Thermodynamic Study of the pH-Dependent Interaction of Chromogranin A with an Intraluminal Loop Peptide of the Inositol 1,4,5-Trisphosphate Receptor

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ABSTRACT: The secretory vesicles of adrenal chromaffin cells have previously been identified as a major inositol 1,4,5-trisphosphate (IP_3)-sensitive Ca^{2+} store, and their Ca^{2+} store role has been attributed to the presence of chromogranin A, a high capacity, low affinity Ca^{2+} binding protein. Chromogranin A has since been shown to exist primarily in a dimeric state at pH 7.5 and primarily in a tetrameric state at the intravesicular pH of 5.5 and has also been shown to interact with the membrane proteins of secretory vesicles at pH 5.5, including a 260-kDa protein reactive to IP_3 receptor antibody [Yoo, S. H. (1994) *J. Biol. Chem. 269*, 12001−12006]. In a recent study, chromogranin A was shown to interact with one of the intraluminal loop regions of the IP_3 receptor at pH 5.5 but not at pH 7.5 [Yoo, S. H., & Lewis, M. S. (1994) *FEBS Lett. 341*, 28−32]. To gain further insight, we have studied the temperature dependence of the pH-dependent interaction of chromogranin A with the intraluminal peptide of the the IP_3 receptor by analytical ultracentrifugation, using multiwavelength scan analysis, and found that four molecules of the intraluminal domain peptide of the IP_3 receptor bound to each chromogranin A tetramer with $\Delta G°$ values ranging from −23.6 to −27.6 kcal mol^{-1} in the absence and presence of 35 mM Ca^{2+}. In the presence of 35 mM Ca^{2+}, the interaction between chromogranin A tetramer and the intraluminal domain peptide of the IP_3 receptor showed $\Delta G°$ values of increasing magnitude as the temperature was increased while the same interaction in the absence of Ca^{2+} showed $\Delta G°$ values of decreasing magnitude. Further, the values for the $\Delta H°$ and $\Delta S°$ of the interaction increased in the absence of Ca^{2+} but decreased in the presence of 35 mM Ca^{2+} as the temperature was increased, indicating the stabilizing effect of Ca^{2+} on the interaction.

The secretory vesicles of adrenal medullary chromaffin cells have been identified as a major IP_3-sensitive[1] intracellular Ca^{2+} store (Yoo & Albanesi, 1990a), and 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) (Yoo & Albanesi, 1990a, 1991). Not only are the secretory vesicles the storage site for chromogranin A and catecholamine, but they also contain high concentrations of Ca^{2+} and H^+, resulting in the intravesicular Ca^{2+} concentration of 40 mM (Winkler & Westhead, 1980; Bulenda & Gratzl, 1985) and the intravesicular pH of 5.5 (Johnson & Scarpa, 1976; Casey et al., 1977). Chromogranin A has previously been shown to undergo pH- and Ca^{2+}-dependent conformational changes (Yoo & Albanesi, 1990b; Yoo & Ferretti, 1993). Moreover, chromogranin A is also known to exist in a primarily tetrameric state at the intravesicular pH of 5.5 and in a primarily dimeric state at the near physiological pH of 7.5 (Yoo & Lewis, 1992), which suggested a dimeric existence of CGA in the endoplasmic reticulum and *cis*-Golgi cisternae, and a tetrameric existence in the *trans*-Golgi network (TGN) and secretory vesicles. The different oligomerization states appeared to be due to the different conformations of CGA at the two pH levels (Yoo & Albanesi, 1991, 1990b; Yoo & Ferretti, 1993; Yoo & Lewis, 1992), i.e., additional sites of interaction are exposed due to the acidic pH-induced conformational changes. Subsequent studies indicated that the conserved C-terminal region is responsible for the dimerization and tetramerization of CGA (Yoo & Lewis, 1993), and the C-terminal region was shown to undergo a drastic conformational change from pH 7.5 to 5.5, changing from 15% α-helical conformation at pH 7.5 to 52% α-helical conformation at pH 5.5 (Yoo & Ferretti, 1993).

Recently it was also shown that CGA interacted with the vesicle membrane at pH 5.5 and was dissociated from it at pH 7.5 (Yoo, 1993) and that the membrane binding ability of CGA is thought to be due to the pH-induced anchor role of the conserved near N-terminal region of CGA (Yoo, 1993). In particular, the pH-dependent interaction of CGA with the vesicle membrane was shown to be due to the interaction between CGA and protein component(s) on the intraluminal side of the vesicle membrane, since tryptic digestion of the cytoplasmic side of the vesicle membrane alone did not affect the CGA−membrane interaction, but tryptic digestion of both intraluminal and cytoplasmic sides of the membrane completely destroyed the membrane's ability to interact with CGA (Yoo, 1993).

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[1] Abbreviations: CGA, chromogranin A; IP_3, inositol 1,4,5-trisphosphate; TGN, *trans*-Golgi network.

Further studies indicated that CGA interacts with several membrane proteins of the secretory vesicle membrane at pH 5.5 and dissociates from them at pH 7.5 (Yoo, 1994). Although it is not clear whether CGA interacts directly with all of these proteins or interacts with only one or two to which the rest are bound, one of the CGA-interacting membrane proteins was tentatively identified as the IP_3 receptor (Yoo, 1994). The fact that the IP_3 receptors are thought to have eight transmembrane domains at the C-terminal region with the C-terminus facing the cytoplasm and to exist as homotetramers in the membrane (Supattapone et al., 1988; Maeda et al., 1990; Chadwick et al., 1990) led to the possibility that the intraluminal region(s) of IP_3 receptor might interact with CGA in a pH-dependent manner. Indeed, it was found in our recent study that one of the intraluminal loops of the IP_3 receptor interacted with CGA at the intravesicular pH of 5.5 but not at pH 7.5 (Yoo & Lewis, 1994). 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. To gain further insight into the mechanism of the interaction, we have studied the pH- and temperature-dependent interaction of CGA with the intraluminal peptide of the IP_3 receptor as determined by analytical ultracentrifugation, using multiwavelength scan analysis (Lewis et al., 1994a,b). We have found that four molecules of the intraluminal domain peptide of the IP_3 receptor bound to each CGA tetramer and that the binding mechanism was governed by the presence or absence of calcium.

EXPERIMENTAL PROCEDURES

Purification of Chromogranin A. Chromogranin A was purified from bovine adrenal chromaffin granules according to the method of Yoo and Albanesi (1990b).

IP_3 Receptor Intraluminal Loop Peptide Synthesis. A peptide with the sequence of DVLRRPSKDEPLFAARVVYD, representing 20 amino acids of an intraluminal loop domain (L2 of Figure 1) of rat type 2 IP_3 receptor (Südhof et al., 1991), was synthesized with the addition of a 5-OH Trp at the N-terminus as a chromophore. The use of 5-OH Trp is necessary since the multiwavelength analysis requires that the reactants have significantly different absorption spectra. The synthesized peptide was purified by high-performance liquid chromatography, and the integrity of the peptide was ensured through analysis by fast atom bombardment mass spectrometry and by amino acid composition analysis. The purity of the peptide was in excess of 98%.

Analytical Ultracentrifugation. Analytical ultracentrifugation was performed in a Beckman XL-A analytical ultracentrifuge at 10 000 rpm and at temperatures of 6, 10, 14, 18, and 22 °C. Three six-channel centerpieces, one cell for each type of buffer, were used. In each cell the upper compartment contained CGA, the lower compartment contained the peptide, and the center compartment contained a mixture of CGA and the peptide having the same concentrations that they had in their respective compartments. The compartment adjoining each sample contained reference buffer. Sample volumes were 0.12 mL, giving column heights of approximately 3 mm. The buffers used were as follows: (1) 20 mM sodium acetate, pH 5.5, 0.1 M KCl; (2) 20 mM sodium acetate, pH 5.5, 0.1 M KCl, 35 mM Ca^{2+}; (3) 20 mM Tris HCl, pH 7.5, 0.1 M KCl. Each of the cells that contained no Ca^{2+} had 2 mM EGTA to ensure the absence of any Ca^{2+} effect.

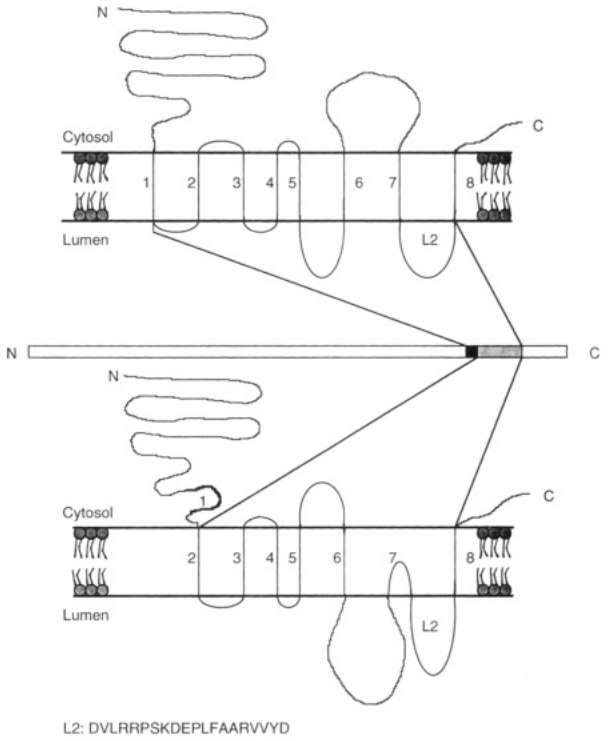

FIGURE 1: Predicted topology of rat type 2 IP_3 receptor showing six or eight transmembrane regions. The predicted topology with eight transmembrane regions (Mignery et al., 1989; Südhof et al., 1991) is shown in the upper half and the one with six transmembrane regions (Michikawa et al., 1994) is shown in the lower half. The eight corresponding regions in both models are numbered 1−8. The amino acid sequence of the intraluminal loop L2 of rat type 2 IP_3 receptor (Südhof et al., 1991) is shown. The amino acid sequence in L2 is identical to that of rat type 1 IP_3 receptor (Mignery et al., 1990) except that there are three conserved changes in L2.

Apparent partial specific volumes, ϕ^*, were calculated for 25 °C from the amino acid sequences using the values of Zamyatnin (1984). A value of $\Delta\phi^*/\Delta T = 0.00035$ cm^3 g^{-1} deg^{-1} was used to calculate the values of ϕ^* at other temperatures. The extinction coefficients of the CGA and the peptide at 280 nm were determined spectrophotometrically, and the extinction coefficients at the other wavelengths used were calculated from the ratios of the concentration gradients at equilibrium measured at those wavelengths to the concentration gradients measured at 280 nm. Measurements were made at 280, 290, 295, 300, 305, and 310 nm, which encompassed a range of wavelengths from those where the CGA absorbance had relative dominance to those where the peptide absorbance had relative dominance.

Multiwavelength Scan Analysis. This method of analysis is described in detail elsewhere (Lewis et al., 1994a,b) and will be described only briefly here. It requires that the two reactants have significantly different absorption spectra over an experimentally appropriate range of wavelengths. These reactants are denoted by the subscripts P and C for the peptide and CGA, respectively.

Assuming that Beer's law is obeyed, the absorbance of a solution at a given wavelength, λ, is the product of the molar concentration and the extinction coefficient of the solute at that wavelength. The optical system of the ultracentrifuge only permits the observation of the total absorbance as a

function of radial position at a particular wavelength, which is given by

$$A_{T,r,\lambda} = E_{P,\lambda}C_{P,T,r} + E_{C,\lambda}C_{C,T,r} \quad (1)$$

where E_P and E_C are the molar extinction coefficients of the peptide and CGA, respectively, and $C_{P,T,r}$ and $C_{C,T,r}$ are the molar concentrations of these reactants. The subscript T denotes total concentration, i.e., free plus complexed.

Consider the case of measuring the absorbance at several wavelengths, but at only one radial position. We now have A at wavelengths λ_i, $i = 1$ to m, and eq 1 becomes a set of m equations in the two unknowns $C_{P,T,r}$ and $C_{C,T,r}$. The m A's form a one-row matrix **A**, the E's form a $2 \times m$ matrix of coefficients, **E**, and the C's form the two-element row matrix, **C**, such that $\mathbf{A} = \mathbf{CE}$. The least-squares solution to this set of equations is $\mathbf{C} = \mathbf{AE}^+$, where $\mathbf{E}^+$ is the Moore–Penrose pseudoinverse of **E** (Strang, 1986).

Extending this to n radial positions, let **A** be an $n \times m$ matrix of absorbancies, such that $A_{i,j}$ is the absorbance for radius r_i and wavelength λ_j. Thus, each column of **A** is the radial distribution of absorbance at a fixed value of λ; each row of **A** represents the absorption spectrum of the solution at discrete wavelengths for a given value of radius over the wavelength range of the values of λ. In similar fashion, **E** is a $2 \times m$ matrix of molar extinction coefficients, where row 1 is for the peptide and row 2 is for CGA. Thus, **C** is an $n \times 2$ matrix computed by $\mathbf{C} = \mathbf{AE}^+$, where the two columns of **C** contain total molar concentrations, uncomplexed and complexed, of the peptide and CGA, respectively, and each column of **C** is a function of r. The matrix **C**, the dependent variables, is now concatenated with a vector of the radial positions, the independent variable, to form a three-column data matrix suitable for analysis by nonlinear, least-squares curve-fitting with appropriate mathematical models.

The values of **C** at any given radial position represent the linear least-squares fit to the corresponding radial values of **A**. Since the transformation is linear, there is no distortion in error distribution; it has been demonstrated for experimental data as well as for simulated data that if the error in **A** is normally distributed, then the error is also normally distributed in **C**. Additionally, if the error of the absorbancies is not normally distributed, the error of the molar concentrations will closely approximate a normal distribution as a result of the transformation.

RESULTS

Analysis of Ultracentrifuge Data. The analysis of multiwavelength data first requires the construction of the **E** and **A** matrices. Construction of the **E** matrix requires obtaining the molar extinction coefficients of the peptide and the CGA at equilibrium at the different experimental wavelengths defined by the XL-A monochromater. Because the gradients of the peptide were so shallow at 10 000 rpm, the mean absorbance of the central 50% of each gradient at each wavelength was obtained and the ratio of that mean absorbance to the mean absorbance at 280 nm was calculated. The operative molar extinction coefficients of the peptide at the wavelengths other than 280 nm were then taken to be the products of those ratios and the spectrophotometrically measured molar extinction coefficient of the peptide at 280 nm (6875 mol^{-1} cm^{-1}). The molar extinction coefficients of the CGA were obtained in a similar manner except that the ratios of the absorbance gradients between the same lower and upper radial positions at each wavelength were used. A value of 56 600 mol^{-1} cm^{-1} at 280 nm was used as the reference molar extinction coefficient for CGA (Yoo & Lewis, 1992). The **E** matrix was then constructed with the values of the molar extinction coefficients of the peptide in the first row and the values of the molar extinction coefficients of CGA in the second row; the columns corresponded to 280, 290, 295, 300, 305, and 310 nm. The $\mathbf{E}^+$ matrix was then obtained by taking the Moore–Penrose pseudoinverse of **E**.

Construction of the **A** matrix required using the absorbancies of the peptide–CGA mixture gradients at equilibrium at those same wavelengths. Exact radial positions for each row of the matrix required using identical lower and upper radial positions and radial increments for each wavelength; these define the radius vector. Since the XL-A sometimes skips an exact radial increment, a small amount of interpolation was required. The columns of the **A** matrix thus are the absorbancies for the same wavelengths as the **E** matrix for the radial positions defined by the radius vector. The **C** matrix was thus the product of **A** and $\mathbf{E}^+$, and the data matrix was constructed by concatenating the radius vector with the **C** matrix.

Development of mathematical models for the binding of the peptide to CGA requires cognizance of two facts: CGA is in a reversible monomer–dimer equilibrium at pH 7.5, in the absence of calcium, and in a reversible monomer–tetramer equilibrium at pH 5.5 with and without calcium. Thus, for pH 7.5, we considered the following possibilities: (1) the peptide might not bind to CGA at all; (2) the peptide might bind only to monomer; (3) the peptide might bind only to dimer; (4) the peptide might bind to both monomer and dimer. Similarly, for pH 5.5, we considered the following possibilities: (1) the peptide might not bind to CGA at all; (2) the peptide might bind only to monomer; (3) the peptide might bind only to tetramer; (4) the peptide might bind to both monomer and tetramer; (5) that binding might induce dimer formation and that the peptide might bind to monomer, dimer, and tetramer.

Appropriate mathematical models were written to test these various possibilities and the criterion of best fit was used to select the optimum models. Best fit was defined by the criteria of minimum sum of squares of the residuals, the most random distribution of the residuals, and minimum standard errors of the parameter values. It was found that at pH 7.5 there was no binding of the peptide to either CGA monomer or dimer. In contrast, at pH 5.5, either in the absence or presence of 35 mM Ca^{2+}, it was found that 4 mol of peptide were bound per mole of CGA tetramer and that no other modalities of binding were viable models.

The mathematical models for the distributions of the peptide and CGA at pH 7.5 are given by

$$C_{r,T,P} = C_{b,P} \exp[A_P M_P (r^2 - r_b^2)] + \epsilon_P \quad (2)$$

and

$$C_{r,T,C} = C_{b,C} \exp[A_C M_C (r^2 - r_b^2)] + \epsilon_C \quad (3)$$

where $C_{r,T}$ is the total molar concentration of either peptide (P) or CGA (C) as a function of radius; C_b is the corresponding concentration at r_b, the radius of the cell bottom; M is

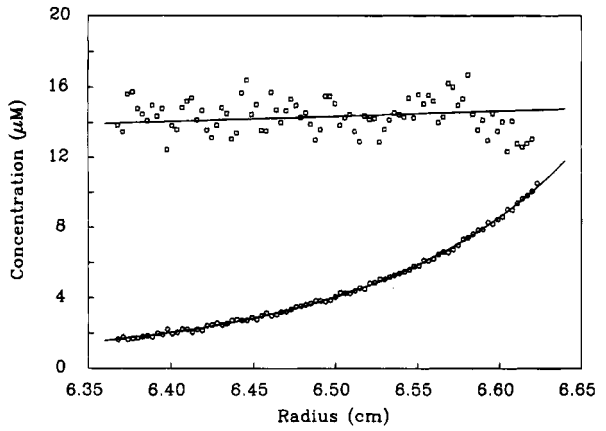

FIGURE 2: Distribution of equilibrium molar concentrations of chromogranin A and an intraluminal peptide of the IP_3 receptor at pH 7.5. Concentration distributions of CGA dimer (lower points, circles) and the intraluminal peptide (L2) of the IP_3 receptor (upper points, squares) in 20 mM Tris-HCl, pH 7.5, and 0.1 M KCl is shown at ultracentrifugal equilibrium at 14 °C in the absence of Ca^{2+} in the buffer. The lines show the best-fitting curves for the model of noninteraction between CGA and the peptide.

the calculated molar mass (2549 for peptide and 48 102 for CGA); $A = (1 - \phi^*\varrho)\omega^2/2RT$, where ϕ^* is the compositional apparent partial specific volume, ϱ is the solution density, ω is the rotor angular velocity, R is the gas constant, and T is the absolute temperature; ϵ is a small baseline error term. If the peptide extinction coefficients comprise the first row and the CGA extinction coefficients comprise the second row of the **E** matrix, then eq 2 is fit to columns 1 and 2 and eq 3 is fit to columns 1 and 3 of the data matrix. Attempts to fit models such as described below by eqs 4 and 5 failed; the returned values of ln K were always the largest negative number the computer could return, thus indicating that $K = 0$ (i.e., no binding). Since there is no binding, it does not matter whether or not simultaneous fitting is performed. The fit of these equations to data from peptide and CGA at pH 7.5 is shown in Figure 2. $C_{b,P}$, $C_{b,C}$, ϵ_C, and ϵ_P are the fitting parameters.

The mathematical models for the distributions of the peptide and CGA at pH 5.5 with binding of four peptides to the CGA tetramer are given by

$$C_{r,T,P} = C_{b,P}\exp[A_P M_P(r^2 - r_b^2)] + 4C_{b,P}{}^4C_{b,C_4} \exp[\ln K + 4(A_P M_P + A_C M_C)(r^2 - r_b^2)] + \epsilon_P \quad (4)$$

and

$$C_{r,T,C} = C_{b,C}\exp[A_C M_C(r^2 - r_b^2)] + C_{b,C_4} \exp[4A_C M_C(r^2 - r_b^2)] + 4C_{b,P}{}^4C_{b,C_4} \exp[\ln K + 4(A_P M_P + A_C M_C)(r^2 - r_b^2)] + \epsilon_C \quad (5)$$

Since there is binding, eqs 4 and 5 are jointly fit to columns 1 and 2 and columns 1 and 3 of the data matrix, respectively. The joint fit of these equations to data from the peptide and CGA at pH 5.5 without Ca^{2+} is shown in Figure 3. $C_{b,P}$, $C_{b,C}$, $C_{b,C4}$, ln K, ϵ_P, and ϵ_C are the fitting parameters. Attempts to fit models involving either more than one or less than one peptide per CGA monomer also failed as indicated by significantly larger RMS errors and significantly larger parameter errors. The fits obtained were also significantly inferior as judged by visual inspection.

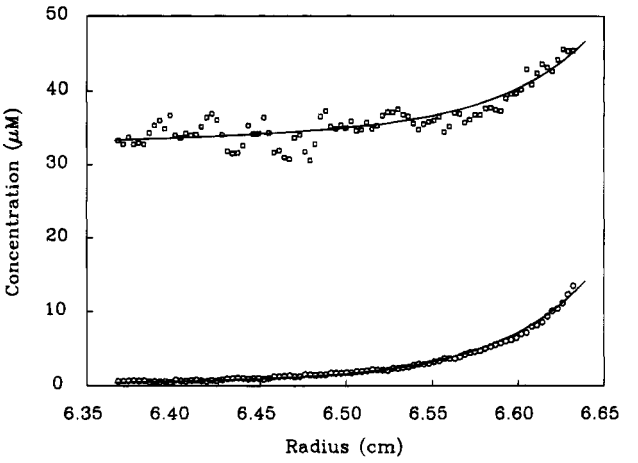

FIGURE 3: Distribution of equilibrium molar concentrations of chromogranin A and an intraluminal peptide of the IP_3 receptor at pH 5.5. Concentration distributions of CGA and the intraluminal peptide (L2) of the IP_3 receptor in 20 mM sodium acetate, pH 5.5, and 0.1 M KCl are shown at ultracentrifugal equilibrium at 14 °C in the absence of Ca^{2+} in the buffer. Distribution of the peptide and the CGA tetramer–peptide complex (upper points, squares), and CGA monomer, CGA tetramer, and the CGA tetramer–peptide complex (lower points, circles). Concentration distributions of CGA and the peptide in the presence of 35 mM Ca^{2+} showed similar results. The lines show the best-fitting curves for the CGA tetramer–peptide interaction model with ln $K = 41.78 \pm 0.23$ and an RMS error of 0.87 μM. It is readily apparent that most of this RMS error comes from the noise in the distribution of peptide and CGA tetramer–peptide complex.

Determination of the Values of the Thermodynamic Parameters. The values of the thermodynamic parameters $T\Delta H°$, $\Delta S°$, and $\Delta C_P°$ were determined by calculating the values of $\Delta G°$ using

$$\Delta G° = -RT \ln K_T \quad (6)$$

and fitting the values of $\Delta G°$ as a function of temperature using a mathematical model derived from standard definitions of the various thermodynamic parameters as follows:

$$\Delta G_T° = \Delta H_T° - T\Delta S_T° \quad (7)$$

$$\Delta H_T° = \Delta H_{T_R}° + \int_{T_R}^{T} \Delta C_{P,T}° \, dT \quad (8)$$

$$\Delta S_T° = \Delta S_{T_R}° + \int_{T_R}^{T} (\Delta C_{P,T}°/T) \, dT \quad (9)$$

$$\Delta C_{P,T}° = \Delta C_{P,T_R}° + \int_{T_R}^{T} (d\Delta C_{P,T}°/dT) \, dT \quad (10)$$

where T_R is a reference temperature, here taken to be 273.15 K, and where it is assumed that $d\Delta C_{P,T}°/dT$ is constant with respect to temperature. When the integrations indicated in eqs 8, 9, and 10 were carried out and the results substituted in eq 7 and simplified, one then obtains

$$\Delta G_T° = \Delta H_{T_R}° + T\Delta S_{T_R}° + T\Delta C_{P,T_R}°[1 - T_R/T - \ln(T/T_R)] + TT_R(d\Delta C_{P,T}°/dT)[T_R/T - T/T_R + 2\ln(T/T_R)]/2 \quad (11)$$

When the data obtained, either without or with calcium present, were fit with eq 11, it was found that the use of the

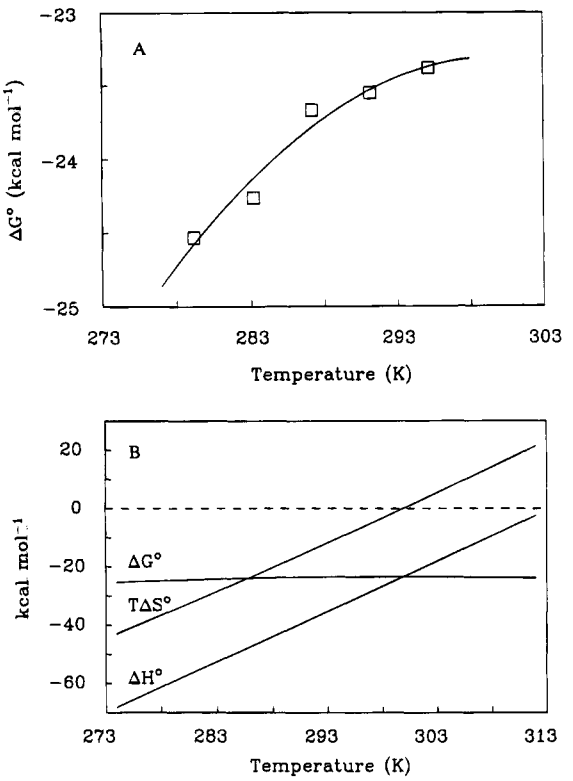

FIGURE 4: (A) Values of $\Delta G°$ as a function of temperature for the interaction of CGA tetramer with four molecules of the intraluminal loop peptide of IP_3 receptor at pH 5.5 in the absence of calcium. The values of $\Delta G°$ were calculated from the values of ln K_T using eq 6, and the data were fit using eq 11. (B) Values of $\Delta G°$, $\Delta H°$, and $T\Delta S°$ as a function of temperature for the interaction of CGA tetramer with four molecules of the intraluminal loop peptide of IP_3 receptor at pH 5.5 in the absence of calcium. The values of $\Delta G°$, $\Delta H°$, and $T\Delta S°$ were calculated using eq 11 and the integrated forms of eqs 8 and 9, respectively, with the values of $\Delta H_{T_R}°$, $\Delta S_{T_R}°$, and $\Delta C_{P,T_R}°$ obtained when fitting the data illustrated in Figure 4A.

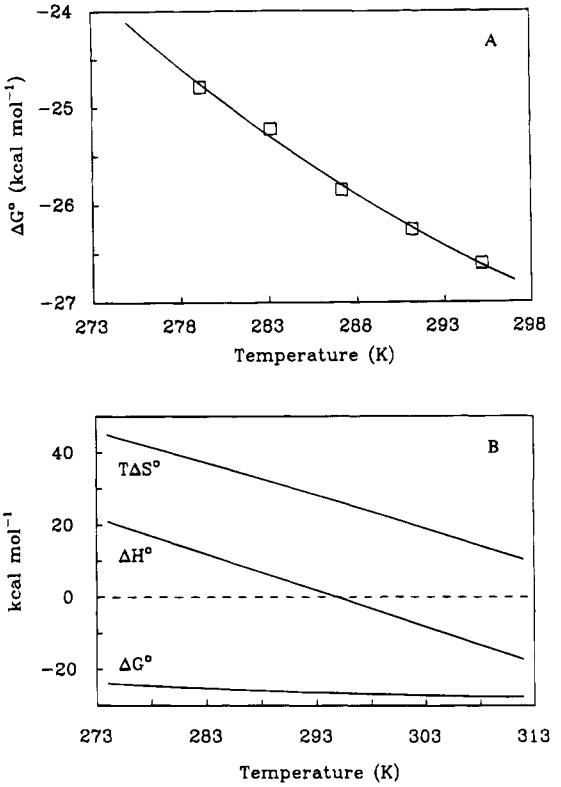

FIGURE 5: (A) Values of $\Delta G°$ as a function of temperature for the interaction of CGA tetramer with four molecules of the intraluminal loop peptide of IP_3 receptor at pH 5.5 in the presence of 35 mM calcium. The values of $\Delta G°$ were calculated as in Figure 4A. (B) Values of $\Delta G°$, $\Delta H°$, and $T\Delta S°$ as a function of temperature for the interaction of CGA tetramer with four molecules of the intraluminal loop peptide of IP_3 receptor at pH 5.5 in the presence of 35 mM calcium. The values of $\Delta G°$, $\Delta H°$, and $T\Delta S°$ were calculated as in Figure 4B.

$d\Delta C_P°/dT$ term did not improve the quality of the fits and gave significantly larger standard errors for the parameters. Accordingly, it was assumed that $\Delta C_P°$ was constant with temperature and eq 11 was terminated after the $\Delta C_P°$ term. Thus, $\Delta H_{T_R}°$, $\Delta S_{T_R}°$, and $\Delta C_{P,T_R}°$ were used as fitting parameters. In order to perform weighted fits, each value of $\Delta G°$ was weighted with the reciprocal of its variance, which was calculated from the standard error of ln K_T obtained when fitting the concentration distribution data for a given temperature.

The plots illustrating the fitting of $\Delta G°$ as a function of temperature using eq 11 for the peptide–CGA tetramer interactions in the absence and presence of 35 mM Ca^{2+} are shown in Figures 4A and 5A. Figures 4B and 5B illustrate the distributions of the calculated values of $\Delta G°$, $\Delta H°$, and $T\Delta S°$ as functions of temperature. The values of the thermodynamic parameters are given in Table 1.

Table 1: Values of the Thermodynamic Parameters for the Interaction of the Intraluminal Loop Peptide of the IP_3 Receptor with Chromogranin A Tetramer in the Absence and Presence of Calcium[a]

	0 mM Ca^{2+}		35 mM Ca^{2+}	
	0 °C	37 °C	0 °C	37 °C
$\Delta G°$ (kcal mol^{-1})	−25.4	−23.6	−23.8	−27.6
$\Delta H°$ (kcal mol^{-1})	−69.7 ± 15.2	−5.70	21.8 ± 9.4	−15.4
$T\Delta S°$ (kcal mol^{-1})	−44.3 ± 14.8	23.3	45.6 ± 9.1	12.2
$\Delta C_P°$ (kcal mol^{-1} K^{-1})	1.73 ± 1.07	1.73	−1.01 ± 0.67	−1.01

[a] $\Delta G°$ values are calculated from the values of the fitting parameters $\Delta H°$, $\Delta S°$, and $\Delta C_P°$. $\Delta C_P°$ is assumed to be constant with temperature since the inclusion of $d\Delta C_P°/dT$ in the mathematical model did not improve the quality of the fits. The standard errors listed for $\Delta H°$, $T\Delta S°$, and $\Delta C_P°$ at 0 °C are the errors obtained for these parameters at the reference temperature when fitting the values of $\Delta G°$ as a function of temperature. The errors in $\Delta G°$, calculated from the fitting errors in ln K, range from 0.20 to 0.75 kcal mol^{-1}. These values give a more realistic assessment of the error of $\Delta G°$ than the values calculated using the statistical formula: var $(\Delta G°)$ = var $(\Delta H°)$ + T var $(\Delta S°)$.

DISCUSSION

It has previously been shown that CGA exists in a predominantly tetrameric monomer–tetramer equilibrium at the intravesicular pH of 5.5 and in a predominantly dimeric monomer–dimer equilibrium at the near physiological pH of 7.5 (Yoo & Lewis, 1992). Further, chromogranin A was also shown to bind to the protein component(s) on the intraluminal side of the vesicle membrane at pH 5.5 (Yoo, 1993), indicating that only tetrameric CGA can interact with the protein component(s) of the vesicle membrane. Moreover, our recent results indicated that CGA interacts with an intraluminal loop of the IP_3 receptor/Ca^{2+} channel at pH 5.5 (Yoo & Lewis, 1994). The interaction appeared to be specifically dependent upon the acidic pH, since there was no interaction at a near physiological pH of 7.5 (Yoo, 1994; Yoo & Lewis, 1994).

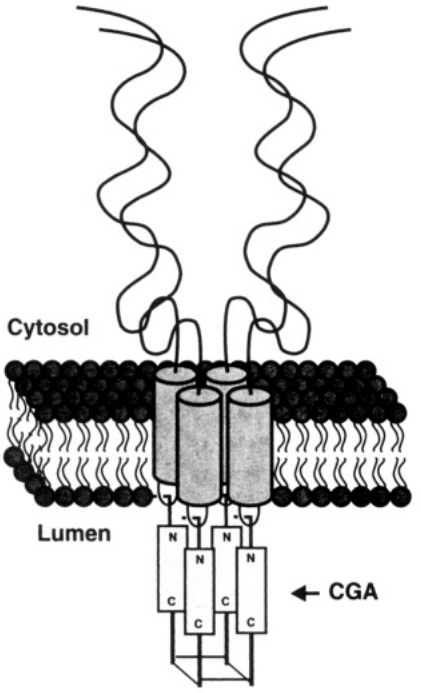

FIGURE 6: Schematic model showing the interaction between the tetrameric IP_3 receptor/Ca^{2+} channel and the tetrameric chromogranin A through the intraluminal loop L2 of IP_3 receptor/Ca^{2+} channel and the near N-terminal region of CGA.

The IP_3 receptor was first cloned from mouse and rat brains with a monomer mass of ~260 kDa (Furuichi et al., 1989; Mignery et al., 1989) and is thought to have six (Michikawa et al., 1994) or eight (Mignery et al., 1989; Südhof et al., 1991) transmembrane regions at the C-terminal end of the molecule (Figure 1). Thus, most of the IP_3 receptor is directed toward the cytoplasm, exposing very little of the receptor to the intraluminal side. Furthermore, in view of the fact that the IP_3 receptors are known to exist as homotetramers in the membrane (Supattapone et al., 1988; Maeda et al., 1990; Chadwick et al., 1990) and the present results indicated an interaction of four molecules of the intraluminal loop peptide of the IP_3 receptor with tetrameric CGA at pH 5.5, it appears that the present interaction supports the concept of the interaction of tetrameric CGA with tetrameric IP_3 receptor in the cell.

Considering that the conserved near N-terminal region of CGA has recently been shown to interact with the vesicle membrane at pH 5.5 (Yoo, 1993) and thus has been proposed to be the anchor region for interaction with the vesicle membrane protein(s), it appears that the intraluminal loop L2 of the IP_3 receptor binds to the conserved near N-terminal region of CGA with a one to one stoichiometry. In addition, given that the C-terminal region of CGA has been shown to be responsible for tetramerization of CGA (Yoo & Lewis, 1993) and the conserved near N-terminal region of CGA is postulated to function as an anchor for the pH-dependent interaction with the vesicle membrane (Yoo, 1993), the interaction between CGA and IP_3 receptor may be proposed as shown in Figure 6. This model incorporates the present result of an interaction between CGA tetramer and four molecules of L2 peptide, along with the proposed roles of the conserved near N-terminal anchor region and the C-terminal oligomerization region of CGA. According to this model, the conformational change of the IP_3 receptor (Mignery & Südhof, 1990), which is known to occur upon IP_3 binding to the receptor, is expected to be transmitted to the linked CGA. The changes in CGA conformation then may alter the affinity of CGA for Ca^{2+} (Yoo & Albanesi, 1991) and free some Ca^{2+} from CGA, thus resulting in the release of Ca^{2+} through the channel. In this regard, the present model may explain the suggestion that binding of even one molecule of IP_3 can mobilize Ca^{2+} from the IP_3-sensitive Ca^{2+} store (Watras et al., 1991).

The interaction of chromogranin A tetramer with four molecules of L2 peptide in the absence of Ca^{2+} showed $\Delta G°$ values of decreasing magnitude as the temperature was increased (Figure 4A), suggesting that the interaction becomes less stable with increasing temperatures. It showed a $\Delta G°$ of −25.4 kcal/mol with a large negative enthalpy change and a moderate negative change in entropy at 0 °C (Table 1), indicating that the interaction is enthalpically driven with moderate opposition by the unfavorable entropy change. However, the same interaction showed a $\Delta G°$ of −23.6 kcal/mol at 37 °C with a moderate positive entropy change and a moderate negative change in enthalpy. These results suggest that the interacting molecules assume more disordered structures as the temperature increases and the interaction of CGA tetramer with four molecules of L2 peptide at 37 °C is driven by favorable enthalpy and entropy changes.

On the other hand, the interaction between CGA tetramer and four molecules of L2 peptide showed markedly different thermodynamic parameters in the presence of 35 mM Ca^{2+}; the magnitude of the $\Delta G°$ values increased as the temperature was increased (Figure 5A), suggesting that the interaction becomes more stable with increasing temperatures although the $\Delta G°$ values of −23.8 to −27.6 kcal/mol are not grossly different from those in the absence of calcium (Table 1). The enthalpy and entropy changes at 0 °C both showed positive values with a $\Delta G°$ of −23.8 kcal/mol, indicating an entropically driven interaction in the presence of calcium. However, the $\Delta H°$ and $\Delta S°$ values decreased with increasing temperatures, and at 37 °C the same interaction showed a moderate negative enthalpy change and a moderate positive entropy change with a $\Delta G°$ of −27.6 kcal/mol, indicating that the interacting molecules assume more ordered structures as the temperature increases and the interaction is driven by comparable contributions from both enthalpy and entropy changes. In view of the fact that the only difference between the two interaction conditions was the presence of 35 mM Ca^{2+} and the presence of Ca^{2+} increased the magnitude of the $\Delta G°$ values of interaction as the temperature was increased to a physiological range, it appears evident that the presence of Ca^{2+} stabilized the interaction between CGA tetramer and four molecules of the loop peptide. Moreover, given that the secretory vesicles contain 35−40 mM Ca^{2+}, the stabilizing effect of 35 mM Ca^{2+} on the interaction appears to underline the role Ca^{2+} play in the interaction between CGA and intact IP_3 receptor in the vesicle.

Considering that a positive enthalpy change of approximately 1 kcal mol^{-1} per residue is observed in the α-helix to coil transition of proteins and peptides (Scholtz et al., 1991; Ooi & Oobatake, 1991), the negative $\Delta H°$ value (−15.4 kcal mol^{-1}) shown for the interaction between CGA tetramer and the loop peptide at 37 °C in the presence of Ca^{2+} suggests that the interaction is accompanied by significant conformational changes, possibly involving coil to α-helix transitions, leading to more ordered structures.

The $\Delta G°$ values per CGA monomer−L2 peptide interaction are approximately −6 to −6.9 kcal/mol, indicating an interaction of moderate strength. In light of these $\Delta G°$ values, it is likely that the interaction between the intact IP_3 receptor and CGA will be significantly stronger. Despite the postulated strength of the interaction between CGA and the IP_3 receptor/Ca^{2+} channel at the intravesicular pH of 5.5, the two molecules dissociate at a near physiological pH of 7.5. This automatic pH-induced dissociation is consistent with the rapid release of CGA into the bloodstream during exocytosis.

The possibility of the interaction of tetrameric CGA with tetrameric IP_3 receptor suggests a well-ordered structural organization of CGA beneath the membrane as schematically drawn in Figure 6. Although a potential interaction between the sarcoplasmic reticulum Ca^{2+} channels and Ca^{2+} storage protein calsequestrin has previously been postulated (Ikemoto et al., 1989), no direct interaction has been demonstrated yet. Hence, the interaction between the IP_3 receptor/Ca^{2+} channel and the Ca^{2+} storage protein chromogranin A represents the first known example of such a direct interaction between either an IP_3 receptor or a Ca^{2+} channel and a Ca^{2+} storage protein.

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REFERENCES

Bulenda, D., & Gratzl, M. (1985) *Biochemistry 24*, 7760−7765.
Casey, R. P., Njus, D., Radda, G. K., & Sehr, P. A. (1977) *Biochemistry 16*, 972−977.
Chadwick, C. C., Saito, A., & Fleischer, S. (1990) *Proc. Natl. Acad. Sci. U.S.A. 87*, 2132−2136.
Furuichi, T., Yoshikawa, S., Miyawaki, A., Wada, K., Maeda, N., & Mikoshiba, K. (1989) *Nature 342*, 32−38.
Ikemoto, N., Ronjat, M., Mészáros, L. G., & Koshita, M. (1989) *Biochemistry 28*, 6764−6771.
Johnson, R. G., & Scarpa, A. J. (1976) *J. Biol. Chem. 251*, 2189−2191.
Lewis, M. S., Shrager, R. I., & Kim, S.-J. (1994a) *Prog. Colloid Polym. Sci. 94*, 46−53.
Lewis, M. S., Shrager, R. I., & Kim, S.-J. (1994b) in *Modern Analytical Ultracentrifugation: Acquisition and Interpretation of Data for Biological and Synthetic Polymer Systems* (Schuster, T. M., & Laue, T. M., Eds.) pp 94−118, Birkhäuser Boston, Inc.
Maeda, N., Niinobe, M., & Mikoshiba, K. (1990) *EMBO J. 9*, 61−67.
Michikawa, T., Hamanaka, H., Otsu, H., Yamamoto, A., Miyawaki, A., Furuichi, T., Tashiro, Y., & Mikoshiba, K. (1994) *J. Biol. Chem. 269*, 9184−9189.
Mignery, G. A., & Südhof, T. C. (1990) *EMBO J. 9*, 3893−3898.
Mignery, G. A., Südhof, T. C., Takei, K., & DeCamilli, P. (1989) *Nature 342*, 192−195.
Mignery, G. A., Newton, C. L., Archer, B. T., III, & Südhof, T. C. (1990) *J. Biol. Chem. 265*, 12679−12685.
Ooi, T., & Oobatake, M. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 2859−2863.
Scholtz, J. M., Marqusee, S., Baldwin, R. L., York, E. J., Stewart, J. M., Santoro, M., & Bolen, D. W. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 2854−2858.
Strang, G. (1986) in *Introduction to Applied Mathematics*, pp 138−139, Wellesley-Cambridge Press, Wellesley, MA.
Südhof, T. C., Newton, C. L., Archer, B. T., III, Ushkaryov, Y. A., & Mignery, G. A. (1991) *EMBO J. 10*, 3199−3206.
Supattapone, S., Worley, P. F., Baraban, J. M., & Snyder, S. H. (1988) *J. Biol. Chem. 263*, 1530−1534.
Watras, J., Bezprozvanny, I., & Ehrlich, B. E. (1991) *J. Neurosci. 11*, 3239−3245.
Winkler, H., & Westhead, E. (1980) *Neuroscience 5*, 1803−1823.
Yoo, S. H. (1993) *Biochemistry 32*, 8213−8219.
Yoo, S. H. (1994) *J. Biol. Chem. 269*, 12001−12006.
Yoo, S. H., & Albanesi, J. P. (1990a) *J. Biol. Chem. 265*, 13446−13448.
Yoo, S. H., & Albanesi, J. P. (1990b) *J. Biol. Chem. 265*, 14414−14421.
Yoo, S. H., & Albanesi, J. P. (1991) *J. Biol. Chem. 266*, 7740−7745.
Yoo, S. H., & Lewis, M. S. (1992) *J. Biol. Chem. 267*, 11236−11241.
Yoo, S. H., & Ferretti, J. A. (1993) *FEBS Lett. 334*, 373−377.
Yoo, S. H., & Lewis, M. S. (1993) *Biochemistry 32*, 8816−8822.
Yoo, S. H., & Lewis, M. S. (1994) *FEBS Lett. 341*, 28−32.
Zamyatnin, A. A. (1984) *Annu. Rev. Biophys. Bioeng. 13*, 145−165.