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Contribution of phytoplankton and bacterial cells to the measured alkalinity of seawater

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Abstract

We report the first direct measurements of the contribution of phytoplankton and bacterial cells to the measured alkalinity of unfiltered seawater. Phytoplankton and bacterial cells suspended in seawater make a significant contribution to the measured alkalinity of unfiltered seawater; their contribution is probably next to that of borate ion in most seawater samples. This nonnegligible contribution of particulate organic matter to the measured alkalinity is due largely to the presence of negatively charged surface groups on the phytoplankton and bacterial cells that react with protons during titration with hydrochloric acid. The contribution of organic particles to the measured alkalinity of unfiltered seawater could potentially be an important factor when evaluating the accuracy of presently available carbonate thermodynamic models using at-sea carbon system parameters that include measured alkalinity.

The concept of the total alkalinity of seawater has evolved in tandem with improvements in analytical techniques and in our understanding of the acid–base processes that occur in seawater (Dickson 1992). Seawater is not a simple solution containing only carbonic and boric acids; it often contains additional bases that react with protons during the titration of seawater with acid. The total alkalinity of seawater (A_T) is thus defined as the number of moles of hydrogen ions equivalent to the excess of bases (proton acceptors) formed from weak acids with pK $\geq$ 4.5 at 25°C and zero ionic strength over acids (proton donors) with pK $<$ 4.5 in 1 kg of seawater sample (Dickson 1981),

$$A_T = [HCO_3^-] + 2[CO_3^{2-}] + [B(OH)_4^-] + [OH^-] + [HPO_4^{2-}] + 2[PO_4^{3-}] + [SiO(OH)_3^-] + [NH_3] + [HS^-] + \cdots - [H^+]_F - [HSO_4^-] - [HF] - [H_3PO_4] - \cdots \quad (1)$$

where all concentrations are micromoles per kilogram (μmol kg^{-1}) and $[H^+]_F$ is the concentration of free hydrogen ions. For most seawater samples, HCO_3^-, CO_3^{2-}, and $B(OH)_4^-$ are the most important bases that contribute to A_T. In open ocean water, the concentrations of NH_3 and HS^- are usually so low that these species make an insignificant contribution to A_T; however, their contributions might be significant in anoxic water. The ellipses represent additional minor acid or base species that are either unidentified or are present in insignificant quantities. The definition of A_T presented in Eq. 1 applies strictly to the homogeneous solution phase and does not include any organic (e.g., phytoplankton or bacterial cells) or inorganic (e.g., $CaCO_3$) particles.

When evaluating titration data for determining A_T values, analytical methods currently in use depend on an accurate chemical model of seawater. This model is based on the understanding of the chemical compositions of seawater contributing to A_T (see Eq. 1) and the associated equilibrium constants; however, this current chemical model of seawater does not adequately treat particulate organic matter, which can cause an error in the calculation of A_T. In particular, in estuarine or biologically productive marine environments, large amounts of organic particles (OM) are suspended in seawater that might contribute significantly to the measured alkalinity of unfiltered seawater (ΔA_{T-OM}).

During the titration of unfiltered seawater with hydrochloric acid, phytoplankton and bacterial cells are potentially important contributors to the measured alkalinity. Such a contribution of organic particles to the measured alkalinity has traditionally been thought be insignificant and, thus, has been ignored. In this study, we show that the presence of phytoplankton and bacterial cells in seawater can contribute significantly to the alkalinity measured on unfiltered seawater samples. We also demonstrate the importance of accounting for ΔA_{T-OM} when evaluating the accuracy of carbonate thermodynamic models.

Methods

Culture of phytoplankton—For measurements of zeta potential and phytoplankton cell surface alkalinity, strains of four phytoplankton species (*Prorocentrum minimum, Heterosigma akashiwo, Skeletonema costatum,* and *Prorocentrum micans*) were collected from the bay of Jangmok in Korea (34.6°N, 128.5°W). The phytoplankton were maintained in stock cultures for several years at the South Sea Institute of the Korea Ocean Research and Development Institute. Each phytoplankton species was incubated at 20°C in a 15-liter polyethylene bottle containing natural seawater (salinity = 30) that had been passed through a 0.2-μm filter and autoclaved. To initiate bloom development, the same

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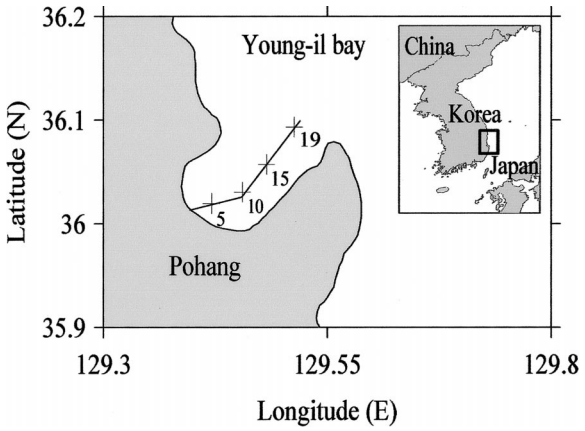

Fig. 1. Sampling locations (1 through 19) in the Young-Il bay of Korea adjacent to the East Sea.

amount of a $NaNO_3$–Na_2HPO_4 mixture was added into each of four containers, yielding initial concentrations of 883 μmol kg^{-1} nitrate, 363 μmol kg^{-1} phosphate, and 107 μmol kg^{-1} silicate (added only to the *S. costatum* culture; Guillard 1975). In addition, to expedite bloom development, the batch cultures were exposed over a 10-d period to continuous light with an incident photon flux density of 15 μmol photons m^{-2} s^{-1}. To minimize the possibility of sampling bias because of inhomogeneity within the batch cultures, the seawater in each culture was mechanically mixed before sampling.

Field seawater samples—To quantify the contribution of phytoplankton and bacterial cells to the measured alkalinity, 19 seawater samples containing varying concentrations of particulate organic matter were collected from the Young-Il bay on the southeast coast of the Korean peninsula (Fig. 1). Phytoplankton populations in Young-Il bay waters are higher than those typically found in open ocean waters.

Determination of surface charge of phytoplankton and bacterial cells—The cell surface charge of phytoplankton and bacteria can be inferred from the zeta potential, which was measured with a zeta meter (Otsuka, ELS-8000; Poortinga et al. 2002). This method determines the electrophoretic mobility (μ_E) of biological cells by measuring the velocity (V_{BP}) of biological particles suspended in seawater under the influence of an applied electric potential (E).

$$\mu_E = V_{BP}/E \tag{2}$$

The Helmholtz–Von Smoluchowski equation then relates the zeta potential (ζ) to μ_E (Kleijn and van Leeuwen 2000),

$$\zeta = 4\pi\mu_E(\eta/\varepsilon) \tag{3}$$

where η is the bulk viscosity (0.89 g cm^{-1} s^{-1} at 25°C) and ε is the dielectric permittivity (78.5 at 25°C) of water. The ζ is the electric potential at the hypothetical interface between a stagnant liquid layer surrounding the particle surface and the bulk liquid moving relative to the stagnant layer during electrophoresis. This interface is usually assumed to be located within several nanometers of the solid particle surface.

In this study, we measured electrophoretic mobility of the four cultured phytoplankton species (*P. minimum, H. akashiwo, P. micans,* and *S. costatum*) as a function of pH at a constant ionic strength of approximately 0.7. To measure cell mobility, samples containing phytoplankton cells were transferred to a quartz cell of a zeta meter thermostatically controlled at 25.0°C ± 0.1°C. An electric potential of 10 V was then applied across the cell. This applied electric field is lower than the potential (80 V) that is usually applied when measurements are performed in Milli-Q water. In seawater, electrolysis of NaCl generates Cl_2. This Cl_2 interferes with the movement of biological particles suspended in the seawater and, therefore, influences zeta potential measurements. To improve reproducibility of the zeta potential measurements, 0.7 mol L^{-1} NaCl was used instead of Mill-Q water to rinse the quartz cell. Triplicate measurements of the zeta potential for each phytoplankton species were performed at each pH. The precision of the measured zeta potentials was on average about ±6 mV for the four phytoplankton strains. A more detailed description of the experimental apparatus can be found elsewhere (James 1991).

Alkalinity determination—The alkalinity values of all seawater samples were determined with a potentiometric titration system similar to one developed by Millero et al. (1993). The titration system used in this study consists of a Metrohm 765 Dosimat titrator and an Orion 720A pH meter controlled by a personal computer. The acid titrant was placed in a water-jacketed burette and the seawater sample in a water-jacketed sample container, with both solutions maintained at a constant temperature of 25.0°C ± 0.1°C with a constant-temperature bath. The titration and recording of the volume of the acid added and the electromotive force of the electrodes was carried out with custom software written in Q-Basic on a computer with RS-232 interfaces. In a typical titration, the electromotive force generated was recorded with a combination glass–reference pH cell after the readings became stable (1 s = ±0.09 mV). Sufficient acid was then added to change the voltage by a preassigned increment (13 mV). In contrast to the delivery of hydrochloric acid in fixed volume increments, this method generates data points that are approximately evenly spaced across the entire range of titration. A full titration consists of approximately 25 points and takes approximately 20 min. The uncertainties in the measured alkalinity associated with the HCl concentration (±0.0001 mol L^{-1}), the mass of the hydrochloric acid delivered (±0.0005 g), and the mass of the seawater sample (±0.01 g) are ±1, ±0.5, and ±0.1 μmol kg^{-1}, respectively. Together, these uncertainties yield a probable error of approximately ±1 μmol kg^{-1} (Millero et al. 1998).

In this study, we determined the alkalinity values of unfiltered seawater and of the same seawater sample passed through filters with nominal pore sizes of 0.7 and 0.45 μm. The contribution of phytoplankton cells to the measured alkalinity of unfiltered seawater ($\Delta A_{T-PLANKTON}$) was determined from the difference in the measured alkalinity between the unfiltered seawater and the same seawater passed through a 0.7-μm filter (GF/F, Whatman), whereas that of bacterial cells ($\Delta A_{T-BACTERIA}$) was determined from the difference in the measured alkalinity between the seawater sam-

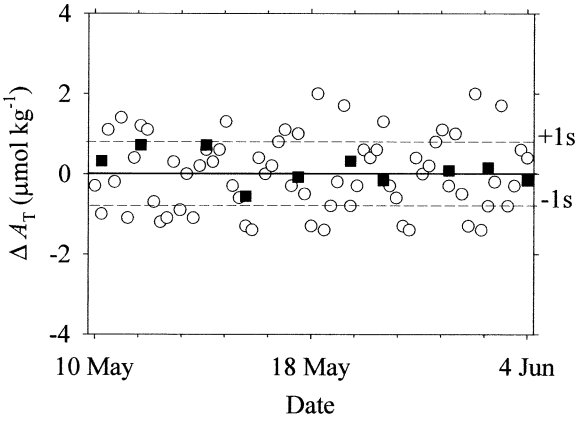

Fig. 2. Comparison of known and measured A_T on certified reference materials (CRMs; filled squares) and Na_2CO_3 solution (open circles). The CRMs and Na_2CO_3 solution have assigned A_T values of 2233.7 μmol kg^{-1} and 2003.8 μmol kg^{-1}, respectively. The thick solid line represents the mean value of measured A_T for the CRMs and Na_2CO_3 solution, and the thin dashed lines represent 1 standard deviation (1s = $\pm$ 1.0 μmol kg^{-1}) from the mean. The A_T measurements were carried out over the 1-month study period between 10 May and 4 June 2005.

ple passed through a 0.7-μm filter and the same sample passed through a 0.45-μm filter (PVDF, Millipore). Throughout a 1-month experimental period, all alkalinity measurements were made with weighed samples in a closed cell with minimum headspace, which ensured negligible loss of the sample by evaporation during titration. The performance of the titration system was monitored daily by titrating certified reference materials (CRMs; provided by A. Dickson of Scripps Oceanographic Institute, San Diego, California) and Na_2CO_3 solution with known total alkalinity values. The precision of the alkalinity measured for these CRMs and Na_2CO_3 solution was approximately $\pm$1.0 μmol kg^{-1} over the study period (Fig. 2). This high precision suggests that our alkalinity measurement protocol was consistent and that the concentration of the acid titrant remained unchanged during the study period.

For determination of the $\Delta A_{T-PLANKTON}$ caused by each cultured phytoplankton species, samples of varying particulate organic carbon (POC) concentrations were prepared by diluting the original batch culture containing a nearly maximum cell population with nutrient-depleted cell-free seawater.

Determination of POC—For POC collections and analysis, suspended particles were collected on glass fiber filters with a nominal pore size of 0.7 μm (Karl et al. 1991; UNESCO 1994). Duplicate samples were taken for the POC determination. During filtration, a slight vacuum ($\sim$20 kPa) was applied to avoid rupture of the cells on the filters, which would increase the measured alkalinity. This increase in alkalinity is probably due to the presence of negatively charged functional groups in cellular fluids that accept protons during the titration of the seawater. After filtration, the wet filters were dislodged from the filter holders with a pair of stainless steel tweezers and placed into a shallow cavity in a clean Plexiglas transport box. The transport box containing the wet filters was then placed in a drying oven and dried at 50°C for 2 days. Immediately before analysis, with the use of a clean pair of tweezers, the dried filters were folded with tinfoil and palletized (Sharp 1974). The CHN elemental analyzer (ThermoFinnigan, EA1112) was used to determine the concentration of POC on the dried filters. The technical details of the CHN analyzer are described elsewhere (Sharp 1974).

Determination of particulate inorganic carbon—To check for the presence of particulate inorganic carbon (PIC; biogenic $CaCO_3$ particles) on the POC-containing 0.45-μm pore size filters, the dried filters were acidified in a CO_2 extraction tower, after which the CO_2 produced was measured coulometrically according to the protocol developed by Johnson et al. (1987). Because the PIC concentration in seawater is low, three dried filters were combined to measure PIC content of the sample. A total of 200 ml of seawater sample was passed through each filter. The precision of our coulometric PIC analysis was determined by measuring the total inorganic carbon content ($C_T = CO_2 + HCO_3^- + CO_3^{2-}$) of CRMs before and after sample analysis for PIC. During a 1-day PIC analysis for all field samples, the precision of four C_T measurements on CRMs was approximately $\pm$0.07% (1 s). All C_T measurements from PIC analysis were normalized to the C_T value from the batch of CRMs (A. G. Dickson pers. comm.). The contribution of biogenic $CaCO_3$ particles suspended in a seawater sample to the measured alkalinity (ΔA_{T-PIC}) is two times the C_T measured on the filters. A detailed description of the C_T measurement technique is given elsewhere (Dickson and Goyet 1994).

Results and Discussion

Charge on phytoplankton and bacterial cell surfaces—Macromolecules in most phytoplankton cell surfaces have charged groups including carboxyl, phosphate, and amino groups (Gordon and Millero 1987; Crist et al. 1990; Gonzalez-Davila and Millero 1990). Such functional groups strongly respond to environmental changes through adsorption of cations and macromolecular components (Poortinga et al. 2002). Charge distribution on phytoplankton and bacterial cell surfaces originates from the dissociation and protonation of ionizable groups; hence, the charge density on the surfaces of these cells can vary with changes in the pH and ionic strength of the seawater in which the cells are suspended (Gonzalez-Davila 1995; van der Wal et al. 1996). The presence of anionic and cationic groups gives the cell surface amphoteric properties. At neutral or high pH, most phytoplankton and bacterial cell surfaces carry a net negative charge because negatively charged carboxyl and phosphate groups are more abundant than positively charged amino groups (Kleijn and van Leeuwen 2000). In contrast, at low pH, they show a net positive charge because of the relative abundance of amino groups and partial or full protonation of carboxyl and phosphate groups.

In this study, we chose four phytoplankton species that are commonly found in Korean coastal waters and measured their electrophoretic mobility as a function of pH at constant ionic strength. For all four phytoplankton species, the zeta

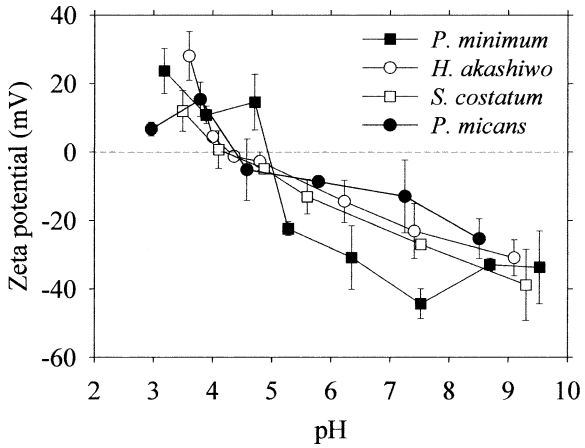

Fig. 3. Measured zeta potentials for the four cultured phytoplankton species *P. minimum, H. akashiwo, S. costatum*, and *P. micans* as a function of the pH of the seawater (salinity = 30). Results represent the means of three separate measurements for each species of phytoplankton, and the error bars represent the standard deviation (1 s = ~6 mV) from the mean.

potentials varied considerably with changes in the pH of the seawater (Fig. 3). At higher pH, the zeta potentials for the four species show negative values, probably because of deprotonation of ionizable groups, whereas the zeta potentials increase with decreasing pH because of increased protonation of those ionizable groups on the cell surface. The pH dependence in the phytoplankton zeta potential could be either characteristic for a given species or caused by characteristic surface areas of different phytoplankton species. In particular, the four phytoplankton species tested here show distinct pH values at which the measured zeta potential is zero (the isoelectric point; Fig. 3). The isoelectric points can be used to infer the relative amounts of anionic and cationic functional groups on phytoplankton cell surfaces (Rijnaarts et al. 1995); when the pH is higher than the isoelectric point, anionic functional groups are probably more abundant than cationic groups, whereas when the pH is lower than the isoelectric point, many of the anionic groups become protonated; therefore, cationic groups become more abundant. The measured isoelectric point for *P. minimum* was about pH 5.0, whereas those for *H. akashiwo, S. costatum,* and *P. micans* were close to 4.2 and were indistinguishable within the uncertainties in the measurements of the zeta potential. Such low isoelectric points for the four species indicate that negatively charged carboxylic and phosphate groups are the dominant functional groups on the cell surfaces of the four phytoplankton species and that these anionic groups react with protons during alkalinity titration of seawater.

We did not separately determine the zeta potential for bacterial cells living in our seawater samples because previous studies have extensively measured the charge distribution of bacterial cell surfaces (van der Mei et al. 1988; Rijnaarts et al. 1999). Like phytoplankton cells, the surfaces of bacterial cells respond dynamically to chemical changes occurring in seawater by adsorbing ions and macromolecular components. The surface charge of bacterial cells is also largely due to dissociation or protonation of carboxyl, phosphate, and amino groups, and it consequently changes with the pH of the seawater (Poortinga et al. 2002). Previous studies have shown that most bacterial strains carry a net negative charge at a pH between 5 and 7 (Busscher et al. 2000).

Contribution of phytoplankton cells to the measured alkalinity ($\Delta A_{T-PLANKTON}$)—The $\Delta A_{T-PLANKTON}$ for each phytoplankton species increases with increasing POC concentration (Fig. 4). This increase in $\Delta A_{T-PLANKTON}$ is due to the presence of negatively charged groups on the surfaces of phytoplankton cells. During the course of titration of unfiltered seawater with hydrochloric acid, these negatively charged functional groups react with protons and, as a result, contribute to the measured alkalinity. The magnitude of $\Delta A_{T-PLANKTON}$ was species dependent (Fig. 5). For the same POC concentration, *P. minimum* had the greatest effect on $\Delta A_{T-PLANKTON}$, followed by *H. akashiwo, S. costatum,* and *P. micans.* This species dependence could potentially be caused either by differences in the ionizable groups on the cell surfaces of different phytoplankton species or by characteristic surface areas of different phytoplankton species. There was little difference in the measured isoelectric points for the four phytoplankton species, indicating that ionizable groups on the cell surfaces of the four phytoplankton species are similar and thus might not be responsible for the difference in the $\Delta A_{T-PLANKTON}$ contribution from different phytoplankton species.

Another possible source of variability in the contributions of different phytoplankton species to the $\Delta A_{T-PLANKTON}$ is differences in their surface-to-volume ratio. This could lead to variations in the net charge density at the phytoplankton cell surface: the higher the surface-to-volume ratio, the greater the cell surface charge density. In other words, more acid would be needed during the titration if the unfiltered seawater contains phytoplankton cells that have more negatively charged functional groups on their surfaces, and this would result in higher $\Delta A_{T-PLANKTON}$ values.

In this study, with a phytoplankton bloom (POC concentration reaching 200 μmol L^{-1}), the $\Delta A_{T-PLANKTON}$ ranged from 3 to 5 μmol kg^{-1}, depending on the phytoplankton species (Fig. 5). However, in the typical oceanic range of POC concentration (1 to 30 μmol L^{-1}; Pilson 1998), the $\Delta A_{T-PLANKTON}$ is likely to be insignificant. The difference in the $\Delta A_{T-PLANKTON}$ contribution from different phytoplankton species is also insignificant until the POC concentration reaches 200 μmol L^{-1} (Fig. 5), but it becomes significant for POC concentrations greater than 200 μmol L^{-1}.

In the eastern coastal waters of Korea, in which the POC concentration ranges from 15 to 80 μmol L^{-1}, the $\Delta A_{T-PLANKTON}$ ranges from <1 to as high as 4 μmol kg^{-1} (Fig. 6a). The measured POC concentrations in Young-Il bay waters are higher than those typically observed in open ocean waters. It is important to note that the contribution of biogenic $CaCO_3$ particles to the measured alkalinity of all bay samples was subtracted from the contribution of phytoplankton and bacterial cells to the measured alkalinity of the same seawater samples. The contribution of $CaCO_3$ particles to the measured alkalinity is described in a following section.

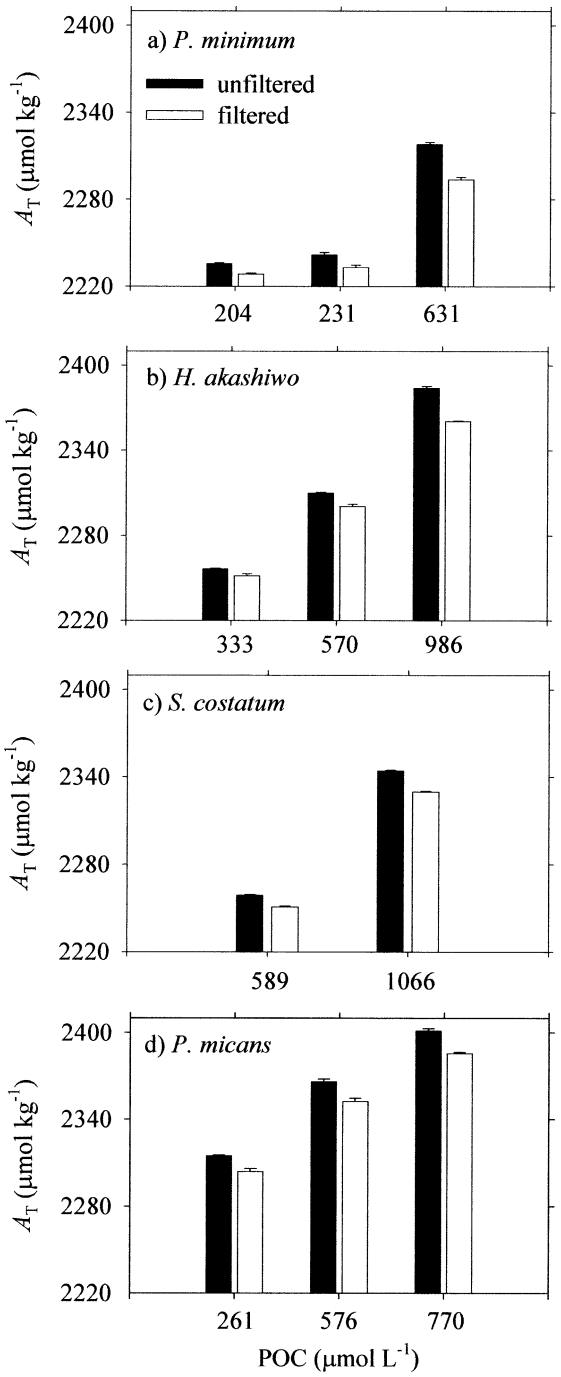

Fig. 4. Measured alkalinity values of unfiltered seawater (filled bars) containing each of the four cultured phytoplankton species (*P. minimum, H. akashiwo, S. costatum,* and *P. micans*) and of the same seawater passed through a 0.7-μm filter (open bars) as a function of POC concentration. For determination of the $\Delta A_{T-PLANKTON}$ caused by each phytoplankton species, cell population in each batch culture containing a nearly maximum cell population was decreased by 100% to 300% by diluting the batch culture with nutrient-depleted cell-free seawater. For direct comparison of alkalinity measurements, all alkalinity values were normalized to a mean salinity of 32.9.

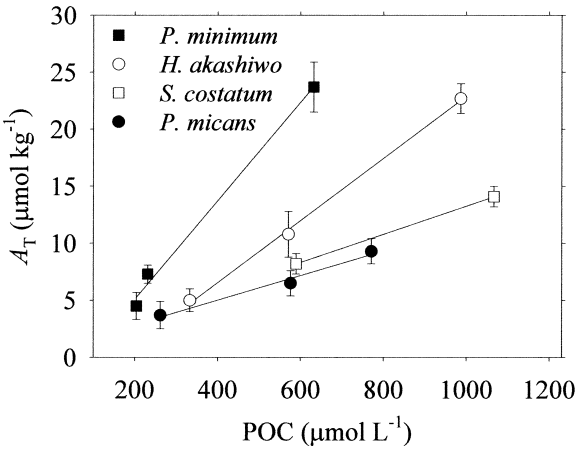

Fig. 5. Measured alkalinity differences ($\Delta A_{T-PLANKTON}$) between unfiltered seawater containing each of the four cultured phytoplankton species and the same seawater passed through a 0.7-μm filter. The solid lines show linear regression plots between $\Delta A_{T-PLANKTON}$ and the POC concentration for four phytoplankton species.

Contribution of bacterial cells to the measured alkalinity ($\Delta A_{T-BACTERIA}$)—Previous findings have shown that bacteria are ubiquitous in the water column and are abundant in suspended particulate matter (Cho and Azam 1988). Bacterial populations ranges from 10^5 cells mL^{-1} in oligotrophic waters to 5×10^6 cells mL^{-1} in coastal waters (Fukuda et al. 1998). Similar to phytoplankton cell surfaces, negatively charged functional groups on bacterial cell surfaces reacted with protons during the titration of unfiltered seawater with hydrochloric acid, causing an increase in the measured alkalinity. This study indicates that, in the eastern coastal waters of Korea, the $\Delta A_{T-BACTERIA}$ in seawater ranges from 1 to as high as 6 μmol kg^{-1} (Fig. 6b). The contribution of bacteria appears to be inversely related to the contribution of phytoplankton, suggesting that the bacterial population could be more important in open ocean waters in which phytoplankton are less abundant. Our results indicate that more alkalinity measurements on unfiltered and filtered (a 0.45-μm filter) samples with low POC concentrations are needed to assess the contribution of bacterial cells to the measured alkalinity.

The seawater samples analyzed in our study probably contained bacterial cells that are small enough to pass through a 0.45-μm filter and therefore are not included in our estimate of the contribution of bacterial cells to the measured alkalinity. Nonetheless, we chose the 0.45-μm pore size for two reasons. First, our alkalinity measurements on a limited number of coastal seawater samples suggested that bacteria smaller than 0.45 but greater than 0.2 μm contributed less than 0.5 μmol kg^{-1} to measured alkalinity. We considered this contribution to be negligible because it is within the precision (± 1 μmol kg^{-1}) of our alkalinity measurements. Second, particulate matter is defined operationally as material that can be collected on a filter, usually one with a pore size of 0.45 μm. Therefore, the contribution to the measured alkalinity of biological particles larger than 0.45 μm is of particular concern to oceanographers. It is also important to note that our estimation of the contribution of bacteria to the

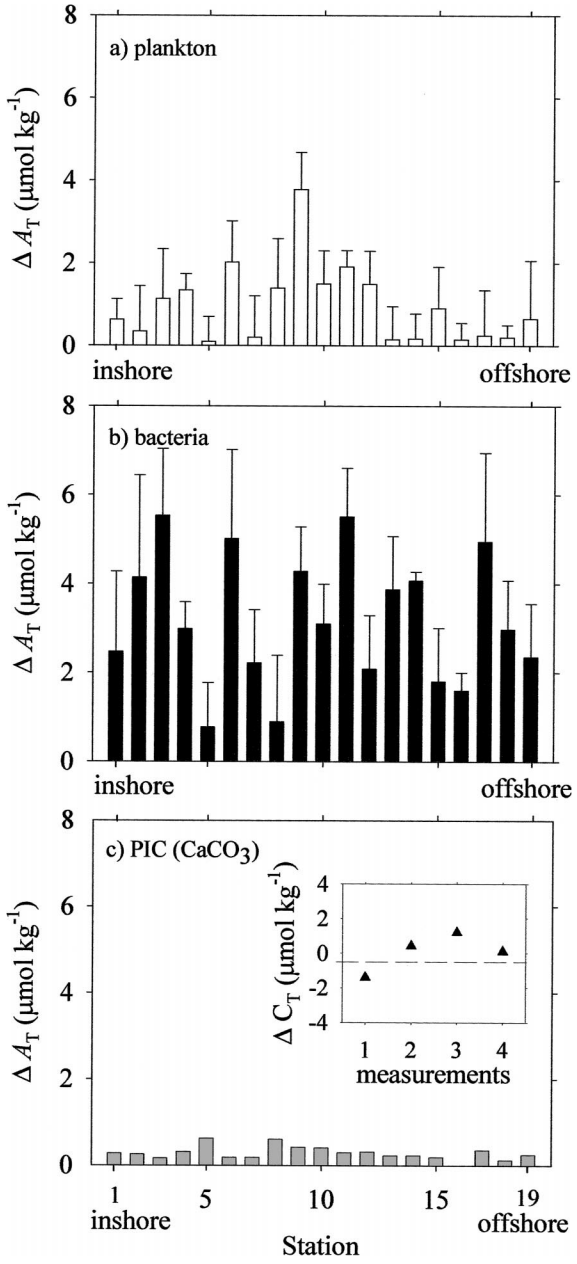

Fig. 6. The contributions of (a) phytoplankton cells, (b) bacterial cells, and (c) biogenic $CaCO_3$ particles to the measured alkalinity of unfiltered seawater. Seawater samples (19) were obtained from the Young-Il bay of Korea adjacent to the East Sea (see Fig. 1). (c) Inset shows four C_T measurements on certified reference materials with an assigned C_T value of 2003.8 μmol kg^{-1}. Two of them were analyzed before sample analysis for PIC and the other two after sample analysis. Results represent the means of four measurements for each sample, and the error bars represent the standard deviation.

measured alkalinity of unfiltered seawater represents a lower bound on the true value because some bacteria might not have been collected by the filtration process.

Contribution of biogenic $CaCO_3$ particles (PIC) to the measured alkalinity (ΔA_{T-PIC})—The inclusion of biogenic

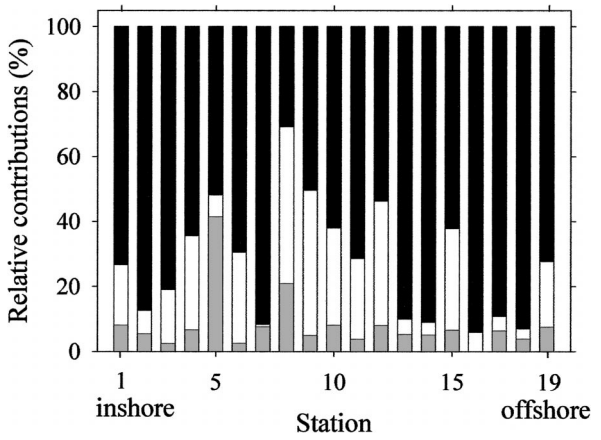

Fig. 7. Relative contributions of phytoplankton ($\Delta A_{T-PLANKTON}$, open bars) and bacterial ($\Delta A_{T-BACTERIA}$, filled bars) cells and of biogenic $CaCO_3$ particles (ΔA_{T-PIC}, gray bars) to the measured alkalinity of unfiltered coastal seawater samples. Seawater samples (19) were obtained from the Young-Il bay of Korea adjacent to the East Sea (see Fig. 1).

$CaCO_3$ particles is the only significant input of alkalinity that has been explicitly recognized as resulting from the widespread habit of measuring alkalinity in unfiltered samples. Therefore, we determined the contribution of biological $CaCO_3$ particles collected on 0.45-μm filters to the measured alkalinity of unfiltered seawater. Two-thirds of the samples analyzed yielded ΔA_{T-PIC} values of less than 0.5 μmol kg^{-1} (Fig. 6c). Our PIC measurements indicated that the ΔA_{T-PIC} accounts for less than 20% of the combined alkalinity contributed by both organic (phytoplankton and bacterial cells) and inorganic (biogenic $CaCO_3$) particles (Fig. 7). As a result, the differences in measured alkalinity between the unfiltered and filtered (0.45 μm) seawater samples were largely because of the presence of negatively charged phytoplankton and bacterial cell surfaces. This observation is perhaps not surprising because diatoms and dinoflagellates, rather than $CaCO_3$ producers, are the dominant phytoplankton species in Korean coastal waters (Kang and Choi 2002).

Geochemical significance of phytoplankton and bacterial cells in the ocean carbonate system—This study highlights the importance of phytoplankton and bacterial cells as potential contributors to measured alkalinity. Although their importance is probably limited to biologically productive coastal or open ocean waters, the ubiquitous presence of bacterial cells in seawater indicates that the $\Delta A_{T-BACTERIA}$ might be nontrivial even in oligotrophic open ocean waters. Further studies should be carried out to check the validity of this hypothesis.

One aspect of the presence of particulate organic matter in seawater is its direct influence on chemical changes in seawater, which subsequently change the total alkalinity (A_T). The activity of phytoplankton in surface ocean depletes nitrate (and phosphate) and utilizes some of the carbon to form soft tissue. The production of this organic matter and its subsequent oxidation by bacteria can be represented by Eq. 4 (Redfield et al. 1963).

$$106CO_2 + 122H_2O + 16HNO_3 + H_3PO_4$$
$$\text{photosynthesis} \Rightarrow$$
$$\Leftrightarrow (CH_2O)_{106}(NH_3)_{16}H_3PO_4 + 138O_2 \quad (4)$$
$$\Leftarrow \text{respiration}$$

During photosynthesis, the removal of nitrate (and phosphate) from seawater to form organic matter raises the A_T of seawater through net transfer of protons in seawater into organic matter, whereas the oxidation of organic matter during respiration results in a net transfer of protons into seawater and consequently decreases the A_T of seawater. Our results indicate that, during the photosynthetic process, the ΔA_{T-OM} due to the presence of organic matter further adds to the measured alkalinity of unfiltered seawater. In contrast, microbial degradation of organic matter, which occurs in deep waters, decreases the magnitude of ΔA_{T-OM} so that its contribution becomes negligible.

Another important aspect of ΔA_{T-OM} is that the widespread practice of determining alkalinity on unfiltered seawater probably results in errors in any CO_2-related calculations based on the measured alkalinity. The CO_2 system in seawater can be defined by specifying the temperature, salinity, and nutrient and boron concentrations, along with two of the four inorganic CO_2 system parameters: CO_2 fugacity (fCO_2), pH ($-\log[H^+]$), C_T, and A_T. The accuracy of available carbonic acid dissociation constants can be evaluated with measurements of more than two of the CO_2 system parameters. The agreement between measurements and calculations is essential to our understanding of important geochemical issues such as the determination of the calcium carbonate compensation depth (Chung et al. 2003; Feely et al. 2004) and the distribution of anthropogenic CO_2 in the oceans (Lee et al. 2003; Sabine et al. 2004). In particular, it is important in numerical ocean circulation models in which surface ocean fCO_2 is calculated from A_T and C_T fields (Sarmiento and Gruber 2002). Different carbonate thermodynamic models yield fCO_2 values that vary by as much as 30 μatm (Lee et al. 2000; Millero et al. 2002).

In thermodynamic calculations with A_T, the carbonate alkalinity ($[HCO_3^-] + 2[CO_3^{2-}]$), which is contributed by the carbon dioxide species, should be calculated after correcting for the contributions from borate, hydroxyl, and other minor species (e.g., HPO_4^{2-}, PO_4^{3-}, and $SiO(OH)_3^-$). All previous thermodynamic studies with field data assumed that the contribution of plankton and bacterial cells to the measured alkalinity of unfiltered seawater was negligible; hence, they did not account for this contribution. When this contribution is ignored, the value of carbonate alkalinity calculated from the measured alkalinity is probably overestimated. This overestimation will result in inaccuracies in the calculated parameters and, more importantly, errors in evaluating the accuracy of different carbonate thermodynamic models. Although the present findings do not change the overall conclusion in terms of the optimal thermodynamic model (Lee et al. 2000; Millero et al. 2002), the potential error in thermodynamic calculations that use alkalinity values measured on unfiltered samples should be recognized.

The results presented here highlight potential biases in calculations of carbonate alkalinity that use at-sea alkalinity measurements on unfiltered seawater samples and, thus, indicate that caution should be exercised when performing or interpreting the results of such calculations. This does not necessarily mean that the contribution of particulate organic matter must be explicitly included in the definition of the total alkalinity of seawater (see Eq. 1). Instead, our observations suggest that at-sea alkalinity measurements should be made on filtered seawater to minimize potential biases in converting measured alkalinity to carbonate alkalinity. More alkalinity measurements on filtered and unfiltered open ocean samples are needed to assess the contributions of phytoplankton and bacterial cells to the measured alkalinity and, with this information, to assess the accuracy of carbonate thermodynamic models.

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