	0.8 ±0.1	4.9 ±0.7 (0.1)	1.3 ±0.2	7.6 ±0.5 (1.0)	0.3 ±0.09	1.7 ±0.3 (0)
Scenedesmus obliquus fresh-water	0.4 ±0.01	3.2 ±0.2 (−)	10.9 ±1.4	91 ±12 (5.3)	0.4 ±0.09	3.6 ±0.5 (4.2)	1.7 ±0.5	9.1 ±1 (1.0)	0.24 ±0.04	2.1 ±0.3 (0)

for *Platymonas*. The ion composition is given in Table 2. In contrast to *Platymonas, Chlorella* possesses a proper cell wall; thus changes in cell volume and of ion concentration with differing external salinity were smaller than those observed in *Platymonas*. In both salinities tested the ion composition did not change greatly. The content and the concentration of the main cation K^+ were lowered slightly with increasing salinity. The same was true for Ca^{2+} and Mg^{2+}.

However, the differences between the two cultures (0.2 and 0.5 M NaCl) were not significant. In contrast the increase of Na^+ was significant while Cl^- remained almost constant.

The phosphate composition (Table 3) was similar to that described for *Platymonas*. The total phosphate concentration of about 130 mM was distributed equally between the TCA and the HCl fractions.

Fresh-Water Algae

The ionic compositions of the two fresh-water species, *Ankistrodesmus* and *Scenedesmus,* were very similar, although the external ion concentrations of the fresh-water media differed (Tables 2 and 3). Ca^{2+}, Mg^{2+}, and Cl^- concentrations were identical. Na^+ concentration was higher in *Anistrodesmus,* perhaps due to the external Na^+ concentration, while K^+ concentration in *Scenedesmus* exceeded that of *Ankistrodesmus*. The composition of phosphate and phosphate compounds was more uniform (Table 3). In general the total phosphate content

Table 3. Concentration (mM) and content (fmol/cell) of phosphate in *Platymonas* and *Chlorella* cells, grown in 0.2 and 0.5 M NaCl, respectively, *Ankistrodesmus* and *Scenedesmus*

Species	P_{total}		P_{TCA}		P_{HCl}	
	fmol/cell	mM	fmol/cell	mM	fmol/cell	Mm
Platymonas subcordiformis (0.2 M NaCl)	73±27	117±31 (3.5)	39±7	63±16	34±5	54±12
Platymonas subcordiformis (0.5 M NaCl)	61±19	93±23 (3.5)	32±8	60±18	23±6	48±16
Chlorella salina (0.2 M NaCl)	57±8	142±28 (3.5)	26±4	64±11	25±5	61±11
C. salina (0.5 M NaCl)	48±9	129±30 (3.5)	27±4	75±13	24±3	65±14
Ankistrodesmus braunii (fresh-water)	11±1.8	72±6 (4.0)	1.3±0.4	8.6±1.4	6.5±1.7	43.3±5
Scenedesmus obliquus (fresh-water)	14.2±2.5	63±18 (1.8)	0.9±0.15	7.4±1.7	6±1.2	49.5±8.6

P_{total} total phosphate content after digestion in $H_2SO_4/HClO_4$; P_{TCA}=TCA extract of algal cells, containing most of the orthophosphate; P_{HCl}=hydrolysate of algal cells with 1 N HCl after TCA extraction, containing most of the polyphosphate. External phosphate concentrations are indicated in parentheses with P_{total} data

was lower compared with that of the marine species. This was attributed to a concentration of about 8 mM phosphate in the TCA fraction, which is much less than that found in *Platymonas* and *Chlorella salina.* The phosphate concentration of the hydrolysate, however, was similar to the concentration of the euryhaline monocells.

Discussion

Comparison of the Ion Composition

It is assumed that *Platymonas* and *Chlorella* were osmotically adapted to their environment because of the constant growth rates in the range of 0.1 to 0.6 M NaCl in the media tested (Kirst, 1975a). As was to be expected, the marine and the fresh-water species differed in their ion compositions. As already emphasized, in *Platymonas* the increase in ion concentration was accounted for largely by the shrinkage of the cell volume, caused by the decrease in water potential with increasing external NaCl concentration. This is in contrast to the walled cells of *Chlorella salina,* which exhibited more constant ion concentrations when cultures grown in low and high saline media were compared (Table 2).

In both species the increased Na^+ concentration was also due to an accumulation of Na^+ per cell. With low salt conditions (0.1 and 0.2 M NaCl), however, the NaCl concentration did not exceed Na^+ concentrations found in fresh-water algae, *Ankistrodesmus* and in *Scenedesmus,* although the external Na^+ concentration, even in 'low' saline media, is much higher than that in fresh-water (Table 2). *Chlorella pyrenoidosa* had the ability to exchange internal K^+ for Na^+ in the absence of external K^+ (Shieh and Barber, 1971; Barber and Shieh, 1973). These ions appear to compete for the same carrier, whose external site has a three or four times higher affinity for K^+ over Na^+. The observed increase in cell Na^+ as K^+/Na^+ in the medium decreased in *Platymonas* and *Chlorella salina* could be explained if these algae possess a similar carrier.

Some giant-celled and thalloid marine algae are known to respond to changes in external salinity by large changes in their ionic composition. The K^+ concentration is greatly affected in *Valonia* (Gutknecht, 1968) and in *Chaetomorpha* (Kesseler, 1964; Zimmermann and Steudle, 1971), where a turgor pressure-controlled K^+-ion pump system regulates the ion uptake. In *Codium,* Cl-concentration seems to be regulated in response to external osmotic pressure, while K^+ concentration appears to be affected solely by external K^+ concentration (Bisson and Gutknecht, 1975). In the experiments reported here, the external K^+ concentration in the medium was constant throughout; the K^+ content of the cells did not change (*Platymonas:* Fig. 1, *Chlorella:* Table 2). These algae thus behave similarly to *Codium.*

Of course the vacuolated cells of the giant and thalloid algae cannot be compared directly with the unicellular microalgae. Considering the ion composition of various unicellular algae, the data of which are summarized from the literature in Table 4, no clear distinction can be made between marine or fresh-water species concerning K^+ concentration. Values for other ions are too scarce to take into account.

Comparing data reported in this paper and in Table 4 with those reviewed for giant and thalloid algal cells (Gutknecht and Dainty, 1968; Raven, 1975; 1976), the generalization is justified that the Cl^- concentration of microalgae is very low. This is true for nonvacuolate cells while the Cl^- content of vacuolate cells is similar to or even exceeds that of the medium. (The following definition is used here: 'Nonvacuolate' cells possess no large vacuole, but little ones distributed in the cell, especially in hypotonic medium; 'vacuolate' cells possess a large central vacuole filling the main part of the cell volume.) The unicellular *Noctiluca milaris* exhibits a large vacuole, and the Cl^- concentration is close to the external Cl^- concentration (Table 4) while the cells of a thalloid marine red alga, *Porphyra perforata,* with no central vacuole, were found to contain 81 mM Cl^- compared with 590 mM in the medium (Eppley, 1958). This is consistent with the finding that in vacuolate algal cells the net influx of Cl^- is, on the average, about 43 to 85 times that of nonvacuolate cells (Raven, 1975).

Further interactions of ion uptake and external ion concentration are described for phosphate. Despite the large variability, the results reported here indicate clearly that the TCA-soluble fraction, i.e., orthophosphate and/or soluble organic phosphate compounds, is higher in marine than in fresh-water species

Table 4. Concentrations of ions in unicellular alga species. External concentrations are in parentheses

Species	Concentration (mM)								Reference
	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	Phosphate			
						total	TCA	HCl	
Cyanophyceae									
Anacystis nidulans	4.6	149			2.8				Dewar and Barber (1973, 1974)
(fresh-water)	(12.7)	(20.5)			(0.2)				
Dinophyceae									
Amphidinium carteri						29–39			Humphrey (1973)[a]
(marine)						(0.07–0.65)			
Nocticula miliaris	487.3	24.1	6.6	2.9	500				Nawata and Sibaoka (1976)
(vacuolar sap)	(440)	(8.0)	(9.5)	(48.2)	(510)				
(marine)									
Chrysophyceae									
Ochromonas malhamensis		10.6–30.2							Kauss et al. (1975)[a]
(fresh water)		(0.185)							
Bacillariophyceae									
Cyclotella cryptica		120–270							Liu and Hellebust (1976)
(marine)		(1–12)							
C. meneghiniana	11–19				9–16				Schobert (1974)
(marine)	(225–525)				(260–560)				
Cylindrotheca closterium						29–39			Humphrey (1973)[a]
(marine)						(0.07–0.65)			
Phaeodactylum tricornutum		140							Overnell (1975)
(marine)		(6.7)							
Chlorophyceae									
Ankistrodesmus braunii						98–145			Ullrich (1972)[a]
(fresh-water)						(4)			
Chlamydomonas	180–300	30–80			70				Okamoto and Suzuki (1964)
spec. (halophil)	(1710)	(0)			(1710)				
Chlorella pyrenoidosa	4.3	142							Shieh and Barber (1971), Barber (1968)
(fresh-water)	(4.0)	(3.0)							
	1.1	108			1.3				
	(1.0)	(6.5)			(1.0)				
						100–125	6–10		Jeanjean (1976)[a]
						(?)			
Scenedesmus obtusiusculus	18–25	62–65							Erdei and Cseh (1971)
(fresh-water)	(0)	(6.1)							
S. spec.	0.8–3.2								Kylin (1966)[a]
(fresh-water)	(1.0)								

[a] Data are recalculated and related to concentration (mM)

Dry weight ≙20% fresh weight – unless other values were given in the literature cited

(Table 3). In the fresh-water red alga *Porphyridium aerugineum* and in the marine red alga *Porphyridium cruentum* the phosphate uptake is enhanced by Na^+ (Ullrich-Eberius and Yingchol, 1974). In the marine species the increase is up to 100 times the rate in the presence of K^+. The ecologic significance of making traces of phosphate available for algae in the presence of high Na^+ concentrations is pointed out by Ullrich-Eberius and Yingchol (1974).

The concentration and content of Ca^{2+}, which plays an important role in the regulation of cell permeability, were higher in the marine species. However, the question arises of whether Ca^{2+} is distributed more in the cell wall and the theca, respectively, or in the cytoplasm. A comparison between *Ankistrodesmus* and *Scenedesmus* (similar cell wall composition: Chlorococcales) indicates an almost equal Ca^{2+} concentration, although external Ca^{2+} concentrations of the media differed remarkably (Table 2). Thus it is suggested for *Chlorella* that the high Ca^{2+} values were not due to the higher external concentration or to precipitations in the cell wall (which would be removed by the washes – see Materials and Methods) but represented internal concentrations. In *Platymonas* a great part of the measured Ca^{2+} is located in the theca (Table 1; Gooday, 1971; Manton et al., 1973). Because of the impossibility of estimating the fraction of Ca^{2+} in the theca and in the cell, no decision could be made for a correction of the Ca^{2+} concentration given in Figure 1. On the other hand it would be sufficient as a first approximation to assume that the Ca^{2+} concentration of *Chlorella* is similar to the real internal Ca^{2+} concentration of *Platymonas* because of the similar cell size and the identical conditions of cultivation. Thus the Ca^{2+} concentration of *Platymonas* would be about 30 mM, increasing with decreasing cell volume, and the remaining 20 mM would be due to the theca. The measurable amounts of Na^+ and Mg^{2+} in the theca may be attributed to Donnan forces arising from fixed negative charges of the galacturonic acid and other compounds of the theca. These effects are small, especially for univalent ions, in solutions of high ionic strength (sea water); therefore K^+ could not be detected in the theca, while the detection of Na^+ can be explained by the high external concentration.

Calculation of the Osmotic Balance

It is generally accepted that ions are not equally distributed over the cell but are accumulated in certain compartments, e.g., Mg^{2+} as a component of chlorophyll and distributed in the matrix of the chloroplasts, phosphate as a metabolically important ion is combined with organic compounds in cell organelles or stored as polyphosphate in special vacuoles. Thus, when considering ions as osmotically active particles K^+, Na^+, and Cl^- will contribute significantly to osmotic equilibrium while Ca^{2+}, Mg^{2+}, and phosphate will do so to a limited extent only. Estimation of the nonosmotic volume, including starch grains, cell wall (theca), oil vacuoles, etc., should quantify the restricted distribution of these ions. Although the nonosmotic compartments obviously contain ions, the concentrations are assumed to be low compared to those

in the cytoplasm and hence can be neglected for a first approach. The nonosmotic volume of *Platymonas* proved to be constant in the range of 0.1 to 0.6 M NaCl in the media (Kirst, 1977). The percentage, however, increases from 45% to about 80% of the total cell volume with increasing salinity due to the shrinkage of the cells. Having this in mind, an appraisal can be made of whether external and internal osmolarity is balanced. As already described, the mannitol content of *Platymonas* is related to external osmotic pressure. In algal cells grown in 0.1 and 0.2 M NaCl media, the mannitol concentration is about 6 to 8 mM (Kirst, 1975b); this is low compared to internal ion concentrations. Calculated on a precentage basis, mannitol contributes about 5% of the osmotically active particles, i.e., in low salinity environments, the internal ions explain osmotic adaptation. However, with increasing salinity, the percentage of mannitol increases to at least 20–30% of the osmotically active particles (0.5 and 0.6 M NaCl).

In *Chlorella salina* the proline level is affected by the external salinity, rising from ca. 6 mM (0.2 M NaCl medium) to about 30 mM (0.5 NaCl medium) (Treichel and Kirst, unpublished results). This is consistent with findings of Hellebust (*Chlorella* sp., marine; 1976) and of Setter and Greenway (*Chlorella vulgaris,* salt tolerant strain; private communication). Concerning the participation of proline to osmotic equilibrium the same is true as was described previously for mannitol in *Platymonas.*

Without any doubt, K^+ is the main osmotically active solute in both *Platymonas* and *Chlorella* because of its high internal concentration. K^+ and associated anions (whose natures remain unclear; phosphate and proteins may contribute to balancing the charges) constitute the basic solute components of the cell; changes in endogenous organic compounds such as mannitol and proline, and in ions such as Na^+ and Cl^- reflect the ability of the algae to adapt to higher salinities. Further investigations of the behavior of the ion concentrations and mannitol content of *Platymonas* after osmotic shocks support this suggestion. In addition there is evidence in the literature that polyols like glycerol and mannitol as well as proline work as protective agents for enzymes exposed to high ion concentrations in the cytoplasm of halophytes (Borowitzka and Brown, 1974).

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References

Barber, J.: Measurement of the membrane potential and evidence for active transport of ions in *Chlorella pyrenoidosa.* Biochim. biophys. Acta (Amst.) **150**, 618–625 (1968)

Barber, J., Shieh, Y.L.: Sodium transport in Na^+-rich *Chlorella* cells. Planta (Berl.) **111**, 13–22 (1973)

Bisson, M.A., Gutknecht, J.: Osmotic regulation in the marine alga, *Codium decorticatum.* I. Regulation of turgor pressure by control of ionic composition. J. Membr. Biol. **24**, 183 (1975)

Bold, H.C.: The cultivation of algae. Bot. Rev. **8**, 70–138 (1942)

Borowitzka, L.J., Brown, A.D.: The salt relations of marine and halophilic species of the unicellular green alga, *Dunaliella*: The role of glycerol as a compatible solute. Arch. Microbiol. **96**, 37–52 (1974)

Cram, W.J.: Negative feed-back regulation of transport in cells. The maintenance of turgor, volume and nutrient supply. In: Encyclopedia of plant physiology, Vol. 2, Transport in plants (U. Lüttge, M.G. Pitman, eds.). Berlin-Heidelberg-New York: Springer 1976

Dewar, M.A., Barber, J.: Cation regulation in *Anacystis nidulans*. Planta (Berl.) **113**, 143–155 (1973)

Dewar, M.A., Barber. J.: Cl-transport in *Anacystis nidulans*. Planta (Berl.) **117**, 163–172 (1974)

Eppley, R.W.: Sodium exclusion and potassium retention by the red marine alga, *Porphyra perforata*. J. gen. Physiol. **41**, 901–911 (1958)

Erdei, L., Cseh, E.: Changes in the K and Na content of *Scenedesmus obtusiusculus*. Ann. Univ. Sci. Budap. Rolando Eötvös Nomina Sect. Biol. **13**, 79–90 (1971)

Findenegg, G.R.: Beziehungen zwischen Carboanhydraseaktivität und Aufnahme von HCO_3^- und Cl^- bei der Photosynthese von *Scenedesmus obliquus*. Planta (Berl.) **116**, 123–131 (1974)

Frank, E.: Vergleichende Untersuchungen zum Ca^{2+}, Phosphat- und K^--Haushalt von Grünalgen. I. Ca^{2+}, K^+ und Phosphat bei *Hydrodictyon* und *Sphaeroplea* in Abhängigkeit von der Belichtung. Flora **152**, 139–156 (1962)

Gerlach, E., Deuticke, B.: Eine einfache Methode zur Mikrobestimmung von Phosphat in der Papierchromatographie. Biochem. Z. **337**, 477–479 (1963)

Gooday, G.W.: A biochemical and autoradiographic study of the role of the Golgi-bodies in thecal formation in *Platymonas tetrathele*. J. expl Bot. **22**, 959–971 (1971)

Gutknecht, J.: Salt transport in *Valonia:* Inhibition of potassium uptake by small hydrostatic pressures. Science **160**, 68–70 (1968)

Gutknecht, J., Dainty, J.: Ionic relations of marine algae. Oceangr. Mar. Biol. Ann. Rev. **6**, 163–200 (1968)

Hellebust, J.A.: Osmoregulation. Ann. Rev. Plant Physiol. **27**, 485–505 (1976)

Humphrey, G.F.: Photosynthetic and respiratory rates, and phosphorous content of algae grown at different phosphate levels. Spl. Publ. mar. biol. Ass. India 74–79 (1973)

Jeanjean, R.: The Effect of metabolic poisons on ATP level and on active phosphate uptake in *Chlorella pyrenoidosa*. Physiol. Plant. **37**, 107–110 (1976)

Kauss, H., Lüttge, U., Krichbaum, R.M.: Änderung im Kalium- und Isofloridosidgehalt während der Osmoregulation bei *Ochromonas malhamensis*. Z. Pflanzenphysiol. **76**, 109–113 (1975)

Kesseler, H.: Die Bedingungen einiger anorganischer Komponenten des Seewassers für die Turgorregulation von *Chaetomorpha linum*. Helgol. wiss. Meeresunters. **10**, 73–90 (1964)

Kesseler, H.: Turgor, Osmotisches Potential und ionale Zusammensetzung des Zellsaftes einiger Meeresalgen verschiedener Verbreitungsgebiete. Bot. Gothoburgensia **3**, 103–111 (1965)

Kirst, G.O.: Wirkung unterschiedlicher Konzentrationen von NaCl und anderen osmotisch wirksamen Substanzen auf die CO_2-Fixierung der einzelligen Alge *Platymonas subcordiformis*. Oecologia (Berl.) **20**, 237–254 (1975a)

Kirst, G.O.: Beziehungen zwischen Mannitkonzentration und osmotischer Belastung bei der Brackwasseralge *Platymonas subcordiformis* (Hazen). Z. Pflanzenphysiol. **76**, 316–325 (1975b)

Kirst, G.O.: The cell volume of the unicellular alga, *Platymonas subcordiformis:* Effect of the salinity of the culture media and of osmotic stresses. Z. Pflanzenphysiol. **81**, 386–394 (1977)

Kuhl, A.: Phosphorus. In: Algal physiology and biochemistry (W.D.P. Stewart, ed.). Oxford-London-Edinburgh-Melbourne: Blackwell 1974

Kylin, A.: Uptake and loss of Na, Rb and Cs in relation to an active mechanism for extrusion of Na in *Scenedesmus*. Plant Physiol. **41**, 579–584 (1966)

Lewin, R.A.: The cell walls of *Platymonas*. J. gen. Microbiol. **19**, 87–90 (1958)

Liu, M.S., Hellebust, J.A.: Effects of salinity and osmolarity of the medium on amino acid metabolism in *Cyclotella cryptica*. Canad. J. Bot. **54**, 938–948 (1976)

Manton, J., Oates, K., Gooday, G.: Further observations on the chemical composition of thecae of *Platymonas tetrathele* West (Prasinophyceae) by means of the X-ray microanalyser electron microscope (EMMA). J. exp. Bot. **24**, 223–229 (1973)

Nawata, T., Sibaoka, T.: Ionic composition and pH of the vacuolar sap in marine dinoflagellate *Noctiluca*. Plant and Cell Physiol. **17**, 265–272 (1976)

Okamoto, H., Suzuki, Y.: Intracellular concentration of ions in a halophilic strain of *Chlamydomonas*. I. Concentration of Na, K and Cl in the cell. Z. allg. Mikrobiol. **4**, 350–357 (1964)
Overnell, J.: Potassium and photosynthesis in the marine diatom *Phaeodactylum tricornutum* as related to washes with sodium chloride. Physiol. Plant. **35**, 217–224 (1975)
Raben, J.A.: Algal cells. In: Ion transport in plant cells and tissue (D.A. Baker, J.L. Hall, eds.), Amsterdam: North-Holland 1975
Raven, J.A.: Transport in algal cells. In: Encyclopedia of plant physiology, Vol. 2, Transport in plants (U. Lüttge, M.G. Pitman, eds.). Berlin-Heidelberg-New York: Springer 1976
Schobert, B.: The influence of water stress on the metabolism of diatoms: I. Osmotic resistance and proline accumulation in *Cyclotella meneghiniana*. Z. Pflanzenphysiol. **74**, 106–120 (1974)
Shieh, Y.L., Barber, J.: Intracellular sodium and potassium concentrations and net cation movements in *Chlorella pyrenoidosa*. Biochem. biophys. Acta (Amst.) **233**, 594–603 (1971)
Simonis, W., Urbach, W.: Photophosphorylation in vivo. Ann. Rev. Plant Physiol. **24**, 89–114 (1973)
Ullrich, W.R.: Untersuchungen über die Raten der Polyphosphatsynthese durch die Photophosphorylierung bei *Ankistrodesmus braunii*. Arch. Mikrobiol. **87**, 323–339 (1972)
Ullrich-Eberius, C.J., Yingchol, Y.: Phosphate uptake and its pH dependence in halophytic and glycophytic algae and higher plants. Oecologia (Berl.) **17**, 17–26 (1974)
Zimmermann, U., Steudle, E.: Effects of potassium concentration and osmotic pressure of sea water on the cell-turgor pressure of *Chaetomorpha linum*. Mar. Biol. **11**, 132–137 (1971)

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