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Refolding and kinetic characterization of the phosphodiesterase-8A catalytic domain

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Abstract

Cyclic nucleotide phosphodiesterase-8 (PDE8) hydrolyzes the second messenger cAMP and is involved in many biological processes such as testosterone production. Although the bacterial and mammalian expression systems have been extensively tried, production of large quantity of soluble and active PDE8 remains to be a major hurdle for pharmacological and structural studies. Reported here is a detailed protocol of refolding and purification of large quantity of the PDE8A1 catalytic domain (residues 480–820) and kinetic characterization of the refolded protein. This protocol yielded about 8 mg of the PDE8A catalytic domain from 2 liter *E. coli* culture, which has at least 40-fold higher activity than those reported in literature. The PDE8A1 catalytic domain has k_{cat} of 4.0 s^{-1} for Mn^{2+} and 2.9 s^{-1} for Mg^{2+}, and the K_M values of 1–1.8 μM. In addition, the PDE8A1 (205–820) fragment that contains both PAS and catalytic domains was expressed in *E. coli* and refolded. This PDE8A1 (205–820) fragment has k_{cat} of 1.1 s^{-1} and K_M of 0.28 μM, but aggregated at high concentration. The K_M of PDE8A1 (205–820) is 2- to 7-fold higher than the K_M values of 40–150 nM for the full-length PDE8s in literature, but about 6-fold lower than that of the catalytic domain. Thus, the K_M difference likely implies an allosteric regulation of the PDE8A activity by its PAS domain.

The second messengers adenosine and guanosine 3′, 5′-cyclic monophosphate (cAMP and cGMP) mediate the response of cells to a wide variety of hormones and neurotransmitters and modulate many metabolic processes [1–6]. Cyclic nucleotide phosphodiesterases (PDEs) hydrolyze cAMP and cGMP to 5′-AMP and 5′-GMP. Human genome contains 21 PDE genes that are categorized into 11 families and express over 100 isoforms of PDE proteins through alternative mRNA splicing [7–10]. PDE molecules are divided into a variable regulatory domain at the N-terminus and a conserved catalytic domain with about 300 amino acids at the C-terminus. Family selective PDE inhibitors have been widely studied as therapeutics for treatment of human diseases, including cardiotonics, vasodilators, smooth muscle relaxants, antidepressants, antithrombotics, antiasthmatics, and agents for improvement of learning and memory [11–18]. A well known example is the PDE5 inhibitor sildenafil (Viagra) that has been approved for treatment of both male erectile dysfunction and pulmonary hypertension [11,14,19].

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Human genome expresses two PDE8 subfamilies (8A and 8B), both of which are cAMP-specific enzymes and have K_M of 40–150 nM for cAMP and >100 μM for cGMP [20–23]. PDE8 is distributed in various human tissues and is abundant in testis [24–27]. Functionally, PDE8 has been reported to be involved in regulation of T-cell activation [28], chemotaxis of activated lymphocytes [29], modulation of testosterone production in Leydig cell [30], and potentiation of biphasic insulin response to glucose [31]. It has been recently reported that the H305P mutation of PDE8B1 is associated with micronodular adrenocortical hyperplasia [32] and PDE8B gene variants are involved in regulation of thyroid-stimulating hormone levels and thyroid function [33]. Molecules of PDE8 contain a Per-ARNT-Sim (PAS) domain that is a structural motif and an environmental protein sensor involved in many biological processes such as response to oxygen partial pressure and redox signaling [34,35]. PDE8 was reported to bind IκBβ, a regulatory protein of transcription factor NF-κB [36], but the binding mode and biological function are unknown.

While crystal structures of the catalytic domains of eight PDE families have been determined [37], PDE8 remains to be one of three PDE families, whose structures of any fragments are not available. The expressions of PDE8 in the baculovirus and *E. coli* systems have been reported [20–23], but such procedures often produced small amounts of the PDE8 enzymes that have low catalytic activity. Lack of an effective protocol for preparation of large quantity of active PDE8 enzymes appears to be a hurdle for the structural study and inhibitor discovery of PDE8. Reported here are the details of the protein expression of the PDE8A catalytic domain in *E. coli*, refolding from the inclusion body, purification and kinetic characterization of the refolded PDE8A1. Our refolding protocol yielded 8 mg of the PDE8A catalytic domain from 2 liter *E. coli* culture, which showed at least 40-fold higher activity than those of PDE8s reported in literature [20–23]. Therefore, this study is valuable not only for basic and structural research, but also for development of PDE8 selective inhibitors.

Materials and methods

Subcloning of the PDE8A1 catalytic domain

The Expressed Sequence Tag cDNA clone of PDE8A1 (GenBank #AF332653) was purchased from American Type Culture Collection (10325182). The cDNA fragments for expression of the PDE8A1 catalytic domain (residues 480–820) and the PDE8A1 fragment (205–820) were amplified by PCR and subcloned into vector pET15b. The following oligonucleotide primers that contain the restriction sites of NdeI and XhoI were used for amplification of the desired genes: 5′-GTGCCGCGCGGCAGCCATATGGCTAGCTCCCTTGATGATGTCCCAC and 3′-GGATCCTCGAGTTACTTCATTTCGTCCAGTCCTTTC. The amplified cDNAs of PDE8A1 and the expression vector pET15b were digested by the restriction enzymes, purified with agarose gel, and ligated together by T4 DNA ligase.

The plasmid pET15b-PDE8A1 was transferred into *E. coli* strain BL21 (CodonPlus) for overexpression. The cell bearing the vector pET15b-PDE8A1 was grown in a modified 2xYT culture medium (16 g tryptone, 10 g yeast extract, and 5 g NaCl per liter) that was autoclaved before addition of 0.4% glucose, 100 mg ampicillin, and 20 mg chloramphenicol per liter. After the cell was grown at 37°C to A600 = 0.7, 0.1 mM IPTG (isopropyl β-D-thiogalactopyranoside) was added to induce the overexpression and the cell culture was transferred to room temperature for further growth overnight.

Protein refolding and purification

About 10 grams of the frozen cells from 2 liter culture were suspended in 40 mL of the extraction buffer and passed through French Press three times at 1200 psi to disrupt them. The extraction buffer was 20 mM Tris-HCl, pH 7.5, 50 mM NaCl, 1 mM β-mercaptoethanol (β-

ME), and 1 mM EDTA. The homogenate was centrifuged at 15,000 rpm in a Beckman JA-20 rotor for 30 min. Unfortunately, the recombinant PDE8A1 fragments mainly existed in the pellet phase and therefore refolding was necessary to obtain soluble and active PDE8A1 protein. The pellet was dissolved in 10 mL buffer of 6 M guanidine, 0.1 M Tris-HCl pH 8.0 under slow orbital shaking at room temperature for 3 hours. The dissolved mixture was centrifuged at 15,000 rpm for 20 min to remove the insoluble debris.

The supernatant was loaded into a Ni-NTA column (φ=2.5 cm, 25 ml QIAGEN agarose beads). The column was washed with 100 mL buffer of 8 M urea, 0.1 M Tris-HCl pH 8.0, and eluted with the same buffer plus 0.5 M arginine. The fractions of the PDE8A1 catalytic domain from the elution were combined. The protein concentration was estimated by the absorption of A280 (1.097 units = 1 mg/ml), as calculated by program ProtParam [38]. Fifty milligrams of the PDE8A catalytic domain at 2 mg/ml (25 ml of protein solution) was added dropwise into 1.7 liters of the refolding buffer under mild stir. The refolding buffer was 0.5 M Tris-HCl pH 7.0, 20 mM $MgCl_2$, 20 mM $MnCl_2$, 20 μM $ZnSO_4$, 0.7 M arginine, 30% glycerol, 10 mM NaCl, 1 mM KCl, and 10 mM DTT. The refolding was carried out at 30 μg/ml protein concentration without shaking at 4°C for 3 days.

To concentrate the refolded PDE8A1, the dilute refolding system was mixed with 15 grams of hydroxyapatite HTP GEL (Bio-Rad) that was presoaked in water. After stirred at room temperature for 15 min, the suspension was filtered with a filter paper. The beads were collected, re-suspended in 100 mL of 20 mM Tris-HCl, pH 8.0, 50 mM NaCl, 1 mM β-ME, and then packed into a column. The column was washed with 50 mL of the same buffer and eluted with 100 mL of 20mM Tris-HCl pH 8.0, 100 mM KH_2PO_4, 1 mM β-ME. The fractions were combined and dialyzed against 0.5 liter of 20 mM Tris-HCl pH 7.5, 50 mM NaCl, 1 mM β-ME twice, 1 hour and overnight.

To remove the His-tag, 2.5 mM $CaCl_2$ and 1 μg/ml bovine thrombin (Haematologic Tech. Inc.) were added for digestion at 25°C for 1 hour. The digested PDE8A1 was loaded into a Q-Sepharose column (GE Healthcare) that was pre-equilibrated with a buffer of 50 mM NaCl, 20 mM Tris-HCl pH 7.5, 1 mM β-ME, 1 mM $MgCl_2$. The column was washed with 200 mL of 20 mM Tris-HCl pH 7.5, 100 mM NaCl, 1 mM β-ME, 1 mM EDTA, and then PDE8A1 was eluted out with the same buffer except for 300 mM NaCl. After being concentrated to about 10 mL, the protein was loaded into a gel filtration column Sephacryl S300 (GE Healthcare) and eluted with a buffer of 20 mM Tris-HCl pH 7.5, 50 mM NaCl, 1 mM β-ME, 1 mM $MgCl_2$. The protein was finally concentrated by Amicon cell and ultrafiltration membrane YM30 and its purity was estimated by the SDS gel.

The fragment of PDE8A1 (205–820) that contains both PAS and catalytic domains was subcloned into pET15b and expressed in *E. coli* under the similar procedure, and refolded in a buffer of 50 mM Tris-HCl, pH 7.0, 1.25 M urea, 1 M arginine, 40 mM $MnCl_2$, 10 mM NaCl, 1 mM KCl, and 10 mM DTT.

Enzymatic assay

The enzymatic activities were assayed by using ^{3}H-cAMP or ^{3}H-cGMP as substrate, as previously reported [39]. The catalytic domain of PDE8A1 was incubated with a reaction mixture of 20 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$ or 4 mM $MnCl_2$, 1 mM DTT, ^{3}H-cAMP or ^{3}H-cGMP (20000–40000 cpm/assay) at 24°C for 15 min. The reaction was terminated by adding 0.2 M $ZnSO_4$ and $Ba(OH)_2$. The reaction product ^{3}H-AMP or ^{3}H-GMP was precipitated out while unreacted ^{3}H-cAMP or ^{3}H-cGMP remained in the supernatant. After centrifugation, the radioactivity in the supernatant was measured in a liquid scintillation counter. The reaction was controlled at hydrolysis of <30% substrate under suitable enzyme concentrations (0.004 to 0.085μg/ml PDE8A1). Twelve concentrations of cAMP or cGMP in a range of 0.04 to 50

μM were used to obtain the kinetic parameters. The enzymatic properties were analyzed by the steady state kinetics [40]. The non-linear regression of the Michealis-Menten equation, as well as Eadie-Hofstee plots were performed to obtain the values of K_M, Vmax, and k_{cat}.

Results

Expression and purification of PDE8A1

To express the soluble catalytic domain of PDE8A1 in *E. coli*, the conditions were extensively tested, including temperatures for *E. coli* cell growth, cell culture mediums (LB, 2xYT, etc), different expression vectors such as pET15 and pET32, various lengths of the PDE8A1 fragments, addition of various chemicals, induction at different stages of *E. coli* growth, etc. However, almost all the conditions mainly produced inclusion body of PDE8A1. The best expression condition yielded a small amount of the soluble protein that appeared to have reasonable activity, but was not enough for crystallization. Thus, refolding of the inclusion body was performed to produce large quantity of the soluble catalytic domain of PDE8A1, as discussed in the next section.

The refolded PDE8A1 was purified by three columns (Table 1). Since refolding was performed in diluted solution, it would be time-consuming if the refolded protein were concentrated by Amicon concentrator and YM30 membrane. Fortunately, the refolded PDE8A1 catalytic domain binds to hydroxyapatite HTP. Thus, mixing hydroxyapatite beads with the diluted protein solution and filtration of the suspension with filter paper quickly concentrated the folded PDE8A1. The PDE8A1 catalytic domain weakly interacted with the ionic Q-Sepharose column and was retained in the column under 100 mM NaCl, but eluted out at 300 mM NaCl. The gel filtration column S300 separated small amounts of oligomers with different molecular weights. The peak from the S300 column appeared to have molecular weight of monomeric PDE8A1. A typical batch of refolding and purification used 50 mg PDE8A1 catalytic domain from the Ni-NTA column and yielded about 8 mg active protein from the S300 column with purity >95%. The overall recovery of the catalytic activity from the purification was about 30% and the protein recovery percentage was 15.5% (Table 1). The SDS gel showed that the protein purity after each step of purification was improved slightly (Fig. 1), thus implying that partially-folded or aggregated PDE8A1 co-existed with the more completely folded enzyme and was removed during the purification.

Refolding of PDE8A1

Refolding conditions were extensively searched to obtain the most active PDE8A1 catalytic domain. Divalent metal manganese was essential for the refolding and its 10 mM or higher concentration reached a plateau of the specific activity (Fig. 2a). Surprisingly, magnesium ion up to 50 mM had little impact on the refolding (Fig. 2a), although it has been mostly used as the catalytic ion in the activity assay (Table 2). DTT was also required for the refolding and enhanced activity by about 3-fold (Fig. 2b). The pH of the buffer was an important factor for the refolding (Fig. 2c). Acidic pHs were better than the basic pHs while pH > 9 yielded inactive PDE8A1. L-arginine was another sensitive factor and at 0.7 M it resulted in 5-fold more activity than the case without L-arginine (Fig. 2d). The protein concentration and temperature were less sensitive, but could yield about 2-fold activity increase (Figs. 2e and 2f). Although the best protein concentration for the refolding was 20 μg/ml at 4°C, 30 μg/ml was more effective to prepare large quantity of PDE8A1 for crystallization. Other tested factors include slow shaking during refolding, folding time, and concentration of glycerol. The slow shaking of the refolding solution in coldroom yielded much lower activity of PDE8A1 than the case without shaking. One day of refolding produced about 90% activity of the sample from three day refolding. Glycerol did not significantly impact the refolding and thus was kept at 30% without further optimization.

Kinetic properties of the refolded PDE8A1

For measurement of the kinetic properties of the refolded proteins, the enzyme concentration in a range of 5.3 to 84.8 ng/ml was tested. As shown in Fig. 3, the activity had essentially a linear relationship with the tested enzyme concentrations. Thus, 10–20 ng/ml PDE8A1 was used in the later measurements of the kinetic properties.

When 4 mM Mn^{2+} was used as the catalytic ion, the refolded PDE8A1 catalytic domain has a K_M of 1.8 μM and a k_{cat} of 4.0 s^{-1} for cAMP, and a K_M of 1.6 mM and a k_{cat} of 1.6 s^{-1} for cGMP (Table 2, Fig. 4). The specificity constant $(k_{cat}/K_M)^{cAMP}/(k_{cat}/K_M)^{cGMP}$ is 2135 fold for the manganese catalysis. When 10 mM Mg^{2+} was used as the catalytic ion, the refolded PDE8A1 catalytic domain has a K_M of 7.0μM and a k_{cat} of 2.9 s^{-1} for cAMP, and a K_M of 1.5 mM and a k_{cat} of 0.4 s^{-1} for cGMP (Table 2). The specificity constant $(k_{cat}/K_M)^{cAMP}/(k_{cat}/K_M)^{cGMP}$ for the Mg^{2+} catalysis is 1680. Thus, the specificity constants measured by using manganese or magnesium consistently indicate the cAMP-specificity of PDE8A1. In addition, the k_{cat} values suggest that manganese is more effective than magnesium as the catalytic ion to catalyze the reaction. This is more obvious at low substrate concentration. At 1 μM cAMP, the specific activity for 4 mM $MnCl_2$ was about 4-fold higher than that of 10 mM $MgCl_2$ (Fig. 4). However, it remains unknown if manganese serves as the physiological catalytic ion.

An unusual observation is that magnesium had K_M of 7 μM, about 4 times bigger than K_M of 1.8 μM for manganese. To study whether the apparent affinity constant K_M is dependent on nature of the divalent ions, the PDE8A1 catalytic domain that was expressed and naturally folded in *E. coli* without addition of divalent ions was partially purified by the similar procedure as that of the refolded and the kinetic parameters were measured. This naturally folded PDE8A1 had the K_M values of 1.0μM for Mg^{2+} and 1.5μM for Mn^{2+} when cAMP was used as the substrate (Table 2). Thus, the K_M difference between the magnesium and manganese catalyses of the refolded PDE8A catalytic domain apparently resulted from the refolding process, but is not biologically relevant.

The kinetic properties of the refolded PDE8A1 catalytic domain are comparable with those of other cAMP-specific PDE families. For example, the full length PDE4D2 and the catalytic domain of PDE7A1 have the K_M values of 1.5 and 0.2 μM, the k_{cat} values of 3.9 and 1.6 s^{-1} for cAMP, and the specificity constants $(k_{cat}/K_M)^{cAMP}/(k_{cat}/K_M)^{cGMP}$ of 500 and 4000, respectively [39]. A notable difference among these three cAMP-specific PDEs is that the k_{cat} values of PDE8A1 for cGMP is 2.5- to 7.9-fold lower than that for cAMP (Table 2), in comparison to comparable or even better k_{cat} for cGMP than that for cAMP in the PDE4D2 and PDE7A1 catalyses [39]. Therefore, the substrate specificity of the three cAMP-specific PDE families is dominantly determined by K_M, while k_{cat} might have some minor contribution in the case of PDE8A1.

Impact of the PAS domain on the catalysis of PDE8A

An unusual observation of this study is that the K_M of our PDE8A1 catalytic domain is significantly larger than those for the full length PDE8. Our catalytic domain of PDE8A1 from the refolding has K_M of 1.0–1.8μM when Mg^{2+} or Mn^{2+} was used as the catalytic ion, which are 7- to 45-fold larger than the K_M values of 40–150 nM for full length PDE8A and PDE8B, as reported by four groups [20–23]. This K_M incomparability of PDE8 is in contrast to our early observations that other PDE families have comparable K_M values between their catalytic domains and the full length proteins, as shown in the cases of PDE4 [39,41], PDE7 [39,42], PDE9 [43], and PDE10 [44,45]. Although the proteins of full-length PDE8s were partially purified and had lower activity, the different K_M values do not appear to be the experimental artifacts. Rather, the different K_M values may reflect the allosteric behavior of PDE8 and the regulation of the catalytic activity by its PAS domain. This argument is supported by the

observation that the PDE8A catalytic domains prepared by either refolding or natural folding in *E. coli* have the similar K_M values. Since the *E. coli* systems have been widely used to express proteins for biochemical and structural studies, the similar K_M values imply that the refolding procedure likely produced biologically relevant conformation of the PDE8A catalytic domain. Indeed, the crystal of the refolded PDE8A1 revealed a topological folding comparable with the structures of other PDE families [46]. The argument of the allosteric catalysis is consistent with the early report that the full-length PDE8A had about 5-fold better activity than its catalytic domain and the binding of the partner protein IκBβ further stimulated catalytic activity about 2-fold [36]. To verify our assumption of the allosteric behavior, a PDE8A1 (205–820) fragment that contains both PAS and catalytic domains was expressed in *E. coli* and refolded using similar protocols. This fragment has K_M of 280 nM and k_{cat} of 1.1 s^{-1} when cAMP is used as the substrate and 4 mM $MnCl_2$ is the catalytic ion. Thus, the K_M of 280 nM for the PDE8A (205–820) fragment is 2- to 7-fold to the K_M values of 40 – 150 nM for the full-length PDE8 [20–23], in contrast to 7- to 45-fold difference between the catalytic domain and full-length PDE8. This comparison implies the impact of the PAS domain on the substrate affinity and the catalysis.

Discussion

PDE8 remains one of three PDE families whose crystal structures of any fragments are not available [35], apparently due to the difficulty in preparation of large quantity of the active enzyme. Several research groups have reported the expression of PDE8A and 8B [20–23], but these protocols yielded proteins with low catalytic activities. For example, the expression of a 545-residue fragment of PDE8A in the baculovirus system showed Vmax of 0.15 μmol/min/mg [20], which is about 40-fold less active than Vmax of 6.1μmol/min/mg for our PDE8A catalytic domain. The fragment of C-terminal 584 amino acids of PDE8B had Vmax of only 0.14 nmol/min/mg [22], which is 40000-fold worse than that of our PDE8A. Thus, the refolding protocol in this paper produces large quantity of highly active PDE8A catalytic domain and is valuable for basic studies of PDE8 and also for discovery of PDE8 inhibitors.

All PDE molecules contain two divalent metals that are separated by about 4 Å [37]. The first metal was identified as zinc ion by the anomalous diffraction experiment [47]. The zinc ion forms an octahedron with four PDE residues and two water molecules and has thus been assumed to play both structural and catalytic roles [48]. The second metal ion remains to be identified, in spite of that almost all structure refinements have used magnesium as the catalytic ion [37]. The second metal ion also forms six coordinations, one with an aspartic acid and five with bound water molecules [37]. Although magnesium has effectively catalyzed most PDE families [7–10], our study showed that manganese has k_{cat} about 1.5-fold better than that of magnesium for the catalysis of the PDE8A1 catalytic domain (Table 2), and the specific activity of manganese is about 4-fold to that of magnesium at concentration of 1 μM cAMP. Thus, our observation, together with the early report that manganese activated PDE9A twice as much as magnesium [49–51], suggest that PDE families may have different preferences for the divalent metal ions, yet to be further studied.

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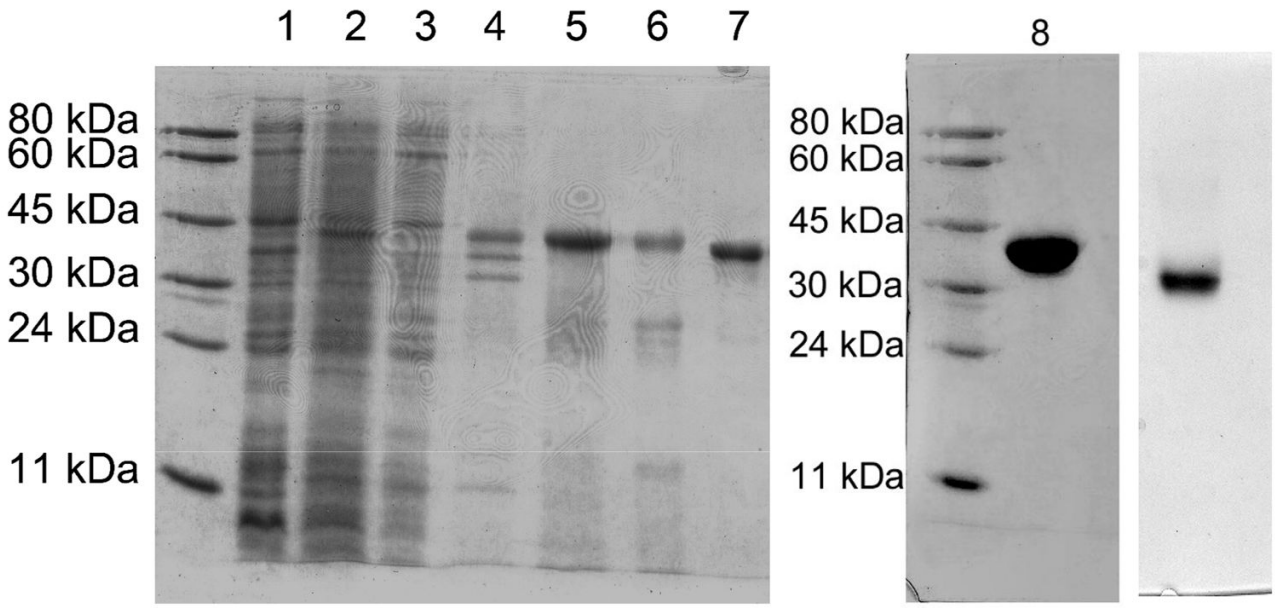


Fig. 1.

Acrylamide gels of the PDE8A1 catalytic domain. Left lane, molecular weight markers; lane 1, *E. coli* cell before induction; lane 2, total cell after induction; lane 3, supernatant; lane 4, pellet; lane 5, proteins from the Ni-NTA column; lane 6, HTP column; lane 7, Q-Sepharose column after thrombin cleavage of the 10 residue tag; lane 8, finally concentrated PDE8A1 from Sephacryl S300 column; right, the native gel of PDE8A1.

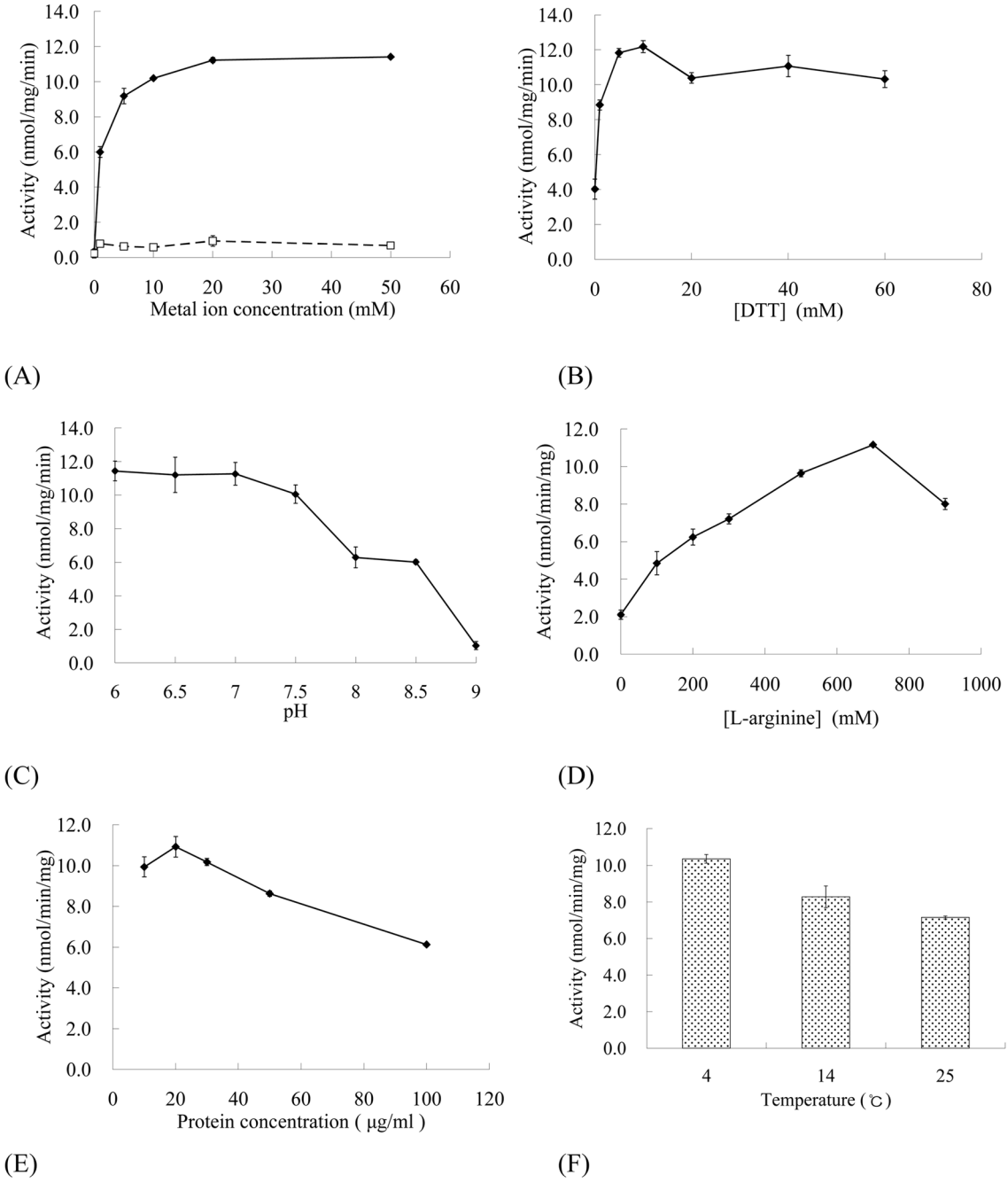


Fig. 2.

Refolding of the PDE8A1 catalytic domain. The following factors were individually optimized while other variables in the final buffer conditions were held constant. (A) Dependence of the catalytic activity on concentration of Mn^{2+} (◆) and Mg^{2+} (□), (B) effect of DTT concentration, (C) pH effect, (D) impact of L-arginine concentration, (E) effect of protein concentration, and (F) effect of temperature. The final buffer for the refolding was 0.5 M Tris-HCl, pH 7.0, 30% glycerol, 0.7 M arginine, 20 mM $MnCl_2$, 20 mM $MgCl_2$, 20μM $ZnSO_4$, 10 mM NaCl, 1 mM KCl, 10 mM DTT.

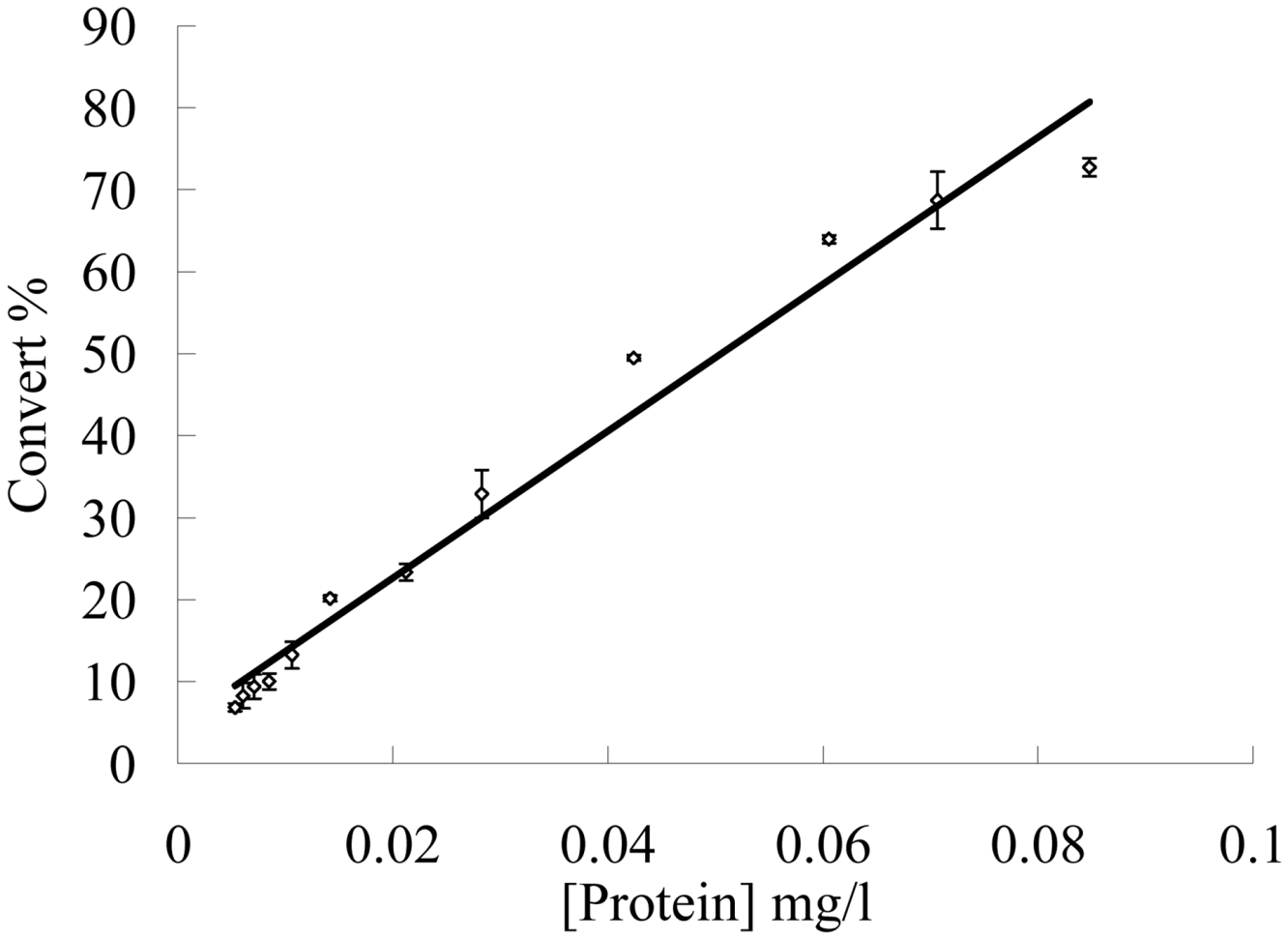
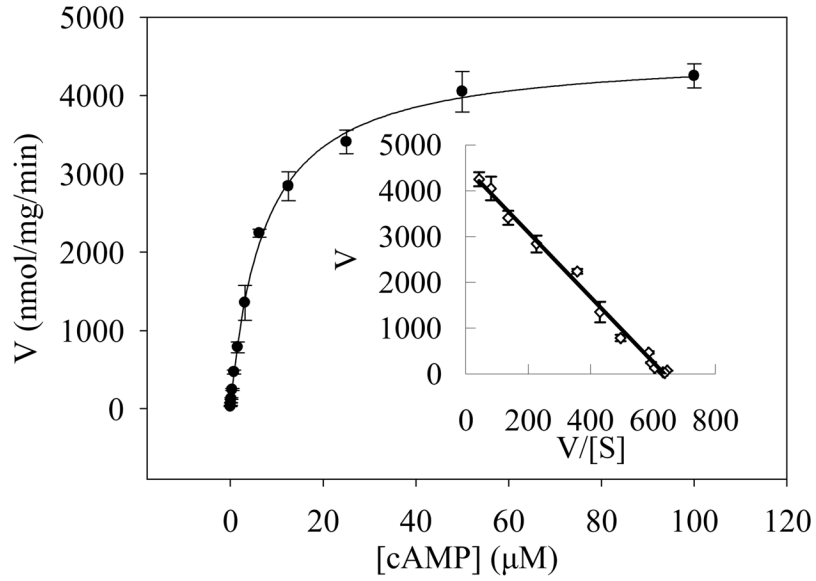
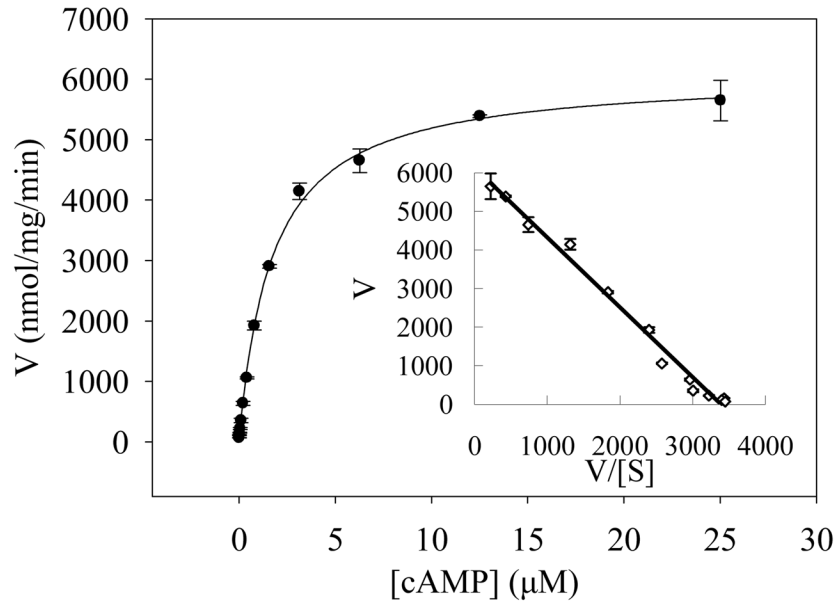


Fig. 3.

Relationship between the PDE8A1 concentration and cAMP conversion. The assays were carried out at a protein concentration range of 5.3 to 84.8 ng/ml and in a buffer of 20 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 1 mM DTT, ^{3}H-cAMP (20000 cpm/assay) at 24°C for 15 min.



(A)



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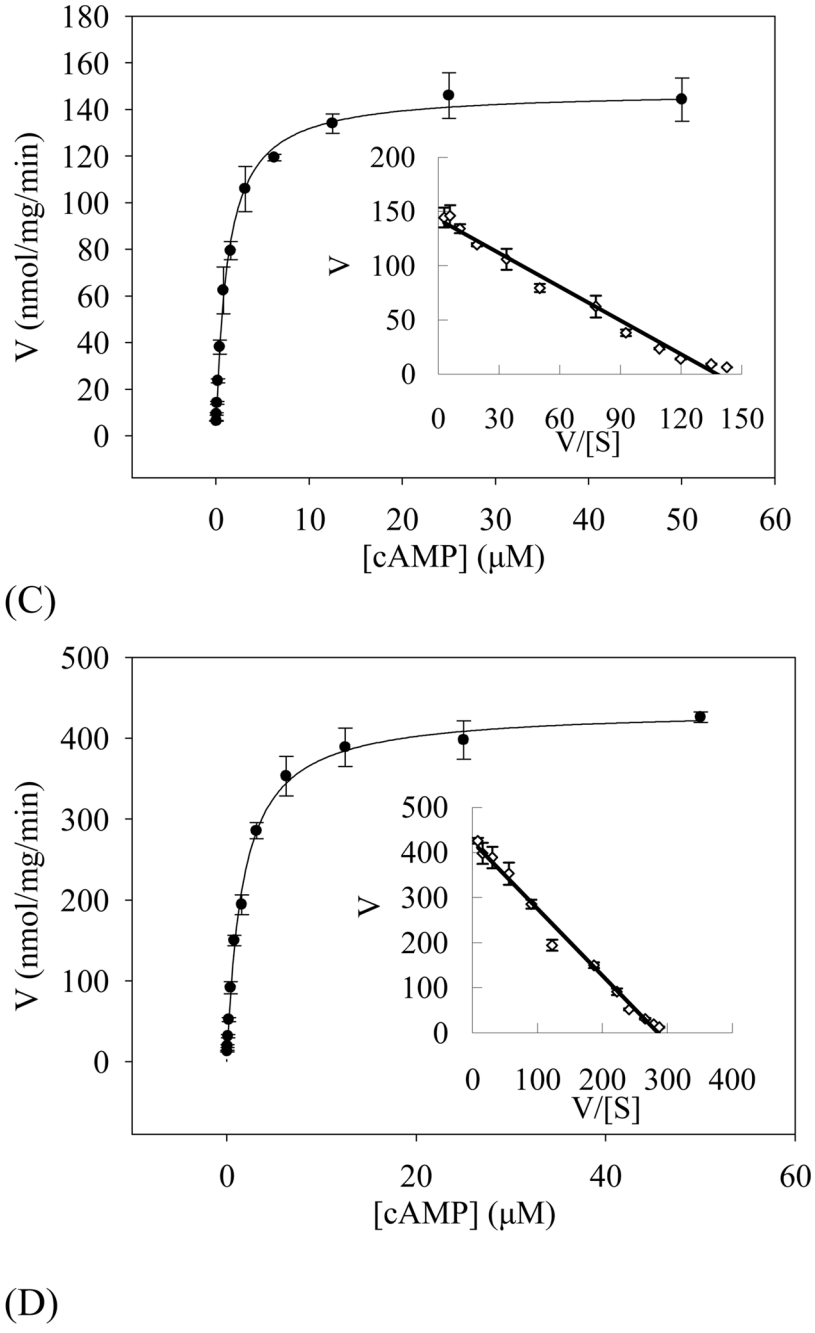

Fig. 4.

Velocity of the PDE8A1 catalysis versus substrate concentrations. The K_M and k_{cat} values were obtained by non-linear regression and Eadie-Hofstie plot (insert). (A) The catalysis of the refolded PDE8A1 by using 10 mM Mg^{2+} as the catalytic ion. (B) The catalysis of the refolded PDE8A1 by using 4 mM Mn^{2+} as the catalytic ion. (C) The catalysis of the supernatant fraction of PDE8A1 from the *E. coli* expression by using 10 mM Mg^{2+} as the catalytic ion. (D) The catalysis of the supernatant fraction of PDE8A1 from the *E. coli* expression by using 4 mM Mn^{2+} as the catalytic ion.

Table 1

PDE8A purification

Purification Steps	Total protein (mg)	Specific activity (nmol/min/mg)	Total activity (nmol/min)	Activity recovery (%)
Refolding*	50.0	5.6	280	100
hydroxyapatite HTP	35.0	6.0	208	74
Q-Sepharose	12.0	7.2	87	31
Sephacryl S300	7.6	11.4	87	31

*Since the PDE8A catalytic domain was expressed as pellet and the cell lysate had trace activity, the pellet from 10 grams of wet cells was dissolved in 6 M guanidine and purified by Ni-NTA affinity column. A total of 50 mg purified protein from Ni-NTA was refolded and the activity from the refolding was used as the starting unit.

Table 2

Table 2a. Enzymatic properties of the PDE8A1 catalytic domain for cAMP as substrate

PDE8A	Metal ion	K_M (μM)	k_{cat} (s^{-1})	Vmax (μmol/mg/min)	k_{cat}/K_M (s^{-1} μM^{-1})
8ACATref	10 mM Mg^{2+}	7.0 ± 0.1	2.9 ± 0.1	4.5 ± 0.1	0.4 ± 0.1
8ACATref	4 mM Mn^{2+}	1.8 ± 0.1	4.0 ± 0.1	6.1 ± 0.1	2.2 ± 0.1
8A205ref	4 mM Mn^{2+}	0.28± 0.01	1.1 ± 0.1	0.93 ± 0.04	3.9 ± 0.1
8ACATnat	10 mM Mg^{2+}	1.0 ± 0.1			
8ACATnat	4 mM Mn^{2+}	1.5 ± 0.2			

Table 2b. Enzymatic properties of the PDE8A1 catalytic domain for cGMP as substrate

PDE8A	Metal ion	K_M (mM)	k_{cat} (s^{-1})	Vmax (μmol/mg/min)	k_{cat}/K_M (s^{-1} μM^{-1})
8ACATref	10 mM Mg^{2+}	1.5 ± 0.2	0.4 ± 0.1	0.6 ± 0.1	$0.3 \pm 0.1 \times 10^{-3}$
8ACATref	4 mM Mn^{2+}	1.6 ± 0.1	1.6 ± 0.2	2.5 ± 0.3	$1.0 \pm 0.1 \times 10^{-3}$

8ACATref and 8ACATnat represent the catalytic domains of PDE8A (residues 480–820) respectively from the refolding and natural folding in *E. coli*.

8A205ref is the PDE8A fragment with residues 205–820 from the refolding.