Interaction between an intraluminal loop peptide of the inositol 1,4,5-trisphosphate receptor and the near N-terminal peptide of chromogranin A

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Abstract The near N-terminal region of chromogranin A (CGA) has been shown to be the secretory vesicle membrane binding region, and tetrameric chromogranin A has been demonstrated to bind four molecules of an intraluminal loop peptide of the inositol 1,4,5-trisphosphate (IP_3) receptor. It was therefore necessary to determine whether the conserved near N-terminal region of CGA interacts with the intraluminal loop region of the IP_3 receptor. In the present study, we found that the proposed anchor region of CGA, the conserved near N-terminal region, does indeed interact with the intraluminal loop region of the IP_3 receptor at the intravesicular pH of 5.5, further strengthening the case for the potential interaction between tetrameric chromogranins and tetrameric IP_3 receptors in the cell.

Key words: Chromogranin A; Inositol 1,4,5-triphosphate receptor; N-terminal peptide

1. Introduction

Since the identification of secretory granules as a major inositol 1,4,5-triphosphate (IP_3)-sensitive intracellular Ca^{2+} store of adrenal medullary chromaffin cells [1], the secretory granules of insulin-containing pancreatic β-cells have also been shown to function as a major IP_3-sensitive Ca^{2+} store of pancreatic β-cells [2]. The secretory granules contain many peptides, ions, hormones, and neurotransmitters in addition to chromogranins [3,4]. In particular, the Ca^{2+} storage function of secretory granules has been attributed to the presence of a high capacity, low affinity Ca^{2+} storage protein, chromogranin A [5].

Chromogranin A (CGA) has been shown to interact with several integral membrane proteins of the secretory vesicle at the intravesicular pH of 5.5 and to dissociate from them at a near physiological pH of 7.5; one of the integral membrane proteins is the IP_3 receptor/Ca^{2+} channel [6]. In addition to the interaction with the integral membrane proteins of secretory vesicles [6], CGA is also known to interact with several secretory vesicle matrix proteins at pH 5.5 and to dissociate from them at pH 7.5 [7]. Like CGA, these vesicle matrix proteins undergo pH- and Ca^{2+}-dependent aggregation, aggregating readily at pH 5.5 in the presence of Ca^{2+} [7]. The pH- and Ca^{2+}-dependent aggregation property appeared to be uniquely that of vesicle matrix proteins in that pH- and Ca^{2+}-dependent aggregation of the mixture of vesicle matrix proteins and non-vesicle matrix proteins such as immunoglobulin G resulted in the exclusion of non-vesicle matrix proteins from the aggregation complex [7]. This selective aggregation and the pH-dependent binding of CGA to the secretory vesicle membrane proteins are considered to be the key steps in selective sorting of the vesicle matrix proteins and the potential vesicle membrane proteins during vesicle biogenesis in the trans-Golgi network [7–10].

It has been shown previously that tetrameric CGA bound to four molecules of the intraluminal loop region of the IP_3 receptor [10]. Although the conserved near N-terminal region of CGA has been proposed to interact with the secretory vesicle membrane as an anchor [9,12], the potential interaction between the conserved near N-terminal region of CGA and the intraluminal loop region of the IP_3 receptor has been demonstrated. In the present study, we demonstrated the interaction of the intraluminal loop region of the IP_3 receptor with the conserved near N-terminal region of CGA, consistent with the proposed anchor role of the near N-terminal region [9,10,12,13].

2. Materials and methods

2.1. CGA peptide and IP_3 receptor loop peptide

The conserved near N-terminal CGA peptide with the sequence of IVEVISDTLSKPSPMPVSKE (residues 18–37) was synthesized with the addition of Trp at the N-terminal as a chromophore [8], and the peptide with the sequence of DVLRRPSKDEPLFAARVVYD, representing 20 amino acids of an intraluminal loop domain of rat type 2 IP_3 receptor (L2 of Fig. 1), was synthesized with the addition of a 5-OH Trp at the N-terminus as a chromophore [10]. The use of 5-OH Trp is necessary since differentiation of these two species in the ultracentrifugal analysis requires that the two reactants have significantly different absorption spectra [14]. The synthesized peptides were purified by high performance liquid chromatography, and the integrity of the peptide was ensured through analysis by amino acid composition analysis. The purity of the peptides was in excess of 98%. The molecular masses of the CGA peptide and the IP_3 receptor peptide, calculated from the amino acid sequences [15–17], were 2343 and 2549, respectively.

2.2. Analytical ultracentrifugation

Analytical ultracentrifugation was performed in a Beckman XL-A analytical ultracentrifuge at 42 000 rpm and over the temperature range 2–30°C with 4°C increments. Three cells with six-channel centerpieces were used, one cell for each type of buffer. In each cell the upper compartment contained the CGA peptide, the lower compartment contained the IP_3 receptor peptide, and the center compartment contained an equimolar mixture of the CGA peptide and the IP_3 receptor peptide having the same concentrations as in their respective compartments. The compartment adjoining each sample contained the reference buffer. Sample volumes were 0.12 ml, giving column heights

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of approximately 3 mm. The buffer used was 20 mM sodium acetate, pH 5.5, 0.1 M KCl, and 1 mM Ca^{2+}.

The compositional partial specific volumes, $\bar{v}$, were calculated for 25°C from the amino acid sequences using the consensus values of Perkin [18]. A value of $\bar{v}/\Delta T = 0.000425$ cm^3 g^{-1} deg^{-1} was used to calculate the values of $\bar{v}$ at other temperatures. The extinction coefficients of CGB and the peptides at 280 nm were determined spectrophotometrically and the extinction coefficients at 295 and 310 nm were calculated from the ratio of the concentration gradients measured at 280 nm. Scans were taken at 280, 295 and 310 nm when the CGB peptide-IP_3 receptor peptide interaction was studied in order to obtain a more rigorous analysis. The data were analyzed by a simplified procedure based on a multi-wavelength procedure [19].

3. Results

3.1. Analysis of ultracentrifuge data

The CGA peptide at pH 5.5 in the presence of Ca^{2+} was found only as an ideal monomer, the concentration distribution of which could be appropriately described by the mathematical model,

$$c_r = c_{b,1}\exp(A_A M_A(r^2 - r_b^2)) + \varepsilon$$

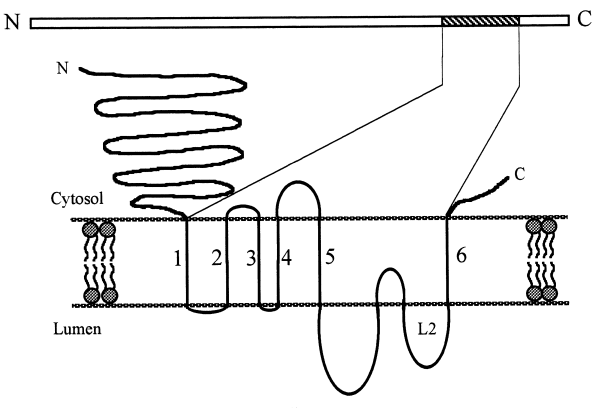
where c_r is the concentration, expressed as absorbance at 280 nm, as a function of radial position; $c_{b,1}$ is the concentration of monomer at the cell bottom r_b; $A_A = (1-\bar{v}\rho)\omega^2/2RT$ for the CGA peptide, where $\bar{v}$ is the compositional partial specific volume, ρ is the solvent density, ω is the rotor angular velocity, R is the gas constant and T is the absolute temperature; M_A is the molecular mass of the CGA peptide monomer which has the value of 2343 Da; ε is a small baseline error correction term.

Likewise, the IP_3 receptor peptide alone at pH 5.5 in the presence of Ca^{2+} was also found as an ideal monomer, the concentration of which could be described by the mathematical model,

$$c_r = c_{b,1}\exp(A_P M_P(r^2 - r_b^2)) + \varepsilon$$

where the subscript P on A and M indicates that these parameters applied to the peptide. The M_P value used was 2549.

In light of the previous observation that CGA interacts with the IP_3 receptor peptide at pH 5.5 [11], we explored



L2: DVLRRPSKDEPLFAARVVYD

Fig. 1. The predicted topology of rat type 2 IP_3 receptor showing six transmembrane regions [25]. The amino acid sequence of the intraluminal loop L2 of rat type 2 IP_3 receptor [17] is shown. The amino acid sequence in L2 is identical to that of rat type 1 IP_3 receptor [26] except that there are three conserved changes in L2.

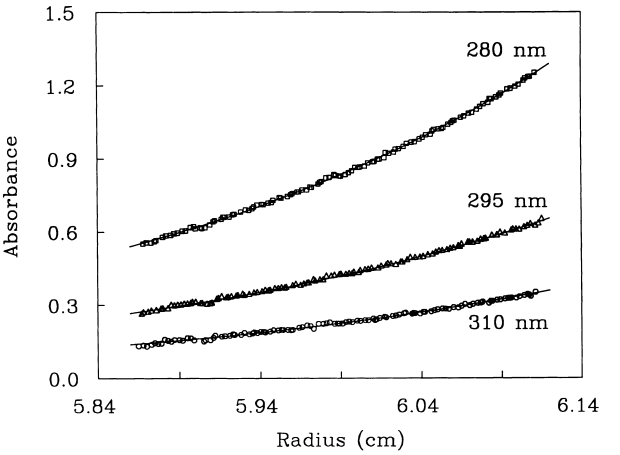


Fig. 2. The distribution of the conserved near N-terminal peptide of CGA and the intraluminal loop peptide of IP_3 receptor in 20 mM sodium acetate, pH 5.5, 0.1 M KCl, and 2 mM Ca^{2+} at three different wavelengths (280, 295, and 310 nm) is shown at ultracentrifugal equilibrium at 10°C. The lines show the best fitting curve for the interaction model of the near N-terminal CGA peptide and the intraluminal loop peptide at each wavelength when fitted globally as described in the text.

the possibility of interaction between the CGA peptide and the IP_3 receptor peptide at pH 5.5 (Fig. 2). Analysis of the concentration distribution data of the equimolar mixture of the CGA peptide and the IP_3 receptor peptide (Fig. 2) indicated that the CGA peptide and the IP_3 receptor peptide interacted with a 1:1 stoichiometry at pH 5.5; no other association scheme approached viability. The mathematical model for this association is given by

$$c_r = c_{b,A,1}\exp(A_A M_A(r^2 - r_b^2)) + c_{b,P,1}\exp(A_P M_P(r^2 - r_b^2))$$
$$+ c_{b,A,1}c_{b,P,1}\exp(\ln K_{(AP)} - \ln E_A E_B/(E_A + E_P))$$
$$+ (A_A M_A + A_P M_P)(r^2 - r_b^2)) + \varepsilon$$

where $\ln K_{(AP)}$ is the logarithm of the molar equilibrium constant for the formation of the heterotetramer, where E_A and E_P are the molar extinction coefficients of the CGA peptide and the IP_3 receptor peptide monomer, respectively, and where the other terms have their usual meanings as described above. The logarithmic term of the extinction coefficients is needed to convert the molar equilibrium constant to an equilibrium constant on an absorbance scale, since this is the unit of concentration actually measured. This equation was used to generate three models for globally fitting the interactions using scans at 280, 295, and 310 nm with appropriate values of E_A and E_P, obtaining $\ln K_{(AP)}$ as a global fitting parameter and the c_As and ε as local fitting parameters.

3.2. Determination of the values of the thermodynamic parameters

The values of the thermodynamic parameters ΔH^O, ΔS^O and ΔC_P^O were determined from the values of ΔG^O as a function of temperature, calculating ΔG^O using:

$$\Delta G^O = -RT \ln K_T \quad (1)$$

Starting with the basic relationships of ΔG^O, ΔH^O, ΔS^O and

ΔC_P^O, ΔG^O as a function of temperature can be written:

$$\Delta G_T^O = \Delta H_{T_R}^O + T\Delta C_{P,T_R}^O(1-T_R/T-\ln(T/T_R))+$$

$$TT_R(d\Delta C_{P,T}^O/dT)(T_R/T-T/T_R+2\ln(T/T_R))/2 \quad (2)$$

where T_R is a reference temperature, here taken to be 273.15 K, and where it is assumed that $d\Delta C_{P,T}^O/dT$ is constant with respect to temperature.

When all of the data obtained were fitted in Eq. 2, it was found that the use of the $d\Delta C_P^O/dT$ term did not improve the quality of the fits and gave significantly larger standard errors for the parameters. Accordingly, it was assumed that ΔC_P^O was constant with temperature and Eq. 2 was terminated after the ΔC_P^O term. Thus, only $\Delta H_{T_R}^O$, $\Delta S_{T_R}^O$, and $\Delta C_{P,T_R}^O$ were used as fitting parameters. In order to perform weighted fits, each value of ΔG^O was weighted with the reciprocal of its variance, which was calculated from the standard error of $\ln K_{(AP)}$ obtained when fitting the concentration distribution data for a given temperature.

The plot illustrating the fitting of ΔG^O as a function of temperature using Eq. 2 for the heterodimer interaction is shown in Fig. 3A. Fig. 3B illustrates the distribution of the calculated values of ΔG^O, ΔH^O, and $T\Delta S^O$ as a function of temperature. The values of the thermodynamic parameters are given in Table 1.

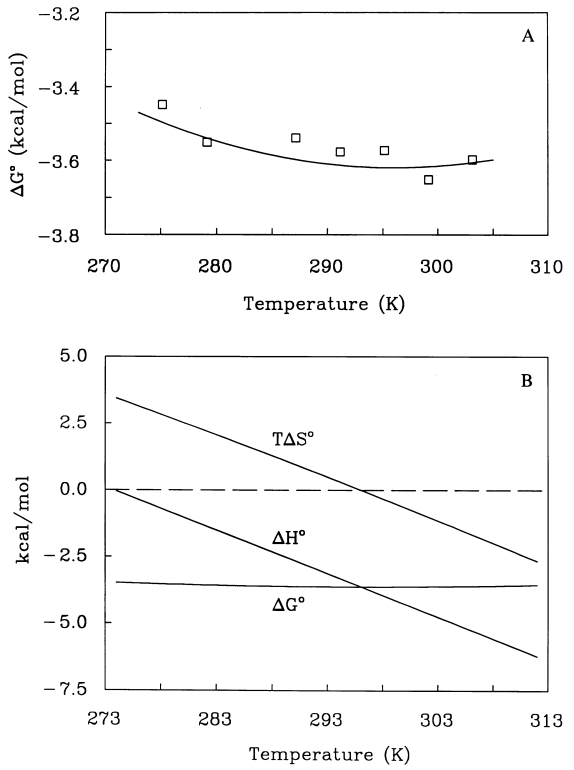


Fig. 3. Thermodynamic parameters as a function of temperature for the formation of the conserved near N-terminal CGA peptide-IP_3 receptor intraluminal loop peptide heterodimer at pH 5.5 in the presence of Ca^{2+}. A: The values of ΔG^O were calculated from the values of $\ln K_T$ using Eq. 1 and the data were fitted using Eq. 2. Since the standard errors whose magnitudes were obtained by Monte-Carlo simulations give error bars approximately the same size as the squares indicating the data points, the error bars have been omitted for simplicity. B: The values of ΔG^O, ΔH^O, and $T\Delta S^O$ were calculated using Eq. 2 and with the values of $\Delta H_{T_R}^O$, $\Delta S_{T_R}^O$, and $\Delta C_{P,T_R}^O$ obtained when fitting the data illustrated in A.

Table 1
Thermodynamic parameters for the interaction of the intraluminal loop peptide of the IP_3 receptor with the conserved N-terminal region of chromogranin A at 310.15 K (37°C)[a]

	pH 5.5, 1 mM Ca^{2+}
ΔG^O (kcal/mol1)	−3.57
ΔH^O (kcal/mol)	−5.91
ΔC_P^O (kcal/mol/K)	−0.16
$T\Delta S^O$ (kcal/mol)	−2.35

[a]The standard state was chosen to be 1 mol/l of each reactant.
ΔG^O values are calculated from the values of the fitting parameters ΔH^O, ΔS^O and ΔC_P^O. ΔC_P^O is assumed to be constant with temperature since the inclusion of $d\Delta C_P^O/dT$ in the mathematical model did not improve the quality of the fits.

4. Discussion

Since the discovery of secretory granules of adrenal medullary chromaffin cells as a major IP_3-sensitive Ca^{2+} store [1], the IP_3-sensitive Ca^{2+} store role of the secretory granules of insulin-containing pancreatic β-cells has also been demonstrated [2]. The IP_3-sensitive Ca^{2+} store role of secretory granules is ascribed to the presence of the high capacity, low affinity Ca^{2+} storage protein, CGA, in the secretory vesicle [1,5]. CGA is known to bind 30–50 mol Ca^{2+}/mol protein with dissociation constants of 2–4 mM [5]. Further, CGA is known to interact with several secretory vesicle membrane proteins including the IP_3 receptor/Ca^{2+} channel at the intravesicular pH of 5.5 and to dissociate from them at a near physiological pH of 7.5 [6]. In addition to the pH-dependent membrane binding property, CGA exists primarily in a dimeric state at pH 7.5 and in a tetrameric state at pH 5.5 [20]. Accordingly, in an experiment carried out with intact CGA and an intraluminal loop peptide of the IP_3 receptor/Ca^{2+} channel, tetrameric CGA was shown to interact with four molecules of the intraluminal loop peptide of IP_3 receptor [11].

Due to the fact that the conservative near N-terminal region of CGA has been shown to interact with the secretory vesicle membrane at pH 5.5 and to dissociate from it at pH 7.5 [9], there was a possibility that the conservative near N-terminal region of CGA might interact with the intraluminal loop region of the IP_3 receptor/Ca^{2+} channel. Consistent with the possibility, the present results demonstrate that the conserved near N-terminal region of CGA interacts with the intraluminal loop region of IP_3 receptor. Although the ΔG^O value of −3.57 kcal/mol (Table 1) suggests a weak interaction, it was nonetheless clear that the N-terminal CGA peptide and the IP_3 receptor loop peptide interact at pH 5.5, and the interaction became more stable as the temperature increased (Fig. 3). In view of the interaction of tetrameric CGA with the intraluminal loop peptide of IP_3 receptor and the proposed anchor role of the conservative near N-terminal region of CGA, the interaction between the conservative near N-terminal region of CGA and the intraluminal loop region of IP_3 receptor was expected. The interaction of the conserved near N-terminal region with the intraluminal loop of IP_3 receptor will free the C-terminal side of CGA to interact with each other or with other secretory vesicle matrix proteins. Indeed, the C-terminal region of CGA has been shown to participate

in the dimerization and tetramerization of CGA at pH 7.5 and 5.5, respectively [21].

Like the conserved near N-terminal region of CGA, the conserved near N-terminal region of CGB also interacted with the intraluminal loop region of IP_3 receptor (Yoo, S.H. and Lewis, M.S., unpublished result). Given that the amino acid sequences of the conserved near N-terminal regions of CGA and CGB are not identical [12,13,15,16,22], it appears that the intraluminal loop region of IP_3 receptor recognizes a certain structural feature, not a fixed series of amino acid residues, of the anchor region on chromogranins for interaction. In light of the formation of CGA_2CGB_2 heterotetramer at the intravesicular pH of 5.5 [23] and the interaction of the conserved near N-terminal region of CGB with the intraluminal loop region of IP_3 receptor (Yoo, S.H. and Lewis, M.S., unpublished result), the present results point to a direct coupling between the heterotetrameric IP_3 receptor/Ca^{2+} channel and heterotetrameric chromogranins. Direct coupling between the IP_3 receptor/Ca^{2+} channel and chromogranins will ensure a direct and rapid communication of IP_3 binding at the N-terminal side of the IP_3 receptor/Ca^{2+} channel to the Ca^{2+} storage proteins, CGA and CGB, for Ca^{2+} release. Since the binding of IP_3 to the N-terminal side of the IP_3 receptor is known to induce a conformational change of the IP_3 receptor [24], the conformational change of the IP_3 receptor will be directly and rapidly transmitted to chromogranins, which in turn are expected to undergo a conformational change, leading to dissociation of some Ca^{2+} for release into the cytoplasm.

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