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Extensive Turnover of Compatible Solutes in Cyanobacteria Revealed by Deuterium Oxide (D_2O) Stable Isotope Probing

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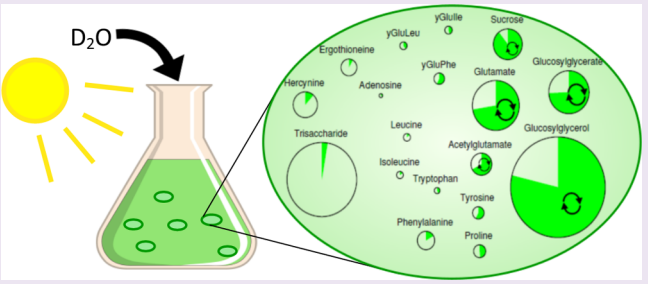
Supporting Information

ABSTRACT: Cyanobacteria are important primary producers of organic matter in diverse environments on a global scale. While mechanisms of CO_2 fixation are well understood, the distribution of the flow of fixed organic carbon within individual cells and complex microbial communities is less well characterized. To obtain a general overview of metabolism, we describe the use of deuterium oxide (D_2O) to measure deuterium incorporation into the intracellular metabolites of two physiologically diverse cyanobacteria: a terrestrial filamentous strain (*Microcoleus vaginatus* PCC 9802) and a euryhaline unicellular strain (*Synechococcus* sp. PCC 7002). D_2O was added to the growth medium during different phases of the diel cycle. Incorporation of deuterium into metabolites at nonlabile positions, an indicator of metabolite turnover, was assessed using liquid chromatography mass spectrometry. Expectedly, large differences in turnover among metabolites were observed. Some metabolites, such as fatty acids, did not show significant turnover over 12−24 h time periods but did turn over during longer time periods. Unexpectedly, metabolites commonly regarded to act as compatible solutes, including glutamate, glucosylglycerol, and a dihexose, showed extensive turnover compared to most other metabolites already after 12 h, but only during the light phase in the cycle. The observed extensive turnover is surprising considering the conventional view on compatible solutes as biosynthetic end points given the relatively slow growth and constant osmotic conditions. This suggests the possibility of a metabolic sink for some compatible solutes (e.g., into glycogen) that allows for rapid modulation of intracellular osmolarity. To investigate this, uniformly ^{13}C-labeled *Synechococcus sp.* PCC 7002 were exposed to ^{12}C glucosylglycerol. Following metabolite extraction, amylase treatment of methanol-insoluble polymers revealed ^{12}C labeling of glycogen. Overall, our work shows that D_2O probing is a powerful method for analysis of cyanobacterial metabolism including discovery of novel metabolic processes.



Cyanobacteria are prokaryotic chlorophotoautotrophs able to thrive in a diverse array of environments.[1] Adaptations to challenging environmental conditions include the biosynthesis of "sunscreens" such as mycosporines or scytonemin[2] to cope with damaging ultraviolet radiation and the biosynthesis of intracellular compatible solutes to compensate for extracellular osmotic potential in environments with higher salinity. Sucrose, trehalose, glucosylglycerol, glucosylglycerate, glycine betaine, or glutamate are common cyanobacterial compatible solutes.[3] Glycine-betaine is most commonly found in extremely halotolerant cyanobacteria like those in the genus *Halothece* or *Halospirulina*, while most marine forms will accumulate glycosylglycerol, and those considered freshwater tend to rely on sucrose or trehalose.[4,5] Intracellular levels of these compounds are usually higher than those of other metabolites and increase with increasing extracellular salinity or upon desiccation.[6] If cells cannot adjust intracellular osmolarity sufficiently rapidly to environmental changes, compatible solutes may also be released upon exposure to a hypoosmotic environment.[7−9] It is also known that osmotic cues can effect changes in the carbon allocation patterns of major intracellular metabolites in cyanobacteria.[10] Conventional thinking based on known metabolic pathways and large pool sizes suggests that compatible solutes (e.g., glucosylglycerol) should not be rapidly turned over in batch culture.[11]

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Previously, we had obtained indications of novel metabolic processes in the marine cyanobacterium *Synechococcus* sp. PCC 7002, including rapid turnover of intracellular glucosylglycerol.[12,13] When the growth medium of ^{13}C-labeled cells was supplemented with a ^{12}C metabolite extract of *Synechococcus* sp. PCC 7002, we observed depletion in the medium and an increase in intracellular levels of some unlabeled metabolites. Depletion of extracellular ^{12}C glucosylglycerol was observed, but not a corresponding increase of intracellular ^{12}C glucosylglycerol. Moreover, the isotopic profiles of a majority of the intracellular metabolites detected shifted toward a slightly higher ^{12}C content.[13] One possible explanation for this set of observations is that there exists a significant turnover of intracellular glucosylglycerol into other cellular metabolites.

Since oxygenic photoautotrophs obtain biosynthetic hydrogen from water oxidation, stable isotope probing with deuterium using deuterium oxide (D_2O, "heavy water") presents a minimally invasive approach to globally probe metabolic flux, which can be monitored by liquid chromatography–mass spectrometry (LC-MS) based metabolomics. The presence of more hydrogen than carbon atoms in reduced organic molecules provides high sensitivity in detection of label incorporation, allowing the use of relatively low concentrations of labeled substrate (10% or less in D_2O). Deuterium stable isotope probing has been established as a clinical method for measuring fractional lipid biosynthesis in mammals[14] or kinetic isotope effects[15] or to analyze spatial heterogeneity of newly biosynthesized lipids in tumors using mass spectrometry imaging.[16] Deuterium thus provides an attractive alternative compared to more commonly used carbon-13 (^{13}C) labeling to measure metabolite turnover.[17,18] One general limitation of using deuterium instead of ^{13}C is the necessity to know the structure of the metabolite in addition to its chemical formula. Only deuteriums which cannot be readily exchanged with protic solvents used during metabolite extraction and analysis should be used in calculations of label incorporation as described below.

In this study, we used deuterium oxide stable isotope probing and LC-MS to measure the deuterium incorporation into compatible solutes compared to that of other intracellular metabolites in cyanobacteria (Figure 1). We selected model unicellular euryhaline cyanobacterium *Synechococcus* sp. PCC 7002[19] and the terrestrial filamentous cyanobacterium *Microcoleus vaginatus* PCC 9802[20] to compare the metabolic activities of these physiologically and phylogenetically divergent cyanobacteria. Cells were grown under standard 12 h light/dark cycles. Then, 10% D_2O was added to the growing culture, and metabolites were analyzed using liquid chromatography mass spectrometry (LC-MS). Glycogen was analyzed using amylase treatment of methanol-insoluble cell fractions and the released glucose analyzed using LC-MS. Deuterium incorporation into nonexchangeable positions in metabolites was determined by fitting isotope distributions and compared for compatible solutes, glycogen, and common metabolites.

RESULTS

To test the feasibility of deuterium oxide (D_2O) probing for comparisons of metabolite turnover, D_2O was added to the medium of *Microcoleus vaginatus* PCC 9802 cultures at approximately 10% final concentration, and its incorporation into intracellular metabolites was followed over 23 days using LC-MS. As expected, deuterium was readily incorporated into metabolites (Supporting Figure 1). Fractional incorporation of

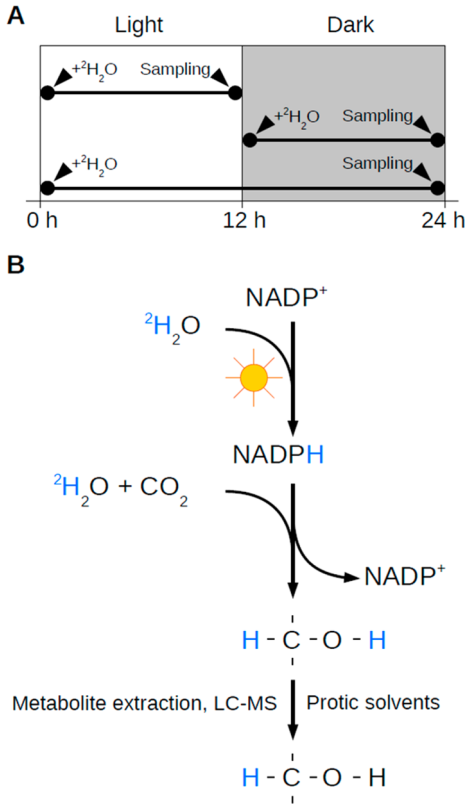

Figure 1. Overview of the deuterium oxide probing experiment (A) and deuterium incorporation into metabolites (B).

deuterium into nonlabile positions (e.g., carbon hydrogen bonds) was calculated as described in the Methods section (Figure 2). Labile hydrogen atoms, such as carboxyl groups, were not considered for fractional incorporation because protic solvents without deuterium were used during metabolite extraction and in the LC mobile phases, which leads to exchange of deuterium incorporated into labile positions for protium (^1H). Naturally occurring deuterium in these solvents was negligible given its low natural abundance (0.01%). We observed rapid labeling for some metabolites during the first 3 days that we speculate reflects a dynamic equilibrium between the biosynthesis of labeled forms, degradation of the pre-existing unlabeled form, and mobilization of unlabeled compounds, enabling high turnover metabolites to reach equilibrium earlier and closer to full theoretical labeling than metabolites with low turnover rates. After 23 days, the deuterium incorporation into nonlabile positions in a dihexose was close to the maximum theoretical level of 10% (essentially in equilibrium with D_2O concentration in the medium). However, for other metabolites such as mercaptohistidine betaine or a C16:0 fatty acid, effectively no deuterium incorporation was detected after 3 days and less than 2% deuterium incorporation after 23 days (Supporting Figure 1).

Microcoleus vaginatus PCC 9802 and *Synechococcus sp.* PCC 7002 were then D_2O probed over shorter periods of time to identify highly turned over metabolites. The probing was performed over 12 h in the light, 12 h in the dark, or 12 h in the light followed by 12 h in the dark (Figure 1A). Differences in the degrees of deuterium incorporation among metabolites as well as between light or dark conditions were observed (Figure 3, Supporting Table S1). In general, and expectedly, higher fractional deuterium incorporations were observed in the light

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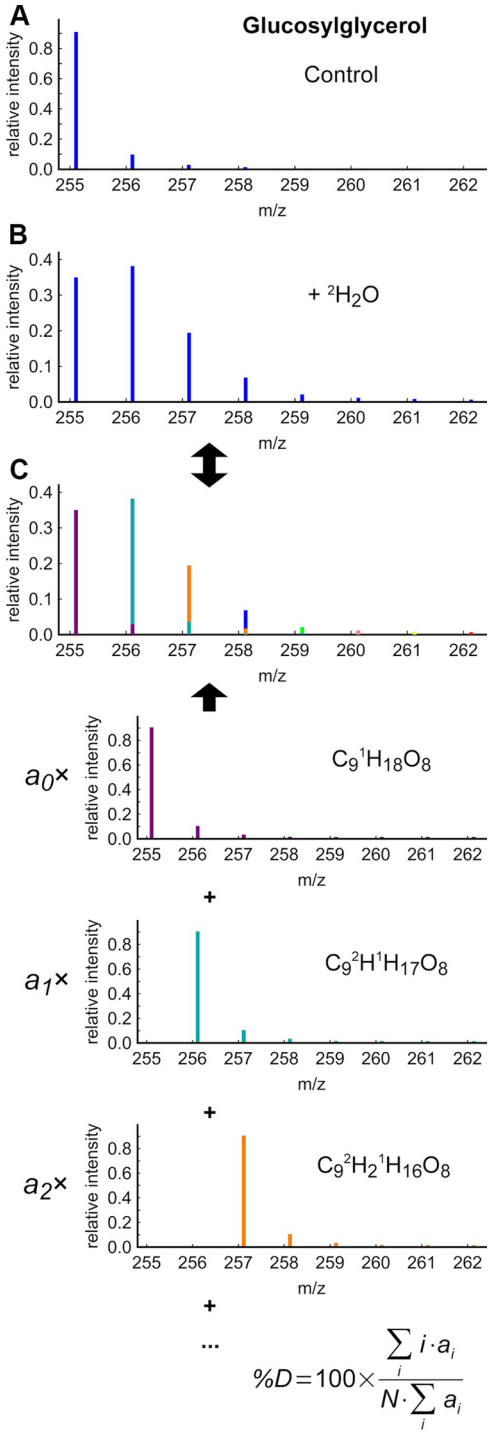

Figure 2. Incorporation of deuterium leads to shifts in relative isotopic abundance profiles of metabolites (A,B). Fractional incorporation of deuterium is calculated from a linear combination of theoretical relative isotopic abundances of isotopologues of a given metabolite containing different numbers of deuteriums (C). *N* is the number of nonlabile hydrogens in the metabolite.

than in the dark conditions for saccharides and glycosides (Figure 3; short-term probing). Smaller differences in turnover between light and dark were observed for amino acids and dipeptides (Figure 3). Metabolites with the highest fractional deuterium incorporation included a dihexose in both strains and glutamate, glucosylglycerol, and glucosylglycerate in *Synechococcus sp.* PCC 7002 (glucosylglycerol and glutamate were not detected in *Microcoleus vaginatus* PCC 9802, which lacks glgC, ggpS, and ggpP required to synthesize glucosylglycerol, Supporting Table S2). Fatty acids in both cyanobacteria and a hexosamine-based trisaccharide from *Synechococcus* sp. PCC 7002 showed dramatically low fractional incorporations of deuterium compared to other metabolites (Figure 3).

High deuterium incorporation into intracellular metabolites is indicative of high metabolic flux through these metabolites. Importantly, since both cyanobacteria grew slowly (doubling time > 24 h), supporting a high flux without accumulation would require a metabolic sink, e.g., catabolism, release into the environment, or condensation into biopolymers. There are several lines of evidence suggesting that compatible solute condensation into biopolymers is most likely: *Synechococcus sp.* PCC 7002 was previously found to release a small number of metabolites into the batch culture media, but glucosylglycerol was not among them.[12] To the contrary, glucosylglycerol was taken up by *Synechococcus sp.* PCC 7002 from a supplemented medium during growth in batch culture.[13] *Microcoleus vaginatus* PCC 9802 was recently found to release a very broad range of metabolites during growth in batch culture. However, again in this strain, the dihexose was not released into the medium and was taken up from supplemented medium.[20] In addition to the absence of glucosylglycerol catabolyzing enzymes from databases of metabolism described below, glucosylglycerol and sucrose have recently been shown to be poor substrates for fermentation in glycogen-biosynthesis-deficient mutants of *Synechococcus sp.* PCC 7002,[21] suggesting that the degradation potential of these metabolites is very limited.

To test if the storage polymer glycogen could act as a sink to create a high flux through sugar metabolites, fractional incorporation of deuterium into glucose subunits was measured after D_2O probing of *Synechococcus* sp. PCC 7002 and culturing for 10 h under light conditions. Glycogen was then hydrolyzed, and the released glucose was extracted and analyzed using LC-MS. A relatively high fractional incorporation of deuterium into glycogen was observed (Figure 4A). To test if glucosylglycerol can serve as a precursor of glycogen, an orthogonal stable isotope labeling approach with ^{13}C was used. Since ^{13}C labeled glucosylglycerol is commercially unavailable, glucosylglycerol incorporation was tracked in *Synechococcus* sp. PCC 7002 by reverse ^{13}C labeling.[13] Fully labeled ^{13}C cultures were grown with unlabeled glucosylglycerol, and cells were collected at three time points (1, 6, and 24 h). Metabolites were extracted, and the remaining biomass was treated with amylase and re-extracted and analyzed using LC-MS. This clearly showed an increase in the ^{12}C over time, indicating glucosylglycerol conversion to glycogen (Figure 4B).

DISCUSSION

Measurements of deuterium incorporation into nonlabile positions of compatible solutes and other cellular metabolites in *Synechococcus sp.* PCC 7002 and *Microcoleus vaginatus* PCC 9802 were used as indicators of turnover of these metabolites. Since metabolites were not quantified, there may be some bias in our results for metabolites with small pool sizes, though generally osmolytes and amino acids are abundant metabolites. High deuterium incorporation identified for dihexoses—probably trehalose in *M. vaginatus* PCC 9802[22] and sucrose in *Synechococcus sp.* PCC 7002[23] (Figure 3)—is surprising given their role as compatible solutes,[3] since there should be no need for extensive turnover of compatible solutes under constant osmotic conditions (batch culture) where they could be

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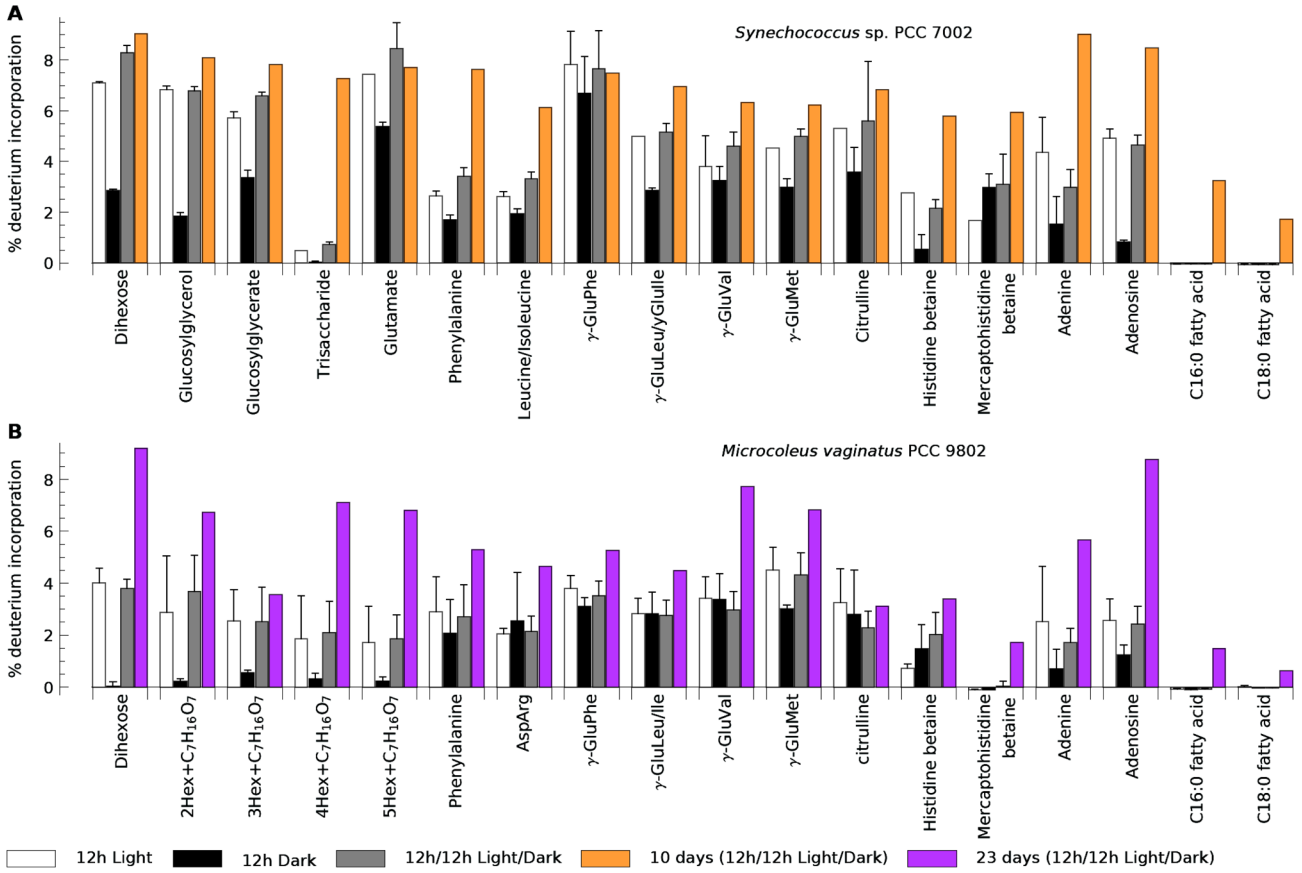

Figure 3. Comparison of fractional deuterium incorporation into selected intracellular metabolites of *Synechococcus* sp. PCC 7002 (A) and *Microcoleus vaginatus* PCC 9802 (B). The cells were probed with 10% D_2O for 12 h in the light (white bars), 12 h in the dark (black bars), and 12 h in the light followed by 12 h in the dark (gray bars). Also shown are measurements from control cultures grown for 10 days (*Synechococcus*) or 23 days (*Microcoleus*) under a 12 h/12 h light/dark cycle. Note: error bars are absent if given metabolite was not detected in two of the three replicate profiles.

biosynthesized in accordance with biomass accumulation. Moreover, the extensive turnover of compatible solutes glucosylglycerol and glucosylglycerate in *Synechococcus sp.* PCC 7002 (Figure 3) is very surprising given the lack of evidence about a possible biodegradation of these glycosides. According to the metabolic databases MetaCyc,[24] KEGG,[25] and BRENDA,[26] glucosylglycerol (and glucosylglycerate) is involved only in a single reaction. This reaction is the biosynthetic reaction catalyzed by glucosylglycerol 3-phosphatase, and the reaction is likely irreversible given that it involves the hydrolysis of a phosphoester bond. The very slow labeling of fatty acids is surprising and difficult to explain. We have observed that these particular species of cyanobacteria both uptake a wide range of metabolites[13,20] and that we detect fatty acids in *Microcoleus vaginatus* spent media,[20] so one highly speculative possibility is that these cultures had accumulated unlabeled exogenous fatty acids pools that were preferentially uptaken, offsetting biosynthesis, consistent with other reports[27] of acyl−acyl carrier protein synthetase enabling lipid recycling in cyanobacteria.

The observed high turnover of compatible solutes in comparison with other intracellular metabolites in batch culture requires a metabolic sink for these molecules. While mutants would be required to determine the exact mechanism, which is beyond the scope of the current study, our results suggest that compatible solutes can serve as a direct conduit toward formation of storage biopolymers, where transglycosylation reactions may provide a link between dihexose(s), glucosylglycerol, and storage polyhexose. This is an intriguing possibility since transglycosylation reactions could imprint a dynamic equilibrium among these compounds using gateways that do not require input of free energy (such as *via* ATP hydrolysis). Glycosyl hydrolases from the GH13 family[28] such as amylosucrase were shown to transfer glucosyl residues from sucrose to glycogen.[29] Glycerol was also shown to act as an acceptor of glucosyl residues from sucrose catalyzed by a putative alpha-amylase.[30] Glycerol is also known to support mixotrophic growth in *Synechococcus sp.* PCC 7002.[31] An alternative to transglycosylation could be phosphorolysis or hydrolysis of oligohexoses or glycosides.[32] It has been shown previously that glucose units of glycosylglycerol can serve as a substrate for fermentation with the glycerol unit being released by *Microcoleus chthonoplastes*.[33] This pathway would, however, require the hydrolysis of additional phosphodiester bonds to synthesize ADP-glucose—the precursor in glycogen biosynthesis.

Our results are consistent with cyanobacterial fixation and storage of carbon during the day as the branched chain carbohydrate glycogen, which at night serves as the primary and often exclusive carbon source for cyanobacterial cells, being rapidly degraded within 2−8 h after cells enter the dark.[34] Specifically, sugars are observed to become nearly maximally labeled during the 12 h day with minimal labeling observed at night (Figure 3). The sugars formed photosynthetically with

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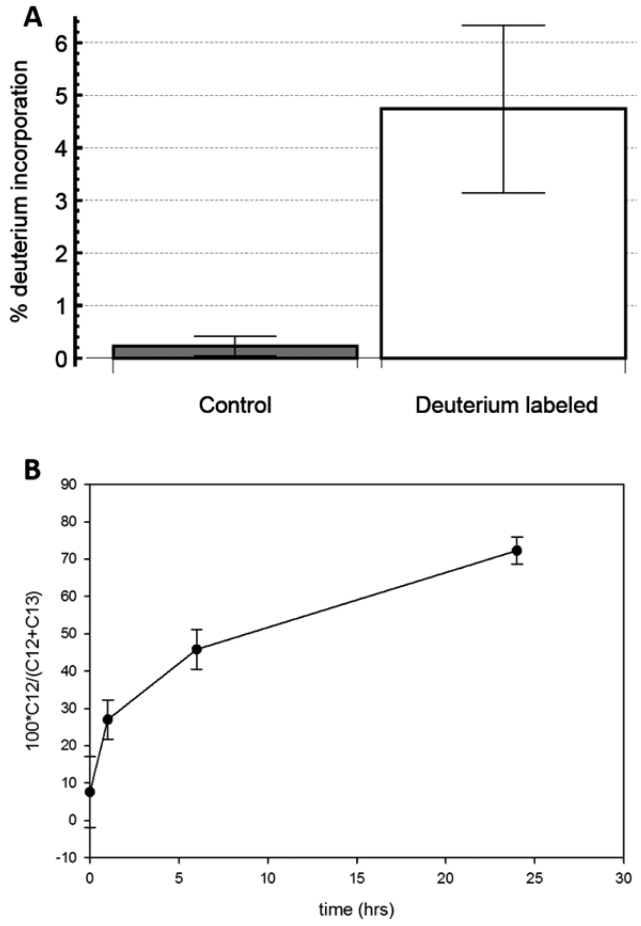

Figure 4. Stable isotope analysis of glucose produced from amylase treatment of insoluble *Synechococcus sp.* PCC7002 biomass. (A) Amylase derived glucose is highly labeled in deuterium treated cells and not in control. (B) Reverse stable isotope labeling experiment shows fractional incorporation of ^{12}C into glucose subunits of glycogen 0, 1, 6 and 24 h following the addition of ^{12}C labeled glucosylglycerol to a uniformly ^{13}C labeled culture of *Synechococcus sp.* PCC 7002. Error bars represent standard deviations (n = 4).

water as a proton rapidly the concentration of D_2O in the growth medium. When the D_2O is added for the 12 h night, minimal additional labeling is observed for the sugars, indicating that they are being made from the unlabeled glycogen, and possibly other biopolymers, accumulated during the preceding light period.

In contrast to the sugars, amino acids, dipeptides, and nucleosides show high levels of labeling when the D_2O is added during both the 12 h day and 12 h night periods (Figure 3). Cyanobacteria primarily process glucose from glycogen degradation *via* the pentose phosphate pathway, and limited work on metabolic flux in cyanobacteria has shown that carbon passing through the pentose phosphate pathway is generally not resynthesized into hexose sugars but is instead passed down through lower glycolysis and the TCA cycle to form precursors for amino acids and nucleotides.[35] The observed labeling results from deuterons from D_2O first becoming incorporated into NADH/NADPH and subsequently into nonexchangeable positions into these compounds during the multiple reduction reactions required to biosynthesize them from glycogen.[16,36] It is interesting to note that amino acid labeling is generally lower at night. This is likely simply a result of protein degradation during the night that mobilizes unlabeled amino acids.

High turnover of glutamate may represent a high flux toward cyanophycin linking the C and the N parts of metabolism. Arginine and aspartate are the building blocks of cyanophycin—the nitrogen-rich cyanobacterial storage polymer.[37] In *Synechococcus sp.* PCC 7002, glutamate and γ-GluPhe show the highest labeling at night and labeling that is comparable to that observed for the compatible solutes during the 12-h day period (Figure 3). An increased biosynthesis rate of glutamate would require higher metabolic flux through phosphoenolpyruvate carboxykinase producing oxaloacetate, the precursor of aspartate (from phosphoenolpyruvate and CO_2), and also arginine through many steps including conversion in the TCA cycle to 2-oxoglutarate, glutamate, and then ornithine.

It has been shown that high turnover rates facilitate rapid response times in transcription networks.[38] High turnover rates of compatible solutes *via* polymer reservoirs make much physiological sense, in that they allow for fast changes in their intracellular levels and intracellular osmotic pressure in response to changes in extracellular osmolarity without incurring a net cellular loss of carbon. An organism can thus respond to a hypoosmotic downshift by increasing reserve polymers at the expense of compatible solutes, rather than releasing carbon to the environment. This carbon can then be repurposed for eventual growth. Conversely, it can respond to upshifts in salinity or to desiccation by rapidly drawing from an available carbon source, rather the by synthesizing the osmolites *de novo*. This is consistent with the observed increased expression of glycogen debranching enzymes by *Microcoleus vaginatus* PCC 9802 upon drying.[39] Only when osmotic stressors overwhelm this capacity, would a net increase in the synthesis of osmolites from central metabolism be needed, as also seen in *M. vaginatus* for trehalose biosynthesis.[39] The dynamic regulation of cellular osmotic pressure becomes thus very nimble to environmental variations in osmotic pressure and may determine the distribution of particular species in nature.[40,41]

METHODS

Chemicals and Strains. Chemicals for growth media preparation and LC-MS grade solvents were purchased from Sigma. Deuterium oxide (D, 99.96%) was purchased from Cambridge Isotope Laboratories. 2-O-α-D-Glucosylglycerol was purchased from Toronto Research Chemicals. *Synechococcus* sp. PCC 7002 was obtained from American Type Culture Collection (ATCC number 27264). *Microcoleus vaginatus* PCC 9802 was our own strain and can also be obtained from the Pasteur Culture Collection (PCC, Pasteur Instititute, France)

Culture Conditions. *Synechococcus* sp. PCC 7002 was cultured in A+ medium[42,43] without TRIZMA base (pH of the medium was adjusted to 8.2 prior to autoclaving). The strains were cultured in a 1925 Shel Lab incubator at 36 °C on a VWR Advanced Digital Shaker (110 rpm). *Microcoleus vaginatus* PCC 9802 was cultured in BG-11 medium without HEPES and without an added inorganic carbon source at room temperature. Both cyanobacteria were cultured under white-light fluorescent tubes with a photon flux of 9 μmol photons·m^{-2}·s^{-1} at the level of culture flasks, under a 12 h light/12 h dark cycle. The growth rate for *Synechococcus sp.* was measured using OD to double ~24 h (Supporting Figure 2), consistent with our previous study,[13] and we estimate that the filamentous *Microcoleus vaginatus* doubles approximately once per week. For stable isotope probing with D_2O, corresponding media were prepared in pure D_2O, filter sterilized, and added to cultures to approximately 10% final fraction of D_2O. At the time of probing, 5 mL of D_2O based culture medium was added to

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45 mL batch cultures (H_2O based) of each strain. For stable isotope probing with ^{13}C, *Synechococcus* sp. PCC 7002 was grown in A+ media supplemented with 0.5 g·dm^{-3} ^{13}C sodium bicarbonate (Cambridge Isotopes) for 1 week prior to the experiment. The ^{12}C 2-O-α-D-glucosylglycerol (Toronto Research Chemicals) was then added to a final concentration of 1 mM to guarantee an excess of glucose units in supplemented glucosylglycerol compared to glucose units in intracellular glycogen at OD(730 nm) of up to 0.32 during the experiment (Supporting Figure 2).[44]

Metabolite Extraction. A total of 50 mL of cell culture was centrifuged for 10 min at 3220*g* using an Eppendorf 5810R centrifuge. Pellets were resuspended in 1 mL of methanol and sonicated in a sonic bath for 10 min. Suspensions were transferred to 2 mL eppendorf tubes and centrifuged for 10 min at 9391*g* using an Eppendorf 5424 centrifuge. Supernatants were transferred to 1.8 mL glass vials (VWR) and dried down using Savant SpeedVac Plus SC110A. Dried down samples were redissolved in 100 μL of methanol and filtered using 0.22 μm PVDF microcentrifuge filters (Millipore) prior to LC-MS analysis.

LC-MS Analysis. Metabolite extracts were analyzed using an Agilent 1200 series capillary LC system coupled to an Agilent 6520 Q-TOF mass spectrometer *via* electrospray ionization (ESI) using a ZIC-HILIC column (150 × 1 mm, 3.5 μm 100 Å, SeQuant) as described previously.[12] The data from D_2O labeling experiments were acquired in fast polarity switching mode, and ^{13}C experiments were acquired in positive mode. Note that unlabeled protic solvents were used for extraction and LC-MS mobile phases to remove labile/exchangeable deuterium (e.g., bound to carboxy or amino groups).

Glycogen Analysis. Relative incorporation of deuterium into *Synechococcus sp.* PCC 7002 glycogen was calculated following glycogen hydrolysis and LC-MS analysis of glucose. Glycogen hydrolysis was based on a previously published protocol.[43] Pellets from 50 mL cultures which were probed with D_2O (10% final fraction) for 10 h under light conditions and from unprobed control cultures were resuspended in 1 mL of methanol and sonicated for 20 s using probe sonicator Qsonica Q125 (amplitude 60%). Suspensions were centrifuged for 10 min at 10 000*g*. The pellet was resuspended in 1 mL of methanol, sonicated for 20 min in a sonic bath (Symphony, VWR), and the centrifugation, methanol extraction, and sonication were repeated one more time to remove small metabolites including free glucose. Pellets were resuspended in 50 μL of 2.5 mM sodium acetate buffer (pH 5.0) containing 0.5 mg·mL^{-1} amyloglucosidase from *Aspergillus niger* (Sigma) and incubated for 1 h at 40 °C with 900 rpm shaking. Following the incubation, 200 μL of methanol was added to each sample. The samples were centrifuged for 10 min at 10 000*g*. Supernatants were filtered using 0.22 μm PVDF microcentrifuge filters (Millipore) and analyzed by LC-MS as described above. Glycogen analysis for samples grown in A+ media containing glucosylglycerol was performed prior to the addition of glucosylglyceraol, 1 h following, 6 h following, and 24 h following. Then, 50 mL cultures were centrifuged for 10 min at 10 000*g* and lyophilized overnight (Labconco). Pellets were then processed as previously stated in these methods.

Data Analysis. Retention time bounds of peaks of metabolites of analyzed cyanobacteria annotated in previous studies[12,45] were located manually using the MathDAMP package[46] or Agilent MassHunter Qualitative Analysis (version B.03.01) software. Peak areas of the monoisotopic peak along with peak areas of corresponding isotopologues were integrated using a ±20 mDa window to calculate relative isotopic abundances (RIA) of all detected isotopologues for each metabolite. Theoretical RIA for each metabolite were calculated using a Matlab algorithm based on the procedure of Rockwood and co-workers,[47] taking into account natural abundances of stable isotopes of all constituent elements with the exception of hydrogen.[15,16] Only the ^1H isotope of hydrogen was considered for these theoretical RIA calculations. A linear combination estimated by the Matlab function for non-negative least-squares of a series of theoretical RIAs shifted by consecutive additions of a mass difference between neighboring isotopic peaks was then calculated to match the measured RIA (Figure 2). The shifted theoretical RIAs represent contributions of isotopologues containing the corresponding numbers of deuterium atoms in the molecule (Figure 2). The fractional incorporation of deuterium into each metabolite was calculated as the weighed sum of contributions of different isotopologues divided by the number of nonlabile (nonexchangeable) hydrogens in the molecule (Figure 2C).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acschembio.6b00890.

Supporting Figures 1 and 2 (PDF)
Supporting Table S1 (XLSX)
Supporting Table S2 (XLSX)

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Notes

The authors declare no competing financial interest.

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