The CyaC protein, a cyanobacterial adenylate cyclase, has a unique primary structure composed of the catalytic domain of adenylate cyclase and the conserved domains of bacterial two-component regulatory systems, one transmitter domain and two receiver domains. In the present work, CyaC was produced in Escherichia coli as a histidine-tagged recombinant protein and purified to homogeneity. CyaC showed ability to autophosphorylate in vitro with the γ-phosphate of [γ-32P]ATP. CyaC derivatives were constructed by site-directed mutagenesis in which the highly conserved phosphorylation sites in the transmitter domain (His572) and receiver domains (Asp60 or Asp895) were replaced by glutamine and alanine residues, respectively. After autophosphorylation of the CyaC derivatives, the chemical stabilities of the phosphoryl groups bound to the derivatives were determined. It was found that His572 is the initial phosphorylation site and that the phosphoryl group once bound to His572 is transferred to Asp895. The enzyme activities of the CyaC derivatives defective in His572 or Asp895 were considerably reduced. Asp895 is phosphorylated by acetyl [32P]phosphate, a small phosphoryl molecule, but Asp60 is not. Acetyl phosphate stimulates adenylate cyclase activity only when Asp895 is intact. These results suggest that the phosphorylation of Asp895 is essential for the activation of adenylate cyclase and that Asp60 functions differently from Asp895 in regulating the enzyme activity.

Cyclic AMP (cAMP) is widely distributed from prokaryotes to eukaryotes as an important signaling molecule. In cyanobacteria, Gram-negative bacteria that are able to perform higher plant-type oxygen-evolving photosynthesis, it has been shown that cellular cAMP levels change in response to changes in environmental conditions such as light-dark, low pH-high pH, oxic-anoxic (1, 2), and nitrogen replete-deplete (3). In a gliding cyanobacterium, Spirulina platensis, extracellular cAMP stimulates the activity of respiration and gliding movement (4). These results suggest that cAMP functions as a signaling molecule in cyanobacteria, although the regulatory mechanism of cellular cAMP is yet to be elucidated.

cAMP is synthesized from ATP by an adenylate cyclase, and the activity of adenylate cyclase is regulated by various mechanisms. Mammalian transmembrane adenylate cyclases are regulated by the stimulatory and inhibitory heterotrimeric G proteins in response to the binding of chemical ligands to appropriate signal receptors (5). Ca2+ and Ca2+/calmodulin also regulate mammalian transmembrane adenylate cyclases (5). The mammalian type V adenylate cyclase is phosphorylated by protein kinase C and protein kinase A, and phosphorylation regulates the activity of the enzyme (6, 7). In budding yeast Saccharomyces cerevisiae, adenylate cyclase is activated by Ras, a small GTP-binding protein (8, 9). In Escherichia coli, a phosphoenolpyruvate:carbohydrate phosphotransferase system regulates the adenylate cyclase activity (10, 11).

Recently, a soluble adenylate cyclase has been isolated from mammalian testis (12). The catalytic domains of this soluble adenylate cyclase are more similar to those of cyanobacterial adenylate cyclases than they are to those of mammalian transmembrane adenylate cyclases (12).

Ten adenylate cyclase genes have been isolated from cyanobacteria (13–17). They have a fairly conserved catalytic domain near the C-terminal region and have characteristically different regulatory domains upstream of the catalytic domains. Among these genes, cyaC encodes a novel protein containing domains homologous to members of the bacterial two-component regulatory system, one transmitter domain and two receiver domains, in addition to the catalytic domain of adenylate cyclase (16). The CyaC protein seems to bind to the membrane of Synechocystis cells, although it has no sufficient hydrophobic regions to pierce the membrane (16).

Bacterial two-component regulatory systems, which provide the dominant modes of signal transduction in bacteria for adaptation to environmental changes, consist of two families of signal transduction proteins, the sensory kinase family and the response regulator family (18, 19). The sensory kinases undergo autophosphorylation at a histidine residue in their transmitter domain in response to environmental stimuli, and the phosphoryl group once bound to the histidine residue is transferred to an aspartate residue in the receiver domain of the cognate response regulator. In most cases, response regulators act as transcriptional factors and control the expression of target genes. CheB in E. coli and RegA in Dictyostelium discoideum exceptionally consist of a receiver domain and a catalytic domain of the enzyme, from methyltransferase and phosphodiesterase, respectively. The enzymes are activated by phosphorylation of their receiver domains (20, 21). Several two-component regulatory proteins, so-called “hybrid sensory kinases,” contain both transmitter and receiver domains (22–25). It can be said that CyaC has a novel structure consisting of both “a hybrid sensory kinase” and a catalytic domain in one molecule. Studies on the function of CyaC are important not only to understand regulation of adenylate cyclase in cyanobacteria, but also to address general issues about the regulation of
enzyme activity by two-component regulatory systems.

In this study, we used recombinant proteins to investigate how CyaC is phosphorylated and whether the phosphorylation state controls the adenyl cyclase activity of CyaC.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains and Growth Media**—The E. coli strains used as hosts were JM101 (recA1, endA1, gyrA96, thi, hsdR17 (rK- mK-)), supE44, relA1, Δ(lac-proAB)F′ (traD36, proAB, lacY1, Δ(lacZΔM15) for cloning and BL21(DE3)pLysS (F-, ompT, hsdS (rK- mB-), dcm, gal-, Δ(lac-proAB)), for expression of recombinant proteins. Bacteria were grown in Luria-Bertani medium (2.5 liters) supplemented with kanamycin (25 μg ml⁻¹) and chloramphenicol (30 μg ml⁻¹). Each recombinant cyaC gene was expressed in exponentially growing cells by adding 1 μM isopropyl-β-D-thiogalactopyranoside. After 6 h, the cells were harvested by centrifugation, washed with 50 mM Tris-HCl (pH 7.5) buffer containing 150 mM NaCl, and resuspended in 50 ml of extraction buffer consisting of 50 mM Tris-HCl (pH 8.0), 10% (v/v) glycerol, 0.5 mM NaCl, 0.05% (v/v) Tween 80, 5 mM imidazole, 1 mM PMSF, and 1 mM β-lysozyme. The cell suspension was incubated on ice for 30 min and then sonicated at 4 °C for 9 min (3 min × 3) using a Kubi model 200H sonicator. The cell extract was centrifuged at 16,000 × g for 10 min, and the supernatant was further centrifuged at 150,000 × g for 40 min. The 150,000 × g supernatant was loaded onto a HiTrap Chelating column (GE Healthcare) with 30 × 0.5 cm bed size, and eluted using an imidazole step gradient of 5, 30, 60, and 200 mM imidazole in Buffer A (50 mM Tris-HCl (pH 8.0), 10% (v/v) glycerol, 0.5 mM NaCl, 0.05% (v/v) Tween 80). The volume of eluent used in each step gradient was 32, 16, and 8.5 ml, respectively. The 200 mM imidazole fraction was concentrated to 0.5 ml using Ultrafree-15 (Millipore). The concentrated eluate was loaded onto a Superose 6 column (Amersham Pharmacia Biotech; 1 × 30 cm) and eluted with Buffer A containing 5 mM imidazole at a flow rate of 0.3 ml min⁻¹.

**Protein Phosphorylation Assay**—Unless otherwise stated, the assay mixture (final volume 15 μl) contained, besides the enzyme, 50 mM Tris-HCl (pH 8.0), 200 mM KCl, 0.1 mM γ-[32P]ATP (～0.7 μCi nmol⁻¹), 1 mM MgCl₂, 2.5 mM DTT, and 1 mM PMSF. To avoid the release of phosphoryl groups, samples were heated immediately at 50 °C for 5 min and applied to an 8% SDS-PAGE gel. The proteins were subsequently blotted onto an Immobilon-P membrane (Millipore) and subjected to autoradiography.

The phosphorylation assay using acetyl [32P]phosphate was essentially the same as that described by Quon et al. (28). The synthesized acetyl [32P]phosphate (7.5 μl) was combined with each purified His-CyaC derivative in 7.5 mM of 50 μM Tris-HCl (pH 8.0) buffer containing 2 mM DTT and 1 mM PMSF. The reactions were run at 30 °C for 15 min and terminated by the addition of 5 μl of 4X SDS-PAGE loading buffer (200 mM Tris-HCl (pH 6.8), 400 mM DTT, 8% SDS, 40% glycerol, and 0.04% bromphenol blue). To avoid the release of phosphoryl groups, samples were heated immediately at 50 °C for 5 min (27) and applied to an 8% SDS-PAGE gel. The proteins were subsequently blotted onto an Immobilon-P membrane (Millipore). The phosphorylated proteins were analyzed using a BAS1000 Imaging Analyzer (Fuji film).

**Adenylate Cyclase Activity Assay**—Unless otherwise stated, the assay mixture (final volume 0.2 ml) contained, besides the enzyme, 50 mM Tris-HCl (pH 8.0), 200 mM KCl, 0.1 mM ATP, 1 mM MgCl₂, 2 mM DTT, 1 mM PMSF, and 0.5 mM NaCl. The reaction was run for 15 min at 30 °C, terminated by the addition of 0.3 ml of 10% perchloric acid, and neutralized with 0.5 ml of 1 M KOH. The mixture was centrifuged at 200 × g for 10 min, and the cAMP content in the supernatants was measured by an enzyme immunoassay system according to the manufacturer’s protocol (EIA system, Amersham Pharmacia Biotech).

**Stability of Phosphate Links in Wild-type and Mutant His-CyaC Proteins**—Wild-type and mutant His-CyaC proteins were phosphorylated and blotted onto an Immobilon-P membrane (Millipore) as described above. The blots were incubated in 1 M HCl, 3 M NaOH, or 50 mM 1 M HCl, 3 M NaOH, or 50 mM HCl, and resuspended in 50 ml of extraction buffer consisting of 50 mM Tris-HCl (pH 7.2) at 25 °C. After incubation for 1 h, the membranes were washed briefly with distilled water, dried in air, and then exposed to an x-ray film.

**RESULTS**

**Purification of Wild-type and Mutant His-CyaC Proteins**—The CyaC protein has a multidomain structure consisting of the catalytic domain of adenylate cyclase and domains that are homologous to those of bacterial two-component regulatory systems. These domains exist in line
was obtained when His-CyaC was incubated with CyaC(H572Q,D60A); His-CyaC(D60A); His-CyaC(D60A,D895A); lane 5 consists of 1202 amino acid residues. The proteins expressed as His-tag fusion proteins. The full-length CyaC protein lane 2 consists of 1202 amino acid residues. The proteins expressed as His-tag fusion proteins. Each protein lane 2 was stained with Coomassie Brilliant Blue R-250.

from the N terminus: a receiver domain (R1), a transmitter domain, a receiver domain (R2), and a catalytic domain. The region between the receiver (R1) domain and the transmitter domain shows similarity to the ETR1 protein that has been identified as an ethylene receptor in plants (29, 30).

Eight kinds of CyaC derivatives were constructed and each protein was fused with a histidine tag (Fig. 1A). The proteins were purified as described under "Experimental Procedures," and the purities were determined by SDS-PAGE (Fig. 1B). One band with a molecular mass of 140 kDa was observed in each lane.

In the previous work, we showed that the catalytic activity of the CyaC protein is stimulated by Mn^{2+} and less extensively by Mg^{2+} (16). The specific activities (Mg^{2+}- or Mn^{2+}-dependent adenylate cyclase activity) of the purified wild-type CyaC protein used in this study were 7.4 and 260 nmol of cAMP formed mg^{-1} min^{-1}, respectively. The specific activities are higher than those used in the previous work (16). This is due to improvements in the purification procedure as described under "Experimental Procedures."

The molecular mass of His-CyaC was estimated to be 590 kDa by Superose 6 column chromatography, a kind of gel permeation column chromatography, suggesting that His-CyaC forms a homotetramer in E. coli.

Autophosphorylation of His-CyaC—Autophosphorylation of His-CyaC was assayed by incubating the proteins with [a-32P]ATP or [γ-32P]ATP and Mg^{2+} or Mn^{2+}. Radiolabeling was obtained when His-CyaC was incubated with [γ-32P]ATP but not with [a-32P]ATP (Fig. 2), indicating that the phosphorylation of His-CyaC is γ-phosphate specific. Mg^{2+} and Mn^{2+} had similar effects on the autophosphorylation activity of His-CyaC (Fig. 2, lanes 2 and 4), although the adenylate cyclase activity of His-CyaC was stimulated by Mn^{2+} (16). Mn^{2+} might activate the catalytic activity of CyaC independently of the autophosphorylation activity.

Autophosphorylation of Mutant His-CyaC Proteins—The His^{572} residue of CyaC corresponds to the highly conserved histidine residue found in sensory kinases of bacterial two-component regulatory systems. The Asp^{60} and Asp^{895} residues of CyaC correspond to a highly conserved aspartate residue among the response regulators of bacterial two-component regulatory systems (16, 18, 19).

To determine the role of these residues in the phosphorylation of CyaC, autophosphorylation activities were assayed using His-CyaC(wild-type), His-CyaC(D60A), His-CyaC(D895Q), His-CyaC(D895A), and His-CyaC(D60A,D895A) (Fig. 1). The results show that His-CyaC(H572Q) is unable to autophosphorylate in vitro (Fig. 3). Thus, His^{572} is thought to be the initial phosphorylation site in CyaC.

The levels of phosphorylation observed in His-CyaC(wild-type) and His-CyaC(D60A) were lower than those observed in His-CyaC(D895A) and His-CyaC(D60A,D895A).

Stability of the Phosphoryl Groups of the Phosphorylated Wild-type and Mutant His-CyaC Proteins—Chemical stability assays have been performed to determine the class of phosphorylated amino acid. N-Phosphate bonds (phosphohistidine or phosphohistidine) are base-stable and acid-labile. Acyl phosphate bonds (phosphoaspartate or phosphoglutamate) are labile to both acid and base, and O-phosphate bonds (phosphoserine, phosphothreonine, or phosphotyrosine) are stable to acid (31, 32). To compare the stability of phosphate links in the phosphorylated wild-type and mutant His-CyaC proteins, each phosphorylated product was subjected to SDS-PAGE and transferred to an Immobilon-P membrane. The membrane strips were treated with acid, base, or neutral buffer.

Compared with the control treated with neutral buffer, His-CyaC(wild-type) and His-CyaC(D60A) retained 64 and 68% of their label after base treatment, whereas His-CyaC(D895A) and His-CyaC(D60A,D895A) retained 94 and 89% of their label, respectively (Fig. 4). When treated with acid, His-CyaC (wild-type) and His-CyaC(D60A) retained 19 and 20%, respectively, of their label, whereas His-CyaC(D895A) and His-CyaC(D60A,D895A) retained no label (Fig. 4). The phosphoryl groups in phosphorylated His-CyaC(wild-type) and His-CyaC(D60A) were partially labile under base conditions and labile under acid conditions, whereas those in phosphorylated His-CyaC(D895A) and His-CyaC(D60A,D895A) were stable under base conditions but labile under acid conditions.
Adenylate Cyclase Activities of Wild-type and Mutant His-CyaC Proteins—The adenylate cyclase activities of His-CyaC derivatives were compared with that of His-CyaC(wild-type) in vitro (Fig. 5). The components in the reaction mixtures and the assay conditions were the same as those used for the autophosphorylation reaction except that the final volume of the reaction mixtures differed. The adenylate cyclase activities of His-CyaC(D60A), His-CyaC(D895A), and His-CyaC(D60A,D895A) were 75, 23, 21, and 11% that of His-CyaC(wild-type), His-CyaC(H572Q), His-CyaC(H572Q,D60A), His-CyaC(H572Q,D895A), and His-CyaC(H572Q,D60A,D895A), respectively. The adenylate cyclase activities of His-CyaC(D60A) and His-CyaC(D895A) were not (Fig. 6). These results show that Asp895 is phosphorylated but Asp60 is not phosphorylated by acetyl phosphate and that these two amino acid residues have somewhat different physiological functions.

Effect of Acetyl Phosphate on the Adenylate Cyclase Activities of His-CyaC Proteins—The adenylate cyclase activities of His-CyaC(H572Q), His-CyaC(H572Q,D60A), His-CyaC(H572Q,D895A), and His-CyaC(H572Q,D60A,D895A) were measured in the presence or absence of acetyl phosphate. Acetyl phosphate greatly activated the adenylate cyclase activities of His-CyaC(H572Q) and His-CyaC(H572Q,D60A) (Fig. 7), while His-CyaC(H572Q,D895A) and His-CyaC(H572Q,D60A,D895A) were largely unaffected. Thus, the presence of Asp895 is essential for the activation of adenylate cyclase activity. Acetyl phosphate might activate the catalytic activity of the adenylate cyclase by phosphorylating Asp895 in His-CyaC.

DISCUSSION

A cyanobacterial adenylate cyclase, CyaC, has been shown to have a unique structure consisting of one transmitter domain and two receiver domains of bacterial two-component regulatory systems in addition to the catalytic domain of adenylate cyclase (15, 16). The possibility has been considered that the autophosphorylation of the transmitter domain and subsequent phosphotransfer to the receiver domains within the molecule regulates the catalytic activity.

In the present study, CyaC was shown to have the ability to autophosphorylate with [γ-32P]ATP in vitro. On the other hand, the CyaC H572Q mutant was shown to be incapable of autophosphorylation. Thus, His872 in the transmitter domain is thought to be the initial phosphorylation site in CyaC. Studies on the stability of phosphate links in phosphorylated CyaC derivatives showed that the wild-type and D60A mutant proteins contain both an acyl phosphate linkage and N-phosphate linkage, whereas the D895A mutant and the D60A,D895A double mutant CyaC contain an N-phosphate linkage alone. The fact that the acyl phosphate linkage in phosphorylated CyaC is lost when Asp895 is replaced by an alanine residue indicates that Asp895 is actually phosphorylated. Thus, after the autophosphorylation of His872 in the transmitter domain, the phosphonyl group bound to His872 is transferred to Asp895 in the receiver (R2) domain. Asp60, the predicted phosphorylation site in the receiver (R1) domain, is not likely to be phosphorylated by the phosphotransfer reaction from the transmitter domain of CyaC. AsgA of Myxococcus xanthus, which is required for the formation of fruiting bodies, consists of a receiver domain on

Phosphorylation of His-CyaC Proteins by Acetyl [32P]Phosphate—Several response regulator proteins have been shown to be phosphorylated at conserved aspartate residue in the receiver domain by small molecular weight phosphodonor such as acetyl phosphate, carbamoyl phosphate, and phosphoramidate (33, 34). It was determined whether Asp60 and Asp895, the highly conserved phosphorylation sites in the receiver domains of CyaC, are phosphorylated by acetyl [32P]phosphate. When His-CyaC(H572Q), His-CyaC(H572Q,D60A), His-CyaC(H572Q,D895A), and His-CyaC(H572Q,D60A,D895A) (Fig. 1) were incubated with acetyl [32P]phosphate, the His-CyaC(H572Q) and His-CyaC(H572Q,D60A) proteins were phosphorylated but the His-CyaC(H572Q,D895A) and His-CyaC(H572Q,D60A,D895A) proteins were not (Fig. 6). These results show that Asp895 is phosphorylated but Asp60 is not phosphorylated by acetyl phosphate and that these two amino acid residues have somewhat different physiological functions.

The phosphorylated proteins were visualized by autoradiography.
Activation of the Adenylate Cyclase Activity of CyaC

phosphorylation of Asp\textsuperscript{895} is required for the full activity of CyaC, consistent with the results that CyaC is phosphorylated at Asp\textsuperscript{895} by acetyl phosphate (Fig. 6) and that the adenylate cyclase activity is stimulated in the presence of acetyl phosphate (Fig. 7). These results indicate that CyaC autophosphorylates first at His\textsuperscript{572} in the transmitter domain, after which the phosphoryl group is transferred to Asp\textsuperscript{895} in the receiver (R2) domain, and this stimulates the adenylate cyclase activity (Fig. 8).

Small phosphorylated molecules, such as acetyl phosphate, phosphoramidate, and carbamoyl phosphate, can act as phosphodonor for the phosphorylation of the receiver domains of response regulators in place of phosphorylated transmitter domains. Each response regulator protein shows different reactivities toward these small compounds (33). Spo0F, a response regulator protein in Bacillus subtilis, is not phosphorylated by acetyl phosphate in vitro while it is phosphorylated by a sensory kinase, KinA (35). In CyaC, the two receiver domains show different responses to acetyl phosphate; Asp\textsuperscript{895} is phosphorylated by acetyl phosphate, while Asp\textsuperscript{60} is not (Fig. 6). This probably reflects that the two aspartate residues have different physiological functions. It is probable that phosphoryl groups may be transferred to Asp\textsuperscript{895} residue of the receiver (R1) domain from the transmitter domains of other sensory kinases that have not yet been identified. The autophosphorylation activity, the phosphotransfer from His\textsuperscript{572} to Asp\textsuperscript{895}, or the adenylate cyclase activity of CyaC may be regulated by the phosphorylation of Asp\textsuperscript{895}.

We note that the phosphorylation levels observed in His-CyaC(wild-type) and His-CyaC(D60A), which are phosphorylated at both His\textsuperscript{572} and Asp\textsuperscript{895}, are lower than those observed in His-CyaC(D895A) and His-CyaC(D60A,D895A). In the receiver domain (R2) derivatives, phosphoryl groups accumulate at His\textsuperscript{572}, because phosphoryl groups bound to His\textsuperscript{572} are not transferred (Fig. 3). The low levels of phosphorylation observed in His-CyaC(wild-type) and His-CyaC(D60A) may be caused by dephosphorylation from phosphorylated Asp\textsuperscript{895}. The phosphoryl groups of several phosphorylated response regulators are known to hydrolyze very rapidly. The half-life of hydrolysis of the phosphoaspartate group in phospho-CheY, a response regulator protein required for chemotaxis, is a few seconds (36). This instability is called autophosphatase activity. In addition, some sensory kinases are known to function to facilitate the rate of dephosphorylation of their cognate response regulators (37). CyaC also must have a mechanism to dephosphorylate its phosphorylated form. The rapid dephosphorylation from phosphorylated Asp\textsuperscript{895} may be required to respond to subsequent signals. However, there is a possibility that the rate of autodephosphorylation decreases when Asp\textsuperscript{895} is phosphorylated.

It is noted that CyaC shows significant adenylate cyclase activity even if Asp\textsuperscript{895} is not phosphorylated. This is similar to CheB and RegA, which are alternative proteins with the catalytic domain of an enzyme beside the receiver domain of the bacterial two-component regulatory systems (20, 21).

**FIG. 6.** Phosphorylation of mutant His-CyaC proteins by acetyl phosphate. Purified mutant His-CyaC proteins (0.5 μg) were incubated with acetyl [\textsuperscript{32}P]phosphate as described under “Experimental Procedures.” Phosphorylated proteins were analyzed using a BAS1800 Imaging Analyzer (Fuji film). The arrowhead indicates the position of the His-CyaC proteins. Lane 1, His-CyaC(H572Q); lane 2, His-CyaC(H572Q,D60A); lane 3, His-CyaC(H572Q,D895A); lane 4, His-CyaC(H572Q,D60A,D895A).

**FIG. 7.** Effect of acetyl phosphate on the adenylate cyclase activities of His-CyaC proteins. Adenylate cyclase activities of His-CyaC derivatives were measured in the presence (+) or absence (−) of acetyl phosphate. Each His-CyaC protein fraction (0.6 μg) was preincubated in 0.1 ml of phosphorylation mixture (50 mM Tris-HCl (pH 8.0), 0.2 mM KCl, 5 mM MgCl\textsubscript{2}, 2 mM DTT, and 1 mM PMSF with (+) or without (−) 10 mM acetyl phosphate) for 30 min at 30 °C. After preincubation, each sample was immediately cooled on ice for 5 min and then 0.1 ml of phosphorylation mixture containing 0.2 mM ATP was added. After incubating for 15 min at 30 °C, the reaction was terminated by adding 0.3 ml of 10% perchloric acid. The cAMP content was measured as described under “Experimental Procedures.” His-CyaC(H572Q), His-CyaC(H572Q,D60A), His-CyaC(H572Q,D895A), and His-CyaC(H572Q, D60A,D895A) were used as protein samples.

the N-terminal side and a transmitter domain on the C-terminal side. The primary structure of the two domains of AsgA is similar to the receiver (R1) domain and the transmitter domain of CyaC. It has been shown that AsgA autophosphorylates in the transmitter domain but that the phosphoryl group once transferred (Fig. 3). The low levels of phosphorylation observed in His-CyaC(wild-type) and His-CyaC(D60A) may be caused by dephosphorylation from phosphorylated Asp\textsuperscript{895}. The phosphoryl groups of several phosphorylated response regulators are known to hydrolyze very rapidly. The half-life of hydrolysis of the phosphoaspartate group in phospho-CheY, a response regulator protein required for chemotaxis, is a few seconds (36). This instability is called autophosphatase activity. In addition, some sensory kinases are known to function to facilitate the rate of dephosphorylation of their cognate response regulators (37). CyaC also must have a mechanism to dephosphorylate its phosphorylated form. The rapid dephosphorylation from phosphorylated Asp\textsuperscript{895} may be required to respond to subsequent signals. However, there is a possibility that the rate of autodephosphorylation decreases when Asp\textsuperscript{895} is phosphorylated.

**FIG. 8.** Proposed mechanism for the stimulation of the adenylate cyclase activity of CyaC. CyaC autophosphorylates at His\textsuperscript{572} (H) in the transmitter domain (white box) using ATP, and the phosphoryl group (circled P) is transferred to Asp\textsuperscript{895} (D) in the receiver domain (dotted oval). Adenylate cyclase activity is stimulated by the phosphorylation of Asp\textsuperscript{895} (shown by the bent arrow). The black box indicates the catalytic domain.
It is likely that the autophosphorylation activity of CyaC is regulated by sensing a specific signal that is transferred from a primary signal sensor of the cell. In sensory kinases, the N-terminal side of the transmitter domain is thought to be a signal input domain (18, 19). The presumed signal input domain of CyaC, which was called the ETR1-like domain in the previous work (16), is similar to the ethylene sensor of Arabidopsis thaliana (ETR1) and to ReaE, the sensor for the complementary chromatic adaptation of Fremyella diplosiphon. This domain also exists in several other sensory kinases of Synechocystis PCC 6803 (17). Although the function of the ETR1-like domain remains unknown, it may participate in the recognition of a specific signal.

A CyaC homologue is found in the cyanobacterium Anabaena sp. strain PCC 7120 (15), in which cellular cAMP levels are reduced in response to a light-on signal. On the other hand, cAMP levels in the disruptant of the cyaC homologue are not affected by the light-on signal (15). It has been suggested that CyaC responds to a light signal; however, CyaC itself would not be a photoreceptor because it has no typical chromophore binding motifs in its amino acid sequence. Recently, a phytochrome that acts as a sensory photoreceptor in plants has been found in the cyanobacterium Synechocystis PCC 6803 (17, 38). The cyanobacterial phytochrome consists of two functional domains, an N-terminal domain homologous to the chromophore attachment domain of the phytochrome and a C-terminal domain homologous to the transmitter domain of the sensory kinase. Such a photoreceptor having phosphorylation capability might control the activity of CyaC through the mechanism of a phosphotransfer reaction.

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