Conformational changes in plant Ins(1,4,5)P₃ receptor on interaction with different myo-inositol trisphosphates and its effect on Ca²⁺ release from microsomal fraction and liposomes

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The interaction of the only reported plant inositol trisphosphate receptor with different myo-inositol trisphosphates (InsP₃ species), namely Ins(1,4,5)P₃, Ins(1,3,4)P₃, Ins(1,5,6)P₃ and Ins(2,4,5)P₃, were studied to assess the extent of Ca²⁺ mobilization from microsomes/vacuoles as well as liposomes in vitro. Ins(1,4,5)P₃ and Ins(2,4,5)P₃ bind with the receptor with comparable affinities, as evidenced from their dissociation constants (Kᵣ approx. 100 nM at 5 °C), whereas the interaction between Ins(1,3,4)P₃/Ins(1,5,6)P₃ and the receptor was not detected even with these ligands at 5 μM. Ins(1,3,4)P₃/Ins(1,5,6)P₃ isomers also do not elicit Ca²⁺ release from liposomes or microsomes/vacuoles. The ability of any InsP₃ to bind the receptor for Ins(1,4,5)P₃ is a prime requirement for Ca²⁺ release. However, the comparison of binding affinities of different isomers in their abilities of Ca²⁺ mobilization released by Ins(1,4,5)P₃ as estimated over a period of 20 s is 3500±200 nM/mg of protein and is about 4-fold higher than that by Ins(2,4,5)P₃ under identical conditions. To understand the role of the receptor conformation in Ca²⁺ release by different isomers, we have probed the conformational change of the receptor when the different isomers bind to it. Accessibility of the tryptophan residues in the free and Ins(1,4,5)P₃/Ins(2,4,5)P₃-bound receptor was monitored by a neutral fluorescence quencher, acrylamide. The resulting Stern–Volmer-type quenching plots of the internal fluorescence indicate a change in the conformation of the receptor on binding to Ins(1,4,5)P₃ and Ins(2,4,5)P₃. It is also detected when far-UV CD spectra (205–250 nm) of the free and ligand [Ins(1,4,5)P₃/Ins(2,4,5)P₃]-bound receptor are compared. The results from CD spectroscopic studies further indicate that the conformational changes induced by the two isomers are different in nature. When thermodynamic parameters, such as enthalpy (ΔH), entropy (ΔS) and free energy (ΔG), for the formation of the two InsP₃–receptor complexes are compared, a major difference is noted in the extent of changes in enthalpy and entropy is noted. All these findings taken together support the proposition that it is the overall interaction leading to the requisite conformational change in the receptor that determines the potency of the InsP₃ isomers in their abilities of Ca²⁺ mobilization from the intracellular stores or reconstituted liposomes.

INTRODUCTION

Considerable progress has been made in understanding the mechanism by which PtdIns(4,5)P₂ produces second-messenger molecules [1]. It has also been established that one of the key events in intracellular signalling is the receptor-mediated hydrolysis of PtdIns(4,5)P₂ by phospholipase C, generating Ins(1,4,5)P₃. It then binds to its receptor (InsP₃R), an Ins(1,4,5)P₃-gated Ca²⁺ channel located at the intracellular Ca²⁺ stores. As a result, Ca²⁺ is released into the cytoplasm [1]. Studies of the receptor from animal systems have characterized the structural domains of the receptor. The Ins(1,4,5)P₃-binding domain is formed largely by residues within the N-terminal region, which is separated by more than 1400 residues from the C-terminal Ca²⁺ channel [2]. Both the N-terminal and C-terminal tails are cytoplasmic, leaving only short loops linking some of the membrane-spanning helices within the lumen of the Ca²⁺ stores. Comparatively little is known about the receptor in plant systems; there have been very few reports on the putative receptor from plants [3,4]. We reported for the first time the isolation and biochemical characterization of a receptor from the mung bean system [5]. The plant receptor (110 kDa per subunit) consists of four subunits, like its animal counterpart (250–300 kDa). It binds to both Ins(1,4,5)P₃ and Ins(2,4,5)P₃ with little difference in their affinities. In contrast, Ins(1,4,5)P₃ is about four times more effective than Ins(2,4,5)P₃ in Ca²⁺ mobilization from microsomes/vacuoles in mung bean. Specific recognition of the receptor by the second messenger and the subsequent release of Ca²⁺ have led to the question of whether there is any correlation between the release and the binding affinity of the receptor for different inositol phosphates. It seems that the receptor requires mainly the motif of the vicinal Ins(4,5)P₂ plus a free 6-OH group and one of the negative charges of the phosphate group at the C-1 position for binding [6]. However, a correlation between the binding affinities of different myo-inositol phosphates to the InsP₃R and effective Ca²⁺ mobilization has not been established [6,7].

The present work is aimed at finding out the nature and extent of conformational changes in the plant InsP₃R after it has bound to the InsP₃ receptors and correlating whether or not the affinity of the InsP₃R for different InsP₃ isomers is directly proportional to intracellular Ca²⁺ mobilization.

MATERIALS AND METHODS

Materials

[¹⁴C]Ins(1,4,5)P₃ (40 Ci/mmol), was obtained from NEN-Dupont. Ins(1,4,5)P₃, Ins(2,4,5)P₃, Ins(1,3,4)P₃, 2-[2-bis-(carboxymethyl)phenoxy]-methyl]-6-methoxy-8-bis(carboxymethyl)aminoquinoline.

Abbreviations used: InsP₃, any of the isomers Ins(1,4,5)P₃, Ins(1,3,4)P₃, Ins(1,5,6)P₃ and Ins(2,4,5)P₃; InsP₃R, InsP₃ receptor; quin-2, 2-[2-bis-(carboxymethyl)phenoxy]-methyl]-6-methoxy-8-bis(carboxymethyl)aminoquinoline.
isomer was added. The concentration of Ca
exhaustively. These vesicles were monitored for Ca
buffer [4 mM Tris
3 mM EDTA/10 mM 2-mercaptoethanol/1 mM PMSF/
1 mM benzamide/10 g/l polyvinyllpyrrolidone/1 g/l BSA]. The homogenate was passed through two layers of cheese cloth. It was then centrifuged at 12000 g for 40 min and the supernatant was finally centrifuged at 80000 g for 1 h at 4° C to pellet the microsomes/vacuoles. The pellet was suspended in 1 % (v/v) Triton X-100 plus buffer B [50 mM Tris/HCl buffer (pH 8.0)/1 mM EDTA/1 mM 2-mercaptoethanol] and the protein was solubilized by stirring the suspension for 1 h at 4° C. This was then centrifuged at 100000 g for 2 h at 4° C. To the supernatant was added sodium chloride to a final concentration of 0.2 M; it was then passed through the heparin–agarose column twice. The receptor was eluted with the buffer C [50 mM Tris/HCl (pH 8.0)/1 mM EDTA/1 mM 2-mercaptoethanol/1 mM PMSF/1 mM benzamide/1 % (v/v) Triton X-100/0.6 M NaCl]. It was desalted, freeze-dried and subjected to PAGE [5 %, (w/v) gel]. When this band was electroeluted and subjected to SDS/PAGE, one protein band (110 kDa) was visible after silver staining, and [3H]Ins(1,4,5)P
-binding activity coincided with this band, as assayed by slicing the gel. The electroeluted protein corresponding to the band also elicited Ca
efflux from the reconstituted proteoliposomes. The yield was approx. 0.08 mg/10 mg of microsomal/vacuolar protein and the purification at the final stage was approx. 1000-fold.

Preparation of proteoliposomes

The receptor obtained (80 µg) was reconstituted in 160 µg of phosphatidylcholine/phosphatidylethanolamine (1:1, w/w) in buffer [4 mM Tris/HCl (pH 8.0)/25 mM NaCl/23 mM KCl/100 µM CaCl2] by sonication. The proteoliposomes were dialysed exhaustively. These vesicles were monitored for Ca
-release experiments as described below.

Evaluation of Ca
uptake and release

For the evaluation of Ca
influx and efflux, microsomal/vacuolar suspension (127 µg/ml) containing 100 µM quin-2, 3 mM NaNO3, 100 µM CaCl2 and 2 mM ATP was placed in a cuvette for fluorescence measurements in Hitachi F-3010 spectrofluorimeter. The addition of ATP initiated the uptake of Ca
by the microsomes/vacuoles, which was monitored by the decrease in fluorescence of quin-2 (λex 339 nm; λem 492 nm). After the process of uptake had reached a steady value, 1 µM of the Ins
phospholipid was added. The concentration of Ca
released over a period of 20 s was calculated from the following equation [8]:

\[ [Ca^{2+}] = K_d (F - F_{min}) / (F_{max} - F) \]  

where \( K_d \), \( F \), \( F_{max} \) and \( F_{min} \) denote the dissociation constant (115 nM) for quin-2–Ca
interaction, the fluorescence of the sample, the maximum fluorescence measured in the presence of 2 mM Ca
and the minimum fluorescence measured in the presence of 5 mM EGTA respectively.

A suspension of proteoliposomes (8 µg of protein) in a volume of 400 µl containing Ca
was incubated with 100 µM quin-2 for 3–4 min at 25° C. To this were added various inositol phosphates; the release of Ca
was monitored as described above.

Evaluation of the binding stoichiometry, dissociation constant and thermodynamic parameters for the interaction of Ins
with the Ins
R

The binding studies were performed in 50 mM Tris/5 mM NaCl containing 0.05 % Triton X-100 (henceforth denoted buffer T). Uncorrected fluorescence spectra are reported here. Appropriate subtractions of the contribution from buffer T were made. The fluorescence of the receptor (λex 295 nm) decreased on the addition of Ins(1,4,5)P
/Ins(2,4,5)P
. The addition of Ins(1,3,4)P
/Ins(1,5,6)P
 up to a concentration of 5 µM did not lead to any significant change in the fluorescence spectrum of the receptor (results not shown), thereby indicating the absence of association between them. A decrease in fluorescence of the receptor provides a method for determining the affinity parameters, binding stoichiometry and dissociation constant for its binding to the isomers Ins(1,4,5)P
/Ins(2,4,5)P
. It was plotted as a function of the input concentration of Ins
R
. The binding stoichiometry was determined from the break-point in the straight lines as obtained. The dissociation constant (\( K_d \)) was evaluated from the ligand-induced quenching of the fluorescence of the receptor by means of the following equation [9]:

\[ 1 / \Delta F = 1 / \Delta F + K_d / (\Delta F_{max} [S]) \]  

where [S] denotes the concentration of the ligand and \( \Delta F \) is the extent of fluorescence quenching at 340 nm as a function of input concentration of the ligand [S]. A least-squares fit of the experimental points was done to get the best-fitting straight line. The ratio of the slope and intercept of the straight line from the plot of 1/\( \Delta F \) against 1/[S] gives the value of \( K_d \). The association constant, \( K_{app} \) (\( = 1 / K_d \)) determined at different temperatures was used for the calculation of the thermodynamic parameters.

The dissociation constant for the interaction of Ins
P
R
 was also determined by a filter-binding assay as follows. Different concentrations of [3H]Ins(1,4,5)P
 (specific radioactivity 0.677 µCi/nmol) were incubated with a fixed concentration (40 nM) of the receptor in buffer T at 5° C. After 15 min the incubation mixture was passed through Whatman GF/C filter paper to separate the free ligand from the receptor-bound ligand. The filter paper was washed with the same buffer and dried for radioactivity measurements. Non-specific binding was checked by using acryl-

Conformations of Ins
R
 alone and in the presence of Ins
P

The conformation of the receptor was ascertained in two ways as follows. In the first method the accessibility of its tryptophan residues under different conditions was checked by using acryl-
amidase, a tryptophan-specific fluorescence quencher [11] that is
commonly used to assess the degree of exposure of an internal
fluorophore such as tryptophan in protein. Quenching of the
protein fluorescence by acrylamide was analysed by the Stern–
Volmer equation [11]: $F_0 / F = 1 + K_{sv}[Q]$, where $F_0$ and $F$ are the
initial and final fluorescence intensities of the receptor, $[Q]$ denotes the input concentration of acrylamide, and $K_{sv}$ is
Stern–Volmer quenching constant. If the accessibility of the
tryptophan residues in a protein changes, it leads to an alteration
in $K_{sv}$. In the present case the value changes between the free
receptor and the InsP₃-bound receptor. The change originates
from an alteration in the conformation of the receptor as a result
of the binding of the ligand. In the second method the CD
spectra of the protein in the far-UV region (205–250 nm) were
measured in the absence and presence of InsP₃. They were
recorded with Jasco J-720 spectropolarimeter in a cuvette of
1 mm path length. The presence of Triton X-100 in the buffer
prevented the recording of the CD spectrum below 205 nm.
Limited solubility and non-specific aggregation of the receptor
prevented us from studying the tertiary structure in the near-UV
region because the longer wavelengths require a higher con-
centration of the protein to achieve a good signal-to-noise ratio.

RESULTS AND DISCUSSION

Affinity parameters for the interaction of InsP₃ isomers with
InsP₃R

Addition of Ins(1,4,5)P₃ or Ins(2,4,5)P₃ (Figure 1) to the receptor
leads to a quenching of the fluorescence of the receptor. Such
quenching of tryptophan fluorescence could originate from two
sources: an alteration in the conformation of the receptor on
binding to the ligand, or the presence of the negatively charged
trisphosphate in the vicinity of the indole fluorophore of the
tryptophan residues. Considering the hydrophobic nature of this
residue, the latter possibility seems remote. However, no significant change in the fluorescence of the receptor occurred in
the presence of 5 $\mu$M Ins(1,3,4)P₃ or Ins(1,5,6)P₃, suggesting
either an absence of its binding or binding with lower affinity ($K_a$
> 5 $\mu$M). The decrease in fluorescence on addition of Ins(1,4,5)P₃
or Ins(2,4,5)P₃ was dependent upon the input concentrations of
InsP₃ until at a point at which the fluorescence reached a plateau
(Figure 2a). This suggests that the quenching occurs as a result
of the association between InsP₃R and InsP₃. Previously the
association had been monitored by the filter-binding assay with
radiolabelled myo-inositol phosphates [5,7]. The result of the
filter-binding assay to evaluate the affinity constant for the
association of Ins(1,4,5)P₃ with InsP₃R is shown in Figure 2(b).
The observed quenching in fluorescence of the receptor provides
an alternative method to evaluate the affinity parameters even
for non-radiolabelled myo-inositol phosphates, e.g. Ins(2,4,5)P₃,
Ins(1,3,4)P₃ and Ins(1,5,6)P₃ in the present case. The dissociation
constant and stoichiometry were calculated either directly from
the linear binding isotherms (Figures 2a and 2b) or from a
double-reciprocal plot [eqn. (2)] from fluorescence measurements
(Figure 3a). Table 1 summarizes the relevant stoichiometry and
dissociation constant values determined by the two methods. The
internal consistency of the values for the dissociation constant
for Ins(1,4,5)P₃ validates the fluorescence method as a means of
evaluating the affinity constant for non-radiolabelled myo-
Table 2 Concentration of Ca\(^{2+}\) released by the Ins\(_P_R\) isomers

The release of Ca\(^{2+}\) from liposomes and microsomes/vacuoles was monitored by the fluorescence assay as described in the Materials and methods section. The values for Ca\(^{2+}\) released from liposomes are the concentrations of Ca\(^{2+}\) released per 8 \(\mu\)g of receptor protein. It was measured over a period of 20 s. The concentration of ligand used was 1 \(\mu\)M in each case. The values for Ca\(^{2+}\) released from microsomes/vacuoles are the concentrations of Ca\(^{2+}\) released per mg of microsomal/vacuolar protein in 20 s. Abbreviation: n.d., not detected.

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Calumosomes released from:</th>
<th>Microsomes/vacuoles released from:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ins(1,4,5)(_R)</td>
<td>36 ± 3</td>
<td>3500 ± 200</td>
</tr>
<tr>
<td>Ins(2,4,5)(_R)</td>
<td>12 ± 1.7</td>
<td>910 ± 100</td>
</tr>
<tr>
<td>Ins(1,3,4)(_R)</td>
<td>n.d.</td>
<td>120 ± 30</td>
</tr>
<tr>
<td>Ins(1,5,6)(_R)</td>
<td>n.d.</td>
<td>50 ± 10</td>
</tr>
</tbody>
</table>

Table 3 Thermodynamic parameters for the interaction of Ins\(_P_R\) isomers with Ins\(_P_R\)

Results were determined from a plot of lnK\(_{app}\) against 1/T (Figure 3b). \(\Delta G\) was calculated by means of eqn. (4) at 25 \(^\circ\)C and corresponds to the mean value of \(\Delta H\).

<table>
<thead>
<tr>
<th>Ligand</th>
<th>(\Delta H) (kJ/mol)</th>
<th>(\Delta S) (J/(K\cdot mol))</th>
<th>(\Delta G) (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ins(1,4,5)(_R)</td>
<td>69.0 ± 14.6</td>
<td>380</td>
<td>-44.3</td>
</tr>
<tr>
<td>Ins(2,4,5)(_R)</td>
<td>23.4 ± 6.3</td>
<td>221</td>
<td>-42.6</td>
</tr>
</tbody>
</table>

Table 1 Dissociation constant and binding stoichiometry for the interaction of Ins\(_P_R\) isomers with Ins\(_P_R\)

Results were determined from the fluorescence quenching data in 50 mM Tris/HCl buffer (pH 8.0)/25 mM NaCl containing 0.05% Triton X-100, by means of eqn. (2). The stoichiometry was estimated as shown in the representative graph of Figure 2(a). The value for \(K_i\) in parenthesis was determined from the curve (Figure 2b) obtained from the filter-binding assay with [\(^3\)H]Ins(1,4,5)\(_R\). Abbreviation: n.d., not determined.

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Dissociation constant, (K_i) (nM)</th>
<th>Stoichiometry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ins(1,4,5)(_R)</td>
<td>82 ± 20 (100)</td>
<td>1.25</td>
</tr>
<tr>
<td>Ins(2,4,5)(_R)</td>
<td>115 ± 30</td>
<td>1.1</td>
</tr>
<tr>
<td>Ins(1,3,4)(_R)</td>
<td>&gt; 5000*</td>
<td>n.d.</td>
</tr>
<tr>
<td>Ins(1,5,6)(_R)</td>
<td>&gt; 5000*</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

* No change in fluorescence of the receptor at 5 \(\mu\)M Ins\(_P_R\) isomers was obtained.

Figure 3 Evaluation of the apparent dissociation constant, \(K_{app}\) and thermodynamic parameters for the Ins\(_P_R\)–Ins(1,4,5)\(_P_R\) interaction

(a) Double-reciprocal plot of 1/\(\Delta F\) (\(\lambda_{ex}\) 295 nm; \(\lambda_{em}\) 340 nm) against 1/[S] to evaluate the dissociation constant for the interaction of Ins(1,4,5)\(_P\) with Ins\(_P\)R at 5 \(^\circ\)C in 50 mM Tris/HCl buffer (pH 8.0)/25 mM NaCl containing 0.05% Triton X-100, by means of eqn. (2). The straight line obtained is from a least-squares fit of the experimental points as in Figure 2(a). (b) Van’t Hoff plot of ln\(K_{app}\) (= 1/\(K_i\)) against the reciprocal of absolute temperature to determine \(\Delta H\) and \(\Delta S\) for the Ins(1,4,5)\(_P\)–Ins\(_P\)R interaction. The straight line obtained is the least-squares fit of the three experimental points.

Results were determined from a plot of lnK\(_{app}\) against 1/T (Figure 3b). \(\Delta G\) was calculated by means of eqn. (4) at 25 \(^\circ\)C and corresponds to the mean value of \(\Delta H\).

Comparison of Ca\(^{2+}\) release from intracellular stores by the Ins\(_P\) isomers

The functional properties of Ins\(_P\) isomers were compared by their abilities to release Ca\(^{2+}\) from intracellular stores. It is clear from Table 2 that the extent of release of Ins(1,4,5)\(_P\) is about 4-fold higher than that for Ins(2,4,5)\(_P\). This ratio is not directly proportional to the Ins\(_P\)R binding affinities for the Ins\(_P\) isomers. A similar trend in the extent of Ca\(^{2+}\) release was noticed with liposomes containing purified receptor (Table 2). Comparatively, Ins(1,3,4)\(_P\) and Ins(1,5,6)\(_P\) cause an insignificant release of Ca\(^{2+}\) because of their poor interactions with the receptor.

Thermodynamic parameters for Ins\(_P\)–Ins\(_P\)R interactions

A mutual compensation of \(\Delta H\) and \(\Delta S\) values might lead to a comparable \(\Delta G\) value, i.e. affinity constant at a particular
temperature ($\Delta G = -RT\ln K_{eq}$). Therefore we measured the thermodynamic parameters to characterize and compare the interactions of the two isomers Ins(1,4,5)$P_3$ and Ins(2,4,5)$P_2$ in particular with Ins$P_R$. Changes in heat content and entropy are also useful parameters for comparing the conformational changes in the receptor as a result of its binding to the isomers. Figure 3(b) shows the representative Van’t Hoff plot [eqn. (3)] for the Ins(1,4,5)$P_3$-Ins$P_R$ interaction. The affinity constants were determined from the double-reciprocal plot such as that shown in Figure 3(a). From a comparison of similar types of report on the thermodynamics of interactions among nucleic acids and proteins with subunits [13], we suggest the following. The linear nature of the Van’t Hoff plot within the temperature range 5–25°C indicates that there is no major change in the quaternary structure of the receptor as a result of the association of each subunit with Ins$P_R$. Table 3 summarizes the thermodynamic parameters. The association with Ins$P_R$ in both cases [Ins(1,4,5)$P_3$/Ins(2,4,5)$P_2$] leads to a positive change in the enthalpy and is therefore entropy-driven. It could be ascribed to two factors: electrostatic interaction between the negative phosphate group of Ins$P_R$ and the positively charged side chain of an amino acid residue in the N-terminal domain of Ins$P_R$, and an alteration in the conformation of the Ins$P_R$. The contribution of the first factor is usually of the order of 8–20 kJ/mol (2–5 kcal/mol). It indicates that the rest of the value for $\Delta H$ is contributed by the conformational change of the receptor on binding to Ins(1,4,5)$P_3$. It implies that Ins(1,4,5)$P_3$ and Ins(2,4,5)$P_2$ induce different degrees and/or natures of conformational change in Ins$P_R$. Similarly, the positive entropy change could be the sum of two factors: $\Delta S = \Delta S_{water} + \Delta S_{configuration}$ [13]. The first term stands for the entropy change due to the release of bound water from the receptor and the second arises mostly from the configurational entropy change of the receptor due to association with Ins$P_R$. A comparison of the entropy change in the two cases (Table 3) shows that there is an additional entropy change $[159 J/(K \cdot \text{mol})]$ when Ins(1,4,5)$P_3$ binds to the receptor. The different extent or nature of the conformational change in the receptor in the two cases is its plausible source. It also supports the earlier proposition made from the trend in enthalpy change.

Acrylamide-quenching probe for conformational change in Ins$P_R$ as a result of binding to Ins$P_R$

The results from the acrylamide quenching studies are shown in the Figure 4. The Stern–Volmer plots show an upward curvature that is characteristic of static quenching [11]. It might occur because the fluorophoric tryptophan residues of the receptor are easily accessible to the quencher. The addition of Ins$P_R$ does not change this trend in static quenching; however, an alteration in $K_q$ results, suggesting that an alteration in the conformation of the receptor occurs without a radical change in the environment of the tryptophan residues. As a second but remote possibility, it could be that $K_q$ increases because bound Ins$P_R$ hinders the access of acrylamide to the tryptophan residues.

Conformational probe for free and Ins$P_R$-bound Ins$P_R$ by CD spectroscopy

Far-UV CD spectra of Ins$P_R$, alone and in the presence of two Ins$P_R$ isomers, are shown in Figure 5. The spectrum of free Ins$P_R$ indicates the presence of ordered $\alpha$-helix and $\beta$-sheet structures from the characteristic bands at 207, 215 and 222 nm [14]. However, it is different from the normal spectrum typical of a model $\alpha$-helix or $\beta$-sheet conformation, possibly because of the transmembrane nature of the protein. Also, the presence of Triton X-100 in the buffer prevented the recording of spectra below 205 nm. Therefore we did not attempt to estimate the relative percentages of the different conformations and confined ourselves to a comparison of the spectrum of free and bound Ins$P_R$. Our suggestion of helical structure in free Ins$P_R$ is consistent with the prediction of the presence of transmembrane helical domains in the Ins$P_R$ reported from rat liver [2]. Binding of either isomer [Ins(1,4,5)$P_3$ or Ins(2,4,5)$P_2$] alters the CD spectrum of Ins$P_R$. The helical domain, mostly consisting of hydrophobic residues, undergoes a conformational change leading to an alteration in the far-UV CD spectrum. The nature of the alteration in the presence of Ins(1,4,5)$P_3$ is not comparable with that of Ins(2,4,5)$P_2$. Addition of Ins(2,4,5)$P_2$ to Ins$P_R$ leads to a decrease in the ellipticity without any significant change in the spectral shape. In contrast, the spectrum of Ins$P_R$ shows a
change in the line shape in the 205–230 nm region in the presence of \( \text{Ins}(1,4,5)P_3 \), which implies that the nature of the conformational changes in \( \text{Ins}P_R \) induced by \( \text{Ins}(1,4,5)P_3 \) and \( \text{Ins}(2,4,5)P_3 \) are different. In accordance with the results from the fluorescence and filter-binding assays, 5 \( \mu \)M \( \text{Ins}(1,3,4)P_3 \) or \( \text{Ins}(1,5,6)P_3 \) do not induce any change in the CD spectrum of the receptor (results not shown). It could be suggested from CD data that the conformational change might involve a membrane-spanning helical domain in \( \text{Ins}P_R \), as required for its property as a \( \text{Ca}^{2+} \) channel. The difference in the extent and nature of the conformational change of the receptor in the presence of, particularly, the two isomers \( \text{Ins}(1,4,5)P_3 \) and \( \text{Ins}(2,4,5)P_3 \) are clearly indicated from a comparison of the far-UV CD spectra, \( K_v \) values and relative values of \( \Delta H \) and \( \Delta S \) characterizing their associations with \( \text{Ins}P_R \). We reasoned that this difference might be the origin of the trend reflected in the relative abilities of the isomers to release \( \text{Ca}^{2+} \) from the liposomes and microsomes/vacuoles.

In an exhaustive study [6] the efficiency of different myo-inositol phosphates to elicit \( \text{Ca}^{2+} \) release in \textit{Xenopus} oocytes was shown to follow a rank order as given: Ins(1,4,5)P_3 > Ins(2,4,5)P_3 > Ins(1,2,4,5)P_3 > Ins(2,4,5)P_3 > Ins(1,2,4,6)P_3 > other InsP. This rank order does not necessarily follow that observed in their binding abilities to \( \text{Ins}P_R \). The present findings support this observation and indicate that it is not only the binding affinity of \( \text{Ins}P_R \) for the receptor but the overall interaction leading to the requisite conformational change in the receptor that determines the potency of the isomers of \( \text{Ins}P_3 \) in their ability to release \( \text{Ca}^{2+} \) from the intracellular stores. It does, however, predict that if the interaction of \( \text{Ins}(1,3,4)P_3/\text{Ins}(1,5,6)P_3 \) with the receptor is promoted by any means, the potentiation of its \( \text{Ca}^{2+} \) mobilization from intracellular stores is possible. It was demonstrated recently in our laboratory that \( \text{Ins}(1,3,4)P_3 \), when complexed with the phytase enzyme, can interact with the receptor, eliciting \( \text{Ca}^{2+} \) efflux from the intracellular stores [15].

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