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FEBS Letters 341 (1994) 28–32

FEBS 13746

FEBS LETTERS

pH-dependent interaction of an intraluminal loop of inositol 1,4,5-trisphosphate receptor with chromogranin A

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Received 20 December 1993

Abstract

The inositol 1,4,5-trisphosphate (IP_3)-sensitive Ca^{2+} store role of the secretory vesicles of adrenal medullary chromaffin cells is attributed to the presence of high capacity, low affinity Ca^{2+} binding protein chromogranin A. Chromogranin A has recently been shown to interact with the protein component(s) on the intraluminal side of the secretory vesicle membrane at the intravesicular pH of 5.5 but to dissociate from them at the near physiological pH of 7.5. Further, one of the chromogranin A-interacting membrane proteins was tentatively identified as the IP_3 receptor. Therefore, the pH-dependent potential interaction of the intraluminal loop domains of the IP_3 receptor with chromogranin A was studied by analytical ultracentrifugation utilizing synthetic intraluminal loop peptides of the IP_3 receptor labeled with 5-hydroxy-tryptophan at the N-terminus as a chromophore. One of the intraluminal loop domains was found to interact with chromogranin A at pH 5.5 but not at pH 7.5, suggesting the importance of the intraluminal loop domain in transmitting Ca^{2+} mobilization signals to chromogranin A.

Key words: pH; Intraluminal loop; IP_3 receptor; Chromogranin A; 5-Hydroxy-tryptophan

1. Introduction

The secretory vesicles of adrenal medullary chromaffin cells, which are the intracellular home for highly concentrated catecholamine, ATP, and Ca^{2+}, in addition to many proteins and peptides (reviewed in [1]), have been identified as a major IP_3-sensitive intracellular Ca^{2+} store [2]. The Ca^{2+} storage function of the secretory vesicle was attributed to the high capacity, low affinity Ca^{2+} binding property of chromogranin A (CGA) [3], which is known to undergo pH- and Ca^{2+}-induced conformational changes [4] and to bind 55 mol of Ca^{2+}/mol of protein at the intravesicular pH of 5.5 with a dissociation constant (K_d) of 4 mM and 32 mol of Ca^{2+}/mol of protein at the near physiological pH of 7.5 with a K_d of 2.7 mM [3]. The pH-induced conformational changes and the differences in the binding capacity and affinity of CGA for Ca^{2+} at different pH levels suggest that the capacity and affinity of CGA for Ca^{2+} are directly influenced by the different CGA conformations.

Furthermore, chromogranin A is also known to exist in a monomer–tetramer equilibrium at pH 5.5 and in a monomer–dimer equilibrium at pH 7.5 [5], which suggests the presence of dimers of CGA in the endoplasmic reticulum (ER) and *cis*-Golgi cisternae, and the presence of tetramers in the *trans*-Golgi network (TGN) and secretory vesicles. Moreover, contrary to the notion of existing only as a soluble matrix protein, chromogranin A has recently been shown to remain bound to the vesicle membrane at pH 5.5 and released at pH 7.5 [6], and the membrane binding ability of CGA was thought to be due to the pH-induced anchor role of the conserved near N-terminal region of CGA [6]. In particular, the pH-dependent interaction of CGA with the vesicle membrane was shown to be due to the interaction between CGA and the protein component(s) on the intraluminal side of the vesicle membrane [6].

Moreover, it was recently found that chromogranin A interacts with several membrane proteins of the secretory vesicle membrane at pH 5.5 and dissociates from them at pH 7.5, and one of the CGA-interacting membrane proteins was tentatively identified as the IP_3 receptor (S.H. Yoo, in preparation). Accordingly, the possibility that the intraluminal region(s) of the IP_3 receptor might interact with CGA in a pH-dependent manner arose. Hence, the potential interaction of the IP_3 receptor intraluminal domain(s) with CGA was studied using synthetic intraloop peptides of the IP_3 receptor, and the pH-dependent interaction of an IP_3 receptor intralumi-

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Abbreviations: CGA, chromogranin A; IP_3, inositol 1,4,5-trisphosphate; 5-OH-Trp, 5-hydroxy-tryptophan.

SSDI 0014-5793(94)00131-E

nal domain with CGA was found. This result potentially provides an unprecedented insight into the relationship between the IP_3 receptor(s) and the internal Ca^{2+} storage protein of an IP_3-sensitive intracellular Ca^{2+} store.

2. Materials and methods

2.1. Purification of chromogranin A

Chromogranin A was purified from bovine adrenal chromaffin granules according to the method of Yoo and Albanesi [4].

2.2. IP_3 receptor intraluminal loop peptide synthesis

Two peptides, each representing 20–21 amino acids of the intraluminal loop domains of rat type 2 IP_3 receptor [7], were synthesized with the addition of a 5-OH-Trp at the N-terminus as a chromophore. The synthesized peptides were purified by high performance liquid chromatography (HPLC) and the integrity of the peptides was ensured through analyses by fast atom bombardment mass spectrometry and by amino acid composition analyses. The purity of the peptides was 98% or higher.

2.3. Determination of peptide concentration

Peptide concentration was determined using 6875 $M^{-1} \cdot cm^{-1}$ at 280 nm as the extinction coefficient.

2.4. Analytical ultracentrifugation

Analytical ultracentrifugation was performed using a Beckman model E analytical ultracentrifuge equipped with a scanning absorption optical system. Data were acquired from the scanner output using a Metrabyte DAS-8 12-bit analog to digital converter in a 286 computer as an acquisition system. Each recorded point was the average of 100 acquired points; the actual data density was 425 points per cm of radial distance in the cell.

The experiments were run for 63 h at a rotor speed of 10,000 rpm. Solutions of the mixture of chromogranin A and the appropriate IP_3 receptor loop peptide were examined in either 20 mM sodium acetate, pH 5.5, 0.1 M KCl, or in 20 mM Tris-HCl, pH 7.5, 0.1 M KCl. One cell at each pH had no Ca^{2+}; the others had 35 mM Ca^{2+}. In each of the cells that contained no Ca^{2+}, 2 mM EGTA was included to ensure the absence of a Ca^{2+} effect.

3. Results

To test the potential interaction of the IP_3 receptor intraluminal loops with CGA, the two major intraluminal loops, i.e. one between the transmembrane domains 5 and 6, DLVYREETLLNVIKSVTRNGR (L1), and the other between the transmembrane domains 7 and 8, DVLRRPSKDEPLFAARVVYD (L2) (Fig. 1), were synthesized with 5-hydroxy-tryptohan (5-OH-Trp) at each N-terminus as a chromophore. Since the other two possible intravesicular loops, with 10 and 5 residues each, are considered too small to interact significantly with CGA, only L1 and L2 appeared to be large enough to have interactions, if any, with CGA. Unlike regular tryptophan-containing proteins whose absorption range extends only up to 300 nm, the 5-OH-Trp-containing proteins have a unique spectroscopic property that their absorption range extends up to 320 nm [8,9]. This extended absorption property of 5-OH-Trp is very useful for following a particular peptide or protein labeled with 5-OH-Trp in a mixture of proteins or of proteins and nucleic acids. Fig. 2 shows the absorption spectra of intact CGA and the loop 2 peptide of the IP_3 receptor with 5-OH-Trp at its N-terminus. The absorbance of CGA peaked at 280 nm, but decreased rapidly in the longer wavelength range above 280 nm, showing virtually no absorbance at 305 nm. However, the absorbance of the 5-OH-Trp-containing IP_3 receptor loop 2 peptide at 305 nm was still very high, absorbing approximately half of the maximal absorption shown at ~275 nm. Utilizing this absorption property of 5-OH-Trp-containing peptides at 305 nm, the potential interaction between CGA and the IP_3 receptor loop regions was studied.

In view of the fact that chromogranin A can interact with the membrane proteins of secretory vesicles only at the intravesicular pH of 5.5 but not at pH 7.5, the potential interaction between CGA and the loop peptides 1 and 2 at pH 5.5 was examined by subjecting the mixture of CGA and each peptide to sedimentation equilibrium study. By centrifuging at a speed (10,000 rpm) high enough to sediment the tetrameric CGA toward the bottom of the cell, but low enough not to sediment the significantly smaller peptide, the oligomeric CGA moved toward the bottom of the cell whereas the loop peptides remained as monomers. As shown in Fig. 3A, the absorbance at 280 nm of the mixture of CGA and the loop 1 peptide at sedimentation equilibrium showed an equilibrium absorbance distribution indicating a monomer–tetramer equilibrium of CGA under this condition [5]. However, the absorbance at 305 nm of the same mixture exhibited a nearly flat absorbance distribution characteristic of the peptide alone, indicating no interaction be-

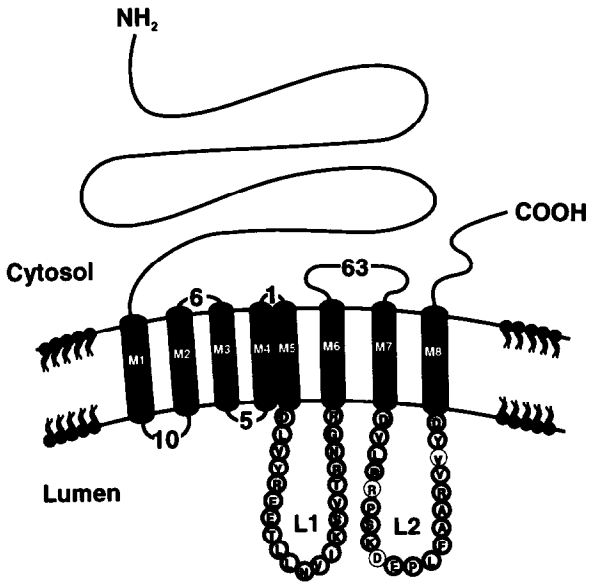

Fig. 1. The predicted topology of rat type 2 IP_3 receptor showing eight transmembrane regions. The amino acid sequences of intraluminal loop 1 (L1) and loop 2 (L2) are shown. The numerals shown between the transmembrane regions indicate the number of amino acid residues in the respective loop regions. The amino acid sequences in L1 and L2 are identical to those of rat type 1 IP_3 receptor except that there are three conserved changes in L2 as noted by the thin circles.

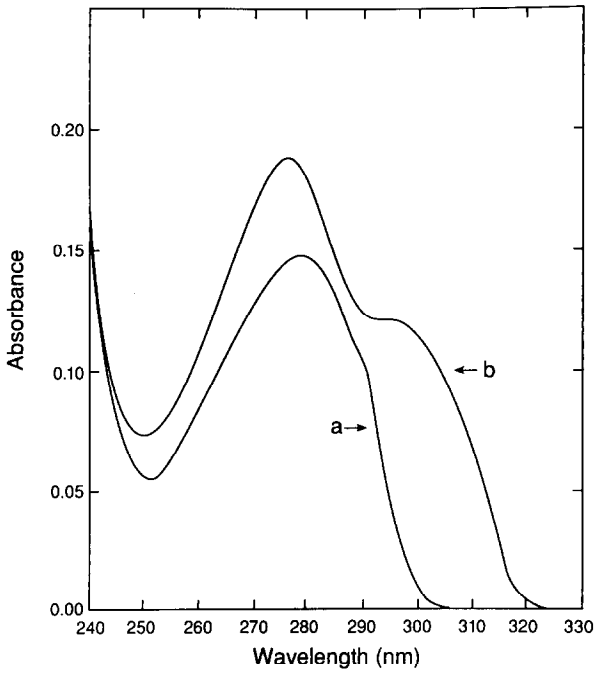

Fig. 2. Absorption spectra of chromogranin A and 5-OH-Trp-containing IP_3 receptor loop 2 peptide. The absorption spectra of chromogranin A (2.6 μM) and the IP_3 receptor loop 2 peptide (26 μM) labeled with 5-OH-Trp at the N-terminus in 20 mM Na-acetate, 0.1 M KCl, pH 5.5, were obtained with Beckman DU-65 spectrophotometer. (a) Chromogranin A; (b) 5-OH-Trp-containing IP_3 receptor loop 2 peptide.

tween CGA and the IP_3 receptor loop 1 peptide. On the other hand, the 280 nm absorbance of the mixture of CGA and the loop 2 peptide at sedimentation equilibrium (Fig. 3B) showed a significantly steeper gradient toward the bottom of the cell compared to that of Fig. 3A, suggesting a possible contribution to the 280 nm absorbance by the CGA-interacting loop 2 peptide. Further, the 305 nm absorbance of the mixture of CGA and the loop 2 peptide showed a gradient characteristic of a CGA tetramer–peptide complex, clearly indicating the interaction of CGA and the loop 2 peptide at pH 5.5.

To determine whether chromogranin A and the loop 2 peptide can interact even at pH 7.5, identical experiments were carried out at pH 7.5. As shown in Fig. 4, the 280 nm absorbance of the mixture of CGA and the loop 2 peptide indicated a monomer–dimer equilibrium distribution of CGA at this pH [5], but the 305 nm absorbance was relatively flat, indicating no interaction between CGA and the loop 2 peptide at pH 7.5. The absence of Ca^{2+} did not change the pH dependency of the interaction although it appeared to change the reaction mechanism (S.H. Yoo and M.S. Lewis, unpublished results).

4. Discussion

The present results indicate that chromogranin A interacts with an intraluminal loop of the IP_3 receptor/Ca^{2+} channel at the intravesicular pH of 5.5. The interaction appeared to be specifically dependent upon the acidic pH, since there was no interaction at a near physiological pH of 7.5. Given that the IP_3 receptor intraluminal loop 1 (cf. Fig. 1) did not interact with CGA even at pH 5.5, the pH-dependent interaction of the IP_3 receptor intraluminal loop 2 with CGA appears to represent a specific interaction between CGA and the loop 2 domain of the IP_3 receptor.

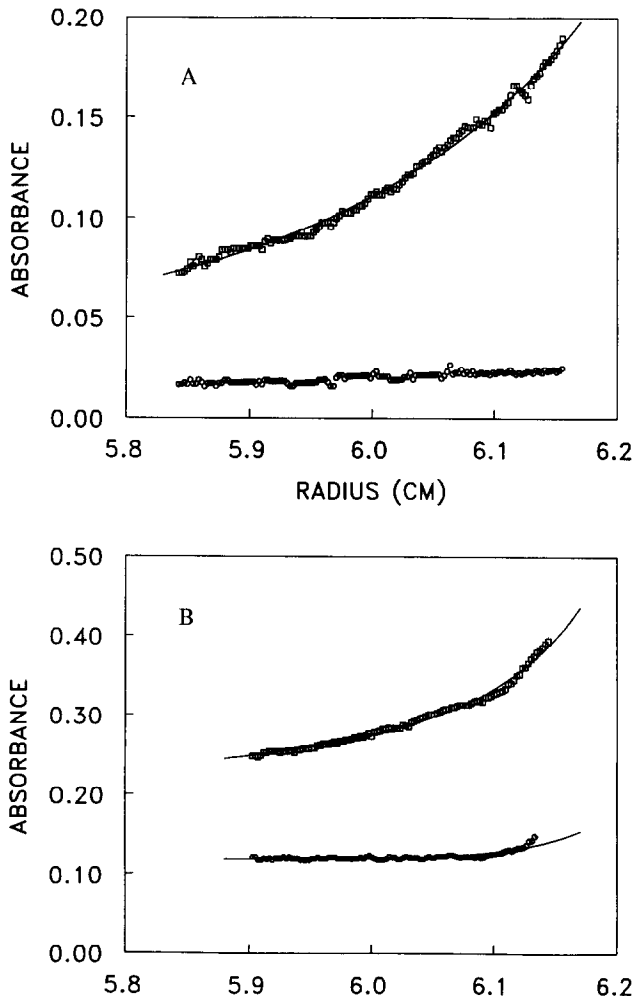

Fig. 3. Distribution of concentration of the mixture of chromogranin A and the IP_3 receptor intraluminal loop peptides at pH 5.5. The mixture of chromogranin A (3 μM) and either of IP_3 receptor intraluminal loop peptide 1 (3 μM) or loop peptide 2 (15 μM) in 20 mM Na-acetate, pH 5.5, 0.1 M KCl is shown at sedimentation equilibrium after 63 h at 10°C in the presence of 35 mM Ca^{2+} at two different wavelengths (280 and 305 nm). (A) Distribution of the concentrations of the mixture of CGA and the IP_3 receptor loop peptide 1 at 280 nm (upper curve) and at 305 nm (lower curve). The fitting line is for a CGA monomer–tetramer equilibrium [5]. (B) Distribution of the concentrations of the mixture of CGA and the IP_3 receptor loop peptide 2 at 280 nm (upper curve) and at 305 nm (lower curve). The fitting line for the upper curve is for a CGA monomer–tetramer equilibrium complexed with the peptide. The fitting line for the lower curve is for a CGA tetramer complexed with the peptide. All of the concentration distributions in the absence of Ca^{2+} at pH 5.5 were very similar to these.

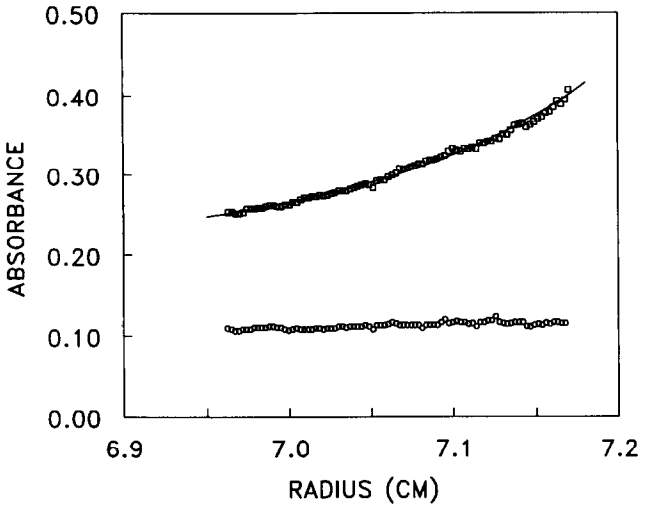

Fig. 4. Distribution of concentration of the mixture of chromogranin A and the IP_3 receptor intraluminal loop peptide 2 at pH 7.5. Distribution of the concentrations of the mixture of chromogranin A (3 μM) and the IP_3 receptor loop peptide 2 (15 μM) in 20 mM Tris-HCl, pH 7.5, 0.1 M KCl is shown at sedimentation equilibrium after 63 h at 10°C in the presence of 35 mM Ca^{2+} at 280 nm (upper curve) and at 305 nm (lower curve). Distribution of the concentrations in the absence of Ca^{2+} at pH 7.5 was very similar to this. The fitting line is for a CGA monomer–dimer equilibrium [5].

The IP_3 receptor was first cloned from rat brain with a monomer mass ~260 kDa [10,11], and is thought to have eight transmembrane regions at the C-terminal end of the molecule [7,12]. Thus, most of the IP_3 receptor is directed toward the cytoplasm, exposing very little of the receptor to the intraluminal side. The segments between transmembrane regions 5 and 6 (intraluminal loop 1; L1) and between transmembrane regions 7 and 8 (intraluminal loop 2; L2) are highly conserved among the sequenced IP_3 receptors [13–15], implying critical roles of these regions. In this regard, the pH-dependent interaction of intraluminal loop 2 of the IP_3 receptor with CGA appears to confirm the implication. Examples of interaction between the intracellular loops of membrane receptor proteins and intracellular proteins are widely known in the signal transduction systems that the intracellular loops of the G-protein-coupled receptors are known to interact with intracellular G-proteins for a variety of signal transduction pathways [16,17].

The direct interaction between the IP_3 receptor/Ca^{2+} channel and chromogranin A suggests that the Ca^{2+} mobilization signals received by the receptor can immediately be transmitted to the Ca^{2+} storage machinery of the IP_3-sensitive Ca^{2+} store, allowing a rapid response by the store. Given that IP_3 binding to the IP_3 receptor is known to change the conformation of the receptor [18], and that the IP_3 receptor is directly coupled to CGA, the conformational changes of the IP_3 receptor are expected to directly change the conformation of CGA, thereby affecting the affinity of CGA for Ca^{2+}. We have shown previously that the affinity and capacity of CGA for Ca^{2+} change as the conformation of CGA changes [3].

Based on the tryptic digestion pattern, we have also demonstrated that CGA consists of a compact N-terminal region and a structurally loose C-terminal region [19], and the overall structure of CGA is more relaxed at pH 5.5 than at pH 7.5, as evidenced by a higher incidence of proteolysis by trypsin at pH 5.5 despite the unfavorable pH condition for tryptic activity [3,19]. This result suggests that chromogranin A is bound to the IP_3 receptor through its structurally compact side [6,19], freeing the loose C-terminal side for self-association [20] or other roles. From a structural standpoint, the interaction of chromogranin A with the IP_3 receptor through its compact region and not through its loose region is clearly more advantageous for the IP_3-mediated Ca^{2+} mobilization machinery. It permits rapid transmission of the conformational changes of IP_3 receptor, which are known to occur after IP_3 binding, to the rest of CGA for rapid dissociation of Ca^{2+} from CGA and release to the outside via the IP_3 receptor/Ca^{2+} channel. Although the secretory vesicles contain 40 mM Ca^{2+} [1], most (> 99.9%) of it stays bound to the intravesicular matrix proteins [21], primarily to CGA [22]. Therefore, Ca^{2+} needs to be freed from CGA first in order to be released to the outside in response to the Ca^{2+} release signals. In this respect, the interaction of the compact N-terminal side of CGA with the IP_3 receptor/Ca^{2+} channel accords well with the rapid mobilization of Ca^{2+} in response to IP_3 [2].

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