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CONFORMATION OF THE ALPHA FORM OF HUMAN CALCITONIN GENE-RELATED PEPTIDE (CGRP) IN AQUEOUS SOLUTION AS DETERMINED BY CIRCULAR DICHROISM SPECTROSCOPY

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Circular dichroism (CD) spectroscopic studies on the α form of human calcitonin gene-related peptide (α-hCGRP) indicate that, in aqueous solution at 4°C, there is some α-helical structure present. This helix involves 8-10 residues of the 28 amino acid, C-terminal tail. The α helix is destabilized by denaturants such as guanidinium hydrochloride and increased temperature and is stabilized by the addition of anionic detergents, such as sodium dodecyl sulphate (SDS). In the presence of SDS and 33% trifluoroethanol, nearly all of the residues in the C-terminal tail are in the α-helical conformation. These studies indicate that there is sufficient helical structure in aqueous solution to suggest that formation of an amphiphilic helix in the C-terminal tail of α-CGRP may be physiologically relevant.

Calcitonin gene-related peptide (CGRP) is a 37 amino acid hormone produced via alternate mRNA splicing[1]. It is involved in modulating a variety of physiological functions, including gastrointestinal[2,3], cardiovascular[4,5], and sensory[6] processes. Structure-activity studies indicate that both the α- and β-forms, arising from different gene products, are nearly equally viable[4,7,8]. Human and rat CGRP, having sequence differences at three positions, are also similar in potency[9]. Finally, the intact molecule appears to be necessary to function in vitro[10]. Despite numerous reports on CGRP, little is known about the relationship between activity and conformation. This investigation represents the first description of the conformation of the alpha-form of human CGRP (α- hCGRP) in aqueous solution, as determined by circular dichroism (CD) spectroscopy.

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MATERIALS AND METHODS

Circular dichroism spectra were obtained on a Jasco J41-C spectrometer interfaced to a Digital Equipment Corp. MINC-11 minicomputer to allow data manipulation and smoothing. Spectra were measured on dilute solutions in quartz, jacketed cells with pathlengths ranging from 0.1 to 0.5 mm. Temperatures were controlled by a circulating temperature bath. Human CGRP (α-hCGRP) was obtained from Peninsula Laboratories, checked for purity by HPLC, and used without further purification. Initial CGRP solution concentrations were determined by amino acid analysis. From this, it was found that a 1mg/ml solution gave an absorbance of 3.6 at 215 nm when placed in a 1 mm pathlength cell, corresponding to a molar extinction coefficient of 3670 M^{-1} cm^{-1} at this wavelength.

RESULTS

Conformation in aqueous solution. Amphiphilic helices are a common secondary structural motif in peptide hormones[11]. Although the amphiphilic helix in calcitonin has received great attention[12-15], its presence in CGRP has only been recently proposed[16]. Kaiser et al. suggested that it may extend from residues 8 through 26[16]. In helix-promoting solvents (50% trifluoroethanol (TFE) / 50% water), the amount of helix was estimated to be 46%, consistent with this hypothesis. Further evidence for the amphiphilic helix was obtained from surface tension measurements and cross-reactivity with the calcitonin receptor.

.....However, many peptides which are helical in detergents or helix-promoting solvents, such as trifluoroethanol (TFE) or hexafluoroisopropanol (HFIP), display no evidence of secondary structure in aqueous solution[17,18]. Further, structures observed in such solvents may not be physiologically relevant . Therefore, the conformation of α-hCGRP in aqueous solution was examined. Upon dissolution in 20 mM sodium phosphate buffer, α-hCGRP displays two strong CD bands in the far UV: a negative shoulder at ~ 220 nm and a sharp negative band at 200 nm (Fig. 1). Such a CD curve is indicative of the presence of both α-helical and unordered secondary structure[19]. Increasing the temperature leads to a decrease in the intensity of the 220 nm shoulder and a marked blue-shift of the negative 200 nm band. This is consistent with a loss, or melting, of the helical structure. A difference spectrum between the high and low temperature forms clearly indicates the disruption of an α helix (see Fig. 2). Considering that the N-terminal region of CGRP is constrained by a disulfide bridge, the α helix must form along the C-terminal (residues 9-37) tail. Estimates of the amount of α helix from the CD spectrum[20] suggest that 20%, or 8-10 residues, are involved in the α helix at 4° C. One possibility is that the helix occurs in the vicinity of Lys-24, as that site is more resistant to cleavage by trypsin than the other three basic sites in α-hCGRP[10].

Conformation in the presence of SDS micelles. A putative, positively-charged amphiphilic helix should interact strongly with anionic detergents. Addition of sodium dodecyl sulphate (SDS) has often been employed to induce an increase in amphiphilic secondary structure[17,21]. In the presence of 0.4 % SDS (0.010 M; this is above the critical micelle concentration[22]) and 20 mM

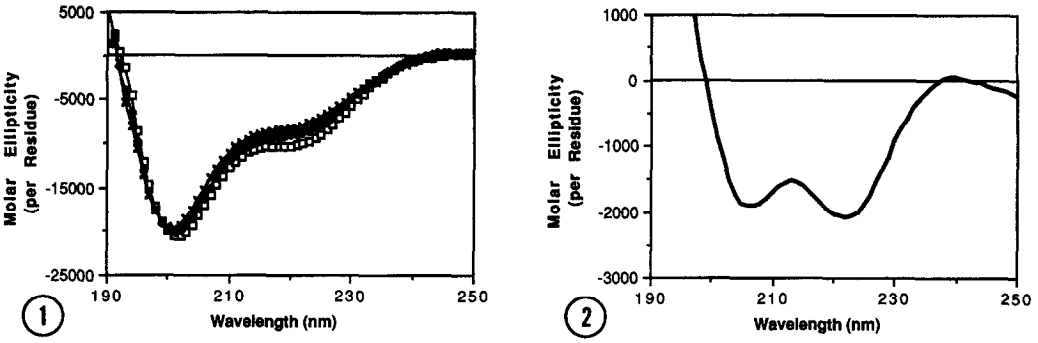

Figure 1. Circular Dichroism (CD) Spectra of α-hCGRP in Aqueous Solution (20 mM phosphate buffer, pH = 7.7) at 4° C (□), 15° C (◆), and 30° C (×).

Figure 2. Temperature Difference CD Spectrum of α-hCGRP in Aqueous Solution (4° C - 30° C).

sodium phosphate (pH 7.7), the amount of α helix increases dramatically to approximately 60%. Such behavior has been noted previously for peptides which adopt a small amount of helical structure in aqueous solution, such as ribonuclease C-peptide[23,24]. Such a high helix content suggests that nearly all of the C-terminal tail exists in an α-helical conformation. In light of the preponderence of "helix-breaking" residues in this region, e.g., Pro-29 and Gly-20/Gly-21, this result is surprising. Possibly, there are three short (~ 8-10 residues) segments, each comprising about two turns of an α helix. Two turns is believed to be the minimum required to produce an α-helix-like CD spectrum[25,26]. Therefore, such an arrangement would also give rise to this type of CD curve (Fig. 3).

It has been proposed that surfactants such as SDS provide a hydrophobic environment which might model a lipid or protein environment. Further, there is some evidence that it is the lipid-bound conformation rather than the solution structure which is recognized by the receptor[11,27].

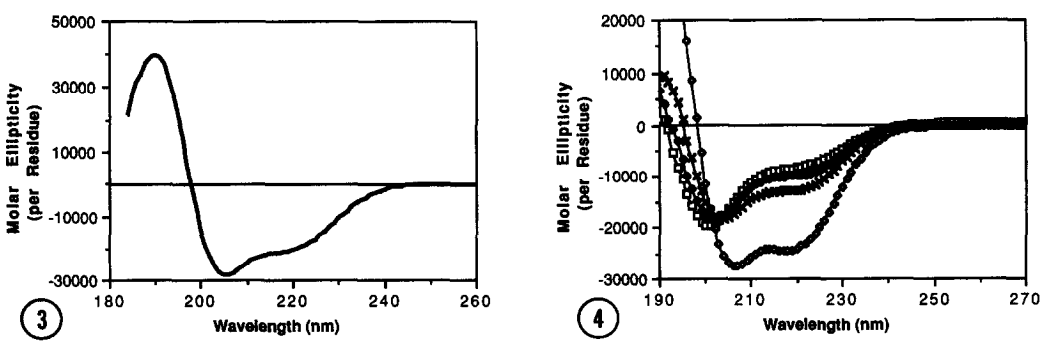

Figure 3. CD Spectrum of α-hCGRP in 20 mM Aqueous Phosphate Buffer (pH = 7.7) Containing 0.27% SDS.

Figure 4. CD Spectra of α-CGRP in 0% (□), 5% (◆), 10% (×), and 33% (◇) trifluoroethanol (TFE) solution.

Considering the basic nature of CGRP and the greater head-group charge of SDS relative to phospholipids, this may not be a suitable model for lipid-CGRP interactions. In any case, SDS does significantly enhance the stability of α-helical structure in α-hCGRP.

Conformation in water/TFE mixtures. It is well established that strong hydrogen-bond donors such as 2,2,2-trifluoroethanol (TFE) or 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) stabilize α helices in homopolypeptide systems[18]. In the presence of 5% TFE (95% 20mM sodium phosphate buffer, pH 7.7), there is little change in the CD spectrum. At 10% TFE, the intensity of the 220 nm shoulder is increased and the 200 nm band is red-shifted (Fig. 4). With TFE concentrations of 33%, the CD spectrum of α-hCGRP is similar to that in SDS solution and, presumably, similar to that obtained by Kaiser et al. for a 50% TFE solution[16]. The intensities of the two negative CD features are nearly equal and the positions are typical of an α-helical polypeptide chain. Helix content in 33% TFE solution is estimated[20] to be ~ 60% (22-24 residues).

DISCUSSION

Based upon studies of calcitonin, the existence of a biochemically relevant, amphiphilic α helix has been postulated for CGRP[16]. However, to date, no experimental evidence for its occurence in aqueous solution has been reported. These results demonstrate that such a structure can exist under physiological conditions, and that its stability is similar to other small, helical peptides. For example,an increase in temperature leads to a loss of ordered structure (Figs. 1 and 2). Analysis of the CD spectra indicates that 8-10 residues of the C-terminal tail of α-hCGRP are in a helical conformation at 4° C. Elevation of the temperature to 30° C leads to a decrease in the amount of helical structure. Similar changes have been observed other small peptides which are helical in aqueous solution, such as the analogs of the C- and S-peptide fragments from ribonuclease[23,24].

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