Dimerization and Tetramerization Properties of the C-Terminal Region of Chromogranin A: A Thermodynamic Analysis

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ABSTRACT: Chromogranin A, which is a high-capacity, low-affinity Ca^{2+} binding protein, has recently been shown to exist in monomer–dimer and in monomer–tetramer equilibria at pH 7.5 and 5.5, respectively [Yoo, S. H., & Lewis, M. S. (1992) *J. Biol. Chem. 267*, 11236–11241]. The pH appeared to be a necessary and sufficient factor determining the types of oligomer formed. In the present study, using 14 synthetic peptides representing various portions of chromogranin A, we have identified a region in chromogranin A which exhibited dimerization and tetramerization properties at pH 7.5 and 5.5, respectively. Of the 14 peptides, only the conserved C-terminal region (residues 407–431), represented by peptide 14, showed the oligomerization property, existing in a dimeric state at pH 7.5 and in a tetrameric state at pH 5.5. The $\Delta G°$ values of tetramerization were approximately −18.0 kcal/mol, and the $\Delta G°$ value of dimerization was −4.6 kcal/mol. Although peptide 14 represented only 6% of the entire sequence, the $\Delta G°$ value of −18.0 kcal/mol accounted for 80–83% of the $\Delta G°$ values (−21.6 to −22.7 kcal/mol) of tetramerization of intact chromogranin A. Unlike the tetramerization mechanisms of intact chromogranin A where the presence of 35 mM Ca^{2+} changed the tetramerization mechanism from an enthalpically driven to an entropically driven reaction, the tetramerization mechanism of peptide 14 remained entropically driven regardless of the presence of Ca^{2+}. Likewise, dimerization of the peptide was also entropically driven. From these results, it was suggested that the conserved C-terminal region has an intrinsic ability to form a dimer at pH 7.5 and a tetramer at pH 5.5 with little or no conformational changes.

Chromogranin A (CGA)[1] had originally been discovered as the major soluble matrix protein of chromaffin granules of adrenal medullary chromaffin cells (Helle, 1966; Smith & Winkler, 1967; Smith & Kirschner, 1967; Blaschko et al., 1967). Subsequent studies showed that CGA is widely distributed in the neuronal and endocrine cells (O'Connor & Frigon, 1984; Fischer-Colbrie et al., 1987; Sietzen et al., 1987; Weiler et al., 1990). The distribution of CGA in the animal kingdom ranges from human to unicellular organisms such as *Paramecium*, implying that CGA is of very ancient origin (Simon & Aunis, 1989). In human, CGA is found in secretory granules of most cells of neuroendocrine origin, including those of brain, olfactory bulb, lung, heart, skin, and thyroid (Cohn et al., 1982; Hartschuh et al., 1989; Simon & Aunis, 1989; Lahr et al., 1990). Therefore, CGA is now used as a marker protein of neuroendocrine cells (Huttner et al., 1991). Chromogranin A is a high-capacity, low-affinity Ca^{2+} binding protein, binding up to 55 mol of Ca^{2+}/mol of protein with dissociation constants (K_d) of 2–4 mM (Yoo & Albanesi, 1991), and is supposed to be responsible for the inositol 1,4,5-trisphosphate-sensitive intracellular Ca^{2+} store function of secretory vesicles (Yoo & Albanesi, 1990a). Also, chromogranin A is known to aggregate and undergo major conformational changes upon binding Ca^{2+} (Yoo & Albanesi, 1990b). Unlike other high-capacity, low-affinity Ca^{2+} binding proteins, i.e., calsequestrin of the sarcoplasmic reticulum (MacLennan & Wong, 1971; Cozens & Reithmeier, 1984) and calreticulin of the endoplasmic reticulum (Ostwald & MacLennan, 1974; MacLennan et al., 1972; Treves et al., 1990), the intravesicular milieu where CGA exists is acidic (Johnson & Scarpa, 1976; Casey et al., 1977), and this acidic milieu appears to play a vital role in shaping CGA for its many physiological functions in the cell.

We have previously shown that CGA undergoes pH-dependent conformational changes, changing from 25% α-helix at a near-physiological pH of 7.5 to 40% α-helix at the intravesicular pH of 5.5 in the absence of calcium, and from 40% α-helix at pH 7.5 to 30% α-helix at pH 5.5 in the presence of 35 mM Ca^{2+} (Yoo & Albanesi, 1990b). Moreover, the Ca^{2+}-dependent aggregation of CGA is also substantially influenced by the pH that CGA aggregates, at a given Ca^{2+} concentration, much faster at pH 5.5 than at pH 7.5 (Yoo & Albanesi, 1990b). Secretogranin II (also called chromogranin C) has also been shown to aggregate upon Ca^{2+} binding at pH 5.2, resulting in the aggregation of secretogranin II from the constitutive protein immunoglobulin G (Gerdes et al., 1989). Hence, it has been proposed that the pH- and Ca^{2+}-dependent aggregation properties of chromograninins play pivotal roles in segregating chromogranins from other non-vesicular proteins (Gerdes et al., 1989; Yoo & Albanesi, 1990b).

Comparison of the amino acid sequences of all the sequenced CGAs revealed two highly conserved regions: one in the near-N-terminal region and the other in the C-terminal region. The N-terminal region, representing residues 1–68, is 87% identical, with 96% conservation among CGA of human (Konecki et al., 1987; Helman et al., 1988), bovine (Benedum et al., 1986; Iacangelo et al., 1986), pig (Iacangelo et al.,

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[1] Abbreviations: CGA, chromogranin A; CaM, calmodulin; EGTA, [ethylenebis(oxyethylenenitrilo)]tetraacetic acid; RMS, root-mean-square.

1988a), rat (Iacangelo et al., 1988b), and mouse (Wu et al., 1991). On the other hand, the C-terminal region, representing residues 391–431, is 90% identical, with 95% conservation among the five sequenced CGAs. The 87–90% sequence identity shows a sharp contrast to the overall 46% sequence identity found in the entire sequence, implying essential roles for these two regions. In fact, residues 40–65 were shown to be involved in the Ca^{2+}-dependent interaction of CGA with calmodulin (CaM) (Yoo, 1992). In the presence of millimolar Ca^{2+}, CGA bound CaM with a 1:1 stoichiometry, and showed a tight binding with a K_d of 17 nM (Yoo, 1992). Similarly, the binding of a synthetic CGA peptide, representing residues 40–65, to CaM also exhibited a 1:1 stoichiometry with a K_d of 13 nM, apparently indicating that residues 40–65 are solely responsible for the Ca^{2+}-dependent binding of CGA to CaM. Furthermore, CGA exists in a monomer–dimer equilibrium at pH 7.5 and in a monomer–tetramer equilibrium at pH 5.5 (Yoo & Lewis, 1992). Extrapolation of the results from the oligomerization studies to 37 °C and 1 mM CGA suggested that CGA is virtually 100% tetramer at pH 5.5 in the presence of 35 mM Ca^{2+} but is 96% dimer at pH 7.5 in the absence of Ca^{2+} (Yoo & Lewis, 1992), the two conditions resembling those seen *in vivo*. These results suggested that CGA is mostly dimer in the ER and *cis*-Golgi area and is essentially all tetramer in the vesicle.

In the present study, 14 peptides representing various regions of CGA were synthesized, and the oligomerization property of these peptides was examined by analytical ultracentrifugation as a part of efforts to identify the region of CGA that might participate in the oligomerization. Among these, only peptide 14, which represents the conserved C-terminal region (residues 407–431) of CGA, exhibited dimerization and tetramerization properties similar to those observed in intact CGA.

EXPERIMENTAL PROCEDURES

Chromogranin A Peptide Synthesis. Fourteen peptides, each representing a 20–27 amino acid sequence of various portions of chromogranin A, were synthesized. The synthesized peptides were purified by high-performance liquid chromatography, and the integrity of the peptides was verified through analyses by fast atom bombardment mass spectrometry and by amino acid composition analyses. The purity of all the peptides was 95% or higher. Since peptides 1–7, 10, 12, and 14 contained no tryptophan, a tryptophan residue was added to the N-terminus of each of these peptides to facilitate analysis by absorption at 280 nm.

Determination of Peptide Concentration. The peptide concentrations were determined using 5500 M^{-1} cm^{-1} as the extinction coefficient at 280 nm.

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 10-MHz 80286 computer as an acquisition system. Using the rapid scan rate, 90 000 data points were acquired in the 18 s required to scan from the outer reference hole to the inner reference hole of the counterbalance. Each recorded point was the average of 100 acquired points; the actual data density was 425 points per centimeter of radial distance in the cell. The inner and outer reference points and the region of interest were selected in the editing of the scan, and the data were saved in the form of millivolts as a function of radial position.

The experiments were run using a six-hole rotor with two counterbalances and four carbon-filled epoxy double-sector cells at a rotor speed of 30 000 rpm. The solutions of chromogranin A peptide 14 (residues 407–431) at concentrations of 0.10 and 0.20 mg/mL either in 20 mM sodium acetate (pH 5.5)/0.1 M KCl or in 20 mM Tris-HCl (pH 7.5)/0.1 M KCl were placed in each cell. Two cells at each pH had no Ca^{2+}; the other two 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. The time required for the attainment of equilibrium at 2 °C with column lengths of approximately 5.5 mm was established by running at the given rotor speed until the scans were invariant; this was achieved by 63 h. The temperature was then increased in 3 °C increments, and scans were taken after 12 h at each temperature until a temperature of 32 °C was attained. It was experimentally verified that this time was sufficient for reequilibration at the increased temperature. The temperature was then reduced to 2 °C again, and scans were taken at this temperature after 40 h in order to evaluate reversibility with temperature. The other peptides (peptides 1–13) were centrifuged at 30 000 rpm for 44 h at 15 °C in 20 mM sodium acetate (pH 5.5)/0.1 M KCl (with the exception of peptides 2 and 3 which were in 20 mM Tris-HCl (pH 7.5)/0.1 KCl due to poor solubility at pH 5.5) with column lengths of approximately 4.5 mm. Only one temperature and one loading concentration of each peptide were used since all the peptides were monomeric. Buffer densities were calculated from the standard table data, and the compositional partial specific volumes were calculated from the amino acid sequence data of the chromogranin A peptides.

Methods of Analysis. At the time of analysis, the data were converted to absorbancies as a function of radial position using a conversion function derived from the data obtained in calibration experiments. Further data manipulation and data analysis by mathematical modeling were performed using MLAB (Knott, 1979) (Civilized Software, Inc., Bethesda, MD) operating on a 33-MHz 80486 computer. MLAB is very well suited for ultracentrifugal data analysis where it is necessary to perform simultaneous weighted nonlinear least-squares curve fits of two or three data sets with a variety of different mathematical models.

Circular Dichroism Spectroscopy. Circular dichroism (CD) spectra were recorded using a Jasco J-600 spectropolarimeter using a cell with a path length of 0.02 cm and a peptide concentration of 50 μM in either 15 mM sodium acetate, pH 5.5, or 15 mM Tris-HCl, pH 7.5. All spectra were taken at 22 °C and were the average of at least two scans.

Chymotrypsin Treatment of Chromogranin A Peptide 14. Chromogranin A peptide 14 (0.3 mg/mL) in 15 mM Tris-HCl (pH 7.5)/0.1 M KCl was mixed with chymotrypsin at a substrate:enzyme ratio of 200:1 (w/w) in a total volume of 1 mL. The reaction mixture was incubated at 25 °C for 1 h; the mixture was then filtered through an Amicon centricon-10 (MW cutoff 10 000) ultrafiltration unit 2 times to separate the peptide from enzyme. The eluate was dialyzed against 300 volumes of either 15 mM sodium acetate (pH 5.5)/0.1 M KCl or 15 mM Tris-HCl (pH 7.5)/0.1 M KCl overnight to remove the cleaved tryptophans.

RESULTS

The mathematical model which was used to obtain the values of the natural logarithms of the equilibrium constants

had the form:

$$c_r = c_{b,1}\exp[AM_1(r^2 - r_b^2)] + c_{b,1}^2\exp[\ln k_{12} + 2AM_1(r^2 - r_b^2)] + c_{b,1}^4\exp[\ln k_{14} + 4AM_1(r^2 - r_b^2)] + \epsilon \quad (1)$$

where $c_{b,1}$ is the concentration of monomer at r_b, the radial position of the cell bottom, M_1 is the molecular mass of the monomer calculated from the amino acid sequence, ϵ is a small base-line error correction term, and $A = (1 - \bar{v}\rho)\omega^2/2RT$, where $\bar{v}$ is the compositional partial specific volume of the monomer calculated from the amino acid sequence (Benedum et al., 1986), ρ is the solvent density, ω is the rotor angular velocity, R is the gas constant, and T is the absolute temperature. In this model, it is assumed that monomer, dimer, and tetramer all have the same partial specific volume. The equilibrium constants for dimer and tetramer formation are defined as $k_{12} = c_{b,2}/c_{b,1}^2$ and $k_{14} = c_{b,4}/c_{b,1}^4$, where $c_{b,1}$, $c_{b,2}$ and $c_{b,4}$ refer to the concentrations of monomer, dimer, and tetramer, respectively, at the radial position of the cell bottom and are given in terms of absorbancy at 280 nm, the scanning wavelength. If a particular species is absent, then the fitting procedure will return a large negative number for that value of ln k appropriate to it. A model involving the presence of octamer was also considered, but analysis of the data indicated that this species was not present under any of the experimental conditions used. Two data sets differing only in initial loading concentrations were fit simultaneously, fitting each set with eq 1 with the values of $c_{b,1}$ and ϵ as local parameters and the values of ln k_{12} and ln k_{14} as global parameters. This is a stringent test for verifying that a reversible chemical equilibrium is present.

The mathematical model ensures that the values of the equilibrium constants are physically meaningful by virtue of having positive values. The use of ln k_{12} and ln k_{14} thus acts as an implicit constraint built into the mathematical model. Fitting for ln k_{12} and ln k_{14} also has the advantage that the logarithms of the molar equilibrium constants, taking 1 mol of the monomer as the standard state, are given by ln K_{12} = ln k_{12} + ln(E_1/2) and ln K_{14} = ln k_{14} + ln(E_1^3/4) where E_1 is the molar extinction coefficient of the monomer (5.5×10^3 M^{-1} cm^{-1} at 280 nm) and where the molar extinction coefficients of the dimer and tetramer are assumed to be respectively 2 times and 4 times that of the monomer. These values are used to calculate the thermodynamic parameters using the equation of Clarke and Glew (1966), and the fitting procedure directly gives the estimated standard error of this parameter, which can then be used to estimate the variance of the value. Finally, the curves describing the calculated values of the sum of squares obtained as a function of the values of the equilibrium constant are asymmetrical about the optimal values of these parameters, indicating that the standard error estimates are poor. However, if the logarithms of the equilibrium constants are used, the sum of squares curves are very nearly parabolic and symmetrical about the optimal values of ln k, indicating that the error estimates are more nearly valid. This test is based on the fact that for a linear model where the data have a normally distributed error and where the data are properly weighted in the fitting procedure, a graph of the sum of squares as a function of the values of a parameter will be a parabola which is symmetrical about the optimal parameter value. This becomes a heuristic test for assessing the quality of the error estimates for the nonlinear case.

For the case where a monomer–dimer is the appropriate model, a large negative value for ln k_{14} is obtained when fitting; where a monomer–tetramer model is appropriate, a larger negative value for ln k_{12} is similarly obtained; where only monomer is present, both ln k_{12} and ln k_{14} have large negative values.

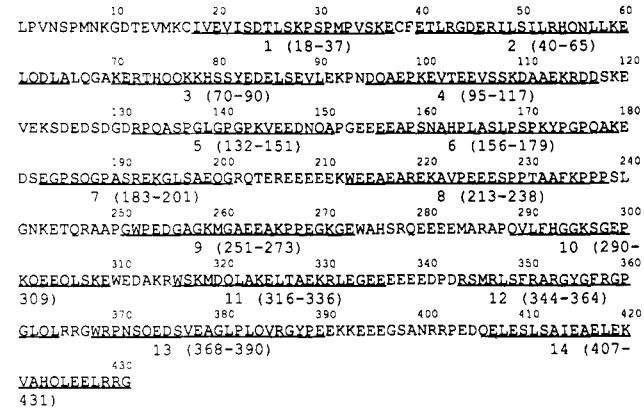

FIGURE 1: Size and location of the synthetic chromogranin A peptides. The amino acid sequence of bovine chromogranin A (Benedum et al., 1986) is expressed in the single-letter code. The peptides were numbered from 1 to 14, and the size of each peptide is indicated by an underline followed by the number of amino acid location in parentheses. For peptides 1–7, 10, 12, and 14 where there was no tryptophan, a tryptophan residue was added at the N-terminus of each peptide to facilitate analysis.

Of the 14 peptides (Figure 1) tested for the presence of any type of oligomerization, only peptide 14 exhibited dimerization and tetramerization properties at pH 7.5 and at pH 5.5, respectively, and all the other peptides showed no sign of oligomerization and were observed only in the monomeric state. The quality of the fits obtained is shown in Figure 2C, which is for peptide 14 at pH 5.5 where we observed a monomer–tetramer equilibrium, and in Figure 3B, which is for peptide 14 at pH 7.5 where we observed a monomer–dimer equilibrium, both at 5 °C. The distribution of the residuals in each condition is shown in Figures 2A, 2B, and 3A; these results demonstrate that the fits are of good quality with low root-mean-square (RMS) errors and no significant systematic deviation.

During the analysis of the pH 5.5 data, we considered the possibility that this might be a monomer–dimer–tetramer association. However, analysis using eq 1 either gave values for ln k_{12} that indicated essentially zero concentration of dimer or, for a few cases, gave a very low value of ln k_{12} and a significantly inferior fit based on the RMS error. Thus, while we cannot absolutely eliminate the possibility that peptide 14 at pH 5.5 undergoes a monomer–dimer–tetramer association, we cannot analyze the data for this association in a meaningful way and can only consider the monomer–tetramer association. One of the examples of the monomeric existence of all the remaining 13 peptides is shown in Figure 4, where CGA peptide 2 (residues 40–65), which is located within the conserved near-N-terminal region and has been shown to bind calmodulin with high affinity (Yoo, 1992), is observed as a monomer.

The temperature dependence of the values of ln K_{12} and ln K_{14} was analyzed by the method of Clarke and Glew (1966) using the Taylor's series expansion about the reference temperature θ:

$$R\ln K = -\Delta G^\circ_\theta/\theta + \Delta H^\circ_\theta(1/\theta - 1/T) + \Delta C^\circ_{p,\theta}[\theta/T - 1 + \ln(T/\theta)] \quad (2)$$

This form of the expression describing the temperature dependence of the equilibrium constant has the desirable property that the fitting parameters ΔG°_θ, ΔH°_θ, and $\Delta C^\circ_{p,\theta}$ are independent of each other. The reference temperature θ was

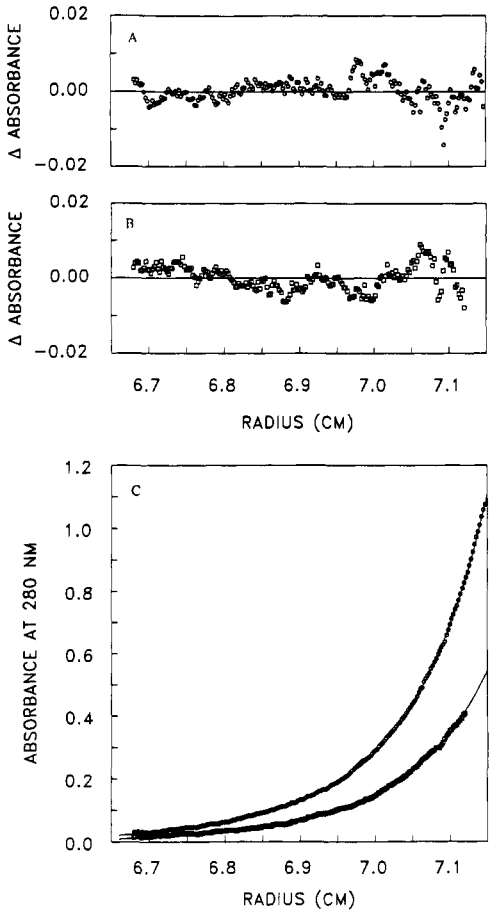

FIGURE 2: Distribution of concentration and residuals of chromogranin A peptide 14 as a monomer–tetramer equilibrium at pH 5.5. Chromogranin A peptide 14 (residues 407–431), at the concentrations of 0.10 and 0.20 mg/mL in 20 mM sodium acetate (pH 5.5)/0.1 M KCl, is shown at ultracentrifugal equilibrium at 10 °C in the absence of Ca^{2+}, where 2 mM EGTA was included in the buffer. Panel A: Distribution of the residuals from the fit of the upper curve of panel C to the monomer–tetramer equilibrium model (see panel C). Panel B: Distribution of the residuals from the fit of the lower curve of panel C under the same conditions. Panel C: Concentration distributions of chromogranin A peptide 14 at the concentrations of 0.20 mg/mL (upper curve) and 0.10 mg/mL (lower curve) in the absence of Ca^{2+}. The lines show the best-fitting curves for the monomer–tetramer equilibrium model. All of the concentration distributions and all of the distributions of residuals for peptide 14 at pH 5.5 in the presence of 35 mM Ca^{2+} were very similar to these.

taken to be 283.15 K in order to facilitate comparison of these values with the values obtained for intact CGA under the same experimental conditions.

When this equation was used to fit the values of ln K as a function of temperature, the data were weighted by the normalized reciprocals of the variances of the values of ln K calculated from the approximate standard errors obtained when fitting the ultracentrifuge equilibrium concentration distributions. The weighted fits for chromogranin A peptide 14 at pH 5.5 in the absence and presence of 35 mM Ca^{2+} and that at pH 7.5 in the absence of Ca^{2+} are shown in Figure 5, and the derived thermodynamic parameters are presented in Table I along with those of chromogranin A. Upon examination, it might appear that the datum at 305 K has been given undue weight when compared to the data at 299 and 302 K. It should be noted that the error bars at these temperatures are approximately 2–3 times greater in magnitude than that at 305 K (and all the other temperatures as well); thus, the weights are approximately one-fourth and one-ninth of the weights at all the other temperatures.

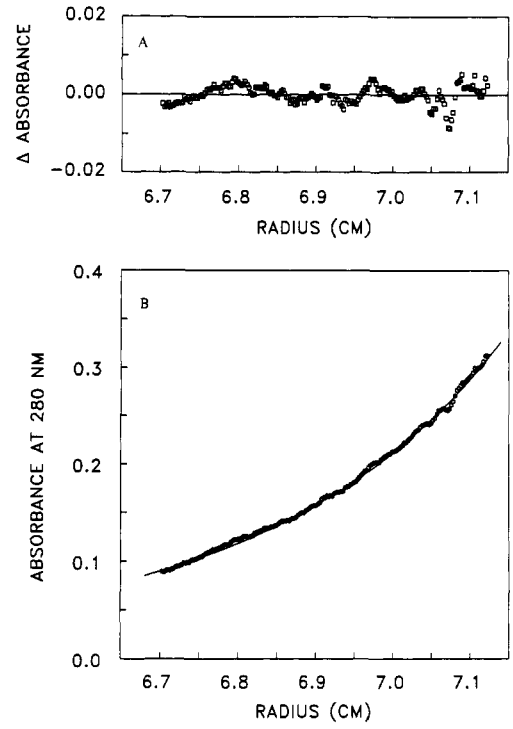

FIGURE 3: Distribution of concentration and residuals of chromogranin A peptide 14 as a monomer–dimer equilibrium at pH 7.5. Chromogranin A peptide 14 at the concentration of 0.10 mg/mL in 20 mM Tris-HCl (pH 7.5)/0.1 M KCl is shown under the same conditions as in Figure 2. Panel A: Distribution of the residuals from the fit to the monomer–dimer equilibrium model in the absence of Ca^{2+} (see panel B). Panel B: Concentration distribution of chromogranin A peptide 14 in the absence of Ca^{2+}. The line shows the best-fitting curve for the monomer–dimer equilibrium model.

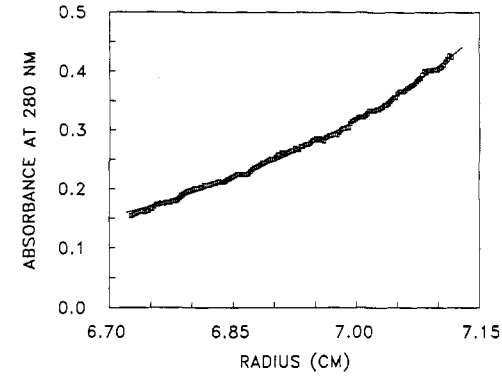

FIGURE 4: Distribution of concentration of chromogranin A peptide 2. Chromogranin A peptide 2 (residues 40–65) in 20 mM Tris-HCl (pH 7.5)/0.1 M KCl is shown at the ultracentrifugation equilibrium at 15 °C in the absence of Ca^{2+} under a similar condition (see Experimental Procedures for details) as in Figure 2. The line shows the best-fitting curve for the monomer model.

In view of the fact that peptide 14 which was synthesized with an extra tryptophan residue at the N-terminus raised a question whether that had an effect on the oligomerization of the peptide, we therefore removed the tryptophan residue by means of chymotrypsin treatment and subjected the peptide to the sedimentation equilibrium study. The self-association of trypsin-stripped peptide 14 at pH 5.5 was examined at the wavelength of 240 nm using a Beckman Optima XL-A analytical ultracentrifuge. In this experiment, peptide 14 without the N-terminal tryptophan exhibited a virtually identical tetramerization property as peptide 14 with the N-terminal tryptophan, having a $\Delta G°$ value of −18.5 kcal/mol at 5 °C in the absence of calcium. This result suggests

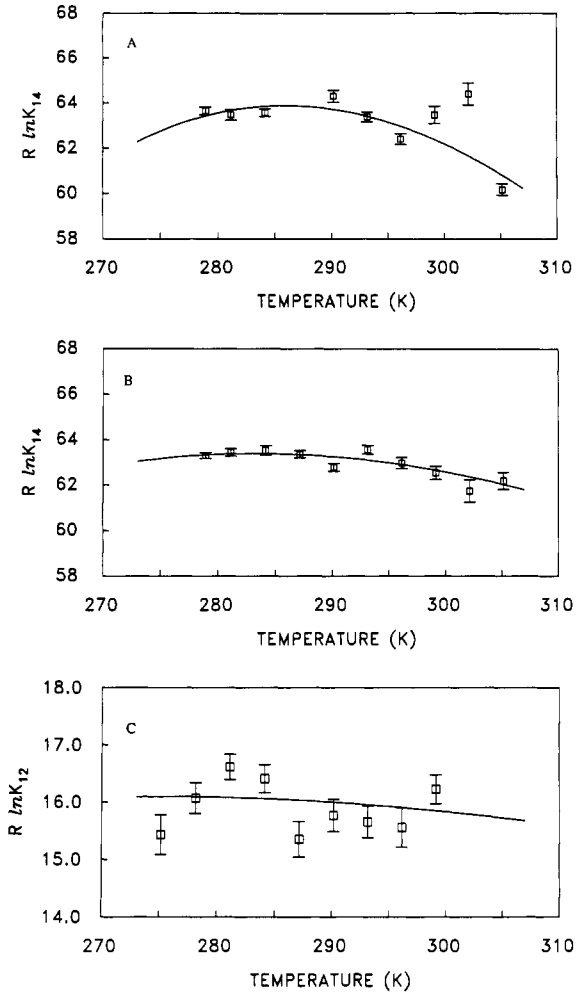

FIGURE 5: Weighted fits of the values of $R \ln K$ as a function of temperature. The temperature dependence of the equilibrium constants for a monomer–dimer or a monomer–tetramer equilibrium as analyzed by the method of Clarke and Glew (1966) using a value of 283.15 K for θ. The error bars show the approximate standard errors obtained when fitting the equilibrium concentration distributions. Panel A: Monomer–tetramer equilibrium at pH 5.5 in the absence of Ca^{2+}. Panel B: Monomer–tetramer equilibrium at pH 5.5 in the presence of 35 mM Ca^{2+}. Panel C: Monomer–dimer equilibrium at pH 7.5 in the absence of Ca^{2+}.

that the extra N-terminal tryptophan has no significant effect on the oligomerization properties of the peptide.

Examination of Figure 5A,B,C shows a similar pattern in all three figures in that not only the tetramer formation but also the dimer formation is not markedly affected by the increase in temperature. The thermodynamic parameters shown in Table I are also similar in that they all show positive $\Delta S°$, small negative $\Delta C_p°$, and near-zero $\Delta H°$ values, suggesting similar reaction mechanisms in all three cases.

DISCUSSION

Despite the high sequence conservation of the C-terminal region of CGA, it was not known what role(s) this region might play in the vesicle. The present results demonstrate that the conserved C-terminal region (residues 407–431) formed a monomer–dimer equilibrium at pH 7.5 and a monomer–tetramer equilibrium at pH 5.5, as had been previously observed with intact CGA. The oligomerization was specific to the conserved C-terminal region. All other synthetic CGA peptides listed as 1–13 in Figure 1 did not exhibit any sign of oligomerization.

Table I: Thermodynamic Parameters Characterizing the Monomer–Dimer and Monomer–Tetramer Associations of Peptide 14 and of Intact Chromogranin A at θ = 283.15 K[a]

peptide 14	chromogranin A[b]	difference
Monomer–Tetramer Association at pH 5.5 and 0 mM Ca^{2+}		
$\Delta G° = -18.1 \pm 0.1$	$\Delta G° = -22.7 \pm 0.2$	$\Delta\Delta G° = -4.6$ kcal mol^{-1}
$\Delta H° = 4.1 \pm 5.5$	$\Delta H° = -58.4 \pm 27.0$	$\Delta\Delta H° = -62.5$ kcal mol^{-1}
$\Delta C_p° = -1.5 \pm 0.7$	$\Delta C_p° = -16.0 \pm 8.2$	$\Delta\Delta C_p° = -14.5$ kcal mol^{-1} deg^{-1}
$\Delta S° = 78.2 \pm 19.5$	$\Delta S° = -126 \pm 95$	$\Delta\Delta S° = -204$ cal mol^{-1} deg^{-1}
Monomer–Tetramer Association at pH 5.5 and 35 mM Ca^{2+}		
$\Delta G° = -18.0 \pm 0.1$	$\Delta G° = -21.6 \pm 0.3$	$\Delta\Delta G° = -3.6$ kcal mol^{-1}
$\Delta H° = 0.1 \pm 2.0$	$\Delta H° = 20.0 \pm 16.5$	$\Delta\Delta H° = 19.1$ kcal mol^{-1}
$\Delta C_p° = -0.5 \pm 0.3$	$\Delta C_p° = 14.4 \pm 5.8$	$\Delta\Delta C_p° = 14.9$ kcal mol^{-1} deg^{-1}
$\Delta S° = 63.7 \pm 7.2$	$\Delta S° = 147 \pm 58$	$\Delta\Delta S° = 83$ cal mol^{-1} deg^{-1}
Monomer–Dimer Association at pH 7.5 and 0 mM Ca^{2+}		
$\Delta G° = -4.55 \pm 0.07$	$\Delta G° = -7.75 \pm 0.02$	$\Delta\Delta G° = -3.20$ kcal mol^{-1}
$\Delta H° = -0.55 \pm 3.07$	$\Delta H° = 6.24 \pm 1.76$	$\Delta\Delta H° = 6.79$ kcal mol^{-1}
$\Delta C° = -0.08 \pm 0.56$	$\Delta C_p° = -1.14 \pm 0.50$	$\Delta\Delta C_p° = -1.06$ kcal mol^{-1} deg^{-1}
$\Delta S° = 14.1 \pm 10.8$	$\Delta S° = 49.4 \pm 6.1$	$\Delta\Delta S° = 35.3$ cal mol^{-1} deg^{-1}

[a] The standard state was chosen to be 1 mol/L of monomer in each solvent. [b] Yoo and Lewis (1992).

The values of $\Delta G°$ for tetramerization of peptide 14 at pH 5.5 were approximately –18 kcal/mol (Table I) while those of intact CGA were approximately –21 to –22 kcal/mol (Yoo & Lewis, 1992). Peptide 14 represents only 6% of intact CGA (Benedum et al., 1986; Iacangelo et al., 1986). Nevertheless, it accounts for 80–83% of the values of $\Delta G°$ for tetramerization of intact CGA. The remaining 17–20% (–3.6 to –4.6 kcal/mol) of the $\Delta G°$ values of tetramerization may thus be attributed to the rest of the molecule (residues 1–406), representing 94% of CGA. Considering that tetramerization of intact CGA involves a large degree of conformational changes whereas that of peptide 14 involves little or no conformational change (see below), it is not difficult to assume that the remaining 94% of CGA accounts for virtually all of the conformational changes occurring during tetramerization. Hence, it is quite likely that the –3.6 to –4.6 kcal/mol change of $\Delta G°$ is due to the conformational changes of the remaining residues of CGA during oligomerization. In addition, the free energy change for dimerization of peptide 14 at pH 7.5 was –4.55 kcal/mol while that of intact CGA was –7.75 kcal/mol. The difference in $\Delta G°$ of dimerization between peptide 14 and intact CGA was –3.2 kcal/mol, which is similar to the $\Delta G°$ difference (–3.6 to –4.6 kcal/mol) observed in the tetramerization of intact CGA and peptide 14. Considering that –3.2 kcal/mol is contributed by the remaining 406 residues of CGA during dimerization, the $\Delta G°$ value of –4.55 kcal/mol for dimerization of peptide 14 correlates well with the –7.75 kcal/mol of dimerization of intact CGA, suggesting the possibility that the C-terminal region of CGA is responsible for the tetramerization of CGA at pH 5.5 and for the dimerization at pH 7.5.

The tetramerization reaction of intact CGA in the absence of 35 mM Ca^{2+} was enthalpically driven with large negative values for $\Delta H°$ and $\Delta S°$ (Yoo & Lewis, 1992). However, the presence of 35 mM Ca^{2+} changed the thermodynamic parameters in the tetramerization reaction to both positive values of $\Delta H°$ and $\Delta S°$, indicating that the tetramerization of intact CGA in the presence of 35 mM Ca^{2+} is entropically driven. Unlike the oligomerization mechanism of intact CGA, the tetramerization of peptide 14 at pH 5.5 did not appear to exhibit different reaction mechanisms in the presence and absence of Ca^{2+} since the reaction is entropically driven both in the presence and in the absence of Ca^{2+}. Moreover, the dimerization of peptide 14 at pH 7.5 is also entropically driven as was the case with intact CGA (Yoo & Lewis, 1992). Although the value of $\Delta G°$ for the dimerization of peptide 14

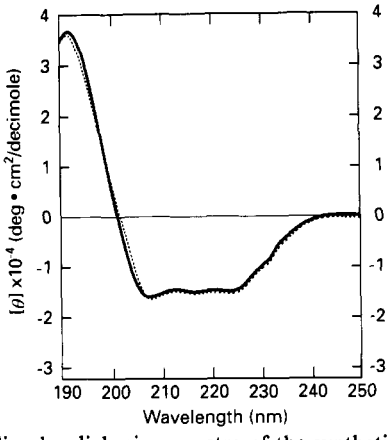

FIGURE 6: Circular dichroism spectra of the synthetic C-terminal peptide (residues 407–431) of chromogranin A. Circular dichroism spectra of the conserved C-terminal region of chromogranin A either in the absence (solid line) or in the presence (dotted line) of 20 mM $CaCl_2$ at the peptide concentration of 50 μM in 15 mM sodium acetate, pH 5.5.

is essentially of entropic origin, the dimerization of intact CGA involves a significantly larger value of $\Delta S°$ because $\Delta H°$ is approximately 6.8 kcal/mol larger. Given that a positive enthalpy change of approximately 1 kcal/mol per residue is observed in the α-helix to coil transition of proteins and peptides (Scholtz et al., 1991; Ooi & Oobatake, 1991), the large positive $\Delta H°$ value (6.24 kcal/mol) shown for the dimerization of intact CGA (Yoo & Lewis, 1992) suggests that the dimerization of intact CGA is accompanied by significant conformational changes, possibly involving α-helix to coil transitions. In contrast, the tetramerization of peptide 14 showed little or no enthalpy change, which might suggest little or no conformational changes. Accordingly, the large differences in $\Delta H°$ values between intact CGA and peptide 14 ($\Delta\Delta H°$) raised a possibility of large conformational changes involving α-helix to coil or coil to α-helix transitions of the remaining residues (residues 1–406) during oligomerization (Table I).

In spite of the small size of peptide 14 compared to that of intact CGA, disproportionately large values of $\Delta S°$ were observed in the tetramerization of peptide 14, contributing to the large negative values of $\Delta G°$ (Table I). In this regard, it is remarkable that peptide 14, representing only 6% of intact CGA, accounted for approximately half of the $\Delta S°$ value of tetramerization of intact CGA in the presence of 35 mM Ca^{2+}. From these results, it appears that the specific conformation adopted by the peptide at pH 5.5 induced the peptide to self-associate to form a tetramer without accompanying conformational changes. A secondary structure estimation of the peptide by circular dichroism spectroscopy at pH 5.5 indicated that there is no detectable structural difference in the absence and presence of up to 20 mM calcium (Figure 6), thereby supporting the notion that tetramerization of peptide 14 at pH 5.5 is entropically driven probably with little or no conformational changes both in the absence and in the presence of 35 mM Ca^{2+}. This result contrasts with our previous secondary structure study of intact CGA by circular dichroism spectroscopy (Yoo & Albanesi, 1990b), which demonstrated clear structural changes in the absence and presence of 15 mM calcium at either pH 5.5 or pH 7.5. Furthermore, there existed striking differences in heat capacity changes between the oligomerization of intact CGA and the peptide (Table I). In view of the observation that positive changes in heat capacity result from interaction of nonpolar groups of proteins or peptides with the aqueous environment and, conversely, negative $\Delta C_p°$ results from withdrawal of nonpolar groups from the aqueous environment (Privalov & Gill, 1988; Spolar et al., 1989; Livingstone et al., 1991), the small negative $\Delta C_p°$ values shown in the tetramerization of peptide 14 suggest little or no movement of nonpolar groups of the peptide during tetramerization. Considering that the magnitude of negative $\Delta C_p°$ values is small, the extent of the transfer of nonpolar groups, if it ever happens, will probably also be small and not affect the overall conformation of the peptide as reflected in the small $\Delta H°$ values. Recently, Livingstone et al. (1991) proposed a relationship between $\Delta C_p°$ and the reduction in water-accessible nonpolar surface area (ΔA_{np}) in protein folding with the expression $\Delta C_p° \approx 0.32\Delta A_{np}$, from which the $\Delta C_p°$ value of protein folding can be estimated given the size of a protein. In view of the fact that intact CGA has 431 amino acid residues, the $\Delta C_p°$ values of 14 to −16 kcal/mol shown for the tetramerization of intact CGA (Yoo & Lewis, 1992) might appear to be somewhat higher than that expected from the folding or unfolding of a protein with 431 amino acids. However, the higher $\Delta C_p°$ values appear to be a natural consequence of the fact that CGA exists in a highly extended structure; the CGA molecule, in a monomer–dimer equilibrium, is eluted in gel filtration chromatography in a volume indicative of a globular protein with a molecular weight of 300 000 (Yoo & Albanesi, 1990b) rather than the maximum value of 96 000 for the dimer. In addition, given that tetramerization of intact CGA involves intermolecular interactions besides the structural changes, it is also conceivable that part of the values of $\Delta C_p°$ might well come from sources other than folding or unfolding. The $\Delta C_p°$ of dimerization of peptide 14 at pH 7.5 also shows a near-zero value, which may suggest little movement of the nonpolar groups during dimerization. In contrast, the large differences in $\Delta C_p°$ values between intact CGA and peptide 14 ($\Delta\Delta C_p°$) may reflect the large movement of nonpolar groups of residues 1–406 which might occur during oligomerization (Table I).

Taken together, the thermodynamic parameters appear to point out the intrinsic ability of the conserved C-terminal region to form a dimer at pH 7.5 and a tetramer at pH 5.5 with little or no conformational changes. The pH-dependent dimerization and tetramerization of the C-terminal peptide of CGA appear to reflect the two conformational states the peptide assumes at each pH. From the fact that intact CGA and the C-terminal peptide each form a tetramer at pH 5.5, it appears that the conformational changes induced by the acidic pH create additional interaction sites for tetramerization. A hydrophobicity plot of the C-terminal region indicates that the first half of the C-terminal peptide (residues 407–418) is hydrophobic while the latter half of the peptide (residues 419–431) is hydrophilic (not shown). Given the α-helicity of the C-terminal region as evidenced by the large magnitude of the molar ellipticity at 222 nm (Figure 6) and the hydrophobicity of the first half of the peptide, it may be that the first half of the peptide is primarily involved in self-association to form oligomers.

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