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Measurement and interpretation of microbial adenosine tri-phosphate (ATP) in aquatic environments

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ABSTRACT

There is a widespread need for cultivation-free methods to quantify viability of natural microbial communities in aquatic environments. Adenosine tri-phosphate (ATP) is the energy currency of all living cells, and therefore a useful indicator of viability. A luminescence-based ATP kit/protocol was optimised in order to detect ATP concentrations as low as 0.0001 nM with a standard deviation of <5%. Using this method, more than 100 water samples from a variety of aquatic environments (drinking water, groundwater, bottled water, river water, lake water and wastewater effluent) were analysed for extracellular ATP and microbial ATP in comparison with flow-cytometric (FCM) parameters. Microbial ATP concentrations ranged between 3% and 97% of total ATP concentrations, and correlated well ($R^2 = 0.8$) with the concentrations of intact microbial cells (after staining with propidium iodide). From this correlation, we calculated an average ATP-per-cell value of 1.75×10^{-10} nmol/cell. An even better correlation ($R^2 = 0.88$) was observed between intact biovolume (derived from FCM scatter data) and microbial ATP concentrations, and an average ATP-per-biovolume value of 2.95×10^{-9} nmol/μm^3 was calculated. These results support the use of ATP analysis for both routine monitoring and research purposes, and contribute towards a better interpretation of ATP data.

1. Introduction

Accurate quantification of microbial abundance and viability in natural aquatic environments is a recognized necessity, with applications amongst others in research (Vives-Rego et al., 2000; Berney et al., 2008), hygiene monitoring (Davidson et al., 2003), and food and beverage quality control procedures (Hoefel et al., 2003; Berney et al., 2008). Numerous different methods that target parameters on single-cell level, such as membrane integrity, enzyme activity, substrate uptake or cell elongation, have been developed and tested in recent years (e.g., Créach et al., 2003; Yokomaku et al., 2000; Berney et al., 2007). It is generally agreed that a combination of methods that focuses on different indicators of viability is

Abbreviations: ATP, Adenosine tri-phosphate; DIC, Differential interference contrast; DMSO, Dimethylsulfoxide; FCM, Flow cytometry; HPC, Heterotrophic Plate Counts; RLU, Relative Light Units; PI, Propidium iodide; SG, SYBR Green I; SGPI, SYBR Green I and Propidium iodide; SSC, Sideward scattered light; TCC, Total cell concentration.

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superior to any individual method, especially when natural microbial communities are assessed (Fairbanks et al., 1984; Berney et al., 2008). Adenosine tri-phosphate (ATP) is a parameter that can be used as an independent, complimentary method for viability assessment. Often described as the "energy currency" of biological cells, ATP is an activated energy carrier that is present in all viable cells (Karl, 1980; Webster et al., 1985). It is therefore not surprising that ATP has for several decades been promoted as a potential indicator for viable biomass estimation (Holm-Hansen, 1969; Thore et al., 1975; Karl, 1980; Van der Kooij et al., 1995). Recent specific applications of ATP analysis on natural communities in aquatic environments include the analysis of drinking water (Delahaye et al., 2003; Hammes et al., 2008; Siebel et al., 2008), groundwater (Ludvigsen et al., 1999; Eydal and Pedersen, 2007; Pedersen et al., 2008; Wang et al., 2008b), biofilters in water treatment plants (Magic-Knezev and van der Kooij, 2004; Velten et al., 2007), and bacteria in biofilms from distribution networks (Boe-Hansen et al., 2002; Lehtola et al., 2002).

A typical method for ATP detection involves the chemical and/or enzymatic extraction of ATP from bacterial cells, followed by the measurement of light emission derived when the dissolved ATP reacts with the Luciferin–Luciferase complex (McElroy, 1947; Webster et al., 1985; Chittock et al., 1998; Sakakibara et al., 1997). The method is fast, robust, easy to perform, affordable and detects both cultivable and uncultivable organisms (Van der Kooij et al., 1995; Venkateswaran et al., 2003; Velten et al., 2007). Considering all the apparent advantages, ATP is used less frequently in both research and routine monitoring than what should be expected. Apart from the need for experienced and trained personnel, this can probably be attributed to three main (perhaps perceived) problems. Firstly, personal experience in our group and communication with practitioners suggested that ATP assays are often not sufficiently sensitive for accurate detection of low cell concentrations that are typical for many aquatic environments. Secondly, ATP measurement protocols usually do not distinguish between extracellular ATP and microbial ATP, and if ignored, this can have a considerable adverse influence on the sensitivity of the assay and interpretation of ATP data (Venkateswaran et al., 2003; Sakakibara et al., 1997; Hammes et al., 2008). The third and probably biggest problem is the correct interpretation of ATP as a parameter of cell viability, and specifically the conversion of ATP concentrations to bacterial cell concentrations. Part of this problem stems from the fact that ATP concentrations are not uniform in bacterial cells. Different bacterial species and different physiological states of bacteria can influence the amount of ATP that is measured for individual cells (Pridmore et al., 1984; Fairbanks et al., 1984; Schneider and Gourse, 2004), while cell size was also suggested as an important contributing factor (Eydal and Pedersen, 2007).

In our group we have previously investigated ATP concentrations relative to total bacterial numbers and different viability indicators in drinking water (Berney et al., 2008; Siebel et al., 2008). Both of the aforementioned studies demonstrated significantly better correlations between ATP and cultivation-independent methods compared to conventional plating methods. However, these studies were limited in the number of samples and the range of different aquatic environments that were investigated, while neither focused in particular on the interpretation of ATP data as such. In the present study we have investigated the use of ATP concentrations as an indicator for viable bacteria in aquatic environments. To this end, we have (1) optimised a commercial ATP assay/protocol to improve the sensitivity for detection of ATP in oligotrophic environments and (2) to distinguish specifically between microbial ATP and extracellular ATP. (3) Furthermore, we compared specifically microbial ATP concentrations to cultivation-independent flow-cytometric measurements of intact cell concentrations and biovolume.

2. Material and methods

2.1. Water samples

A total of 102 water samples from various sources were processed over a period of 5 months. Surface water was sampled from seven lakes and two oligotrophic streams in the vicinity of Zürich (CH). The lake water samples were filtered (8.0 μm; SCWP 02500, Millipore, Billerica, MA, USA) to remove particles and algal biomass prior to measurements. Groundwater was collected from seven alpine springs (Sachseln, CH) and artificial recharge sources (Basel, CH) used for drinking water treatment. Non-chlorinated drinking water samples were taken from multiple household taps and public fountains in the vicinity of Zürich, covering several different treatment plants and distribution networks. Wastewater effluent was sampled from two local treatment plants (Dübendorf, CH). All samples were collected in sterile 1 L borosilicate glass bottles with tight-sealing screw caps, transported under cold storage (5 °C) to the laboratory, and processed within 4 h of sampling. Commercially available bottled water (14 different brands of still water) was sampled directly from 1.5 L bottles that were purchased locally.

2.2. Adenosine tri-phosphate (ATP) analysis: protocol optimisation

ATP was measured using the BacTiter-Glo™ Microbial Cell Viability Assay (G8231; Promega Corporation, Dübendorf, CH) and a GloMax® 20/20 Luminometer (Turner BioSystems, Sunnyvale, CA, USA). The BacTiter-Glo™ reagent was prepared according to the manufacturers guidelines, with a 2 h period of "burn-off" to reach maximum sensitivity with low background signals. The prepared reagent was stored in 1 mL aliquots in the dark at −20 °C until use, but never exceeding 2 weeks of storage. This particular ATP kit combines the ATP releasing agents and the luciferase enzymes in a single reagent, and generates a constant light intensity during the ATP reaction. The manufacturer's protocol for this product prescribes reagent-to-sample volumes of 100 μL:100 μL (alternatively 25 μL:25 μL), undefined "room temperature" for sample incubation, and 5 min incubation for the reaction time. The main aspects of this protocol (temperature and reaction volumes) were investigated individually: (a) the impact of increased sample volume on the sensitivity of the assay was tested by adding different volumes (50, 100, 200, 300, 400 and 500 μL) of a tap water sample to a set volume