RESEARCH

Unveiling the impact of glycerol phosphate (DOP) in the dinoflagellate Peridinium bipes by physiological and transcriptomic analysis

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Abstract

Background: The ability to use dissolved organic phosphorus (DOP) is important for survival and competition when phytoplankton are faced with scarcity of dissolved inorganic phosphorus (DIP). However, phosphorus availability to the freshwater dinoflagellate Peridinium bipes has received relatively little attention, the efficiency of glycerol phosphate use by phytoplankton has rarely been investigated, and the regulatory molecular mechanisms remain unclear.

Result: In the present study, cultures of the freshwater dinoflagellate Peridinium bipes were set up in 119 medium (+DIP), DIP-depleted 119 medium (P-free), and β -glycerol phosphate-replacing-DIP medium (+DOP). Gene expression was analyzed using transcriptomic sequencing. The growth rate of cells in DOP treatment group was similar to that in DIP group, but chlorophyll a fluorescence parameters RC/CS₀, ABS/CS₀, TR₀/CS₀, ET₀/CS₀ and RE₀/CS₀ markedly decreased in the DOP group. Transcriptomic analysis revealed that genes involved in photosynthesis, including *psbA*, psbB, psbC, psbD, psaA and psaB, were downregulated in the DOP group relative to the DIP group. Glycerol-3-phosphate dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase, rather than alkaline phosphatase, were responsible for β -glycerol phosphate use. Intercellular gluconeogenesis metabolism was markedly changed in the DOP group. In addition, genes involved in ATP synthases, the TCA cycle, oxidative phosphorylation, fatty acid metabolism and amino acid metabolism in *P. bipes* were significantly upregulated in the DOP group compared with the DIP treatment.

Conclusions: These findings suggested that β -glycerol phosphate could influence the photosynthesis and metabolism of *P. bipes*, which provided a comprehensive understanding of the phosphorus physiology of *P. bipes*. The mechanisms underlying the use of β -glycerol phosphate and other DOPs are different in different species of dinoflagellates and other phytoplankton. DIP reduction may be more effective in controlling the bloom of *P. bipes* than DOP reduction.

Keywords: Freshwater dinoflagellate, Peridinium bipes, Photosynthesis, Metabolism, Transcriptome

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Phosphorus is a vital element involved in many biochemical reactions and metabolic processes, including cell membrane synthesis, signal transduction, photosynthesis, nucleic acid metabolism and energy metabolism [1-4]. As an essential nutrient for growth, phosphorus availability not only constrains phytoplankton productivity but also plays a very important role in the community structure of phytoplankton [4]. Therefore, phosphorus

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Background

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is regarded as a limiting factor in marine and freshwater ecosystems [5].

In aquatic environments, dissolved inorganic phosphorus (DIP) and dissolved organic phosphorus (DOP) are the two major total dissolved phosphorus (TDP) pools [6]. DIP can be directly used by phytoplankton cells [7]. However, DIP is rapidly consumed and shows very low replenishment from external sources, often resulting in growth-limiting concentrations in aquatic environments [8–10]. However, DOP is relatively abundant in aquatic environments. In natural waters, DOP is derived from soil and sediment, excretion by live organisms, and decay of dead organisms [11]. DOP also comes from organic compounds discharged from industrial, agricultural and domestic drainage [12]. According to Benitez-Nelson [8], in coastal marine environments and in the open ocean, the DOP pool can be up to 50% or 75% of the TDP pool, respectively. Other studies have shown that in the North Atlantic Ocean, DOP often accounts for >80% of the TDP pool [13–15]. The DOP pool has even been found to exceed DIP by an order of magnitude in some environments, such as the Sargasso Sea, where DOP:DIP ratios in surface waters can exceed 100 [13, 16]. Bogé et al. [17] observed that the highest DOP concentration in Toulon Bay, France, was 0-0.33 µM, while DIP concentration was approximately 0-0.19 µM.

Because DOP can be only assimilated by phytoplankton cells with the assistance of hydrolases [6, 18], the ability to use DOP is a potential driving factor in both phytoplankton species composition and the initiation and maintenance of harmful algal blooms (HAB) [9]. Glycerol phosphate is an important component of DOP because it is widely used in biology as well as in medicine and other fields of human endeavor [19–21]. However, the efficiency of glycerol phosphate use by phytoplankton has rarely been investigated, and the regulatory molecular mechanisms remain unclear.

Dinoflagellates are an important functional component of the phytoplankton community in marine and freshwater ecosystems [22, 23]. Many dinoflagellates, such as *Ostreopsis* [24], *Akashiwo* [25–27], *Alexandrium* [28] and *Karenia* [6, 29], have been reported to cause HABs in marine ecosystems [30, 31]. Phosphorus is believed to affect the abundance, volume, toxin synthesis and motile form of these dinoflagellates [32–34] and to be one of the primary factors influencing the frequency of red tides [35]. Numerous studies have indicated that dinoflagellates can assimilate various types of DOP when DIP is insufficient [18, 36–38]. However, relative to marine ecosystems, the dinoflagellate species in freshwater ecosystems are little studied.

Recently, species of *Peridinium* have occurred in dense blooms in freshwater reservoirs and lakes throughout the world. During the bloom of *P. cinctum*, the organism comprises more than 90% of the phytoplankton biomass in Lake Kinneret, Israel and Lake Torrens, Australia [39, 40]. A bloom of *P. bipes* was also observed in the meso-trophic Huanglongdai Reservoir (biomass 5138 μ g L⁻¹), China, and Juam Reservoirs, South Korea [41, 42]. High-biomass blooms have caused mass mortalities of a variety of aquatic organisms through predation, starvation, shading, or creation of anoxic conditions [43–46].

Wynne et al. [39] suggested that the rapid decline of *Peridinium* in Lake Kinneret in June is brought about by a combination of physical and chemical factors, such as pH, temperature, irradiation and limitation of P, N or other micronutrients. Previous studies have been proposed that phosphorus not only affects the abundance, toxin synthesis and motile form of these dinoflagellates [32–34], but also regulates the frequency of dinoflagellate savailability to the freshwater dinoflagellate *P. bipes* has received relatively little attention. Thus, the aims of this study were (1) to evaluate the growth and photosynthetic responses of *P. bipes* to DIP and DOP; and (2) to identify potential genes and pathways involved in the use of DIP and DOP by comparative transcriptomic analysis.

Materials and methods

Algal strain and culture conditions

The strain of Peridinium bipes used in this study was isolated from a tributary of Three Gorges Reservoir, China. This strain was grown in 119 medium (http://algae.ihb. ac.cn/Products/ProductDetail.aspx?product=10) at 25 ± 1 °C under white light with intensity 50 mmol photons $m^{-2}s^{-1}$ and a 12-h light/12-h dark cycle. A mixture of ampicillin (final concentration 200 μ g mL⁻¹), kanamycin (final concentration 100 μ g mL⁻¹) and streptomycin (final concentration 100 μ g mL⁻¹) was used to inhibit the growth of bacteria to <1% of the culture biomass, which was confirmed using a Nikon CE-I fluorescence microscope (Nikon, Tokyo, Japan). Before the experiment, all glassware was soaked in 0.1 mol L^{-1} HCl for 24 h and then rinsed with ultrapure water to prevent external phosphorus contamination. Cells in the logarithmic growth phase (after growth for about 10 days) were collected by centrifugation at $2683 \times g$ for 5 min, washed three times with P-free medium (119 medium without phosphorus), and then inoculated in P-free medium for 5 days to remove excess phosphate [6].

When the DIP concentration in the culture was below the detection limit of 0.2 μ mol L⁻¹, the cells were inoculated into 500-mL glass flasks containing 250 mL 119 medium. Three different conditions were used: (i) 119 medium without P (P-free medium); (ii) P-free 119 medium supplemented with 52 μ mol L⁻¹ K₂HPO₄ (DIP treatment, as the control); and (iii) P-free 119 medium with 52 µmol L⁻¹ β-glycerol phosphate (DOP treatment). All phosphorus reagents were purchased from Sigma–Aldrich (USA; >99% purity, analysis- or chromatography analysis-grade). The initial concentration of *P. bipes* in each treatment was 0.37×10^4 cells L⁻¹.

Cell density and specific growth rate

After inoculation, cell density was determined using a hemocytometer chamber under a Nikon CE-I microscope. Specific growth rate (μ) was calculated as $\mu = (\ln N_1 - \ln N_0)/(t_1 - t_0)$, where N_0 and N_1 are the biomass at times t_0 and t_1 , and t_0 and t_1 are the times that the exponential growth phase started and ended.

Polyphasic Chl *a* fluorescence transient and energy pathway model

Samples in logarithmic growth were dark-adapted for 20 min before conducting chlorophyll *a* fluorescence measurement using a Plant Efficiency Analyzer (Hansatech Instruments Ltd., UK) with an actinic light of 3000 µmol photon $m^{-2}s^{-1}$. Fluorescence signals were recorded within a time scan of 10 µs to 2 s. Baesd on the theory of energy flux in PSII, several fluorescence parameters were obtained from the polyphasic Chl *a* fluorescence transient (OJIP) curve [47]. An energy pathway model of photosynthesis in *P. bipes* was established according to the energy flow model of Appenroth et al. [48] with specific activity parameters of the unit area of photosynthetic apparatus and the unit active reaction centers (RC) of *P. bipes*.

DIP concentration and alkaline phosphatase activity

DIP concentrations were measured by the phosphorus molybdenum blue method [49]. The supernatant in samples was used to determine the activity of extracellular phosphatases. Alkaline phosphatase activity (APA) was detected with p-nitrophenyl phosphate (Amresco, USA) as the substrate [50].

Transcriptome analysis

After 11-day inoculation, samples from the DIP and DOP treatments were harvested by suction filtration. The supernatant was discarded, and the centrifuge tube was immersed in liquid nitrogen to freeze the cell pellet for later processing. RNA isolation, library preparation and sequencing referred to the methods of Dong et al. [51]. Transcriptome measurement was undertaken by Novogene Company (Beijing, China). The data were submitted to NCBI (Accession No. PRJNA608149).

Clean data (clean reads) were obtained by removing reads containing adapters or poly-N and low-quality reads from the raw data. Q20, Q30, GC-content and sequence duplication level of the clean data were calculated. All downstream analyses were based on highquality clean data. Gene function was annotated based on the following databases: Nr (NCBI non-redundant protein sequences), Nt (NCBI non-redundant nucleotide sequences), Pfam (Protein family), KOG/COG (Clusters of Orthologous Groups of proteins), Swiss-Prot (a manually annotated and reviewed protein sequence database), KO (Kyoto Encyclopedia of Genes and Genomes Ortholog database), and GO (Gene Ontology). Transcript relative abundance was aligned to annotated gene models using the HTSeq method (HTSeq v0.6.1) and was expressed as the number of reads. Fragments per kilobase of exon per millions of fragments mapped (FPKM) values were normalized to obtain the gene expression levels from RNA-Seq. Then, analysis of differential expression in different treatments was conducted using the DEGseq method in the DESeq R package (1.18.0). The cutoff value for analyzing gene transcriptional activity was determined based on the 95% confidence interval for all FPKM values. Fold expression changes between different samples were calculated using transcript relative abundance log₂ ratios. A differentially gene expression was accepted when genes showed at least q value < 0.005 and $|\log_2 FoldChange| > 1$.

Statistical analysis

All experiments were performed in triplicate. Data obtained in this study were analyzed using a least significant difference test with one-way analysis of variance (ANOVA) in SPSS software version 17.0 (IBM, USA). Statistical significance was determined at p < 0.05. Data were plotted using Origin software version 6.1 (Origin-Lab Corporation, USA).

Results

Growth

The growth of *P. bipes* under different treatments after culture for 20 days is shown in Fig. 1a. Compared with the DIP group, a significant decrease was found in the P-free treatment (p < 0.01, ANOVA). However, no significant difference was determined when cells were cultured in DIP and DOP (the β -glycerol phosphate group). After culture for 20 days, the cell density reached 1.95×10^4 cells L⁻¹ (DIP group) and 1.79×10^4 cells L⁻¹ (DOP group). A significant decrease in specific growth rate was observed in the P-free treatment compared with the DIP and DOP treatments (Fig. 1b) (p < 0.01, ANOVA). Specific growth rates (μ) in the P-free, DIP and DOP treatments were 0.06 day⁻¹, 0.12 day⁻¹, and 0.11 day⁻¹, respectively.



When *P. bipes* was inoculated into the DIP-containing medium, the concentration of DIP showed a rapid depletion by the 10th day compared with day 1 (p < 0.01), while it was consistently low in the P-free group. In contrast, the DIP concentration in the DOP group increased sharply to reach 0.91 mg L⁻¹ on the 5th day (p < 0.01), and then declined slowly (Fig. 2a). On day 15, there was about 0.41 mg L⁻¹ residual DIP in the DOP culture. APA was barely detectable on the first day in all three groups (Fig. 2b). On day 5 and thereafter, APA increased in the P-free group. In contrast, APA remained low in the other two groups.

Chlorophyll a fluorescence of PSII

OJIP curves for *P. bipes* treated in P-free, DIP and DOP conditions are shown in Fig. 3. Phase J increased clearly from the normalized OJIP curve in the P-free and DOP groups compared with that for the DIP group. A total of 18 parameters calculated from chlorophyll *a* fluorescence induction curves are shown in Additional file 1: Table S1. The values of PI_{abs} (performance index based on absorption of light energy), S_m (multiple turnover in the closure of the RCs), N (Q_A has been reduced in the time span from 0 to t_{Fmax}), and $\psi_{(E_0)}$ (probability [at t=0] that a trapped exciton moves an electron into the electron transport chain beyond Q_A) exhibited a remarkable

decrease in the DOP group compared with those in the DIP group (p < 0.05). The values of $V_{\rm J}$ (relative variable fluorescence in the J-step) and M_0 (approximate initial slope of the fluorescence transient) increased in the DOP group compared with that in the DIP group (p < 0.05). Compared with the DIP group, a significant decline in PI_{abs}, $S_{\rm m}$, N, $\psi_{(E_0)}$, and $\varphi_{(E_0)}$ (quantum yield of electron







transport), and an increase in $V_{\rm J}$ and M_0 was found in the P-free group (p < 0.05).

A simple energy flow model is shown in Fig. 4. Referring to the RC in the membrane and thus dealing with specific energy flux ABS/RC (absorption flux per RC), TR_0/RC (trapped energy flux per RC) and DI_0/RC (dissipated energy flux per RC) increased significantly, but ET_0/RC (electron transport flux per RC) and RE_0/RC (reduction of end acceptors at the PSI electron acceptor side per RC) declined markedly in the P-free group compared with those in the DIP group (p < 0.05). Similarly, a significant difference in TR_0/RC , ET_0/RC and RE_0/RC was also found in the DOP group compared with that in the DIP group (p < 0.05). However, a significant decline in RC/CS₀ (density of PSII RCs), ABS/CS₀ (absorption flux per cross-section), TR_0/CS_0 (trapped energy flux per cross-section), ET_0/CS_0 (electron transport flux per cross-section), DI_0/CS_0 (dissipated energy flux per cross-section), and RE_0/CS_0 (reduction of end acceptors at the PSI side per cross-section) in the P-free and DOP treatments was observed in the algal model in contrast to the treatment of DIP. The most striking decease was the number of active RCs in the model.

Transcriptomic analysis

GO analyses revealed 31 downregulated genes when *P. bipes* was cultured in DOP compared with DIP that were mostly distributed in categories membrane,





photosynthesis (photosynthetic electron transport chain, photosynthesis light reaction, and electron transport chain), generation of precursor metabolites and energy, thylakoid, and chlorophyll binding (Fig. 5a; Additional file 2). For example, the downregulated genes involved in photosynthesis included psbA (encoding PSII P680 RC D1 protein), psbD (encoding PSII P680 RC D2 protein), *psbC* (encoding PSII CP43 chlorophyll apoprotein), psbB (encoding PSII CP47 RC protein), psbW (encoding PSII RC W protein), petD (encoding electron transport protein cytochrome b (C-terminal)/ b_6), psaA (encoding PSI P700 chlorophyll a apoprotein A1), and psaB (encoding PSI P700 chlorophyll a apoprotein A2), which respectively showed 2.65-, 2.07-, 2.43-, 2.12-, 2.87-, 1.94-, 2.09- and 1.99-fold downregulation in P. bipes cultured in DOP compared with these genes of cells cultured in DIP (p < 0.05) (Fig. 5b).

One hundred and forty-eight genes were significantly upregulated in DOP treatment compared with DIP treatment (Additional file 3). Significant shifts in gluconeogenesis were observed, and the genes encoding phosphoenolpyruvate carboxykinase, glycerol-3-phosphate dehydrogenase (GPDH), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), malate dehydrogenase, fructose-1,6-bisphosphatase (FBP), and isocitrate lyase were respectively upregulated 4.70-, 9.64-, 4.52-, 4.17-, 3.73- and 5.13-fold in the DOP culture compared with these genes in DIP culture (p < 0.05). In addition, changes were observed in expression of genes involved in the TCA (tricarboxylic acid) cycle, oxidative phosphorylation, sulfur metabolism, membrane transport, ribosome and transcription (p < 0.05) (Fig. 6).

Discussion

Mechanism of β -glycerol phosphate uptake and use

In the face of DIP limitation, algae have evolved a set of mechanisms to obtain sufficient P [18, 52], including enhancement of the ability to assimilate low abundance inorganic phosphorus via high-affinity phosphate transporters [53]; decreasing the demand for phosphorus by replacing membrane phospholipids with non-phosphorus lipids and accelerating the turnover of phospholipid to provide a short-term phosphorus supply [54–56]; bypassing phosphorus-consuming processes in glycolysis reactions and lipid recycling [57–59]; and obtaining P from organic phosphorus, which is the major alternative phosphorus supply to DIP in ecosystems [34, 60–63], via alkaline phosphatase (AP) and other hydrolytic enzymes [6, 18, 64].

Oh et al. [36] found that *Alexandrium tamarense* grew poorly on glycerophosphate, while *Gymnodinium catenatum* was able to use glycerophosphate as well as DIP, suggesting that dinoflagellates show different responses to glycerophosphate. DIP can be directly absorbed and used by cells, but the application of DOP requires cells to consume more energy to break bonds to obtain active phosphorus [62, 65]. In the present study, *P. bipes* grew well using β -glycerol phosphate—only slightly lower growth was found in the DOP group compared with the DIP group (Fig. 1), this indicates that glycerophosphate could be used as the sole phosphorus source to support the growth of *P. bipes*.

Measurement of PSII activity can provide information about photosynthetic apparatus, the absorption and distribution of energy, and the transportation of electrons in PSII of intact algal cells [66, 67]. Chlorophyll fluorescence is a very useful method to analyze the energy transfer and photochemical events of PSII [47, 68, 69]. In this study, a remarkable decrease in density of reaction centers (RC/CS_0), absorption flux per cross-section (ABS/CS_0) , dissipated energy flux per cross-section $(DI_0/$ CS_0 , trapped energy flux per cross-section (TR_0/CS_0), electron transport flux per cross-section (ET_0/CS_0) , and reduction of end acceptors at the PSI electron acceptor side per cross-section (RE_0/CS_0) was found in P-free and DOP conditions compared with the culture grown in DIP (Additional file 1: Table S1; Figs. 3 and 4), indicating that photosynthesis decreased significantly in P-free and DOP conditions [70-72].

Moreover, an increase in the closure of active RCs (V_j) and the maximum rate at which Q_A was reduced (M_0) , and a decrease in the energy required for Q_A to be fully reduced (S_m) and for Q_A to be reduced in the time span from 0 to $t_{\rm Fmax}$ (N), was also observed in the P-free and DOP groups (Additional file 1: Table S1; Figs. 3 and 4), suggesting that the probability that an electron residing on Q_A^- would enter the transport chain was reduced; which in turn reduced the proportion of quanta in the electron transport chain [47, 68, 69]. These findings indicated that photosynthesis was inhibited in the DOP culture of *P. bipes* due to blockage of the electron transport chain from Q_A^- to Q_B .

Transcriptomic analyses revealed that 31 genes, including many encoding photosynthesis-related proteins such as *psbA*, *psbD*, *psbC*, *psbB*, *psbW*, *petD*, *psaA* and *psaB*, were downregulated when *P. bipes* was cultured in DOP compared with that in DIP (Fig. 5a; Additional file 2). The RC of PSII consists of proteins D1 (encoded by *psbA*) and D2 (encoded by *psbD*), which provide the binding sites for the electron transfer chain cofactors [73]. Protein D1 is connected with RC P680, the primary electron acceptor pheophytin, and the secondary electron acceptor $Q_{\rm B}$, while the D2 protein provides a binding site for the electron acceptor $Q_{\rm A}$ [74, 75]. Due to strongly oxidative chemistry of PSII water splitting, the D1 protein is prone to constant photodamage, whereas most of the other PSII



Fig. 5 Down-regulated expression genes in *P. bipes* between DOP and DIP condition. Results are summarized for the three main GO categories: biological processes, cellular component and molecular function (**a**). Differential gene expression related to photosynthesis in *P. bipes* between DOP and DIP conditions (**b**)



subunits ordinarily remain undamaged [76]. When subjected to environmental stress, the degradation rate of D1 and D2 proteins is greater than their synthesis rate, which drives destruction of the RC and blocks electron transfer [77]. In this study, both psbA and psbD were downregulated in the DOP group (Fig. 5a; Additional file 2), suggesting that the synthesis rate of D1 and D2 proteins might be impeded, resulting in damage to the RCs and blocked electron transfer. CP43 and CP47 are important subunits of PSII, which together form the central lightharvesting pigment complex and are closely connected to the RC [78]. Expression of psbC and psbB was downregulated (Fig. 5b) in the DOP treatment, indicating that the ability to capture light was reduced. These data collectively indicated that PSII activity was inhibited in the DOP-cultured P. bipes.

Previous studies have proposed that some phytoplankton can excrete AP to hydrolyze organic phosphorus when DIP is scarce [79, 80]. It is clear that in our study, β -glycerol phosphate was hydrolyzed because the concentration of DIP in the medium increased (Fig. 2a). Luo

et al. [6] proposed that three mechanisms might underlie the use of DOP: (1) extracellular DOP hydrolysis is slow and coupled tightly to uptake, leaving no residual DIP; (2) DOP is absorbed directly without extracellular hydrolysis; and (3) both extracellular hydrolysis and direct uptake of DOP or its hydrolysis products occur. In the present study, however, there was no significant increase in APA when *P. bipes* was cultured in DOP (Fig. 2b), suggesting that *P. bipes* did not employ AP for β -glycerol phosphate hydrolysis. Huang et al. [81] showed that APA increased only when low-molecular-weight DOP was exhausted in marine microalgae. Luo et al. [6] found that the dinoflagellate Karenia mikimotoi did not release AP to hydrolyze DOP when it was cultured in ATP, and 5'-nucleotidase enzymatic activity was responsible for the utilization of ATP in K. mikimotoi [6], Prochlorococcus MED4 [82], Thalassiosira pseudonana [63] and Emiliania huxleyi [83]. These results suggested that AP was not the only enzyme to hydrolyze DOP. Therefore, the mechanism of DOP use in *P. bipes* needs to be further studied in the future.

Modulation of metabolic pathways by β -glycerol phosphate

One hundred and forty-eight significantly upregulated genes were detected in transcriptomic analyses on growth of P. bipes in DOP compared with DIP (Fig. 6; Additional file 3). ATP synthases are membrane-embedded rotary motors that produce or consume ATP and control the pH within cells [84]. The Na^+/K^+ -ATPase is an ion pump belonging to the P-type ATPase family. Using energy derived from ATP hydrolysis, Na⁺/K⁺-ATPase generates electrochemical gradients for Na⁺ and K⁺ across plasma membranes, as required for electrical excitability, cellular uptake of ions, nutrients and neurotransmitters, and regulation of cell volume and intracellular pH [85]. Kdp is a P-type ATPase of unique structure, a complex of three membrane proteins (KdpFABC); this complex is a highaffinity ATP-dependent potassium uptake system, which plays a fundamental role in the steady supply of potassium to cells [86]. The Ca^{2+} -transporting ATPase is the most abundant in the sarcoplasmic reticulum membrane [87]. It couples the energy derived from hydrolysis of ATP to transport of Ca²⁺ ions across the membrane against a concentration gradient [87]. In the present study, genes encoding these P-type ATPases were upregulated in the DOP group (Fig. 6; Additional file 3), suggesting that DOP affected the regulation of ion transport and osmotic pressure of cells. F-type ATPases are highly conserved enzymes used primarily for the synthesis of ATP [84]. Upregulation of the genes encoding the F-type synthase (ATPeF1A, ATPeF1B) (Fig. 6; Additional file 3) indicated increased ATP synthesis and energy metabolism in the DOP group.

The metabolism of glycerol-3-phosphate (G3P) is important for environmental stress responses of eukarvotic microalgae. All cells contain polar lipids with a glycerol backbone; the glycerol backbone in the polar lipids of bacteria and eukaryotes is G3P [88]. G3P can be used in various metabolic pathways via glycolysis [88]. GPDH activity is important for glycerol production via the metabolism of G3P to provide osmotic stress tolerance or for lipid synthesis in many organisms [89, 90]. There is a G3P shuttle in mitochondria, consisting essentially of two components: an NAD⁺-GPDH (EC 1.1.1.8) in the cytosol, and a membrane-bound FAD-GPDH (EC 1.1.99.5) located in the inner mitochondrial membrane. The NAD⁺-GPDH reduces dihydroxyacetone phosphate (DHAP) to G3P using NADH as a reducing agent, while the FAD-GPDH catalyzes the conversion of G3P to DHAP and forms FADH₂ which enters the electron transport chain [91]. In this study, the expression of *glpA* encoding the FAD-GPDH was 9.64-fold higher in the DOP treatment than in the DIP treatment (Additional file 3), indicating the enhancement of DHAP generation.

The structure of β -glycerol phosphate used as the DOP in the experiments in this study is similar to that of G3P. Thus, GDPH might be an important enzyme for catalysis of conversion of β -glycerol phosphate to DHAP. In the DOP culture group, the genes encoding GAPDH and FBP were significantly upregulated. GAPDH and FBP are key enzymes in gluconeogenesis. GAPDH can reversibly catalyze the oxidation and phosphorylation of 1,3-diphosphoglycerate to glyceraldehyde-3-phosphate to form Pi [92]. FBP (encoded by FBP) and phosphoenolpyruvate carboxykinase (encoded by pckA) regulate the irreversible steps of gluconeogenesis [93, 94]. In the present study, the expression of FBP and pckA in P. bipes was significantly upregulated in the DOP group compared with the DIP group (Fig. 6; Additional file 3), suggesting that the gluconeogenesis pathway was fueled by β -glycerol phosphate. As photosynthesis was downregulated on culture in DOP, intracellular organic synthesis and insufficient supply of glucose would be replenished by gluconeogenesis. Moreover, gluconeogenesis releases Pi, resulting in the observed increase of DIP in the culture medium (Fig. 2). The enhanced Pi could make up for the deficiency in the intracellular phosphorus supply if DOP could not be directly used, or the use was delayed.

The TCA cycle is the final common catabolic pathway for the oxidation of fuel molecules. In this cycle, succinyl-CoA synthetase converts succinyl-CoA to succinate and yields a high-energy phosphate bond [95]. Malate dehydrogenase, encoded by the gene MDH2, is responsible for the regeneration of oxaloacetic acid and a new turn of the TCA cycle, and catalyzes conversion of malate to oxaloacetate and generates NADH. Oxidative decarboxylation is an important stage in the TCA cycle. Complex I (NADH-Q reductase), Complex II (succinate-Q reductase), Complex III (cytochrome c reductase), Complex IV (cytochrome c oxidase) and Complex V (ATP synthase) are involved in oxidative phosphorylation to catalyze the transfer of electrons coupled with protons across the membrane using substrates generated in the TCA cycle [96]. In this study, genes encoding succinyl-CoA synthase (e.g. LSC1, LSC2) and malate dehydrogenase (e.g. MDH2) were significantly upregulated in the DOP group compared with the DIP group (Fig. 6; Additional file 3). Moreover, genes involved in oxidative phosphorylation, such as those encoding cytochrome *c* reductase (GO: 0004553) and cytochrome c oxidase (e.g. CYC, COX1), also showed significantly higher expression in the DOP group compared with the DIP group (Fig. 6; Additional file 3). These data indicate that P. bipes could enhance the production of energy to support metabolism when it was cultured in DOP.

The gene *Amt* is associated with ammonium transport and is induced substantially when ammonium is limiting for growth. The assimilation of ammonium is regulated by the combined activities of glutamine synthetase, encoded by *glnA* [97]. In this study, *Amt* and *glnA* were respectively upregulated 6.77- and 3.92-fold in the DOP group compared with the DIP group (Additional file 3), suggesting that the enhancement of ammonium transport is possibly resulted from the decline of the N:P ratio due to increased P concentration in the DOP group. Similar results have been observed in other dinoflagellates, such as *K. mikimotoi* [6]. In addition, regulation of ammonium transporters is not only a function of substrate availability resulting from external supply, but also the extent of internal metabolic pathways that are ammonium-generating, namely photorespiration and the extent of mixotrophic nutrition [98].

Krüßel et al. [99] demonstrated that sulfur dioxygenase (encoded by *ETHE1*) plays an important role in plant cysteine catabolism. Sulfur dioxygenase can oxidize persulfide to sulfite and is involved in metabolic homeostasis in mitochondria. It can also bind to nuclear transcription factors [100]. The cysteine desulfurase IscS can catalyze formation of alanine and sulfane sulfur from cysteine, and provides sulfur for iron–sulfur cluster synthesis [101]. The genes *ETHT1* and *IscS* were upregulated 3.66- and 7.23-fold, respectively, in the DOP group in our study compared with the DIP group (Additional file 3), suggesting that DOP improved sulfur metabolism and respiratory electron transport [99].

Conclusions

The present study combined physiological and transcriptome sequencing analyses to determine the response of P. bipes to different phosphorus conditions. The data indicate that the RC of PSII was damaged and the electron transfer in PSII was blocked when P. bipes was cultured in DOP conditions, due to the downregulation of photosynthesis genes. GPDH and GAPDH are most likely responsible for β -glycerol phosphate use, and alkaline phosphatase activity was an indicator of DIP stress, but not an exclusive indicator for DOP. Moreover, gluconeogenesis played a vital role in β -glycerol phosphate use. In addition, ATPases, the TCA cycle, oxidative phosphorylation, and fatty acid, ammonium, sulfur and amino acid metabolism were significantly upregulated in DOP conditions compared with culture in DIP, suggesting their involvement in the use of β-glycerol phosphate or its hydrolysis products in P. bipes. Collectively, these findings provided a comprehensive understanding of the phosphorus physiology of P. bipes. In addition, our results underscore that GPDH and GAPDH should be studied further, and that the mechanisms underlying the use of β -glycerol phosphate and other DOPs are different in different species of dinoflagellates and other phytoplankton, suggesting that the control of dinoflagellate blooms by targeted nutrient reduction is largely dependent upon the dominant species. DIP reduction may be more effective in controlling the bloom of *P. bipes* than DOP reduction.

Supplementary information

Supplementary information accompanies this paper at https://doi. org/10.1186/s12302-020-00317-6.

Additional file 1: Table S1. Parameters calculated using data extracted from the O-J-I-P fast fluorescence transient.

Additional file 2. Information on down-regulated genes under different phosphorus treatments.

Additional file 3. Information on up-regulated genes under different phosphorus treatments.

Abbreviations

DIP: Dissolved inorganic phosphorus, it also represents K₂HPO₄ here; DOP: Dissolved organic phosphorus, it also represents glycerol phosphate here; TCA: Tricarboxylic acid: TDP: Total dissolved phosphorus: OJIP: The polyphasic Chl a fluorescence transient; RC: Active reaction centers; APA: Alkaline phosphatase activity; FPKM: Fragments per kilobase of exon per millions of fragments mapped: Plabs: Performance index based on absorption of light energy: S_{m} Multiple turnover in the closure of the RCs; N: Q_A has been reduced in the time span from 0 to t_{Fmax} ; $\psi_{(E_0)}$: Probability [at t=0] that a trapped exciton moves an electron into the electron transport chain beyond Q_{Δ} ; V_{i} : Relative variable fluorescence in the J-step; M_0 : Approximate initial slope of the fluorescence transient; $\varphi_{(E_0)}$: Quantum yield of electron transport; ABS/RC: Absorption flux per RC; TR₀/RC: Trapped energy flux per RC; Dl₀/RC: Dissipated energy flux per RC; ET₀/RC: Electron transport flux per RC; RE₀/RC: Reduction of end acceptors at the PSI electron acceptor side per RC; RC/CS₀: Density of PSII RCs; ABS/CS₀: Absorption flux per cross-section; TR₀/CS₀: Trapped energy flux per cross-section; ET₀/CS₀: Electron transport flux per cross-section; Dl₀/CS₀: Dissipated energy flux per cross-section; RE₀/CS₀: Reduction of end acceptors at the PSI side per cross-section; GPDH: Glycerol-3-phosphate dehydrogenase; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; FBP: Fructose-1,6-bisphosphatase; AP: Alkaline phosphatase; G3P: Glycerol-3-phosphate; DHAP: Dihydroxyacetone phosphate.

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Authors' contributions

Each author has participated sufficiently in the work to take public responsibility for all the content. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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