Crystal Structures of Phosphodiesterases 4 and 5 in Complex with Inhibitor 3-Isobutyl-1-methylxanthine Suggest a Conformation Determinant of Inhibitor Selectivity*

Qing Huai‡, Yudong Liu‡, Sharron H. Francis§, Jackie D. Corbin§, and Hengming Ke‡

From the ‡Department of Biochemistry and Biophysics and Lineberger Comprehensive Cancer Center, the University of North Carolina, Chapel Hill, North Carolina 27599-7260 and the §Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, Tennessee 37232-0615

Cyclic nucleotide phosphodiesterases (PDEs) are a superfamily of enzymes controlling cellular concentrations of the second messengers cAMP and cGMP. Crystal structures of the catalytic domains of cGMP-specific PDE5A1 and cAMP-specific PDE4D2 in complex with the nonselective inhibitor 3-isobutyl-1-methylxanthanine have been determined at medium resolution. The catalytic domain of PDE5A1 has the same topological folding as that of PDE4D2, but three regions show different tertiary structures, including residues 79–113, 208–224 (H-loop), and 341–364 (M-loop) in PDE4D2 or 533–566, 661–676, and 787–812 in PDE5A1, respectively. Because H- and M-loops are involved in binding of the selective inhibitors, the different conformations of the loops, thus the distinct shapes of the active sites, will be a determinant of inhibitor selectivity in PDEs. IBMX binds to a subpocket that comprises key residues Ile-336, Phe-340, Gln-369, and Phe-372 of PDE4D2 or Val-782, Phe-786, Gln-817, and Phe-820 of PDE5A1. This subpocket may be a common site for binding nonselective inhibitors of PDEs.

Cyclic nucleotide phosphodiesterases (PDEs)1 hydrolyze cAMP and cGMP to 5’-AMP and 5’-GMP. The second messengers, cAMP and cGMP, mediate the response of cells to a wide variety of hormones and neurotransmitters and modulate many metabolic processes such as cardiac and smooth muscle contraction, glycogenolysis, platelet aggregation, secretion, lipolysis, ion channel conductance, apoptosis, and growth control (1–6).

The human genome encodes 21 PDE genes and over 60 PDE isoforms categorized into 11 families (7–16). PDE molecules contain three regions: an N-terminal splicing region, a regulatory domain, and a catalytic domain near the C terminus. The 11 PDE families share a conserved catalytic domain of about 300 amino acids but rare homology in other regions across families. The function of the N-terminal splicing region of the PDE families is unknown. The regulatory domains of PDEs contain various structural motifs such as a calmodulin-binding domain in PDE1, upstream conserved region in PDE4, PAS (period clock protein, aryl hydrocarbon receptor nuclear translocator, and single-minded protein) domain in PDE8, GAF (cGMP-specific PDE, adenylyl cyclase, and Fh1A) domain in PDE2, -5, -6, -10, and -11. The regulatory domains have been shown to play roles in the regulation of the catalytic activity of PDEs or to participate in cross-talk with other signaling pathways (16–18).

PDEs share high degree (25–49%) of amino acid conservation in the catalytic domains, implying a similar three-dimensional structure of the catalytic domains. However, PDE families and isoforms within the respective family have varying substrate preferences for cAMP and cGMP. The PDE4, -7, and -8 families prefer to hydrolyze cAMP, whereas PDE5, -6, and -9 are cGMP-specific. PDE1, -2, -3, -10, and -11 show activities toward both substrates but have distinct Km values for cAMP and cGMP (16). In addition, many PDE families possess selective inhibitors that bind to the conserved active site. For example, rolipram is a PDE4-selective inhibitor, and sildenafil (VIAGRA®) is a PDE5-selective inhibitor (19).

In the past 3 decades, selective inhibitors of PDEs have been widely studied as therapeutics such as cardiotonics, vasodilators, smooth muscle relaxants, antidepressants, antiinflammatories, and agents for improving cognitive functions such as learning and memory (20–24). Many PDE inhibitors have been in clinical trials or have already entered the marketplace. For example, the PDE3-selective inhibitor cilostazol (Pletal®) is a drug for the reduction of symptoms of intermittent claudication, and the PDE5 inhibitors sildenafil (VIAGRA®), vardenafil (Levitra®), and tadalafl (Cialis®) are used for treatment of male erectile dysfunction. Selective inhibitors of PDE4 have been studied as anti-inflammatory drugs for asthma and chronic obstructive pulmonary disease (24–30).

Extensive biochemical, pharmacological, and clinical studies have been performed on PDEs and their selective inhibitors. However, the mechanisms by which the conserved active sites of PDEs recognize a common inhibitor and distinguish selective inhibitors remain mysteries. Structural studies on the catalytic domain of unligated PDE4B (31), PDE4D in complex with inhibitor zardavarine (32), PDE4D2 in complex with rolipram (33) and AMP (34), and PDE5A in complex with sildenafil (35) have provided preliminary understanding of inhibitor selectivity. Here we report the structures of the catalytic domains of human PDE5A1 and PDE4D2 in complex with a nonselective inhibitor 3-isobutyl-1-methylxanthine (IBMX). These structures reveal a common subpocket in the active site of PDEs for

* This work was supported by National Institutes of Health Grants GM59791 (to H. K.) and DK40029 and DK58277 (to J. D. C.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The atomic coordinates and structure factors (code 1RKO and 1RKp) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

† To whom correspondence should be addressed. Tel.: 919-966-2244; Fax: 919-966-2838; E-mail: hke@med.unc.edu.

‡ The abbreviations used are: PDE, phosphodiesterase; IBMX, 3-isobutyl-1-methylxanthine.
Fig. 1. Structures of the PDE-IBMX complexes. A, ribbon diagram of PDE4D2-IBMX. The α-helices are colored as cyan, and blue color represents 3_10 helices. The first metal ion is interpreted as zinc, as discussed previously (31, 33), whereas the second metal ion (Me2) is ambiguous. B, ribbon diagram of PDE5A1-IBMX. The second metal ion was assigned as magnesium because 0.2 M MgSO4 was used in the crystallization buffer. C, the structural superposition between PDE4D2 and PDE5A1. The cyan ribbons represent the conserved core structures between PDE4D2 and PDE5A1. The variable regions are drawn in gold for PDE4D2 and green for PDE5A1. D, the correspondence of amino acid sequence to the secondary structures.
Structural comparison between PDE4 and PDE5 suggests that conformational difference, in addition to the specific amino acid determinants, is another mechanism of inhibitor selectivity.

**EXPERIMENTAL PROCEDURES**

**Protein Expression and Purification**—The catalytic domain of human PDE4D2 (BF059733) was expressed in *Escherichia coli* and purified as described previously (33). The cDNA of the catalytic domain of human PDE5A1 was generated by site-directed mutagenesis of the bovine PDE5A1 gene. The coding regions for amino acids 535–860 of PDE5A1 were amplified by PCR and subcloned into the expression vector pET15b. The resultant plasmid pET-PDE5A1 was transformed into *E. coli* strain BL21 (Codonplus) for overexpression. The *E. coli* cell carrying pET-PDE5A1 was grown in LB medium at 37 °C to an A600 of 0.7, and then 0.1 mM isopropyl-β-D-thiogalactopyranoside was added for further growth at 15 °C overnight. Recombinant PDE5A1 was purified by nickel-nitrilotriacetic acid affinity column (Qiagen), thrombin cleavage, Q-Sepharose (Amersham Biosciences), and Sephacryl S300 (Amersham Biosciences) columns. The PDE5A1 protein had purity greater than 95% as shown by SDS-PAGE. A typical batch of purification yielded over 10 mg of PDE5A1 from a 2-liter cell culture. The fragment of human PDE5A1 expressed in *E. coli* had a catalytic activity of about 2 μmol/min/mg, which is comparable with that of the protein expressed in a baculovirus system (36).

**Crystallization and Data Collection**—Crystals of PDE4D2-IBMX and PDE5A1-IBMX were grown by hanging drop. The catalytic domain of 15 mg/ml PDE4D2 (amino acids 79–438) in a storage buffer of 50 mM NaCl, 20 mM Tris/HCl (pH 7.5), 1 mM -mercaptoethanol, and 1 mM EDTA was mixed with 5 mM IBMX. The PDE4D2-IBMX complex was crystallized against a well buffer of 0.1 M HEPES (pH 7.5), 20% PEG3350, 30% ethylene glycol, 10% isopropyl alcohol, and 5% glycerol at 4 °C. The well buffer was used as the cryosolvent for freezing the crystals in liquid nitrogen. Diffraction data were collected on beamline 14C at Advanced Photon Source (Table I). The PDE4D2-IBMX crystal has the space group P212121 with cell dimensions of a = 99.3, b = 112.5, and c = 160.9 Å. The catalytic domain of 10 mg/ml PDE5A1 (amino acids 535–860) was mixed with 5 mM IBMX and crystallized against a well buffer of 0.1 M Tris-base (pH 7.5), 20% PEG3350, 30% ethylene glycol, 10% isopropyl alcohol, and 5% glycerol at 4 °C. The well buffer was used as the cryosolvent for freezing the crystals in liquid nitrogen. Diffraction data were collected on beamline 14C at Advanced Photon Source (Table I). The PDE4D2-IBMX crystal has the space group P212121 with cell dimensions of a = 99.3, b = 112.5, and c = 160.9 Å. The catalytic domain of 10 mg/ml PDE5A1 (amino acids 535–860) was mixed with 5 mM IBMX and crystallized against a well buffer of 0.1 M Tris-base (pH 7.5), 20% PEG3350, and 0.2 mM MgSO4 at room temperature. To freeze the crystals in liquid nitrogen, the...
Structures of PDE5-IBMX and PDE4-IBMX

TABLE I

Statistics on diffraction data and structure refinement

| Data collection      | PDE5A1-IBMX | PDE4D2-IBMX |
|----------------------|-------------|-------------|
| Space group          | P32,21      | P21,2,2     |
| Unit cell (a, b, c, Å) | 74.5, 74.5, 130.1 | 99.7, 111.7, 159.4 |
| Resolution (Å)       | 2.05        | 2.1         |
| Total measurements   | 211,071     | 754,083     |
| Unique reflections   | 27,018      | 94,246      |
| Completeness (%)     | 100.0 (100.0) | 91.0 (64.4) |
| Average I/σ          | 13.4 (4.9)  | 18.9 (3.3)  |
| Rmerge               | 0.063 (0.475) | 0.074 (0.440) |

Structure refinement

| R-factor              | 0.229        | 0.296       |
| B-factor              | 0.243        | 0.259       |
| Resolution (Å)        | 50–2.05      | 50–2.1      |
| Reflections           | 25,797       | 89,970      |
| r.m.s.d. for bond     | 0.0059       | 0.0059      |
| r.m.s.d. for angle    | 1.20(5)      | 1.09(8)     |
| Average B-factor (Å²) | 36.6 (2674)  | 32.8 (10,987) |
| All atoms             | 36.6 (2541)  | 32.8 (10,726) |
| Protein               | 27.5 (16)    | 58.9 (64)   |
| IBMX                  | 35.1 (1333)  | 27.8 (189)  |
| Waters                | 32.1 (2)     | 42.0 (8)    |
| Metals                |              |             |

R = 0.243, 0.259

* The numbers in parentheses are for the highest resolution shell.

** r.m.s.d., root mean square deviation.

The number of atoms is listed in the parentheses.

cryosolvent was prepared by mixing 20% glycerol with the well buffer. Diffraction data of PDE5A1-IBMX were collected on beamline X20C at National Synchrotron Light Source (Table I). It has the space group P32,21 with cell dimensions of a = b = 74.5, and c = 130.1 Å. All data were processed by program HKL (37).

Structure Determination—The PDE4D2 tetramer from the PDE4D2-rolipram structure (33) was directly applied to solve the structure of PDE4D2-IBMX, and the orientation of the tetramer was optimized by rigid body refinement of CNS (38). The structure of PDE5A1-IBMX was solved by molecular replacement program AMoRe (39), using the catalytic domain of PDE4D2 as the initial model and a data set collected on Raxis IV++ to 2.5 Å resolution. The translation search yielded a correlation coefficient of 0.162 and R-factor of 0.526 for 3247 reflections between 4 and 8 Å resolution. The amino acid differences between PDE5A1 and PDE4D2 were replaced, and the atomic model was rebuilt by program O (40) against the electron density map that was improved by the density modification package of CCP4 (41). The structure was refined by CNS (Table I). The coordinates have been deposited in the Protein Data Bank (accession codes 1RKO and 1RKP).

RESULTS AND DISCUSSION

Overall Structures—The crystallographic asymmetric unit of PDE4D2 in complex with IBMX contains four catalytic domains (residues 79–438) that form a tetramer. The monomer of PDE4D2-IBMX consists of 16 helices (Fig. 1) that fold into three subdomains as described previously in the structures of PDE4B (31) and PDE4D2-rolipram (33). The catalytic domain of PDE5A1 (residues 535–860) contains 16 helices and has the same folding topology as that of PDE4D2 (Fig. 1). The crystal of PDE5A1 contains one catalytic domain in the crystallographic asymmetric unit, which apparently exists as a monomer. This is consistent with the biochemical studies that the monomeric fragment of bovine PDE5A (residues 508–865) has similar catalytic activity as the full-length PDE5A (36). The electron density showed that residue 778 of PDE5A1 is better modeled with leucine instead of isoleucine in the wild type PDE5A1 sequence. The interpretation of this observation is not clear, but it may be caused by spontaneous mutation during PCR.

The superposition of the catalytic domains of PDE5A1 over PDE4D2 showed an root mean square deviation of 1.5 Å for the Ca atoms of 257 superimposable residues. However, the root mean square deviation increases to 4.8 Å if the entire catalytic domain is compared (312 residues). Three regions show different tertiary structures and are not superimposable, in addition to residues 289–298 in PDE4D2, which become a short connection with deletion of 6 residues in PDE5A1. The N-terminal regions of residues 79–113 in PDE4D2 and 535–566 in PDE5A1 contain two helices but have a totally different three-dimensional arrangement. The region of residues 208–224 in PDE4D2 and 661–676 in PDE5A1, which is labeled as H-loop in Fig. 1, shows differences of over 7 Å for the Ca atom positions of two short helices H8 and H9. Besides, the H-loop of PDE5A1 is flexible, although traceable in electron density, as shown by an average B-factor of 71.8 Å² in comparison with 36.8 Å² for all atoms of the structure. The region of residues 341–364 in PDE4D2 or 787–812 in PDE5A1 (M-loop in Fig. 1) shows dramatic conformational differences. Nine residues 341–349 at the tail of helix H14 in PDE4D2 are unfolded into coil or random conformations in PDE5A1. Residues 793–807 in PDE5A1 are not traceable due to lack of electron density and presumably exist in random conformation, in contrast to the ordered conformations of the corresponding sequences in PDE4D2.

It is interesting to note that our structure of PDE5A1-IBMX shows some conformational differences from those of PDE5A in complex with the inhibitors such as sildenafil. Although the structure of PDE5A-sildenafil is not available for a detailed comparison, the paper by Sung et al. (35) reported that residues 665–675 of the H-loop are disordered, and the M-loop is ordered in the PDE5A-sildenafil structure. In contrast, our structure of PDE5A1-IBMX shows that the H-loop is traceable, but the M-loop is disordered. One possible interpretation of these differences may be that binders of each of the different inhibitors selectively affect the conformations of these loops. Besides, we note that the catalytic activity of our recombinant fragment 535–860 of PDE5A1 (2 μmol/min/mg) is about 800 times higher than that of the fragment 537–860 for the PDE5A-sildenafil structure (0.0025 μmol/min/mg) (35). Nevertheless, the structural studies by the two groups imply that the conformational flexibility of H- and M-loops may play roles in inhibitor selectivity.

Metal Binding—Two metal-binding sites have been assigned in the crystal structures of PDE4B and PDE4D2 as follows: one is zinc and another is ambiguous (31, 33, 34). Two metal sites are also found in the structure of PDE5A1 and occupy the same location as in the PDE4 structures (Fig. 1). In the PDE5A1 structure, the strongest peak in the 2Fᵣ − F map has about 10 times the background and is assigned as zinc, despite the fact that no zinc was added during expression, purification, and crystallization of PDE5A1. This site coordinates with His-617, His-653, Asp-654, Asp-764, and two bound water molecules. These six coordination forms an octahedron and are the same as in PDE4 (31, 33). The role of the equivalent residues His-607 and His-643 of bovine PDE5 in metal coordination was established by site-directed mutagenesis (42). The second metal ion forms an octahedron with Asp-654 and five bound water molecules and has the same coordinations as in PDE4D2. The second metal ion is assigned as magnesium in the crystal because 0.2 M MgSO₄ was used in crystallization. The crystallographic refinement showed that both metal ions have the B-factors comparable with the overall average B-factor of the protein atoms (Table I), in support of the assignment. However, the physiological metal ions await to be identified. Biochemical studies have suggested that zinc is the optimal divalent cation for supporting catalysis in PDE5A1 (43).

IBMX Binding—The electron density maps that were calculated from the PDE4D2 and PDE5A1 structures without IBMXs show that IBMX binds to a subpocket of the active site of PDE5s (Fig. 2). The majority of IBMX-binding residues are conserved between PDE4 and PDE5. Thus, the xanthine ring of IBMX stacks against Phe-372 of PDE4D2 or Phe-820 of
PDE5A1 on one side and contacts hydrophobic residues Ile-336 and Phe-340 of PDE4D2 or Val-782 and Phe-786 of PDE5A1 on another side. IBMX forms a hydrogen bond with the side chain of Gln-369 in PDE4D2 or Gln-817 in PDE5A1, in addition to hydrophobic interactions with Leu-319 and Asn-321 of PDE4D2 or Leu-765 and Ala-767 of PDE5A1.

On the other hand, IBMX shows some distinct interactions in the two PDE structures. First, O-6 of IBMX forms a hydrogen bond with Ne-2 of Gln-817 in PDE5A1, but with a water molecule in PDE4D2. Second, the xanthine ring of IBMX shows a translational shift of about 2 Å when the protein structures of PDE4D2 and PDE5A1 are superimposed (Fig. 2). Third, the

Fig. 2. IBMX binding. Stereoview of the electron density for IBMX bound to PDE4D2 (A) and PDE5A1 (B). The $2F_o - F_c$ maps were calculated from the structures omitted IBMX and contoured at 1.5$\sigma$ for PDE4D2 and 2.0$\sigma$ for PDE5A1. C, chemical structure of IBMX. D, IBMX binding to the active site of PDE4D2. The xanthine group stacks against Phe-372 and forms hydrogen bond with Gln-369 (dotted lines). E, IBMX binding to the active site of PDE5A1.
forms hydrophobic interactions with the cyclopentane ring of the PDE4-selective inhibitor rolipram (33), indicating its role in binding of the selective inhibitor. In contrast, the corresponding residues 787–812 of PDE5A1 show significantly different conformations: the unfolding of C-terminal end of helix H14 and disordered conformation of 793–807 in PDE5A1 (Fig. 1). Therefore, the M-loop would have different contact patterns when interacting with PDE5 inhibitors from the mode in PDE4. In summary, our structural study suggests that both the conformation of the active site and the chemical nature of the residues in contact with the inhibitors contribute to the selectivity.

Acknowledgments—We thank Dr. Howard Robinson and beamlines X21C at National Synchrotron Light Source and 14-BM-C at Advanced Photon Source for collection of diffraction data.

REFERENCES

1. Antoni, F. (2000) Front. Neuroendocrinol. 21, 103–132
2. Pelligrino, D. A., and Wang, Q. (1996) Prog. Neurobiol. 51, 1–18
3. Carvajal, J. A., Germain, A., and Chambard, J.-P., and Wei, C. P. (2000) J. Cell. Physiol. 184, 409–420
4. Lucas, K. A., Pitari, G. M., Kaszman, S., Ruiz-Stewart, I., Park, J., Schulz, S., Chapsenik, R. F., and Waldman, S. A. (2000) Pharmacol. Rev. 52, 375–414
5. Klein, C. (2002) Cell. Signal. 14, 493–498
6. Chin, K. Y., Yang, W. L., Riba, R., Rits, T., Reitman, E., Vettori, D., Cvicj, M. E., Shin, M., and Iacono, L. (2002) Ann. N. Y. Acad. Sci. 968, 49–64
7. Manganuillo, V. C., Taira, M., Degerman, F., and Belfrage, P. (1995) Cell. Signal. 7, 445–455
8. Muller, T., Engels, P., and Fozard, J. R. (1996) Trends Pharmacol. Sci. 17, 294–298
9. Houssay, M. C., Sullivan, M., and Bolger, G. B. (1998) Adv. Pharmacol. 44, 225–243
10. Houssay, M. D., and Adams, D. R. (2003) Biochem. J. 370, 1–18
11. Turphey, T. J. (1998) An. J. Respir. Crit. Care Med. 157, 351–370
12. Corbin, J. D., and Francis, S. H. (1999) J. Biol. Chem. 274, 13729–13732
13. Soderling, S. H., and Beavo, J. A. (1990) Curr. Opin. Cell Biol. 12, 174–179
14. Francis, S. H., Turko, I. V., and Corbin, J. D. (2001) Prog. Nucleic Acids Res. Mol. Biol. 65, 1–52
15. Conti, M., Richter, W., Mehta, C., Livera, G., Park, J. Y., and Jin, C. (2003) J. Biol. Chem. 278, 5493–5496
16. Mehta, C., Andersen, C. E., Filipanti, M., Jin, S. L., and Conz, M. (2002) Trends Endocrinol. Metab. 13, 29–35
17. Manganuillo, V. C., Murata, T., Taira, M., Belfrage, P., and Degerman, F. (1995) Arch. Biochem. Biophys. 322, 1–13
18. Degerman, F., Belfrage, P., and Manganuillo, V. C. (1996) Biochem. Soc. Trans. 24, 1010–1013
19. Corbin, J. D., and Francis, S. H. (2002) Int. J. Clin. Pract. 56, 453–459
20. Nicholls, R. A., and Beavo, J. A. (2000) Eur. J. Pharmacol. 392, 903–907
21. Truss, M. C., Stief, C. G., Eckert, S., Becker, A. J., Wafer, J., Schultzleiss, D., and Jonas, U. (2001) World J. Urol. 19, 344–350
22. Liu, X., Shaker, Y., Yoshitake, M., and Kambyashi, J. J. (2001) Cardiovasc. Drug Rev. 19, 369–386
23. Huang, Z., Ducharine, Y., MacDonald, D., and Robinhain, A. (2001) Curr. Opin. Chem. Biol. 5, 432–438
24. Botella, D. P. (2002) Nat. Rev. Drug Discovery 1, 674–682
25. Barnette, M. S. (1999) Prog. Drug Res. 53, 193–229
26. Souness, J. E., Aldous, D., and Sargent, C. (2000) Immunopharmacology 47, 127–162
27. Barnette, M. S., and Underwood, D. C. (2000) Curr. Opin. Pulmonary Med. 6, 164–169
28. Piaz, V. D., and Giovannoni, P. (2000) Eur. J. Med. Chem. 35, 463–480
29. Giembycz, M. A. (2002) Monaldi Arch. Chest Dis. 57, 48–64
30. Sharpe, T. J., and Fitzpatrick, D. (2000) Chest 121, S192–S196
31. Xu, Y. S., Hassell, A. M., Vanderwall, D., Lambert, M. H., Holmes, W. D., Luther, M., Rocque, W. J., McMillan, M. V., Zhao, Y., Ke, H., and Nolte, R. T. (2000) Science 288, 1822–1825
32. Liu, X., Markowitz, J. D., and Lee, H. (2002) PERS Lett. 330, 53–58
33. Hui, Q., Wang, H., Sun, Y., Kim, H. Y., Liu, Y., and Ke, H. (2003) Structure 11, 865–873
34. Hui, Q., Colilleii, J., and Ke, H. (2000) Biochemistry 41, 13200–13206
35. Song, S. J., Huang, K. Y., Xiong, J. Y., Yang, W. L., Riba, R., Rits, T., Reitman, E., Vettori, D., Cvicj, M. E., Shin, M., and Iacono, L. (2002) Nature 425, 58–102
36. Piek, T. L., Francis, H., Ruedy, A., Karin, K. A., and Corbin, J. D. (1999) J. Biol. Chem. 274, 24613–24620
37. Owinsowski, Z., and Minor, W. (1997) Methods Enzymol. 276, 307–326
38. Ertz, A. T., Adams, P. D., Clarke, G. M., DeLano, W. L., Grosse-Kunstleve, R. W., Wang, J. S., Kuszewski, J., Nilges, M., Pannu, N. S., Read, R. J., Rice, L. M., Simonsson, T., and Warren, G. L. (1998) Acta Crystallogr. Sect. D. Biol. Crystallogr. 54, 905–921
39. Navaza, J., and Salvador, P. (1994) Methods Enzymol. 276, 581–594
40. Jones, T. A., Zou, J. Y., Cowan, S. W., and Kjeldgaard, M. (1991) Acta Crystallogr. Sect. A 47, 110–115
41. Collaborative Computational Project, Number 4 (1994) Acta Crystallogr. Sect. D. Biol. Crystallogr. 50, 760–763
42. Francis, S. H., Turko, I. V., Grimes, K. A., and Corbin, J. D. (2000) Biochemistry
39, 9591–9596
43. Francis, S. H., Colbran, J. L., McAllister-Lucas, L. M., and Corbin, J. D. (1994) J. Biol. Chem. 269, 22477–22480
44. Beavo, J. A., Rogers, N. L., Crofford, O. B., Hardman, J. G., Sutherland, E. W., and Newman, E. V. (1970) Mol. Pharmacol. 6, 597–603
45. Sekhar, K. R., Grondin, P., Francis, S. H., and Corbin, J. D. (1996) in Phosphodiesterase Inhibitors (Schudt, C., Dent, G., and Rabe, K. F., eds) pp. 135–146, Academic Press, New York
46. Alvarez, R., Daniels, D. V., Shelton, E. R., Baecker, P. A., Fong, T. A. T., Devens, B., Wilhelm, R., and Eglen, R. M. (1996) in Phosphodiesterase Inhibitors (Schudt, C., Dent, G., and Rabe, K. F., eds) pp. 161–171, Academic Press, New York