Solution Structure of Atypical Protein Kinase C PB1 Domain and Its Mode of Interaction with ZIP/p62 and MEK5*

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Atypical protein kinase C (aPKC) has been implicated in several signaling pathways such as cell polarity, cell survival, and cell differentiation. In contrast to other PKCs, aPKC is unique in having the PB1 (Phox and Bem 1) domain in the N terminus. The aPKC PB1 domain binds with ZIP/p62, Par6, or MEK5 through a PB1-PB1 domain interaction that controls the localization of aPKC. Here, we determined the three-dimensional structure of the PB1 domain of PKCγ by NMR and found that the PB1 domain adopts a ubiquitin fold. The OPCA (OPR, PC, and AID) motif inserted into the ubiquitin fold was presented as a ββαβ fold in which the side chains of conserved Asp residues were oriented to the same direction to form an acidic surface. This structural feature suggested that the acidic surface of the PKCγ PB1 domain interacted with the basic surface of the target PB1 domains, and this was confirmed in the case of the PKCγ-ZIP/p62 complex by mutational analysis. Interestingly, in the PKCγ PB1 domain a conserved lysine residue was located on the side opposite to the OPCA motif-presenting surface, suggesting dual roles for the PKCγ PB1 domain in that it could interact with either the conserved lysine residue or the acidic residues on the OPCA motif of the target PB1 domains.

As part of the ongoing study of atypical protein kinase C (aPKC), a isoform PKCγ was first cloned in 1988 (1), followed by the cloning of isoform PKCα (2, 3). Now, aPKC has been suggested as playing a crucial role in signal transduction pathways such as cell polarity, cell differentiation, and cell survival (4, 5). In contrast to conventional PKC, aPKC contains only a single C1 domain but is devoid of a C2 domain in the N-terminal regulatory region. A tandem repeat of the C1 domain is known to be a target of diacylglycerol, and the C2 domain is a binding site for Ca2+. However, because of the lack of a C2 domain and the incomplete C1 domain in aPKC, neither Ca2+ nor diacylglycerol activates aPKC. Instead, aPKC contains a PB1 domain at the N terminus.

In PKC signaling, the regulation of both the catalytic activity and the spatial localization of PKC is essential. PKC is known to translocate to a specific region in response to activation signals and then phosphorylate specific target proteins. Such spatial localization of PKC in cells is partially regulated by either PKC-binding scaffold proteins or anchoring proteins (6). In particular, aPKC binds with PB1 domain-containing proteins such as ZIP/p62 (7–9), Par6 (10–13), or MEK5 (14) through the PB1-PB1 domain interaction. Thus, the PB1-mediated interaction between aPKC and the scaffolding or anchoring proteins plays a crucial role in exerting the biological functions of aPKC (4).

ZIP/p62 homologues were isolated in different biological contexts as ZIP, p62, and EBIAP (7, 15, 16). Recently, it has been demonstrated that ZIP/p62 is involved in the NFκB activation pathway in association with aPKC. ZIP/p62 is a scaffold protein of aPKC for the activation of NFκB downstream of extracellular signals such as tumor necrosis factor α, interleukin-1, and the nerve growth factor (17–19). Par6 has been identified as a product of par-6, one of the par genes, which is essential for asymmetric cell division in the early embryo of Caenorhabditis elegans (20). The aPKC-Par6 complex is conserved from nematodes to mammals as a cell polarity regulator (5, 21) and is involved in various cell polarity-related processes, such as asymmetric cell division (22, 23), directed cell migration (24, 25), axon specification (26), and tight junction formation (27–31), wherein the aPKC-Par6 complex shows a specific localization in each signaling process. A defect in PB1-PB1 interaction between aPKC and Par6 results in incomplete localization (32).

The PB1 domain is evolutionarily conserved from yeasts to humans, especially in the context of cell polarity-related proteins (9, 33) and is classified into two types, type I and type II (34). Type I contains a motif of 28 amino acid residues with highly conserved acidic and hydrophobic residues that is named a PC motif (35). This name is derived from the fact that the motif occurs in mammalian p40phox and budding yeast Cdc24p. The PC motif is also known as OPR (octicosapeptide repeat) (36) or AID (atypical PKC interaction domain) (4) and is generically named the OPCA (OPR, PC, and AID) motif (33). Type I utilizes acidic residues on the OPCA motif as an interaction site, whereas type II utilizes a conserved lysine residue on the side opposite to the OPCA motif-presenting surface as its interaction site, suggesting that the PB1-PB1 interaction is mainly electrostatic (9, 37) but also highly specific and exclusive (9). Moreover, some PB1 domains such as aPKC and ZIP/

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The atomic coordinates and structure factors (code 1VD2) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/)

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†The abbreviations used are: PKC, protein kinase C; aPKC, atypical PKC; GST, glutathione S-transferase; MBP, maltose-binding protein; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase kinase; NOE, nuclear Overhauser effect; OPCA, OPR, PC, and AID; PB1, Phox and Bem 1.

This paper is available on line at http://www.jbc.org
p62 contain the OPCA motif and the conserved lysine residue and are classified as type I and type II (Fig. 1) (34). The OPCA motif of aPKC and the conserved lysine residue of ZIP/p62 were reported to be responsible for aPKC-ZIP/p62 complex formation (38, 39). On the other hand, aPKC uses the conserved lysine residue for interaction with the OPCA motif of MEK5 (32), suggesting that the aPKC PB1 domain functions as type I and type II.

To elucidate the structural properties of the PB1 domain of aPKC and the interaction mode with its binding partners on the basis of the tertiary structure, we determined the solution structure of the PB1 domain of aPKC, PKC\_6, by NMR and discussed the biological implications of the PB1-PB1 interaction.

**EXPERIMENTAL PROCEDURES**

**Preparation of PKC\_6 (16–99)—Complementary DNA fragments encoding PKC\_6 and ZIP/p62 were prepared as described previously (9). Human PKC\_6 (16–99) was expressed in *Escherichia coli* strain BL21 (DE3) cells as a glutathione S-transferase (GST) fusion protein. PKC\_6 (16–99) was first purified by affinity chromatography using glutathione-Sepharose 4B resin (Amersham Biosciences). Following cleavage with PreScission protease (Amersham Biosciences), PKC\_6 (16–99) was purified by gel filtration on a Superdex75 prep grade column (Amersham Biosciences). Following cleavage with PreScission protease (Amersham Biosciences), PKC\_6 (16–99) was concentrated to 1 mM in 90% H\_2O/10% D\_2O or 100% D\_2O containing 50 mM phosphate (pH 7.0), 150 mM NaCl, and 5 mM dithiothreitol, and 0.05% (w/v) NaN\_3.

**Solution Structure of aPKC PB1 Domain**

To elucidate the structural properties of the PB1 domain of aPKC and the interaction mode with its binding partners on the basis of the tertiary struc-

**RESULTS AND DISCUSSION**

**Identification of the aPKC PB1 Domain Boundaries Required for Interaction with ZIP/p62**—Although a minimum sequence of the PB1 domain of PKC\_6 corresponding to 17–98 amino acids was carried out using the Olivia (objective, chemical shift library-based and stereo view assignment) software developed in our laboratory. The crystallography and NMR system software program (43) with the ARIA module (44) was used to assign NOE peaks and calculate structures. A total of 48 \( \psi \) and \( \phi \) values were estimated on the basis of the characteristic NOE patterns. The restraints used for structural calculations are summarized in Table 1. The structural calculations in combination with iterative NOE peak assignments were performed for nine cycles, and a total of 100 structures was finally obtained. The mean coordinates were obtained by averaging the coordinates of the 20 lowest energy structures. The coordinates of PB1 (16–99) have been deposited in the Protein Data Bank (accession code 1VD2).

The figures of the superimposed 20 structures were prepared using the MOLMOL program (46). The molecular surface model was generated by the GRASP program (47), and the ribbon model was generated using the PyMOL program (pymol.sourceforge.net).

In *Vitro* Pull-down Binding Assay—The constructs of various mutants of PKC\_6 (16–99) and rat ZIP/p62 (1–98) were prepared by PCR-mediated site-directed mutagenesis. The PCR products of PKC\_6 variants were ligated to pGEX6P (Amersham Biosciences), and those of ZIP/p62 variants were ligated to pMAL (New England Biolabs). All of the constructs were sequenced to confirm their identities. The proteins were expressed in *Escherichia coli* strain BL21 (DE3) cells and purified with glutathione-Sepharose 4B (Amersham Biosciences) resin for a GST fusion protein or with amylose resin (New England Biolabs) for a maltose binding protein (MBP) fusion protein, respectively. For *in vitro* pull-down binding assays, the purified PKC\_6 variants and ZIP/p62 variants were mixed in phosphate-buffered saline and incubated at 4 °C for 3 h. Subsequently, a slurry of glutathione-Sepharose 4B was added, followed by further incubation at 4 °C for 2 h. After washing three times with phosphate-buffered saline containing 0.1% Triton X-100, proteins were eluted with 20 mM glutathione in 50 mM Tris-HCl buffer (pH 8.0). The eluates were subjected to 10% SDS-PAGE and stained with Coomasie Brilliant Blue. We evaluated the binding affinity by densitometric analysis of the bands in the SDS-PAGE using NIH Image software as described previously (37).
is expected from the sequence alignment of the PB1 domain (Fig. 1), the binding assays for an aPKC-ZIP/p62 complex formation have been performed using a much longer construct of PKC\textsubscript{c}(1–107) \cite{7, 9, 32, 38, 39}. Interestingly, p40\textsubscript{phox} PB1 required more C-terminally extended residues out of the alignment in order to interact with p67\textsubscript{phox} PB1, whereas Cdc24p PB1 did not require such a residual region to bind with Bem1p PB1 \cite{35}. These results imply diversity and mutual specificity in the PB1-PB1 interaction. Thus, we expressed both constructs of PKC\textsubscript{c}(16–99) and PKC\textsubscript{c}(1–107) in E. coli strain BL21 (DE3) cells as GST fusion proteins. The results of an in vitro pull-down binding assay revealed that PKC\textsubscript{c}(16–99) bound with ZIP/p62 to an extent similar to that of PKC\textsubscript{c}(1–107). Thus, PKC\textsubscript{c}(16–99) was considered to be a functionally minimum region. This view was consistent with the experimental evidence that the degradation of PKC\textsubscript{c}(1–107) occurred soon after the removal of GST by treatment with protease (data not shown). Furthermore, the \textsuperscript{1}H,\textsuperscript{15}N heteronuclear single quantum correlation spectrum of PKC\textsubscript{c}(16–99) was well dispersed, suggesting that PKC\textsubscript{c}(16–99) corresponds to a structural domain (Fig. 2B). We concluded that any region of PKC\textsubscript{c} that extended out of the PB1 domain did not contribute to the interaction with ZIP/p62, and, thus, PKC\textsubscript{c}(16–99) is more suitable for structure determination than PKC\textsubscript{c}(1–107).

![Image](98x217 to 523x737)

**Fig. 2. Identification of aPKC domain boundary.** A, in vitro pull-down binding assay. MBP-ZIP PB1 was mixed with GST-PKC\textsubscript{c}(1–107) (lane a) or GST-PKC\textsubscript{c}(16–99) (lane b) and incubated with glutathione-Sepharose 4B resin. After washing with PBS, the eluates were subjected to 18% SDS-PAGE and stained by Coomassie Brilliant Blue. B, a two-dimensional \textsuperscript{1}H,\textsuperscript{15}N heteronuclear single quantum correlation spectrum of PKC\textsubscript{c} PB1. The cross-peaks are labeled with one-letter codes of amino acids and residue numbers (sc denotes side-chain resonances).
we started to determine the tertiary structure of PKC\textsubscript{H9259}\textsubscript{-(16–99)} (hereafter referred to as PKC\textsubscript{H9259} PB1) in solution by NMR. Recently, the NMR assignment of PKC\textsubscript{H9259}\textsubscript{-(1–113)} was reported (48). In that \textsuperscript{1}H\textsuperscript{-15}N heteronuclear single quantum correlation spectrum, the cross-peaks corresponding to the regions outside of PKC\textsubscript{H9259}\textsubscript{-(16–99)} were either absent or located at the random coil region, a finding that is consistent with our result that the PKC\textsubscript{H9259}\textsubscript{-(16–99)} is a structural domain.

Structure of the PKC\textsubscript{H9259} PB1 Domain—The assignments of \textsuperscript{1}H, \textsuperscript{15}N, and \textsuperscript{13}C NMR resonances were accomplished through the analyses of a suit of the NMR spectra described under “Experimental Procedures.” The structure of PKC\textsubscript{H9259} PB1 was determined on the basis of 1492 NOE-derived interproton distance restraints, 56 dihedral angle restraints, and 27 hydrogen bond restraints. A total of 100 structures was calculated, and the 20 lowest energy structures were evaluated. The residues derived from the PreScission protease cleavage site (N-terminal five residues, GPLGS) and the loop between C\textsubscript{\alpha} and C\textsubscript{\beta} were flexible because of a lack of long range NOEs. For residues 38–49, 74–84 and 92–98, the root mean square deviation from the mean structure was 0.41 Å for the backbone C\textsuperscript{\alpha}, C\textsuperscript{\beta}, and N atoms, indicating that the backbone of PKC\textsubscript{H9259} PB1 was well defined, as illustrated in Fig. 3A. The number of the experimental restraints and the structural statistics of PKC\textsubscript{H9259} PB1 are summarized in Table I.

The PKC\textsubscript{H9259} PB1 domain contains two \alpha-helices (\alpha1, residues 38–49; \alpha2, 74–84) and a mixed \beta-sheet that consists of five \beta-strands (\beta1, residues 17–22; \beta2, 27–32; \beta3, 58–62; \beta4, 68–70; \beta5, 92–97). The inner two strands, \beta1 and \beta5, are parallel, whereas \beta2, \beta3, and \beta4 run in an antiparallel manner. PKC\textsubscript{H9259} PB1 takes on a ubiquitin fold on which the OPCA motif is presented. The OPCA motif of PKC\textsubscript{H9259} (Leu-56–Leu-83) has a \beta/\beta'/\beta fold, and each of the side chains of Trp-61 and Pro-68 is located on the opposite side of the \beta-sheet, which forms a hydrophobic core. The \beta-hairpin between \beta3 and \beta4 forms a characteristic turn, referred to as a type I \beta\textsubscript{1}G1 \beta-bulge, so that the side chains of the two conserved Asp residues (Asp-63 and Asp-67) orient to the same side to form an acidic surface. Additional residues Glu-64, Glu-65, Glu-76, and Glu-79 in the OPCA motif also contribute to form this acidic surface, the characteristic interaction site of the type I PB1 domain. The tertiary structures of the PKC\textsubscript{H9259} and Cdc24p PB1 domains confirm that the OPCA motif has not only sequence similarity but also structural similarity, and, accordingly, PKC\textsubscript{H9259} PB1 appears to serve as a type I PB1 domain.

Mutational Analysis to Test for Interactions between aPKC and ZIP/p62—The present structural analysis suggests that
whereas the K20A mutant of PKC
Asp-63 of PKC in the type I PB1 domain was known to be an essential residue, down binding assay (Fig. 4). Because the conserved Asp residue in vitro Thus, we performed mutational analysis using an ability to bind with PKC the other hand, the K7A mutant of ZIP/p62 PB1 disrupted the PB1 served as a type I PB1 domain. On the type II PB1 domain. Recently Wilson et al. and Lamark et al. independently reported the results of a binding assay of aPKC-ZIP/p62 and their mutants that were similar to ours (38, 39). Both independently reported the results of a binding assay of aPKC-ZIP/II PB1 domain. The mobility of the K20A mutant of PKC-(16–99) was retarded as compared with that of the wild-type protein. The mutant protein would be expected to undergo a mobility shift in the opposite direction, because abnormal retardation of migration on SDS-PAGE is usually considered to be associated with a high proportion of basic amino acids (49). Although the reason for this discrepancy is presently unknown, it may be due to intramolecular hydrophobic interactions, which can sometimes affect the mobility of proteins in SDS-PAGE (49). Therefore, ZIP/p62 PB1 binds with PKC as a type

### Table I

| Structure calculation restraints and structural statistics |
|----------------------------------------------------------|
| Abbreviations used here that are not defined elsewhere are: r.m.s.d., root mean square deviation; vdw, van der Waals; cdih, constrained dihedral (energy). |
| Number of experimental restraints                           |
|----------------------------------------------------------|
| Total unambiguous distance restraints                      | 1315 |
| Intra-residual                                            | 654  |
| Sequential (| i - j | = 1)                                   | 201  |
| Medium range (| 2 ≤ | j - i | ≤ 4)                                 | 97   |
| Long range (| 5 ≤ | j - i |)                                          | 363  |
| Ambiguous distance restraints                              | 177  |
| Dihedral angle restraints                                  | φ, 48; ψ, 8 |
| Hydrogen bonds                                            | 27   |
| Structural statistics                                      |
|----------------------------------------------------------|
| R.m.s.d. from mean coordinate<sup>a</sup>                  | 0.41 |
| Backbone (N, Ca, C) (Å)                                   | 0.85 |
| Heavy atoms (Å)                                           | 0.85 |
| R.m.s.d. from experimental restraints                     | 0.006 ± 0 |
| NOE distances (Å)                                         | 0.161 ± 0.034 |
| Dihedral angles (*)                                       | 0.001 ± 0.003 |
| R.m.s.d. from idealized geometry                          |
| Bonds (Å)                                                | 0.001 ± 0.003 |
| Angles (*)                                                | 0.263 ± 0.003 |
| Improper (* )                                             | 1.22 ± 0.08 |
| Final energies                                            |
| E<sub>vanx</sub> (kcal/mol)                               | 68.34 ± 1.96 |
| E<sub>bonds</sub> (kcal/mol)                              | 1.30 ± 0.09 |
| E<sub>angle</sub> (kcal/mol)                              | 27.08 ± 0.54 |
| E<sub>improp</sub> (kcal/mol)                             | 1.71 ± 0.24 |
| E<sub>ster</sub> (kcal/mol)                               | 35.45 ± 1.48 |
| E<sub>apex</sub> (kcal/mol)                               | 2.72 ± 0.49 |
| E<sub>cdih</sub> (kcal/mol)                               | 0.09 ± 0.04 |
| Ramachandran analysis<sup>a,b</sup> (analyzed by PROCHECK-NMR) |
| Residues in most favored regions (%)                      | 79.8 |
| Residues in additionally allowed regions (%)              | 19.0 |
| Residues in generously allowed regions (%)                | 1.2 |
| Residues in disallowed regions (%)                        | 0.0 |

<sup>a</sup> Analysis is applied to the residues with the exception of the protease cleavage site (11–16), the flexible loops (85–91), and the C-terminal (99) region.

<sup>b</sup> Non-Gly/Pro residues for the mean coordinate. The program PROCHECK-NMR (52) was used to assess the quality of the structure.

FIG. 4. Mutational effect of PKC<sub>1</sub> PB1 and ZIP PB1. GST-PKC<sub>1</sub> PB1 (wild-type), GST-PKC<sub>1</sub> PB1 K20A, or GST-PKC<sub>1</sub> PB1 D63A was incubated with MBP-ZIP PB1. MBP-ZIP PB1 K7A or MBP-ZIP PB1 D67A was incubated with GST-PKC<sub>1</sub> PB1. Proteins pulled down with glutathione-Sepharose 4B resin were subjected to SDS-PAGE and stained with Coomassie Brilliant Blue.

FIG. 5. Interaction surface of PKC<sub>1</sub> PB1 as the type I PB1 domain. A, electrostatic surface potential of PKC<sub>1</sub> PB1. Red indicates negative charge, and blue indicates positive charge. One-letter amino acid codes are used with position numbers. B, effects of mutation of ZIP PB1. MBP-ZIP PB1 D67A, MBP-ZIP PB1 R21A/R22A, or MBP-ZIP PB1 R94A is incubated with GST-PKC<sub>1</sub> PB1. Proteins pulled down with glutathione-Sepharose 4B resin were subjected to SDS-PAGE and stained with Coomassie Brilliant Blue.

II PB1 domain. The electrostatic surface potential of PKC<sub>1</sub> PB1 as shown in Fig. 5A. The conserved acidic residues in the OPCA motif form two
acidic surfaces, wherein Asp-63, Glu-64, Glu-65, and Asp-67 form one acidic surface, and Glu-76 and Glu-79, located on the N-terminal region of α2, form the other. These two acidic surfaces are common features of the type I PB1 domain (34). Because the interaction surface of PKC\(_{β1}\) PB1 is highly acidic, the cognate binding region of ZIP/p62 PB1 should present a basic surface. Considering the alignment and topology of the ZIP/p62 PB1 domain, basic residues on α2 and α5 surround the conserved lysine residue, Lys-7, on β1, suggesting that such basic residues on β2 and β5 seem to assist the PB1-PB1 interaction. Thus, we prepared several mutants where the basic residues on α2 and α5 were replaced by alanine residues, namely the R94A mutant and the R21A/R22A double mutant of ZIP/p62 PB1. We evaluated the effects of these mutations on the formation of the PKC\(_{α}\)-ZIP/p62 complex. The R21A/R22A double mutant of ZIP/p62 PB1 showed an apparent decrease in binding affinity with PKC\(_{β1}\) PB1, suggesting that Arg-21 or Arg-22 or both are involved in the interaction between PKC\(_{β1}\) PB1 and ZIP/p62 PB1. The R94A mutant also showed an appreciably weaker interaction with PKC\(_{α}\) PB1, but the D67A mutation had no effect. These results, taken together, support the notion that ZIP/p62 PB1 serves as a type II PB1 domain and that the basic residues on β2 and β5 (Arg-21 and Arg-22 on β2 and Arg-94 on β5) as well as the Lys-7 of ZIP/p62 PB1 interact with the acidic residues on the OPCA motif of PKC\(_{β1}\). Interaction Surface of aPKC PB1 as a Type II PB1 Domain— aPKC PB1 is known to bind with MEK5 PB1, where aPKC PB1 accepts the OPCA motif in MEK5 PB1, thus serving as a type II PB1 domain in the aPKC-MEK5 complex (32). The structure of PKC\(_{β1}\) PB1 demonstrates that the conserved lysine residue (Lys-20) is exposed, which enables Lys-20 to interact with the OPCA motif of MEK5 PB1 (Fig. 6). The interaction surface of the type II PB1 domain presented on Bem1p PB1 (37) and p67\(^{phox}\) PB1 (38) is mainly composed of α1, Ile-27 and Ile-29 on β2, and acidic residues on α1 (Glu-44 and Asp-47). α1 in PKC\(_{β1}\) PB1 uniquely presents a negatively charged surface in contrast to those of Bem1p PB1 (37) and p67\(^{phox}\) PB1 (38), which present positively charged surfaces (Fig. 6). Thus, in the case of
PKCβ1, neither self-oligomerization using both the OPCA motif and Lys-20 occurs, nor is Lys-20 involved in an interaction with ZIP/p62 as the type II PB1 domain. In this respect, the negatively charged α1 of PKCβ may confer its specificity toward MEK5. Further structural study is required to elucidate this specific interaction.

**Structural Comparison among the PB1 Domains**—The type I and type II PB1 domains form a complex using different regions regardless of their high structural similarity. We compared the structure of the PKCβ1 PB1 domains with those of the Cdc24p and Bem1p PB1 domains and also with ubiquitin using the DALI search engine (50) (Fig. 7). PKCβ1 PB1 is highly similar to both Cdc24p PB1 (root mean square deviation 1.4 Å) and Bem1p PB1 (root mean square deviation 1.7 Å). All form a ubiquitin-like β-groasp fold. Despite the overall structural similarity among the PB1 domains, the structure of the OPCA motif comprising the β1α fold and the turn classified as the type I + G1 β-bulge is unique to the PKCβ1 and Cdc24p PB1 domains, which are classified as type I PB1 domains.

On the other hand, the opposite surface defined as the interacting portion of the type II PB1 domain is quite similar to the equivalent portion of the type I PB1 domain and is also similar to the equivalent region of ubiquitin. However, the lysine residue in β1 is absolutely conserved only in type II PB1 domains and is substituted with arginine in type I PB1 domains (Fig. 1). It should be noted that aPKC PB1 and ZIP/p62 PB1, containing both the OPCA motif and the conserved lysine residue, can be expected to serve as type I and type II PB1 domains.

**Specificity of PB1-PB1 Interaction and Its Biological Implication**—The electrostatic surface of the OPCA motif in Cdc24p presents two acidic surfaces split by the hydrophobic residues Val-826 and Val-836. Consistent with its surface, the expected interaction surface of Bem1p is composed of two basic surfaces on β1 and α1 divided by the hydrophobic residues of β2. Thus, the interaction surfaces between the type I and type II PB1 domains are complementary (34). Although the interaction between two acidic surfaces in the type I PB1 domain and the two basic surfaces in the type II PB1 domain is canonical, the PB1-PB1 interaction uses additional interaction surfaces. The recently determined structure of the p40\textsuperscript{hoss} PB1-p67\textsuperscript{hoss} PB1 complex revealed that the C-terminal extension out of the p40\textsuperscript{hoss} PB1 domain is involved in the interaction with p67\textsuperscript{hoss} PB1, where an extensive region that includes β5 of p67\textsuperscript{hoss} PB1 is used as a binding site (38) in addition to the canonical interaction. In the aPKC-ZIP/p62 PB1 complex, β5 of the ZIP/p62 PB1 domain probably interacts with the OPCA motif of aPKC, because the region extending out of the aPKC PB1 domain is not required for the interaction. This implies that the interaction between aPKC and ZIP/p62 may differ from that of p40\textsuperscript{hoss} and p67\textsuperscript{hoss}. Such diversity in interactions in the PB1-PB1 complex gives specificity to the PB1-PB1 interaction.

The identification of the type I and type II PB1 surfaces on the opposite sides of aPKC implies that aPKC may bind with both ZIP/p62 and MEK5 in a ternary complex. MEK5 and aPKC form a complex in an epidemical growth factor-dependent manner, and the formation of the complex is required to activate extracellular signal-regulated kinase 5 (ERK5) (14). ZIP/p62 is also reported to be involved in ERK5 activation downstream of TrkA in NGF (51). Thus, the biological relationship in vivo and the PB1-PB1 interaction in vitro suggest that ZIP/p62, and MEK5 form a ternary complex through the PB1-PB1 interaction and function cooperatively upstream of ERK5.

**Conclusion**—The structure of aPKC PB1 has the typical features of type I PB1 domains in that the conserved acidic residues in the OPCA motif form two conserved acidic surfaces. Moreover, mutational analysis confirmed that aPKC served as the type I PB1 domain and that ZIP/p62 served as the type II PB1 domain in the aPKC-ZIP/p62 PB1 complex formation. The acidic surface in the type I PB1 domain and the basic surface in the type II PB1 domain are utilized for the canonical interaction in the PB1-PB1 complex formation. However, additional sites are used for specific interactions. This gives diversity and specificity to the PB1-PB1 interaction.

The aPKC is known to serve as type II PB1 domain in the aPKC-MEK5 PB1 complex. The structure of aPKC PB1 shows that the conserved lysine residue in the type II PB1 domain is exposed to interact with the OPCA motif. Notably, α1 of aPKC presents an acidic surface in contrast to the basic surface in Bem1p PB1 or p67\textsuperscript{hoss} PB1 (Fig. 6). Such variation in the electrostatic surface potential may also contribute to the specificity of the PB1-PB1 interaction. In addition, the surface potential of aPKC eliminates the possibility of self-oligomerization of the aPKC PB1 domain.
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