Cooperative Formation of the Ligand-binding Site of the Inositol 1,4,5-Trisphosphate Receptor by Two Separable Domains*

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Limited trypsin digestion of mouse cerebellar membrane fractions leads to fragmentation of the type 1 inositol 1,4,5-trisphosphate receptor (IP3R1) into five major components (Yoshikawa, F., Iwasaki, H., Michikawa, T., Furuichi, T., and Mikoshita, K. (1999) J. Biol. Chem. 274, 316–327). Here we report that trypsin-fragmented mouse IP3R1 (mIP3R1) retains significant inositol 1,4,5-trisphosphate (IP3) binding activity that is comparable to the intact receptor in affinity, capacity, and specificity. This is despite the fact that the IP3-binding core (residues 226–578), which is close to the minimum for high affinity binding, is completely split into two tryptic fragments at the Arg-343 and/or Arg-345, around the center of the core. Furthermore, we have examined whether binding activity could be complemented in vitro by mixing two distinct glutathione S-transferase (GST) fusion proteins, which were respectively composed of residues 1–343 and 341–604, almost corresponding to two split binding components, and separately expressed in Escherichia coli. The GST-fused residues 1–343 (GN) showed no binding affinity for IP3, whereas the GST-fused residues 341–604 (GC) displayed weak but definite activity with an affinity >100-fold lower than that of the native receptor. Upon mixing of both GN and GC, a high affinity site comparable to the native site appeared. We suggest that the IP3-binding pocket consists of two non-covalently but tightly associated structural domains each of which has a discrete function: the C-terminal domain alone has low affinity for IP3, whereas the N-terminal one alone is incapable of binding but is capable of potentiating binding affinity.

Inositol 1,4,5-trisphosphate (IP3)1 is a second messenger that mediates Ca2+ release from intracellular stores by binding to the IP3 receptor (IP3R) which is a tetrameric IP3-gated Ca2+ release channel (1, 2). Cerebellum has an extraordinary density of specific IP3-binding sites (3) and purified cerebellar IP3R protein binds IP3 in a stoichiometric manner, namely one subunit for one IP3 (4–6). Ca2+ release experiments using various synthetic inositol phosphates have suggested that molecular recognition of IP3 is markedly stereospecific (7). Type 1 IP3R (IP3R1) is the neuronal type and predominates in cerebellar Purkinje cells (8–10). It is 2749 amino acids long (molecular mass about 313 kDa) and is structurally divided into three parts as follows: a large N-terminal cytoplasmic arm region (residues 1–2275), a putative six membrane-spanning region clustered near the C terminus (residues 2276–2589) which is thought to constitute an ion channel by forming a tetramer, and a short C-terminal cytoplasmic tail region (residues 2590–2749) (11). A series of deletion mutants showed that the IP3R1 binds IP3 within the N-terminal 650 amino acids, independently of tetramer formation (12, 13).

We previously demonstrated the structural basis for molecular recognition of IP3 by mouse IP3R1 (mIP3R1) (14). The minimum region for high affinity binding has been localized within the 353 residues, 226–578, so that it appears to be close to the binding “core.” Within the core region, we have identified 10 important basic amino acid residues all of which are well conserved in all IP3R family proteins cloned to date: three (Arg-265, Lys-508, and Arg-511) are critical and the other seven are required for specific binding. Nahorski and Potter (7) predicted that ionic interactions of positive charges on a binding site with negative charges on the three phosphate groups of IP3 would make major contributions to specific recognition and binding. Thus, we have proposed that the IP3-binding core forms a pocket with a positively charged inner surface lining of these basic residues which recognizes and binds a negatively charged IP3 ligand. Interestingly, all members of the IP3R family share extensive homology in the core sequence, except that the IP3R1 has an alternative splicing SI region (15 residues, 318–332) (9, 15), adjacent to which are 30 residues forming the longest stretch of characteristic diversity within the family. An internal deletion of residues 316–352 leads to loss of binding (13), although neither the presence nor the absence of the SI segment (mIP3R1SI+ nor mIP3R1SI− subtype, respectively) significantly affects the binding, suggesting that the diversified stretch is not a prerequisite for binding but that its boundary should be strictly defined.

Recently, we have shown that limited trypsin digestion of mouse cerebellar membrane fractions causes fragmentation of the mIP3R1 into five major trypsin-resistant polypeptides and that these five tryptic fragments I–V have tight structural-functional coupling because of the following: (i) co-sedimentation...
characterized the IP₃ binding properties of trypsin-fragmented soybean trypsin inhibitor and 0.1 mM PMSF. Tor. To analyze further the split IP₃ binding components, we have separately synthesized two glutathione S-transferase (GST) fusion proteins with these two components, and we have shown that high affinity binding site can be reconstituted in vitro by complementation with both distinct fusion proteins, each of which alone has no (N-terminal component) or low affinity (C-terminal component) binding. From these data, we propose that the functional structure of the IP₃-binding pocket consists of two well folded structural domains that are non-covalently but tightly associated and a diverse loop-like structure between these two domains.

EXPERIMENTAL PROCEDURES

Materials—N-Tosyl-l-phenylalanyl chloromethyl ketone-treated bovine pancreas trypsin, soybean trypsin inhibitor, and γ-globulin were purchased from Sigma; [³²P]IP₃ was from NEN Life Science Products; d-myo-inositol 1,4,5-trisphosphate was from Daido; d-myo-inositol 2,4,5-trisphosphate and d-myo-inositol 1,3,4,5-tetrakisphosphate were from Calbiochem; polyethylene glycol (PEG) 6000 was from Wako Pure Chemical; Solvable and Atomlight were from Packard; reduced glutathione and mercaptoethanol were from Bio-Rad; reduced glutathione-S-transferase (GST) fusion proteins were from PBL Bioresearch; Rapid Blue Protein Assay kit was from Kabi; and monoclonal antibodies 10A6 and 18A10 have been described (18).

Preparation of Membrane Fractions from Mouse Cerebellum—ddY mice (8–10 weeks old; Nippon SLC, Japan) were anesthetized and then decapitated, and cerebella were quickly dissected. The cerebella were homogenized by 10 strokes (850 rpm) in an ice-chilled glass Teflon Potter homogenizer containing 9 volumes of 0.32 M sucrose, 1 mM EDTA, 100 μM phenylmethylsulfonyl fluoride (PMSF), 10 μM pepstatin A, 10 μM leupeptin, and 5 mM Tris-HCl, pH 7.4. The homogenate was centrifuged at 1,000 × g for 15 min at 4 °C. The supernatant was re-centrifuged under the same conditions to completely remove P₁ fraction. The second supernatant was centrifuged at 105,000 × g for 60 min at 2 °C. The precipitate (crude microsome) was resuspended in phosphate-buffered saline and disrupted by sonication on ice. After centrifugation at 30,000 × g for 60 min at 2 °C, the supernatants were collected and subjected to a GST purification step through glutathione-Sepharose 4B column chromatography according to the manufacturer’s protocol. The purified GST fusion proteins were subjected to SDS-PAGE analysis and IP₃ binding assay.

RESULTS

Characterization of IP₃ Binding Activity of the Trypsin-fragmented mIP₃R₁—We previously showed that limited trypsin digestion of mouse cerebellar membrane fractions fragmented the mIP₃R₁ into five major polypeptides (fragments I–V), in which the IP₃-binding region was split into two fragments I and II (18). Intriguingly, these completely fragmented mIP₃R₁ still retained strong activity for IP₃-induced Ca²⁺ release comparable with that of the intact receptor, suggesting that even the split IP₃-binding site was sufficient to couple with gating the IP₃ receptor. In this study, we described the structure-function relationships among these components by complementation with both distinct fusion proteins, each of which alone has no (N-terminal component) or low affinity (C-terminal component) binding. From these data, we propose that the functional structure of the IP₃-binding pocket consists of two well folded structural domains that are non-covalently but tightly associated and a diverse loop-like structure between these two domains.
FIG. 1. Effects of trypsinization on IP₃ binding of the mIP₃R₁. Mouse cerebellar microsomal fractions (1.5 mg of protein) were subjected to Western blotting and IP3 binding assay. The Triton-treated mixtures were centrifuged at 20,000 g for 60 min at 2 °C to collect the insoluble membrane fractions. The pellet was resuspended in 0.5 ml of the binding buffer, solubilized by the addition of 10% (w/v) Triton X-100 to give a final detergent concentration of 1%, and rotated for 30 min at 4 °C. The Triton-treated mixtures were centrifuged at 105,000 g for 60 min at 2 °C to obtain the supernatants (Triton extracts). We confirmed by Western blotting that almost all of the tryptic fragments and the intact receptor were collected in these extracts (data not shown). The Triton X-100 extracts (2.5 mg of protein) were digested with 5 μg/ml trypsin for 4 min at 35 °C in the presence (Trypsin +) and the absence (Trypsin −) of 50 μg/ml trypsin inhibitor and 0.1 mM PMSF. The digested samples were centrifuged at 105,000 × g for 60 min at 2 °C to collect the insoluble membrane fractions. The pellet was resuspended in 0.5 ml of the binding buffer, solubilized by the addition of 10% (w/v) Triton X-100 to give a final detergent concentration of 1%, and rotated for 30 min at 4 °C. The Triton-treated mixtures were centrifuged at 20,000 × g for 60 min at 2 °C to obtain the supernatants (Triton extracts). We confirmed by Western blotting that almost all of the tryptic fragments and the intact receptor were collected in these extracts (data not shown). The Triton extracts were subjected to Western blotting and IP3 binding assay. A, the Triton X-100 extracts (2.5 mg of protein) were separated by 8% SDS-PAGE and probed with N1, N3, 10A6, anti-(1718–31), 1ML1 and 18A10 antibodies which specifically recognize the Ia/b, II, III, IVa/b and V major tryptic fragments, respectively. Intact IP3R and major tryptic fragments are indicated by an arrowhead and arrows, respectively. B, specific [3H]IP3 binding to the Triton extracts (45 μg of protein) was measured in the presence of 2 μM cold IP3. Values are the mean ± S.D. of four experiments.

FIG. 2. Binding characteristics of the fragmented mIP₃R₁ with limited trypsin digestion. All samples used (Trypsin –) and Trypsin + were the same as those in Fig. 1. A, competition of specific [3H]IP3 (9.6 nm) binding to the Triton extracts (45 μg of protein) by various inositol phosphates: (1,4,5)IP3 (filled circles), (2,4,5)IP3 (open triangles), and (1,3,4,5)IP4 (filled squares). Values are normalized to 100% of controls measured without competitor. Nonspecific binding was measured in the presence of 2 μM IP3. Each point is the mean of two experiments. B, Scatchard plots of inhibition of specific [3H]IP3 (4.8 nm) binding to the Triton extracts (45 μg of protein) by cold (1,4,5)IP3. Nonspecific binding was measured in the presence of 10 μM IP3. The results of a typical experiment are shown. Kᵦ and Bmax of each sample shown are the means of two experiments.

Results in complete fragmentation of the mIP₃R₁ into five tryptic fragments that were detectable with the site-specific antibodies (Trypsin +), but when pre-mixed with 50 μg/ml trypsin inhibitor and 0.1 mM PMSF prior to digestion, no apparent mobility change was observed in the intact mIP₃R₁ band (Trypsin −). The IVa/b fragments were only detected at very low levels, since the epitope for anti-(1718–31) located in the alternative splicing SI region (40 residues 1692–1731) of these fragments is most labile to trypsinolysis (18). The size difference between the Ia and Ib fragments has been thought to be due to alternative splicing at the SI region (15 residues 318–332), the former derived from the SI+ subtype and the latter from the SI − subtype (15, 18).

By using the Triton extracts containing these tryptic fragments, the following [3H]IP3-binding experiments were carried out. Although the IP₃-binding core was completely separated into two fragments I and II, which were recognized by the N1 and N3 antibodies, respectively (Fig. 1A), the fragmented mIP₃R₁ (Trypsin +) still retained specific IP₃ binding activity equivalent to that of the intact receptor (Trypsin −) (Fig. 1B). In [3H]IP₃ binding competition experiments with various inositol phosphates, the fragmented mIP₃R₁ (Trypsin +) showed comparable ligand binding specificity to the intact receptor (Trypsin −).
Complementation of Split IP₃ Receptor-binding Site

FIG. 3. Temporal profiles of IP₃ binding activity of fragments I and II in soluble and insoluble membrane fractions upon prolonged trypsin digestion. A, mouse cerebellar microsomal fractions (1 mg/ml protein) were incubated with 10 µg/ml trypsin for 0, 5, 10, 20, and 40 min at 35 °C in trypsinizing buffer (closed). The reactions were quenched with 100 µg/ml trypsin inhibitor and 0.1 mM PMSF. As a control (open symbols), microsomal fraction was supplemented with trypsin inhibitor and PMSF followed by the addition of trypsin and then incubated for 40 min at 35 °C. The insoluble and soluble fractions were separated by centrifugation at 105,000 × g for 60 min at 4 °C. The pellet was then solubilized in 1% Triton X-100 solution in the same way as described in Fig. 1. Equivalent volumes of soluble fractions (circles) and Triton extract of insoluble fraction (squares) were subjected to the [³H]IP₃ binding assay. Nonspecific binding was measured in the presence of 2 µM IP₃. B, equivalent volumes of soluble fraction (sup) and Triton extract of insoluble fraction (ppt) prepared as described above were subjected to immunoblotting with the N1 and N3 antibody. An asterisk shows a nonspecific band.

extended to beyond 20 min, no more marked changes were observed. These temporal profiles appear to parallel the immunoblotting patterns (Fig. 3B); as the digestion time was extended to 10 min, the levels of the Ia/b and II fragments, containing the epitopes for the N1 and N3, respectively, were decreased in the insoluble fraction but increased in the soluble fraction, and thereafter (at 20 and 40 min) no apparent change was seen in both fractions. However, more extensive digestion caused loss of binding activity and of these immunoreactive fragments (data not shown). These data indicated that the two tryptic Ia/b and II fragments were tightly associated with the tryptic mIP₃R₁-membrane complex and that some portions released by the prolonged digestions appeared to retain conformation for specific binding by interaction between fragments.

Reconstitution of IP₃ Binding Activity by Complementation between Two Recombinant Binding Components—To examine the functional interaction between the two tryptic fragments I and II containing the split IP₃-binding core, we tried to express recombinant proteins corresponding to these fragments in an E. coli system. However, it was difficult to obtain sufficient amounts of soluble proteins, especially for the fragment II (data not shown). To solve this, we constructed GST fusion proteins as shown in Fig. 4. The GN was composed of GST fused to the N-terminal residues 1–343, corresponding to almost the entire N terminus. We previously showed that Arg-265 within the IP₃-binding core is one of three basic amino acid residues for which Gln substitutions (R265Q) caused a complete loss of binding activity, even though both have a large extra GST moiety at the N terminus. The GC consisted of a GST fusion protein with residues 341–604, corresponding to almost all of the functional interaction between the two tryptic fragments I and II (residues 226–578; solid line) and the alternative splicing SI segment (residues 318–332; open box) are indicated. Boxes represent GST fusion proteins. All constructs were made in pGEX-2T; GN, GST + residues 1–343; GN(R265Q), R265Q mutant of GN; GC, GST + residues 341–604; GST, pGEX-2T vector alone as a control.

and the GC complemented each other to retrieve IP₃ binding activity, even though both have a large extra GST moiety at the N terminus.

To characterize further this structural and functional reconstitution of an IP₃-binding site by mixing two separately synthesized GN and GC, we carried out Scatchard analysis as shown in Fig. 6. The GN alone exhibited no binding activity as the control GST. On the other hand, the GC alone could form a very low affinity site with a Kₐ value of 4.7 µM. It was of particular interest that the mixture of GN + GC displayed a plot fitted by two binding sites; the low affinity site appeared to be consistent with that of the GC alone, and the high affinity site had a Kₐ value of 11 nM, about 400-fold lower than that of the low affinity site and comparable to that of the intact mIP₃R₁. Judging from the Bₘₐₓ value of 40 pmol/mg protein, it was estimated that about 2% of the total GC (about 1800 pmol/mg of protein) was involved in reconstitution of this high affinity site by complementation with the GN.

DISCUSSION

Mild trypsin digestion of a cerebellar membrane fraction generates five major tryptic fragments, including the N-termi-
nal two fragments Ia/b (40/37 kDa) and II (64 kDa), that share the entire IP₃-binding site and are tightly associated with the insoluble membrane fraction (18). In the present study, we have shown that the trypsinized IP₃-binding site of mIP₃R1 retains significant affinity, specificity, and binding capacity for IP₃, comparable to those of the intact one. These results indicate that the trypsinized IP₃-binding site may retain functional tertiary structure. Prolonged trypsinization caused concomitant loss of IP₃ binding activity and release of fragments I and II from the insoluble mIP₃R1-membrane complex to the soluble fractions. These results demonstrate that the folded conformation of the IP₃-binding site is stably retained in either the insoluble or soluble form by a possible inter-fragment interaction. Joseph et al. (17) previously reported that prolonged trypsin digestions of rat cerebellar microsomes caused release of a 68-kDa fragment of IP₃R with concomitant appearance of higher IP₃ binding activity in the soluble fraction, whereas with mild digestion the 68-kDa fragment was retained in the membrane-bound insoluble form with lower activity. In the present study, however, we have shown that the affinity of the insoluble fraction from the mildly trypsinized samples is slightly higher than that of un-trypsinized samples. We consider that the 68-kDa fragment is likely to correspond to the 64-kDa fragment II identified in the present study and that the binding activity observed by Joseph et al. (17) could be attributed to a native-like interaction between fragments I and II which are simultaneously released, although Joseph et al. (17) did not note fragment I in both the soluble and insoluble fractions. This discrepancy between our results and the previous study (17) may be due to differences in preparation of membrane fractions, trypsin digestions, and/or binding assays (filtration versus PEG precipitation).

Neither the SI plus (mIP₃R1SI⁺) nor minus (mIP₃R1SI⁻) subtype showed any significant alteration in IP₃ binding activity, whereas deletions of any other region within the IP₃-binding core tested so far completely abolished the activity (12–14), suggesting that the SI region is the only redundant part of the binding core. Arg-343 and Arg-345, which lie between fragments I and II and are close to the alternative splicing SI region (residues 318–332), are very susceptible to

![Fig. 5. Complementation of the IP₃ binding activity by two separately expressed GST-fused IP₃-binding domains. A, SDS-PAGE analysis of recombinant GST-fused IP₃-binding domains. Two μg of GST fusion proteins, GN, GN(R265Q), and GC, and GST (see Fig. 4) were separated by 10% SDS-PAGE and stained with Coomassie Brilliant Blue R-250. Sizes of molecular makers in kDa are shown in the right. B, specific [³H]IP₃ (9.6 nM) binding to the GST fusion proteins. IP₃ binding of GST fusion proteins (7 μg) were individually analyzed: GST, GN, GN(R265Q), and GC. Two GST fusion proteins were mixed (7 + 7 μg), and then the IP₃ binding activity of the mixtures was analyzed: GN + GC, GN(R265Q) + GC, GST + GC, GN + GST, and GN(R265Q) + GST. Nonspecific binding was measured in the presence of 2 μM IP₃. Values are the mean ± S.D. of three experiments.](image)

![Fig. 6. Scatchard analysis of the specific IP₃ binding to the GST fusion proteins. Scatchard plots of inhibition of specific [³H]IP₃ (9.6 nM) binding to the GST fusion proteins by cold (1,4,5)IP₃. Each protein (7 μg) was analyzed individually: GC, GN, and GST. The mixture of GN (7 μg) and GC (7 μg) was analyzed: GN + GC. In the plot of GN + GC, the quantity of protein is tentatively expressed as that of the GC regardless of the GN, since the GN alone has no significant activity. Nonspecific binding was measured in the presence of 200 μM IP₃. An inset in the GN + GC plot shows an enlargement of a plot for a high affinity site.](image)
Complementation of Split IP$_3$ Receptor-binding Site

![Fig. 7. Schematic model of the domain structure of the IP$_3$-binding site. Trypsin-resistant fragments I and II of the mIP$_3$R1 are designated as the binding domains I (residues 1 or N-terminal to 317) and II (residues 346 to 923). The N terminus of domain I in the native mIP$_3$R1 could not be determined by amino acid sequencing (18). Around the region including the SI segment (residues 318 to 346), Arg-265, Lys-508, and Arg-511 are critical residues for the specific IP$_3$ binding, all of which are well conserved within IP$_3$R family.](image)

limited trypsinolysis. Within the IP$_3$-binding core of all members of the IP$_3$R family, the most divergent sequences are found around the SI region. Thus, we suggest that in the vicinity of the SI region, Arg-343 and Arg-345 may form a flexible loop between two domains, exposed to the outside, thereby being hypersensitive to limited trypsinolysis. The domain II consists of at least two subdomains of the N-terminal 38 kDa (probably residues 346 to 604) and the C-terminal 38 kDa (probably residues 605 to 923), which are cleaved off with a more extensive trypsinolysis (18). The IP$_3$-binding core has been experimentally determined to reside within residues 226 to 578, and therefore there are at least two putative core domains, I (residues at least 226 to 317) and II (residues 346 to 604). The inner surface of the core domains may be lined with 10 basic amino acid residues for which Gln substitutions caused significant reduction of the binding activity (14). Of them, Arg-265, Lys-508, and Arg-511 are critical residues for the specific IP$_3$ binding, because their single amino acid substitutions to either Gln or Ala caused a loss of the activity (14). The functional roles for the ligand binding of the domain I, core domains I and II, and the region between residues 1 and ~225 are indicated in italics as described under “Discussion.”

The present study provides evidence for the modular construction of the IP$_3$-binding region with discrete functional domains. We suggest that the low affinity core domain II is a prototype of the IP$_3$-binding structure (Fig. 7). The binding domain I, although it has no binding capacity by itself, could contribute to form a high affinity site cooperatively with the core domain II. Thus, the domain I seems to act as a “modulator” to potentiate the affinity of the IP$_3$-binding prototype, core domain II. The functional property of this N-terminal binding domain I, however, is a little complicated as described previously (14); a short deletion of the N-terminal 31 amino acids from the N-terminal 734 amino acid region (T734) resulted in a significant reduction in the binding activity, although the resultant mutant included the entire IP$_3$-binding core sequence. Such contradictory mutational effects were also found in serial N-terminal deletions up to residue 215. However, the authentic binding activity was markedly recovered, when deleted up to the first N-terminal 220, 223, or 225 amino acids, thereby indicating that the N-terminal boundary of IP$_3$-binding core is at most residue 226. Notably, the mutant lacking the first N-terminal 223 amino acids showed more than 10-fold higher affinity for IP$_3$ than that of the parental T734. Furthermore, a substitution of GST (26 kDa) for the first 223 amino acids of the N-terminal 604 amino acids (T604) significantly increased the affinity, as compared with the parental T604. These lines of peculiar evidence led us to hypothesize that the N-terminal first ~225 amino acids are not directly responsible for the binding but are somewhat related to its suppression. Then, as shown in Fig. 7 we hypothesize that there are at least three functional modules, two modulators in the domain I (N-terminal suppressor and C-terminal enhancer (core domain I)) and one proto-IP$_3$-binding site in the core domain II. Co-operative regulation among these modules for ligand binding may influence the channel gating.

Mignery et al. (12) reported that the N-terminal cytoplasmic region of rat IP$_3$R1 expressed in COS cells displayed an altered mobility (apparent decrease in mass of ~50 kDa) on gel chromatography in the presence of IP$_3$ and suggested that any conformational change induced upon binding to IP$_3$ might be involved in coupling the ligand binding to the channel gating. We suppose that relative movement of the core domains I and II non-covalently associated with each other would occur upon ligand binding as described below. We previously demonstrated the significance of the 10 basic amino acid residues (Arg or Lys) in specific IP$_3$ binding, all of which are well conserved within the IP$_3$R family (14). Of these, Arg-265, Lys-508, and Arg-511 are critical. We thus suggested that these 10 basic residues, especially Arg-265, Lys-508, and Arg-511, contribute to form a positively charged pocket for binding to the negative charges on the three phosphate groups of IP$_3$. Four of them (Arg-241, Lys-249, Arg-265, and Arg-269) are positioned in the core domain I and the other six (Arg-504, Arg-506, Lys-508, Arg-511, Arg-568, and Lys-569) in the core domain II (Fig. 7). Thus, the IP$_3$-binding pocket constituting two core domains may be relatively expanded due to repulsion among the positive charges on the inner surface in the nonligand-bound state (open) and become narrow due to neutralization of the positive charges by interactions with the negative charges on IP$_3$ in the ligand-bound state (closed). Finally, studies on the higher order struc-
tures of the IP$_3$-binding site and the possible relative movement upon IP$_3$ binding will shed light on the molecular basis of the gating of the mIP$_3$R1 channel as well as the ligand binding.

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**REFERENCES**

1. Berridge, M. J. (1993) *Nature* **361**, 315–325
2. Furuichi, T., and Mikoshiba, K. (1995) *J. Neurochem.* **64**, 953–960
3. Worley, P. F., Baraban, J. M., Colvin, J. S., and Snyder, S. H. (1987) *Nature* **325**, 159–161
4. Supattapone, S., Worley, P. F., Baraban, J. M., and Snyder, S. H. (1988) *J. Biol. Chem.* **263**, 1530–1534
5. Maeda, N., Ninobe, M., and Mikoshiba, K. (1990) *EMBO J.* **9**, 61–67
6. Maeda, N., Kawasaki, T., Nakade, S., Yokota, N., Taguchi T., Kasai, M., and Mikoshiba, K. (1991) *J. Biol. Chem.* **266**, 1109–1116
7. Nahorski, R., and Potter, B. V. L. (1989) *Trends Pharmacol. Sci.* **10**, 139–144
8. Furuichi, T., Yoshikawa, S., Miyawaki, A., Wada, K., Maeda, N., and Mikoshiba, K. (1989) *Nature* **342**, 32–38
9. Mignery, G. A., Newton, C. L., Archer, B. T., III, and Sudhof, T. C. (1990) *J. Biol. Chem.* **265**, 12679–12685
10. Yamada, N., Makino, Y., Clark, R. A., Pearson, D. W., Mattei, M.-G., Gue ´net, J.-L., Ohama, E., Fujino, I., Miyawaki, A., Furuichi, T., and Mikoshiba, K. (1994) *Biochem. J.* **302**, 781–790
11. Michikawa, T., Hamanaka, H., Otsu, H., Yamamoto, A., Miyawaki, A., Furuichi, T., Tashiro, Y., and Mikoshiba, K. (1994) *J. Biol. Chem.* **269**, 9184–9189
12. Mignery, G. A., and Sudhof, T. C. (1990) *EMBO J.* **9**, 3893–3898
13. Miyawaki, A., Furuichi, T., Ryu, Y., Yoshikawa, S., Nakagawa, T., Saiteh, T., and Mikoshiba, K. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88**, 4911–4915
14. Yoshikawa, F., Morita, M., Monkawa, T., Michikawa, T., Furuichi, T., and Mikoshiba, K. (1996) *J. Biol. Chem.* **271**, 18277–18284
15. Nakagawa, T., Okano, H., Furuichi, T., Aruga, J., and Mikoshiba, K. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88**, 6244–6248
16. Ehlers, M. D., Zhang, S., Bernhardt, J. P., and Huganir, R. L. (1996) *Cell* **84**, 745–755
17. Joseph, S. K., Pierson, S., and Samanta, S. (1995) *Biochem. J.* **307**, 859–865
18. Yoshikawa, F., Iwasaki, H., Michikawa, T., Furuichi, T., and Mikoshiba, K. (1999) *J. Biol. Chem.* **274**, 316–327