Crystal Structure of the GAF-B Domain from Human Phosphodiesterase 10A Complexed with Its Ligand, cAMP*

Noriko Handa1, Eiichi Mizohata1, Seiichiro Kishishita1, Mitsutoshi Toyama1, Satoshi Morita1, Tomomi Uchikubo-Kamo1, Ryogo Akasaka5, Kenji Omori5, Jun Kotera5, Takaho Terada5, Mikako Shirouzu5, and Shigeyuki Yokoyama1*2

From the 1Department of Biophysics and Protein Biology, Graduate School of Science, The University of Tokyo, Tokyo 113-0033, Japan

Cyclic nucleotide phosphodiesterases (PDEs) catalyze the degradation of the cyclic nucleotides cAMP and cGMP, which are important second messengers. Five of the 11 mammalian PDE families have tandem GAF domains at their N termini. PDE10A may be the only mammalian PDE for which cAMP is the GAF domain ligand, and it may be allosterically stimulated by cAMP. PDE10A is highly expressed in striatal medium spiny neurons. Here we report the crystal structure of the C-terminal GAF domain (GAF-B) of human PDE10A complexed with cAMP at 2.1-Å resolution. The conformation of the PDE10A GAF-B domain monomer closely resembles those of the GAF domains of PDE2A and the cyanobacterium Anabaena cyaB2 adenyl cyclase, except for the helical bundle consisting of α1, α2, and α5. The PDE10A GAF-B domain forms a dimer in the crystal and in solution. The dimerization is mainly mediated by hydrophobic interactions between the helical bundles in a parallel arrangement, with a large buried surface area. In the PDE10A GAF-B domain, cAMP tightly binds to a cNMP-binding pocket. The residues in the α3 and α4 helices, the β6 strand, the loop between 310 and αe, and the loop between α4 and β5 are involved in the recognition of the phosphate and ribose moieties. This recognition mode is similar to those of the GAF domains of PDE2A and cyaB2. In contrast, the adenine base is specifically recognized by the PDE10A GAF-B domain in a unique manner, through residues in the β1 and β2 strands.

cAMP and cGMP act as second messengers for many cellular functions (1). Their intracellular levels are strictly determined by synthesis and degradation, catalyzed by adenylyl/guananyl cyclase and phosphodiesterase (PDE), respectively. In humans, 21 PDE genes have been identified and categorized into 11 different families, based on their substrate specificity, sensitivity to inhibitors and endogenous regulators, kinetic properties, and amino acid sequence similarity (2).

PDEs contain variable N-terminal regulatory domains and a highly conserved C-terminal catalytic domain. About half of the PDE families, namely PDE2, -5, -6, -10, and -11, have tandem GAF domains (named for cGMP-specific and -stimulated PDEs, Anabaena adenylyl cyclases, and Escherichia coli FhA) (3). The GAF domain is one of the widespread families of small molecule-binding domains. It is present in all living organisms, and is especially prominent among bacteria (4–6). In mammals, almost all of the GAF domains are found in the PDE families. The GAF domains of PDE2, -5, -6, and -11 bind cGMP (7–11). PDE2 and PDE5 are allosterically stimulated by cGMP binding to the GAF domains (8, 9). Experiments using a chimeric construct of the PDE10A GAF domain and cyanobacterial adenylyl cyclase suggested that PDE10A is the only mammalian PDE that binds cAMP, and that PDE10A is allosterically stimulated by cAMP binding to the GAF domain (11). The crystal structure of the tandem GAF domains of mouse PDE2A revealed that the GAF-A domain functions as a dimerization locus, and that the GAF-B domain binds cGMP (7). The crystal structure of the tandem GAF domains from cyanobacterium Anabaena cyaB2 adenylyl cyclase showed that both the GAF-A and GAF-B domains are involved in cAMP-binding and dimerization, and that the tandem GAF domains form an antiparallel dimer (12).

PDE10A can hydrolyze both cAMP and cGMP (13–15). PDE10A is the sole member of the PDE10 family, and two major N-terminal variants, PDE10A1 and PDE10A2, along with some additional variants, have been identified in human (13, 16). PDE10A has the most restricted distribution, and its transcripts are particularly abundant in brain and testis (13, 17). Immunohistochemical studies revealed that PDE10A expression is high only in the dopaminergic medium spiny neurons of the striatum, the principal input site to the basal ganglia (18, 19). Furthermore, PDE10A knock-out mice show decreased explor-

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1 Present address: MPC Group, Division of Molecular Biosciences, Imperial College London, Exhibition Road, London SW7 2AZ, UK and the Membrane Protein Lab (MPL), Diamond Light Source, Harwell Science and Innovation Campus, Didcot, Oxfordshire OX11 0DE, United Kingdom.

2 To whom correspondence should be addressed: Systems and Structural Biology Center, Yokohama Institute, RIKEN, 1-7-22 Suehiro-cho, Tsurumi, Yokohama 230-0045, Japan. Fax: 81-45-503-9195; E-mail: yokoyama@biochem.s.u-tokyo.ac.jp.

3 The abbreviations used are: PDE, cyclic nucleotide phosphodiesterase; cNMP, cyclic nucleotide monophosphate; BisTris, 2-(bis(2-hydroxyethyl)amino)-2-(hydroxymethyl)propane-1,3-diol.


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Activity and delayed acquisition of conditioned avoidance behavior, suggesting that PDE10A is involved in regulating striatal output, possibly by reducing the sensitivity of medium spiny neurons to glutamatergic excitation (20). Papaverine, which inhibits PDE10A, improves the behavior of animal models of psychosis, and therefore the inhibition of PDE10A may have the potential to treat psychosis (21, 22).

In this study, we determined the crystal structure of the human PDE10A GAF-B domain complexed with cAMP. The PDE10A GAF-B domain forms a parallel dimer mediated by hydrophobic interactions between helical bundles. The dimer interface is completely different from those of the GAF domains in PDE2A and cyaB2. Interestingly, the cAMP molecule tightly binds to a cNMP-binding pocket. The residues in the β1 and β2 strands specifically recognize the adenine base in a unique manner.

EXPERIMENTAL PROCEDURES

Protein Expression and Purification—The GAF-B domain (residues 246–427) of human PDE10A1 was produced as a 212-amino acid protein with an N-terminal histidine affinity tag and a tobacco etch virus protease cleavage site. The selenomethionine-substituted protein was synthesized by the Escherichia coli cell-free system, using the dialysis method (23–26). The reaction solution was centrifuged at 16,000 × g at 4 °C for 20 min. The supernatant was loaded on a HiTrap Chelating (GE Healthcare) column (5 ml), previously equilibrated with 20 mM Tris-HCl buffer (pH 8.0) containing 1M NaCl and 15 mM imidazole, and was eluted with 20 mM Tris-HCl buffer (pH 8.0) containing 500 mM NaCl and 500 mM imidazole. The sample buffer was exchanged to 20 mM Tris-HCl buffer (pH 8.0) containing 1 M NaCl and 15 mM imidazole, with a HiPrep 26/10 desalting column. The histidine affinity tag was cleaved by 250 μl of tobacco etch virus protease (4 mg/ml) at 25 °C for 3 h and was removed by a second passage through the HiTrap column. The protein sample was desalted on a HiPrep 26/10 desalting column, and was eluted with 20 mM Tris-HCl buffer (pH 8.5) containing 10 mM NaCl and 5 mM β-mercaptoethanol. Next, the protein sample was loaded on a HiTrap Q (GE Healthcare) column (5 ml), previously equilibrated with 20 mM Tris-HCl buffer (pH 8.5) containing 10 mM NaCl and 5 mM β-mercaptoethanol, and was eluted with a linear gradient of 10 mM to 1.0 M NaCl in 20 mM Tris-HCl buffer (pH 8.5) with 5 mM β-mercaptoethanol. Finally, the protein sample was loaded on a HiLoad 16/60 Superdex 75 (GE Healthcare) column, previously equilibrated with 20 mM Tris-HCl buffer (pH 8.0) containing 300 mM NaCl and 2 mM dithiothreitol, and was eluted with this buffer. The native protein prepared for the analytical ultracentrifugation experiments was synthesized and purified in the same manner as the selenomethionine-substituted protein.

Crystallization and Data Collection—The best crystals of the selenomethionine-substituted protein were grown at 20 °C by the sitting-drop vapor-diffusion method by mixing equal volumes of the protein (2.8 mg/ml in 20 mM Tris-HCl buffer, pH 8.0, containing 300 mM NaCl, 2 mM dithiothreitol, and 4 mM sodium 3’,5’-cAMP), and reservoir solutions (0.1 M BisTris buffer, pH 5.5, containing 0.2 M ammonium sulfate and 25% PEG 3350). The crystals belong to the space group P3,21, with unit cell constants of a = b = 74.58 Å, c = 146.68 Å. There is one dimer in the asymmetric unit. X-ray diffraction data for the MAD method were collected at three different wavelengths at BL26B2 of SPring-8 (Harima, Japan). All data were processed using the HKL2000 and SCALEPACK programs (27). The redundancy-independent merging R factor (R_{i.m.}) and the precision-indicating merging R factor (R_{o.i.m.}) were calculated using the program REFMAC5 (28, 29). The data processing statistics are summarized in Table 1.

Structure Determination and Refinement—The positions of the selenium atoms and the initial MAD phases were determined using the program SOLVE (30), and the MAD phases were improved with RESOLVE (31). The resulting electron density map was clear. Two cAMP molecules in the asymmetric unit were traced unambiguously. The model was built with the program TURBO-FRODO, and multiple cycles of model building and refinement were performed. The model was refined using CNS 1.1 (32). TLS refinement with 8 groups, defined by the TLSMD server, was used in Refmac5 for the final refinement stage (33–35). In the electron density map, the N-terminal artificial linkers and residues 423–427 in molecule A, and residues 246–247 and 420–427 in molecule B were disordered. The final model has good geometry, as examined by MolProbity (36): 97.4% of the residues have φ/ψ angles in the “favored region” of the Ramachandran plot, and 99.8% are in the “allowed regions.” The refinement statistics

| TABLE 1 | Data collection, phasing, and refinement statistics |
|-----------------------------------------------|-------------------|
| Data set | Peak | Edge | Remote |
| Wavelength (Å) | 0.9790 | 0.9793 | 0.9640 |
| Resolution range (Å) | 50–2.1 | 50–2.1 | 50–2.2 |
| Unique reflections | 28,345 | 28,386 | 24,772 |
| Measured reflections | 280,100 | 277,995 | 248,793 |
| Redundancy | 9.9 | 9.8 | 10.0 |
| Completeness (%) | 99.9 (99.4) | 99.9 (99.3) | 99.9 (99.9) |
| I/σ(I) | 21.1 (4.2) | 28.1 (3.8) | 26.3 (5.7) |
| R_{sym} (%) | 8.2 (35.4) | 6.7 (38.2) | 6.9 (33.0) |
| R_{fom} (%) | 8.5 (36.4) | 7.1 (39.4) | 7.1 (34.5) |
| R_{free} (%) | 2.7 (12.1) | 2.2 (13.2) | 2.2 (11.1) |

* Statistics for the highest resolution shell are given in parentheses.

** Figure of merit after SOLVE phasing.
are summarized in Table 1. The ribbon, tube, ball-and-stick, and solvent-accessible surface models in the figures were drawn using PyMOL (DeLano Scientific, Palo Alto, CA).

Analytical Ultracentrifugation—All analytical ultracentrifugation experiments were carried out with a Beckman Optima XL-I analytical ultracentrifuge. The sample buffer was 20 mM Tris-HCl (pH 8.0), 300 mM NaCl, and 5 mM β-mercaptoethanol, and all experiments were performed at 4 °C. The solvent density (ρ = 1.0129 g/ml) and the protein partial specific volume (v = 0.728 ml/g) were estimated with SEDNTERP (37).

Sedimentation velocity data were obtained at 40,000 rpm using an Epon double-sector centerpiece, with loading concentrations of 1.0 mg/ml of native protein. The data were collected every 5 min for 500 min. The data were analyzed with the program SEDFIT (38).

RESULTS

Overall Structure of the GAF-B Domain of PDE10A—The GAF-B domain monomer structure of human PDE10A contains a six-stranded antiparallel β-sheet (β3-β2-β1-β6-β5-β4) sandwiched by a three-helix bundle (α1, α2, and α5) and three short helices (α3, α4, and 310) on each side (Fig. 1A). The topology is similar to those of the published GAF domain structures; namely, the GAF domains of mouse PDE2A, cyanobacterium Anabaena adenylyl cyclase cyaB2, yeast YKG9, and Deinococcus bacterial phytochrome (7, 12, 39, 40). A structure-based sequence alignment of the PDE10A GAF-B domain with other PDE GAF domains and the cyaB2 GAF domains is shown in Fig. 2.

The GAF-B domain of human PDE10A forms a dimer in the asymmetric unit of the crystal (Fig. 1, C and D). Each GAF-B domain has a bound cAMP. The sedimentation velocity data from the analytical ultracentrifugation experiments at 4 °C showed that the PDE10A GAF-B domain sedimented as a single major peak, with an estimated s-value of 2.2013 S (Fig. 1B). The corrected s-value under water conditions at 20 °C is 3.3957 S. The molecular weight was estimated to be 41,444, which is in agreement with the formation of a dimer, with a calculated molecular weight of 42,430.

The two molecules (A and B) superimpose each other well in the region of the six-stranded β-sheet and the three short helices (Fig. 1A), with a root mean square deviation of 0.74 Å for the 104 Ca atoms of residues 284–387. In contrast, the structure of the helical bundle is different: the α1 helix of molecule B is much shorter than that of molecule A (16 and 9 residues in molecule A and B, respectively); and the α5 helix has a kink at Phe404 only in molecule B (Fig. 1, A and E).

Dimer Interface—Most of the dimer interface involves the three-helix bundle (Figs. 1, C and D, and 2). The interaction between the molecules buries a total surface area of 3,061 Å², calculated with the AREAIMOL program in the CCP4 suite (41). The homodimer interaction is asymmetric. The α1 and α2 helices of molecule B interact with the α5 helix of molecule A,
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The homodimer interface is stabilized mainly by hydrophobic interactions (Fig. 1E).

**cAMP-binding Pocket**—The bound cAMP molecules were unambiguously identified in the electron density map (Fig. 3A). cAMP is located between the β-sheet and the α4 helix of each GAF-B domain molecule (Figs. 1A and 3B). Because the structures of the cAMP-binding sites and cAMP recognition of the two molecules in the asymmetric unit are essentially identical, we will describe only molecule A. The cAMP molecule is buried in the binding pocket, and only the C2 carbon of cAMP is visible through an opening on the protein surface (Fig. 3A). Furthermore, the B factors for the cAMP atoms (21.1–24.6 Å²) are similar to the average B factor for the protein molecule (21.4 Å²). Thus, the interaction between the protein and cAMP is tight. This may explain the fact that even if cAMP was never added during either the purification or crystallization, cAMP complex crystals of PDE10A GAF-B, which diffract to 3.0-Å resolution, were obtained. The bound cAMP might have originated from the *E. coli* extract and remained bound tightly to the protein. A crystal diffracting to 2.1-Å resolution was obtained when cAMP was added in the crystallization drop.

The adenine base of cAMP is sandwiched between the aromatic ring of Phe304 and the aliphatic side chain of Val385 (Fig. 3B). The N1 forms a hydrogen bond to the side chain of Arg286, and the side chain of Arg286 is sandwiched between the aromatic ring of Tyr362 and the main chain of Ile306 (Fig. 3B). The N3 forms water-mediated hydrogen bonds to the side chains of Asp357 and Thr280. The N6 forms hydrogen bonds to the main chain of Cys277 and Asp305. The O2' forms a hydrogen bond between the two molecules (Fig. 1E).

**FIGURE 3. Recognition of cAMP by the GAF-B domain of PDE10A.** A, a semi-transparent surface representation, viewed from the opening of the cAMP-binding pocket. The bound cAMP molecule is represented by a yellow ball-and-stick model, with oxygen, nitrogen, and phosphorus atoms shown in red, blue, and purple, respectively. A simulated annealing omit Fo-Fc map was calculated without the cAMP molecule atoms to 2.1-Å resolution, and was contoured at 3.0σ. B, stereo diagram showing the cAMP recognition. The bound cAMP (yellow) and the interacting residues (white) are shown by ball-and-stick models, with oxygen, nitrogen, phosphorus, and sulfur atoms shown in red, blue, purple, and orange, respectively. Two water molecules are shown as red spheres. In the ribbon model, the β strands are cyan, the α helices are salmon, the α5 helix is green, and the random coils are gray. Hydrogen bonds between cAMP and the protein are indicated by broken red lines.

but only the α1 helix of molecule A interacts with the α5 helix of molecule B (Fig. 1, C and D). The α1 helix of molecule B also interacts with the β5–β6 loop, the β5 strand, and the α3–β4 loop of molecule A, but the α1 helix of molecule A interacts only with the β5–β6 loop and the β5 strand, and not with the α3–β4 loop of molecule B (Fig. 1C). There is an intensive interaction between the α5 helices (Fig. 1E). The α5–α5 interaction surface is also asymmetric, with a slight translation along the long axis of the helices, and the conformations of the side chains of the corresponding amino acids are different between the mice PDE5A, -6A, and -6B, and GAF-As of human PDE5A, -6A, -6B, -6C, and -11A. The seven sequences of the lower lines are mouse PDE2A GAF-A, GAF-As of human PDE5A, -6A, -6B, -6C, and -11A. The seven sequences of the lower lines are mouse PDE2A GAF-A, GAF-As of human PDE5A, -6A, -6B, -6C, and -11A. Helices and β-strands are indicated by pink and green boxes, respectively, where the three-dimensional structure has been determined by x-ray crystallography. Strictly conserved and similar residues are represented by red and violet characters, respectively, in blue boxes (comparing all the sequences) and in red boxes (comparing the sequences in the upper lines). Residues that recognize the cAMP bases with their main chains and side chains are indicated by open boxes and triangles, respectively. Residues that recognize the phosphate group and/or the ribose with their main chains and side chains are indicated by filled boxes and triangles, respectively. The types of interactions are indicated as green for hydrophobic or van der Waals interaction, red for hydrogen bond, and pink for water-mediated hydrogen bond. Residues involved in homodimerization of the PDE10A GAF-B domain are indicated by purple (molecule A) and cyan (molecule B) asterisks. The highly conserved NNKDFE motif is shown below the alignment. The alignment was produced by Clustal X (47) and was manually modified. The figure was generated using ESPript (48).
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to the side chain of Thr\textsuperscript{364}, and water-mediated hydrogen bonds to the main chains of Asn\textsuperscript{353} and Asp\textsuperscript{357}. The oxygen atoms of the phosphate group form hydrogen bonds to the main chains of Ile\textsuperscript{330}, Ala\textsuperscript{331}, and Asn\textsuperscript{353}, and to the side chain of Gln\textsuperscript{383} (Fig. 3B).

**DISCUSSION**

Comparison with Structures of Other cNMP-binding GAF Domains—Two crystal structures of GAF domains bound to a cNMP have been reported previously: the tandem GAF domains of PDE2A complexed with cGMP (7), and the tandem GAF domains of cyaB2 cyclase complexed with cAMP (12). In PDE2A, the GAF-A domain is involved in dimerization, and the GAF-B domain binds cGMP (7). In cyaB2, both the GAF-A and GAF-B domains are involved in dimerization and cAMP binding (12). The superposition of the PDE10A GAF-B domain over the GAF-A and GAF-B domains of PDE2A yielded root mean square deviation values of 2.9 and 4.4 Å for 149 and 156 C atoms, respectively. The superposition over the GAF-A and the GAF-B domains of cyaB2 yielded root mean square deviation values of 3.5 and 3.1 Å for 154 and 157 Ca atoms, respectively. The main difference in the conformation exists in the helical bundle, whereas the conformations of the β sheet and the three short helices are similar. The PDE10A GAF-B domain also contains the conserved NNKFD motif, which is reportedly essential for nucleotide binding (11, 12). The conformation and the mutual interaction network of the motif are similar to those in PDE2A and cyaB2 (data not shown).

The PDE10A GAF-B domain dimer and the PDE2A GAF-A domain dimer are parallel, whereas the cyaB2 dimer is antiparallel. Although the homodimer interaction mainly occurs at the helical bundle in both the PDE10A GAF-B and PDE2A GAF-A domains (7), the dimer interaction mode is completely different. The PDE10A GAF-B domain homodimer interaction is asymmetric, whereas the PDE2A GAF-A domain homodimer interaction is symmetric. The buried area of the PDE10A GAF-B domain is much broader than that of the GAF-A domain of PDE2A (3,061 and 1,338 Å\textsuperscript{2}, respectively). In addition, the connecting helix between the GAF-A and GAF-B domains of PDE2A is also critically involved in the homodimer interaction, which buries 1,476 Å\textsuperscript{2}, and the PDE2A GAF-B domain is not involved in dimerization (7). The interaction of the cyaB2 antiparallel dimer mainly occurs through the connecting helices, the two N-terminal helices of GAF-A, and the one C-terminal helix of GAF-B (12). In PDE5, homodimerization occurs between the GAF-A domains and the GAF-B domains, and the connecting region between the GAF domains also contributes to the stability (42, 43). In retinal rod PDE6, the catalytic core is a heterodimer formed by the α and β subunits (43), and the GAF-A domains of PDE6α and PDE6β determine the specificity of dimerization (44). The amino acid residues corresponding to the helical bundle in the GAF domains of the PDE family proteins are weakly conserved (Fig. 2). It seems that the dimer interaction mode differs among the PDE GAF domains, although the helical bundle is the main interacting portion in all of the PDE GAF domains studied thus far.

cNMP Recognition by GAF Domains—All of the bound cNMPs in the GAF domains of PDE10A, PDE2A, and cyaB2 have an anti conformation and a C3’-endo ribose. The recognition manner of the sugar and phosphate group of cNMP is similar among the three crystal structures (Fig. 4A). The O2’ forms a hydrogen bond to the strictly conserved Thr residue (Fig. 4A, indicated in *sky blue*). In contrast, the corresponding residues of the non-cNMP-binding GAF domains of the PDE families are all aliphatic residues (Fig. 2). Therefore, this Thr residue may be one of the major determinants of the cNMP binding of the GAF domains (12). In the cases of PDE10A and cyaB2 cyclase, the phosphodiester ring is in the chair conformation, and the phosphate group forms two hydrogen bonds to the backbone amides of the Ile/Phe-Ala sequence in the α3 helix, and a hydrogen bond to the side chain of Gln (Fig. 4A, indicated in *green*). In the PDE2A GAF-B domain, although Ile\textsuperscript{458}, Ala\textsuperscript{459}, and Glu\textsuperscript{512}, corresponding to Ile\textsuperscript{330}, Ala\textsuperscript{331}, and Gln\textsuperscript{383} in PDE10A, respectively, are at similar positions to those of PDE10A, the phosphodiester ring of the bound cGMP is in the boat conformation (7). The cNMP is sandwiched between the side chains of Val/Leu and Phe/Ile on one side and the side chains of Val/Ile on the other (Fig. 4A, indicated in *orange*), except for the GAF-A domain of cyaB2. These residues are highly conserved among the GAF domains of the PDE families (Fig. 2). The N3 of cAMP or cGMP is recognized through water-mediated hydrogen bonds by the side chains of the conserved Thr/Asn and Asp residues in the α4 helix (Fig. 4A, indicated in *pink*).

The GAF domains specifically recognize the 1 and 6 key positions of the purine ring in different manners. In PDE10A, the side chain of Arg\textsuperscript{386} forms a hydrogen bond to the N1 of cAMP (Figs. 3B and 4B). These interactions are similar to those of the cyaB2 complex, where the side chain of Arg\textsuperscript{453}/Arg\textsuperscript{291}, equivalent to Arg\textsuperscript{386} in PDE10A, forms a hydrogen bond to the N1. In contrast, the side chain of Asp\textsuperscript{439} forms a hydrogen bond to the N1 in PDE2A (Fig. 4B).

The remarkable difference in cAMP recognition is the N6 recognition. The main chain carboxy1 of Cys\textsuperscript{287} forms a hydrogen bond to the N6 in PDE10A (Fig. 4B). In cyaB2, the side chain of Thr\textsuperscript{105}/Thr\textsuperscript{291}, just after the corresponding residues of Cys\textsuperscript{287} in PDE10A, hydrogen bonds with the N6 and N7 of cAMP (Fig. 4B). In PDE2A, the side chain of the corresponding Ser\textsuperscript{424}, at the same position as Thr\textsuperscript{105}/Thr\textsuperscript{291} in cyaB2, also hydrogen bonds with the N7 of cGMP (Fig. 4B). The residue equivalent to these residues in the PDE10A GAF-B domain is Ala\textsuperscript{288}. The N6 atom is also recognized by the PDE10A GAF-B domain and the cyaB2 GAF-A domain through hydrogen bonds to the carbonyl oxygens of Asp\textsuperscript{305} and Ala\textsuperscript{122}, respectively (Fig. 4B). The corresponding residue in PDE2A is Asp\textsuperscript{439}, which forms hydrogen bonds to the O6 and the N1 through the main chain amide and the side chain, respectively, and contributes to the cGMP-specific recognition (Fig. 4B). Whereas Asp\textsuperscript{439} in PDE2A is at the β2–β3 turn, Asp\textsuperscript{305} in PDE10A is within the β2 strand (Fig. 2). The backbone directions of these Asp residues are completely different between the two structures (Fig. 4B). The side chain of Asp\textsuperscript{305} in PDE10A hydrogen bonds with the main chain
amide of Gly307 (Fig. 3B). This interaction fixes the conformation of Asp305, and may contribute to the base recognition.

**Tight and Specific Interaction with cAMP**—The expression of PDE10A is restricted to the brain and testis, and high levels of expression are only found in the GABAergic medium spiny neurons in the striatum (13, 17–19). In Huntington disease transgenic mice, PDE10A mRNA and protein levels decrease in the striatum prior to motor symptom development (45). Experiments using a chimeric construct of the PDE10A GAF domain suggested that the PDE10A GAF-B domain weakly binds cAMP (11). To the contrary, we found that cAMP is bound tightly and specifically in the binding pocket of the PDE10A GAF-B domain in the crystal. The weak binding estimated by the experiments might be due to the pre-bound cAMP, which is bound during protein preparation. It has also been suggested by the experiments using a chimeric construct that cAMP binding stimulates the catalytic activity of PDE10A, like PDE2A and PDE5 (11). The best crystals were obtained only when cAMP was added in the protein solution, suggesting that the cAMP-binding pocket of the purified PDE10A GAF-B domain sample was not fully occupied at low cAMP concentrations. In addition, Na\(^+\) inhibits the activity of the cyaB1 and cyaB2 adenylyl cyclases by binding to GAF domains at less than 1 \(\mu\)M cAMP, and this regulation is conserved in mammalian GAF domains specific to cGMP (46). Allosteric regulation of PDE10A may occur at relatively low cAMP concentrations. The tight and specific binding of cAMP to the PDE10A GAF domain may be important to critically control the basal ganglia circuit of the mammalian brain.

PDE10A inhibitors may have the potential to treat schizophrenia and Parkinson disease (21, 22). PDE10A is the only mammalian PDE family protein in which the GAF domain ligand is cAMP. Although the targets of structure-based drug design are usually the catalytic sites, the regulatory GAF-B domain of PDE10A is also an attractive target. The crystal structure presented here can be used for the development of drugs treating such neuropsychiatric disorders.

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**REFERENCES**

1. Beavo, J. A., and Brunton, L. L. (2002) Nat. Rev. Mol. Cell Biol. 3, 710–718
2. Omori, K., and Kotera, J. (2007) Circ. Res. 100, 309–327
