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A novel method for the measurement of dissolved adenosine and guanosine triphosphate in aquatic habitats: applications to marine microbial ecology

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Abstract

A novel method for the measurement of dissolved adenosine-5′-triphosphate and guanosine-5′-triphosphate (D-ATP and D-GTP, respectively) in marine and freshwater habitats was developed and applied to samples collected from the oligotrophic North Pacific Ocean. Both D-ATP and D-GTP are co-precipitated by authigenically formed $Mg(OH)_2$ and can be concentrated by factors greater than 200-fold, for subsequent measurement by the firefly luciferin–luciferase bioluminescence reaction. The detection limit for this method was 2–3 pmol ml^{-1} of concentrated sample (equivalent to an in situ concentration of 10 pM) with a 5% precision at concentrations of 10 pmol ml^{-1} or above.

A significant positive correlation ($P < 0.001$) was observed between particulate ATP (P-ATP) and D-ATP in water samples collected from Station ALOHA (22.75°N, 158°W; depth profiles 0–1000 m). The highest concentrations of dissolved nucleotides were found in the euphotic zone (0–175 m) below which the concentrations were low and relatively invariant. The dissolved nucleotide pools generally exceeded their corresponding particulate pools.

Using radioisotopic tracer techniques and the new concentration method, turnover times for both particulate and dissolved nucleotides can be determined. The ability to measure concentrations and follow nucleotide tracers accurately in a very dilute environment provides a unique opportunity to address questions on microbial community metabolism, nutrient dynamics and energy flux. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: ATP; GTP; Microbial ecology; Nucleotides

1. Introduction

Since the pioneering research of Levin et al. (1964) and Holm-Hansen and Booth (1966), the measurement of adenosine-5′-triphosphate (ATP) has been widely used within the field of microbial ecology (see reviews by Karl, 1980; Karl and Dobbs, 1998). The most frequently employed detection system, the firefly bioluminescence assay, has several advantages especially high sensitivity (detection limits in the femtomolar [10^{-15} M] range; Karl and Holm-Hansen, 1976). However, the enzyme-mediated reaction is strongly inhibited by a variety of anions and cations (McElroy, 1956; Karl and LaRock, 1975), rendering it virtually impossible to measure ATP in seawater samples without prior concentration and desalting.

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In aquatic environments, dissolved nucleic acids, nucleotides and nucleotide derivatives are ubiquitous components of the dissolved organic matter (DOM) pool (Minear, 1972; Azam and Hodson, 1977; Karl and Bailiff, 1989). Dissolved ATP (D-ATP) has been reported to occur in a variety of aquatic habitats ranging from eutrophic, freshwater lakes to temperate marine coastal regions and Antarctic seawaters (Azam and Hodson, 1977; Hodson and Azam, 1977; Riemann, 1979; Hodson et al., 1981a; McGrath and Sullivan, 1981; Maki et al., 1983; Nawrocki and Karl, 1989). Due to the universal occurrence of nucleotides in the environment and their obligate role in all living organisms, coupled with the possibility to quantify both intra- and extracellular concentrations, nucleotide compound inventories and fluxes are potentially useful in marine microbial ecology. Despite this potential, few studies have been carried out in marine environments presumably due to the lack of a reliable, user-friendly methodology.

In this study, we present a novel, straightforward method for the measurements of D-ATP and dissolved guanosine-5′-triphosphate (D-GTP) in seawater samples. The method is also readily applicable to freshwater environments. This new method employs a modified version of the magnesium-induced co-precipitation (MAGIC) procedure (Karl and Tien, 1992), originally devised as a high-sensitivity technique for the measurement of soluble reactive phosphorus (SRP) in aquatic environments, and was applied to natural seawater samples from the oligotrophic North Pacific gyre. The ecological implications of the ability to quantify and determine fluxes of nucleotides and their usefulness in nutrient dynamics studies are discussed.

2. Materials and methods

2.1. Station location and sample collections

Samples analyzed for D-ATP and D-GTP were collected between June 1998 and February 1999, during monthly cruises of the Hawaii Ocean Time-series (HOT) program (HOT-94, -95, -96, -100, -101 and -102) to Station ALOHA (22.75°N, 158°W), an oligotrophic North Pacific Ocean site (Karl and Lukas, 1996). Seawater was collected from 11 depths (5, 25, 45, 75, 100, 125, 150, 175, 250, 500 and 1000 m), transferred to acid washed high-density polyethylene bottles and vacuum filtered through 0.2 μm polycarbonate (PC) membrane filters (Nuclepore or Poretics) at a pressure differential between 5 and 15 cm Hg to remove particles. Duplicate samples were typically collected for the top two to three depths; all others were single sample measurements. The filtrate (450 ml) was stored frozen (−20 °C) until analyzed for D-ATP and D-GTP, usually within 10 days of collection.

Samples for particulate ATP (P-ATP) were 1–2 l seawater filtered onto glass fiber filters (Whatman GF/F, Ø 47 mm) and extracted in 5 ml boiling Tris buffer (20 mM, pH 7.4; Karl and Holm-Hansen, 1978).

2.2. Preparation of the firefly lantern extract (FLE) for the bioluminescence assay

Several different modifications to the commercially available firefly lantern extract (FLE; Sigma, St Louis, MO) were tested to achieve an enzyme preparation with low background light emission, low ionic strength and, therefore, high sensitivity. The crude firefly lantern extract, FLE-50, was exclusively used throughout this study. One vial containing 50 mg FLE was reconstituted in 5 ml dH_2O and allowed to age at room temperature (22 °C) for 6–8 h before dilution with Tris (20 mM, pH 7.4; Sigma, MO # T-4003) and arsenate (0.1 M, pH 7.4; Mallinckrodt) buffers. The FLE preparation was filtered through a GF/F filter (Whatman) and the filtrate was amended with dithiothreitol (DTT) and luciferin (Sigma, MO # D-9779, and # L-6882, respectively) to ensure enzyme stability and maximum sensitivity. The FLE preparation routinely used for natural samples consisted of reconstituted FLE:Tris:As-buffer in a ratio of 1:7:7, DTT to 1 mM and luciferin to 33 μM final concentrations.

2.3. D-ATP and D-GTP co-precipitation by $Mg(OH)_2$

Seawater samples were amended with ATP or GTP to a final concentration of 1 μM. Aliquots received increasing volumes of 1 M NaOH, ranging

from 0.05% to 2.5% (vol:vol) distributed over 14 samples, to induce variable mass of $Mg(OH)_2$ formation. The samples were thoroughly mixed and centrifuged at $1000 \times g$ for 1 h. Subsamples taken from the supernatant were diluted 10-fold with Tris buffer and subsequently analyzed for remaining ATP or GTP using the firefly bioluminescence reaction. The $Mg(OH)_2$ scavenging efficiency was further tested by using ATP labeled at the γ position with ^{32}P (ICN #35001X, specific activity 167 TBq $mmol^{-1}$). Radiolabeled ATP was also used to assess the effectiveness of the passive precipitation technique using 450 ml seawater as described below for natural samples. In both cases, the samples were labeled to an activity of 1.1 kBq ml^{-1} seawater.

2.4. Preparation and analysis of D-ATP and D-GTP in natural samples

After thawing the frozen samples, 2.25 ml of 1 M NaOH (Fluka BioChemika #71689) was added to 450 ml of sample to effect a 0.5% (vol:vol) MAGIC precipitation (Karl and Tien, 1992; Thomson-Bulldis and Karl, 1998). The samples were mixed and allowed to stand for 3 h at room temperature. After the authigenically produced $Mg(OH)_2$ had settled, the supernatant was carefully aspirated without disturbing the precipitate, and the remaining volume was transferred to 50 ml polypropylene tubes and centrifuged at $1000 \times g$ for 1 h to pellet the $Mg(OH)_2$ which contained the co-precipitated nucleotides. The supernatant was aspirated and the pellets were dissolved by titration with 5 M HCl until the solution turned from opaque to clear (150–200 μl depending on sample). The samples were then brought up to exactly 2.0 ml final volume in Tris buffer (20 mM, pH 7.4) thereby effecting a 225-fold concentration of the original sample volume. The concentrated samples were stored frozen at −20 °C until analyzed for ATP by peak height of bioluminescence and for GTP by the bioluminescence integral method (Karl, 1979). Sample stability over time was tested on selected concentrates after 3, 5 and 7 months of storage at −20 °C.

The samples were analyzed using an ATP-photometer (SAI Technological, model 2000) equipped with an automated injection unit, and interfaced to a computer for the measurement of both peak height (highest value 0–3 s, after injection), and the integrated counts of the light emission decay curve (15–75 s, after injection). To assure proper, reproducible mixing, 800 μl of the FLE mixture was automatically injected directly into the reaction vial containing 100 μl of concentrated sample.

ATP standards were prepared the same way as natural samples by using 0.2 μm filtered seawater from 1000 m that contained only a minimal background nucleotide concentration < 4 pM. Usually, a set of standards included 7–8 different concentrations ranging from 10 to 600 pM ATP (equivalent to 2.5 to 150 pmol ATP ml^{-1} concentrated sample). A "zero concentration" blank was produced using the supernatant of $Mg(OH)_2$ precipitated samples, essentially D-ATP and D-GTP free, and then treated the same way as the other samples. The slope of the standard curve was used to determine the nucleotide concentrations of the natural samples where the blank sample concentration was deducted. Standard samples for GTP were similarly prepared but were not included on a routine basis.

2.5. Verification of the dissolved nature of measured D-ATP pool

Two independent separation techniques were tested to verify that sample processing did not artifactually produce dissolved nucleotides from the particulate fraction. In the first of these, total ATP (T-ATP; i.e., sum of D-ATP and P-ATP) was measured by direct acid extraction of unfiltered seawater using concentrated H_2SO_4 added to a 0.1 M final concentration (HOT-101, -102). Samples were prepared in triplicate and included sample sets spiked with known amounts of ATP, serving as internal standards, to monitor recovery. Samples for T-ATP were also collected from several depths for comparison to P-ATP and D-ATP samples collected on the same occasion. The samples were stored frozen. After thawing, the acidified seawater samples were titrated to pH 8.2 with NaOH (10 M) prior to the standard co-precipitation method, as described above. The T-ATP concentrations were determined and corrected for losses by hydrolysis and salt interference using internal ATP standards.

A second method employed a vortex flow filtration (VFF) system (Membrex-Benchmark GX)

equipped with a 200-cm^2, 0.2-μm polypropylene filter cartridge. Approximately 3 l, equal to about six times the VFF's vessel volume, of seawater was allowed to pass through the filtration system prior to collecting discrete samples. Spikes were also introduced to assess losses due to non-specific binding of ATP with the components of this system. All VFF samples were compared to 0.2 μm PC filtrates collected by vacuum filtration using the same batch of seawater.

2.6. Verification of GTP

Most estimates of GTP concentration reported herein were based on the difference between ATP concentration calculated using the peak height vs. "ATP" concentration in the same sample using integrated light emission values (Karl, 1978a). If only ATP is present in the sample, both estimates are identical (i.e. the difference is zero). If non-adenine nucleotide triphosphates (NA-NTP) are present, the integral ATP (ATP_{int}) estimate will exceed the peak ATP (ATP_{peak}) when the assay employs FLE-50 preparations (Karl, 1978a). This is a result of the presence of the enzyme nucleoside diphosphokinase (NDPK) in the crude enzyme preparation and the time-dependent production of ATP from NA-NTP according to the reaction: NA-NTP + ADP → ATP + NA-NDP. Furthermore, Karl (1978a) showed that the difference between these two measurements, termed ΔATP ($\Delta ATP = [ATP_{int} - ATP_{peak}]$), is highly correlated to GTP concentrations for a variety of natural samples. To verify that ΔATP at Station ALOHA was a valid approximation for GTP, a specific enzyme assay was employed. The assay relies on the selective enzymatic hydrolysis of ATP in mixed nucleotide extracts using hexokinase (HK) and glucose-6-phosphate dehydrogenase (G6P-DH) in the presence of glucose and NADP to catalyze the coupled reactions: ATP + glucose → G6P + ADP and G6P → gluconate-6P (Karl, 1978b). The remaining GTP can be measured by the firefly bioluminescence assay. Several modifications of the original assay were required, including omitting $MgCl_2$ in the enzyme mixture, substituting phosphate buffer for Tris buffer (20 mM, pH 7.4), dilution of the samples (2:1, sample:dH_2O) to avoid salt interference, and extending the incubation time to 2 h at 30 °C.

3. Results

3.1. Nucleotide magic

A range of NaOH additions was tested to identify the minimum concentration necessary to scavenge D-ATP and D-GTP reproducibly. It was determined that a ratio of 0.5% (vol:vol) NaOH (1 M):seawater consistently co-precipitated ≥ 90% of the dissolved ATP and GTP from 50 ml samples (Fig. 1). While quantitative (100%) recovery can be achieved at NaOH:seawater ratios of ≥ 1% (vol:vol; Thomson-Bulldis and Karl, 1998), the lower ratio of 0.5% (vol:vol) was selected as a compromise to maximize the D-ATP removal while keeping $Mg(OH)_2$ formation to a minimum, and, therefore, also the residual salt concentration low. The co-precipitation procedure routinely employed throughout this study, using

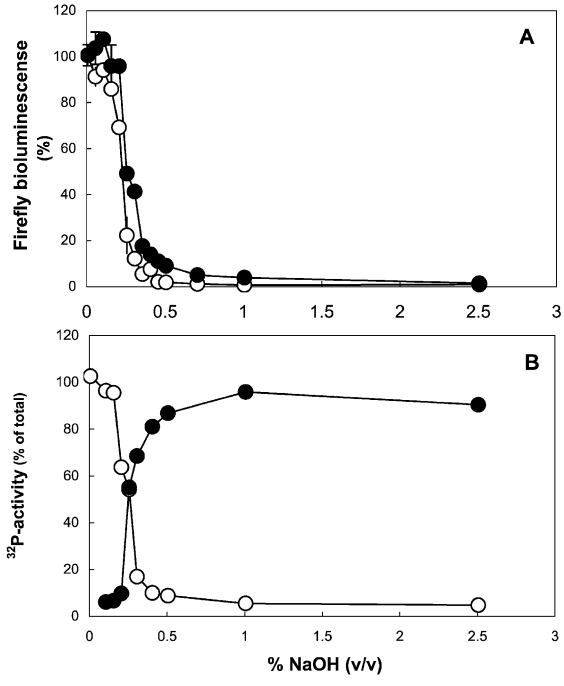


Fig. 1. Scavenging of D-ATP and D-GTP with increasing additions (vol:vol) of NaOH (1 M). (A) Nucleotides remaining in supernatant measured by firefly bioluminescence, as % of bioluminescence at 0% NaOH addition. Filled symbols are ATP, open symbols are GTP. (B) ^{32}P activity, as % of the total activity added, remaining in supernatant and recovered in pellet of MAGIC precipitated samples of $AT^{32}P$. Filled circles represent pellets, open circles supernatant activities.

Table 1
Comparison of dissolved, particulate and total adenosine-5′-triphosphate (ATP) measurements in the subtropical North Pacific gyre

Cruise and date	Depth (m)	Measured P-ATP[a] (pM)	Measured D-ATP[b] (pM)	Sum P + D-ATP (pM)	Measured T-ATP[c] (pM)
HOT 101 (Jan. 1999)	45	53 ± 8[d]	75 ± 14	128 ± 16	170 ± 10
HOT 102 (Feb. 1999)	5	61 ± 12	36 ± 1	97 ± 12	131 ± 7
	25	59 ± 9	32 ± 2	92 ± 9	183 ± 8
	45	66 ± 7	27 ± 2	92 ± 7	120 ± 16
	75	60 ± 8	–	–	170 ± 4
	100	84 ± 12	44 ± 0	129 ± 12	178 ± 4
	125	62 ± 11	44 ± 2	106 ± 11	158 ± 0

[a] P-ATP = particulate adenosine-5′-triphosphate. P-ATP concentrations were measured on particles collected on GF/F filters and extracted in Tris buffer.
[b] D-ATP = dissolved adenosine-5′-triphosphate. D-ATP concentrations were measured on filtrates (0.2 μm PC membranes) and concentrated by MAGIC.
[c] T-ATP = total adenosine-5′-triphosphate. T-ATP concentrations were measured on acid extracted, unfiltered seawater samples and concentrated by MAGIC.
[d] Values are mean ± 1 S.D.

450 ml samples and passive settling of the precipitate, proved to recover > 75% of the ATP, as determined using γ-^{32}P-labeled ATP (21% remaining in supernatant 1, 0.5% in supernatant 2, 78.3 ± 1.7% in pellet, $n = 3$). The single centrifugation procedure, with its higher recovery and shorter processing times, makes it desirable over the passive precipitation method and could preferably be used for environmental samples containing high (> 50 pM) D-ATP concentrations. However, processing large volume samples in the field may preclude the use of a centrifuge, and the passive precipitation technique can easily be used without compromising the analyses. The routine preparation of D-ATP standard curves using an identical procedure as used for the samples assures that any variations in D-ATP and D-GTP recovery and subsequent ionic interference of the bioluminescence assay is accounted for.

Although salt interference is the primary concern for these samples, pH is also a consideration. However, at the mixing ratio employed here (1:8, sample:FLE, by volume), the original sample was sufficiently buffered by the FLE enzyme mixture and little to no negative impact on the resulting light emission was observed over a sample pH range of 2 to 10. The post-mixing pH was buffered to pH 7.4 by the FLE mixture, which is optimal for the light emission reaction.

The detection limit for the passive precipitation method is 2.5 pmol ml^{-1} concentrated sample with a precision of < 5% at ≥ 40 pM D-ATP in the original water sample. Concentrated samples were stable for at least 5 months when kept at −20 °C.

3.2. Verification of dissolved ATP and GTP

To verify that our routine filtration was not producing D-ATP, two alternative sample treatments were tested. First, T-ATP extractions were compared to the sum of D-ATP, measured on 0.2-μm membrane filtrates, and P-ATP concentrations. The direct acid extraction recovered ≥ 75% of D-ATP as determined from the sample spikes. The acid extracted T-ATP concentrations consistently exceeded, by approximately 30%, the sum of the combined dissolved and particulate pools obtained from filtered samples (Table 1).

Table 2
Comparison of dissolved adenosine-5′-triphosphate (D-ATP) measurements by two independent methods

Date	Cruise	Depth (m)	D-ATP (pM)		*n*
			VFF[a]	Filtrate[b]	
Jan. 1999	HOT 101	25	27 ± 7[c]	26 ± 2	3
Feb. 1999	HOT 102	45	13 ± 2	19 ± 1	3

[a] VFF = Vortex flow filtration.
[b] Filtrate = seawater passed through a 0.2-μm PC membrane by vacuum filtration.
[c] Values are mean ± 1 S.D.

The second method used to compare the D-ATP concentrations derived from vacuum filtered samples was the VFF, a procedure generally considered non-destructive to microbial cells (Paul et al., 1991). D-ATP concentrations in VFF samples were not significantly different from the routine membrane filtration procedure at 27 ± 7 pM ($n = 3$), compared to 26 ± 2 pM ($n = 3$). On another occasion, VFF samples were lower than the routinely processed samples by about 30%; however, the samples differed by only a few pM due to the overall low ATP concentrations in these samples (Table 2). Sample spikes of D-ATP were recovered with the same efficiency by either VFF or vacuum filtration, indicating that the VFF polypropylene filter cartridge did not bind D-ATP.

3.3. Verification of GTP by selective hydrolysis of ATP

Evaluation of enzymatic hydrolysis of ATP and GTP in Tris buffer showed that ATP was completely

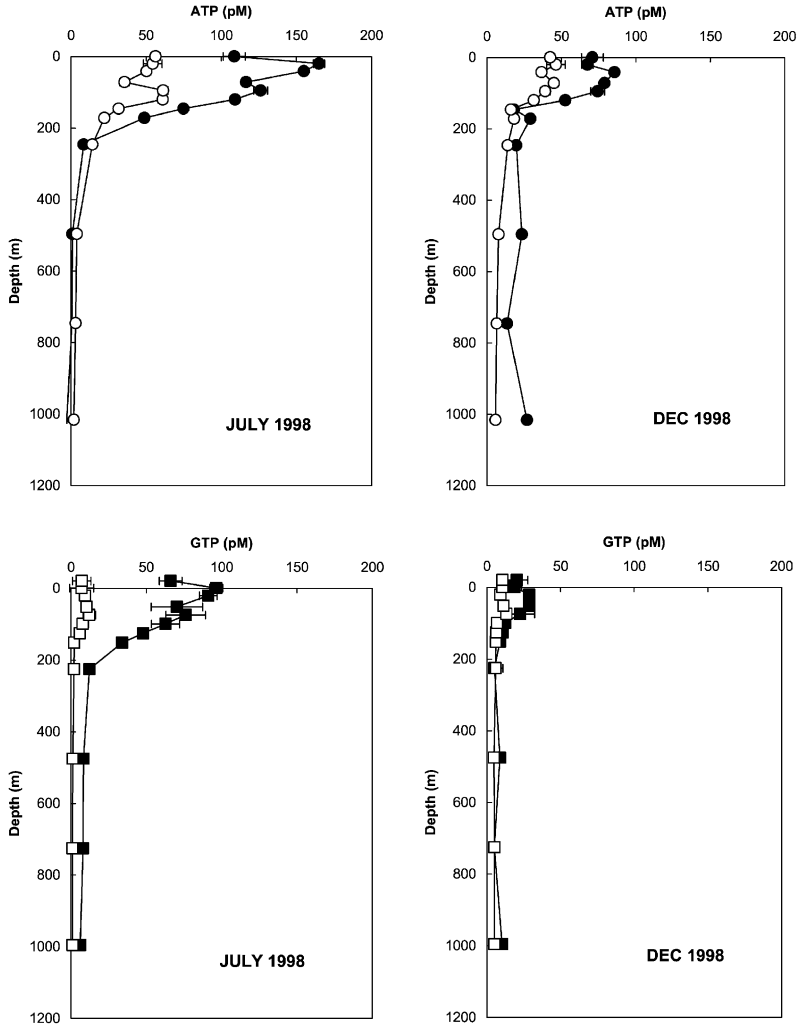


Fig. 2. Representative depth vs. concentration profiles of particulate and dissolved ATP and GTP at Station ALOHA for summer (HOT 95) and winter (HOT 100) conditions. Open symbols are particulate nucleotides, filled symbols are dissolved nucleotides. Circles represent ATP and squares GTP.

hydrolyzed over a range of concentrations, whereas GTP remained intact under the conditions applied here. When using samples concentrated by MAGIC, the hydrolysis was incomplete and about 30% of the D-ATP remained in the sample. D-GTP, however, was unaffected and recovered to 100%. In samples with both ATP and GTP added in equal amounts, a slight offset from the estimated GTP content and that calculated as ΔATP was observed. At low GTP concentrations, the two estimates corresponded well, but ΔATP tended to underestimate GTP above 50 pM. The same was true for natural samples tested.

3.4. Dissolved and particulate nucleotide concentrations at Station ALOHA

A significant positive correlation ($P < 0.001$) was observed between particulate P-ATP and D-ATP. D-ATP and P-ATP concentrations measured during the period June 1998 to February 1999 at Station ALOHA revealed that the dissolved pools consistently exceeded the particulate pools, except in February 1999. Similarly, D-GTP concentrations were generally higher than those observed in the respective particulate fractions, except in June 1998. The highest D-ATP and D-GTP concentrations were found in the euphotic zone (0–175 m), similar to the P-ATP distribution, in both summer and winter. Below the euphotic zone, the dissolved and particulate nucleotide concentrations were low and relatively invariant with season (Fig. 2).

4. Discussion

When separating particulate and dissolved nucleotide fractions using membrane filtration, there is potential criticism about procedural artifacts, especially cell damage and subsequent leakage. This concern derives from laboratory observations of primarily eukaryotic cell damage during the filtration process (Goldman and Dennet, 1985; Nagata and Kirchman, 1990). However, recent studies have shown that cells in the bacterial size range also may be susceptible to losses from their intracellular pools upon membrane filtration (Kiene and Linn, 1999). In previous studies of D-ATP in seawater, there was no evidence for increased inventories of ATP in the dissolved fraction when filtering through a 0.2-μm Nuclepore filter at pressure differentials between 5 and 37.5 cm Hg, and filtration results were comparable to those obtained using dialysis bags (Azam and Hodson, 1977). Our results from VFF were comparable to those obtained by membrane filtration. Also, in incubation experiments where a ^{32}P-orthophosphate tracer was added, the specific ^{32}P activity of the D-ATP pool was approximately one third of the corresponding P-ATP (HOT 104; 30 vs. 94 Bq pM ATP^{-1}), implying that the D-ATP concentrations measured were not likely an artifact of filtration. The systematically higher inventories of T-ATP observed when applying the direct acid extraction compared to the sum of D-ATP and P-ATP may be due to a more effective extraction when using acid. It has been demonstrated that the phosphorylation state of the intracellular total adenine pool may change rapidly upon filtration, i.e., ATP may hydrolyze to ADP + Pi or AMP + PPi, thus reducing the ATP signal in the sample without any actual losses to the extracellular pools (Karl and Holm-Hansen, 1978).

Azam and Hodson (1977) first developed a method for the measurement of D-ATP in seawater using a mixture of activated charcoal and celite as an adsorption matrix, ammonical-ethanol solvent elution, and evaporation to desalt and concentrate D-ATP prior to measurement by firefly bioluminescence. Due to this labor-intensive procedure and relatively low and variable recoveries of ATP (typically 10–50%), this method has been applied only rarely in marine ecological studies (Hodson et al., 1981a; McGrath and Sullivan, 1981; Nawrocki and Karl, 1989), despite the great potential of D-ATP as a model compound. These studies were all conducted in relatively nutrient-rich, high-productivity regions with P-ATP concentrations ranging from 400 to >2000 pM, and D-ATP commonly well above 200 pM. By comparison, P-ATP concentrations in the subtropical North Pacific Ocean rarely exceed 100 pM even in the most productive layers of the water column (e.g., HOT 1–100; 0–100 m, mean = 68.4 pM, s.e. = 30.7 pM, median 61.3 pM, $<15\%$ of distribution >100 pM, $n = 493$). Consequently, it was anticipated that larger volumes of seawater would be required to detect D-ATP, precluding the use of the existing methodology in these low biomass regions of the ocean. An alternative, and perhaps more efficient,

method for D-ATP detection would thus be desirable. The presently described method relies on a pH-induced formation of the mineral brucite ($Mg[OH]_2$), which co-precipitates a variety of organic and inorganic phosphorus-containing compounds from seawater (Karl and Tien, 1992; Karl and Yanagi, 1997; Thomson-Bulldis and Karl, 1998). D-ATP and D-GTP are among the compounds that are quantitatively co-precipitated (Fig. 1).

The depth distribution of D-ATP and D-GTP suggests production and accumulation within the euphotic zone, results that are consistent with a local biological source and, hence, the positive correlation to P-ATP pool concentrations. Below 200 m, we routinely measured low and relatively uniform concentrations of D-ATP (below detection, 20 pM; Fig. 2). These subeuphotic zone concentrations are lower, by at least a factor 4, than D-ATP concentrations reported by Azam and Hodson (1977) for samples collected at 200 m or deeper in the San Diego Trough. Considering the rapid biodegradation of D-ATP in marine ecosystems (Azam and Hodson, 1977; Björkman et al., 2000), it is conceivable that in order to maintain high concentrations of D-ATP and D-GTP at depth, there has to be a local source capable of producing high amounts of dissolved nucleotides. Given the low biomass at depth (usually < 10 pM P-ATP), it appears unlikely that the extant microorganisms can be responsible for maintaining an order of magnitude higher D-ATP concentration than reported by Azam and Hodson (1977). Although mixing processes and scavenging onto particles may contribute to higher than expected D-ATP concentrations at depths, a gradient in D-ATP would still be anticipated rather than a uniformly high concentration with increasing depth.

Both ATP and GTP play integral roles in the regulation of metabolic activities in all living cells. ATP functions in the energy transfer for anabolic and catabolic cellular processes and, together with ADP and AMP, for controlling the energy status of the cell (Karl, 1980). However, many biosynthetic reactions rely on energy derived from non-adenine nucleotide triphosphates. For example, GTP is required in protein synthesis as the energy donor in RNA transcription, tRNA binding and translocation, and the intracellular levels of GTP appear to be positively correlated to the rate of protein synthesis and thus growth (Karl, 1979). GTP and ATP are also necessary components in the biosynthesis of both RNA and, in their deoxyribose forms, DNA. Karl (1978a, 1979) found that the particulate GTP/ATP ratio in microorganisms was positively correlated to growth rate, suggesting that the P-GTP/P-ATP ratio could be useful in comparing microbial community growth rates in natural samples.

In an environment presently believed to be under P-control (Karl, 1999; Karl et al., 2001), nucleotides may be important in sustaining the P-cycle. Recent studies in the North Pacific subtropical gyre have shown nucleotides to be among the most bioavailable dissolved organic phosphorus compounds and release of orthophosphate from dissolved nucleotides could exceed the rate of phosphorus uptake by as much as 50-fold (Björkman et al., 2000). This indicates a potentially significant role for nucleotides in P dynamics in these oligotrophic environments. Consequently, dissolved nucleotides may be important in microbial metabolism and the measurement of the fluxes of ATP and GTP through the microbial community could be a powerful tool for understanding interactions among the particulate and dissolved matter fractions, especially, but not exclusively, to P pool dynamics

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