Journal of Pharmaceutical & Biomedical Analysis
Vol. 11, No. 2, pp. 89–93, 1993
Printed in Great Britain

0731–7085/93 $6.00 + 0.00

Conformation of human calcitonin gene-related peptide (8–37) in aqueous solution as determined by circular dichroism spectroscopy

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Abstract: Circular dichroism (CD) studies on CGRP(8–37) indicate that there is some latent α-helical structure in aqueous solution. However, the amount is quite small (~10% at 5°C), which is substantially less than for CGRP itself (~15–20%). Upon addition of helix-promoting materials, such as trifluoroethanol and sodium dodecyl sulphate, the helix content increases dramatically. No evidence for helix stabilization upon the addition of zinc was observed.

Keywords: *Circular dichroism spectroscopy; CGRP(8–37); antagonists; secondary structure; calcitonin gene-related peptide.*

Introduction

Calcitonin gene-related peptide (CGRP) contains 37 amino acid residues and is a potent vasodilator. It appears to be involved in the control of cardiovascular function [1–4], and has potential in the treatment of subarachnoid haemorrhage [5, 6]. Two distinct structural features have been identified within CGRP, a disulphide-bridged ring between positions 2 and 7 and an extended C-terminal tail section. Recently, the tail fragment, containing residues 8–37 of CGRP, was found to act as an antagonist of CGRP [7–9]. Earlier structural studies in our laboratory found that the tail of intact CGRP adopted an α-helical conformation, even in aqueous solution [10]. Addition of anionic detergents and non-aqueous solvents have been shown to stabilize the α helix of CGRP [10–13].

It has been proposed that the receptor recognizes the amphiphilic α-helical structure of the tail [10]. Consequently, it is of importance to determine whether the antagonist, CGRP(8–37), adopts a similar conformation in aqueous solution and whether such a structure is stabilized to a similar degree by the addition of nonaqueous solvents and detergents. Should CGRP(8–37) display similar α-helical structure, that would suggest that the CGRP receptor recognizes the solution conformation. However, if the structures are quite different, it would indicate that other factors (interaction with lipid membrane, non-specific interaction with the receptor) might be needed to induce the proper conformation for antagonism to occur. For these studies, circular dichroism (CD) spectroscopy was employed to monitor the secondary structure of CGRP(8–37) in solution. Previously, CD spectroscopy has been shown to be a valuable analytical method for assessing both protein stability [14, 15] and structure [10, 16, 17].

Materials and Methods

Circular dichroism spectra were measured using an Aviv 62DS spectrophotometer equipped with a thermoelectric temperature control unit. Temperatures were regulated to within ±0.1°C. Spectra were obtained on samples in quartz cuvettes with pathlengths ranging from 0.5 to 10 mm. CGRP(8–37) was obtained from Bachem and was used without further purification. The pH of the samples ranged from 5.0 to 6.0 while the concentrations ranged from 10 to 100 μg ml^{-1}.

Results and Discussion

Conformation of CGRP(8–37) in aqueous solution

The far ultraviolet (UV) CD spectra of CGRP(8–37) in aqueous solution (pH 5.5) at 5

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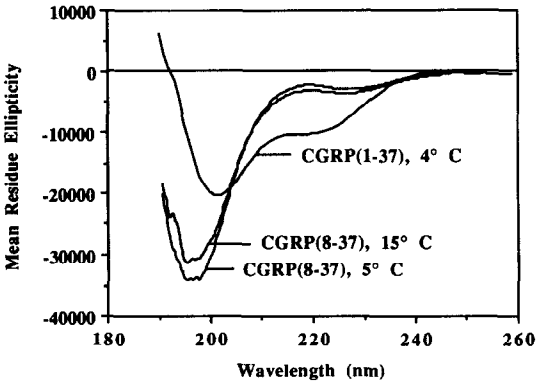

Figure 1
Far UV CD spectra of CGRP(8–37) at 5 and 15°C and CGRP(1–37) at 4°C. The spectra have been smoothed.

and 35°C are shown in Fig. 1. The weak negative feature near 220 nm and the strong negative band at 200 nm suggests that the secondary structure is mostly unordered with a small amount of α helix present. Based on the intensity at 222 nm [18], the α-helix content is only 5–10% at 5°C, corresponding to one or two turns of an α helix. This method assumes that a α helix will display an intensity of $[\theta]_{222}$ of −30,300 deg cm^2 dmol^{-1} and a random coil structure an intensity of +2340 deg cm^2 dmol^{-1}. The helix content is then calculated by equation (1):

$$[\theta]_{222} = -30{,}300\, f_H - 2340, \qquad (1)$$

where f_H is the fraction of the polypeptide which exists in an α-helical conformation.

Other methods for the estimation of secondary structure [19, 20] were unable to provide accurate determinations of the α-helix content of CGRP(8–37). Least squares methods have been found to have difficulty in deconvoluting the CD spectra of small peptides in trifluoroethanol–water mixtures [21]. Increasing the temperature from 5 to 35°C disrupts the helix in CGRP(8–37), although at 15°C, the amount of α helix is nearly the same as at 5°C (Fig. 1). A difference spectrum shows that the lost intensity upon heating to 35°C is due almost entirely to melting of an α helix (data not shown). The disruption of the α-helical structure by increasing the temperature to 35°C is similar to the behaviour of the intact hormone [10].

Contrary to CD spectrum observed for CGRP(8–37), intact CGRP displays a much stronger 220 nm band [10–13], suggesting CGRP possesses a significantly greater α-helix content (Fig. 1). Earlier work suggests an α-helix content of 15–20% for CGRP at 4°C and this has been verified by other workers [11, 12]. It appears that the N-terminus of CGRP helps stabilize the α-helical structure of the tail portion. However, the exact nature of this interaction is still unknown. Recent NMR studies on CGRP in 50% (v/v) trifluoroethanol (TFE), have not revealed any significant interactions between the two domains [22], although there were close contacts between the γ-methyl protons of Val-8 and residues in the disulphide loop. The structures obtained from the NMR study showed that residues 8–18 adopted a stable α-helical conformation. Most likely, the helix observed in aqueous solution for CGRP(8–37) occurs within this region.

Both calcitonin and CGRP belong to a superfamily of peptide hormones which appear to adopt some amphiphilic helical structure related to their biological activity [23–25]. One homologous peptide with CGRP is amylin (46% homologous), which is responsible for the amyloid deposits observed in patients with type 2 diabetes melittus. The CD spectrum of amylin has been reported to display a negative band near 220 nm and a more intense negative maximum at 200 nm [13]. However, some discrepancy exists about the actual far UV CD spectrum of amylin [12, 13, 26]. Overall, amylin appears to possess at least as much helical structure as CGRP. Considering that CGRP(8–37) can antagonize both CGRP and amylin effects [27], it appears that a similar type of extended secondary structure exists for all three and may be responsible for receptor recognition.

In order to assess the intrinsic stability of the α helix in CGRP(8–37), various methods were used which are known to enhance α-helix stability. Both sodium docecyl sulphate (SDS) and TFE are known to stabilize nascent α helices [19, 26–32] and have been evaluated for CGRP [10, 13]. The effects of both of these materials on the helix stability of CGRP(8–37) were examined.

Due to the homology between CGRP, amylin and insulin, it has been postulated that the histidine residue at position 10 may function as a ligand for zinc, leading to discrete aggregation states for CGRP in much the same way as insulin forms dimeric and hexameric structures [13]. Therefore, the effect of zinc

upon the far UV CD spectrum of CGRP(8–37) was investigated.

Effect of trifluoroethanol (TFE) on the secondary structure of CGRP(8–37)

Addition of TFE is known to stabilize latent helices in small peptides [10–13, 28–32]. Hubbard and co-workers have described some of these effects on CGRP, CGRP fragments, and on amylin [12]. However, they did not show a far UV CD spectrum CGRP(8–37). They reported the helix content of CGRP(8–37) to be similar to that of CGRP itself (nearly 20% in water), in contrast to our results. As seen in Fig. 1, the CD spectrum of CGRP(8–37) definitely shows less helical structure than CGRP itself.

Upon addition of TFE, a significant increase in α-helix content is observed (Fig. 2). A difference spectrum for samples containing 0% TFE and 50% TFE clearly demonstrates that the CD spectral changes are due to α-helix formation (Fig. 3), displaying distinct negative bands at 222 and 207 nm and a strong positive band near 193 nm.

Above TFE concentrations of 35% (v/v), the helix content no longer increases and remains at its maximum of 55% α helix. Titration of CGRP(8–37) with TFE produced a sigmoidal curve with a midpoint at a concentration of 20–25% TFE (Fig. 4). These results are similar to those obtained by Hubbard *et al.* [12]. A midpoint of 20–25% is indicative of the relative propensity of the peptide to adopt a α-helical conformation and is similar to the concentrations needed for other peptides of similar size [28–32]. Together, they indicate that the helix in CGRP(8–37) is relatively stable.

Upon raising the temperature for CGRP(8–37) in 50% (v/v) TFE, the helix melts in a cooperative fashion. The cooperativity can be seen in that there is an isodichroic point near 203 nm, indicating the transition can be approximated by a two-state process with no evidence of an intermediate (Fig. 5). The melting behaviour is similar to that observed for helices based upon fragments of bovine growth hormone [29]. Note that even at 60°C, the α-helix content is still significantly greater than for CGRP(8–37) in water alone. Taking the difference between spectra taken at 5 and 60°C, demonstrates that the lost CD intensity is entirely due to melting of an α helix (Fig. 6).

Effect of sodium dodecyl sulphate (SDS) on the secondary structure of CGRP(8–37)

For marginally stable cationic amphiphilic helices such as that found in CGRP, inter-

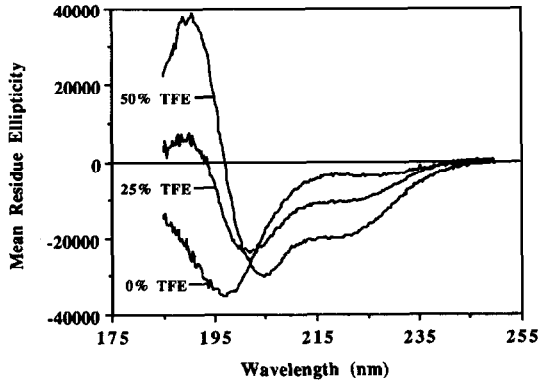

Figure 2
Effect of the addition of TFE on the far UV CD spectrum of CGRP(8–37). The TFE concentrations are given in terms of v/v percentage.

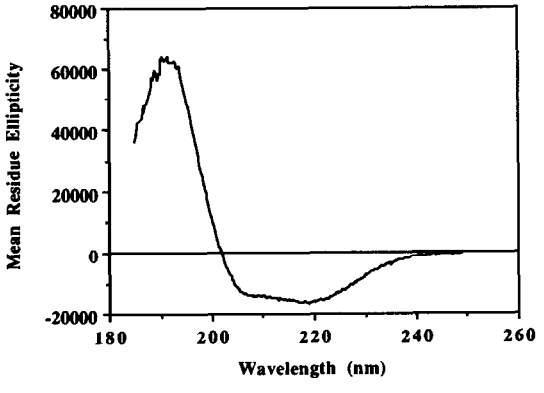

Figure 3
Difference spectrum between CGRP(8–37) in 50% TFE–water (v/v) and CGRP(8–37) in water alone.

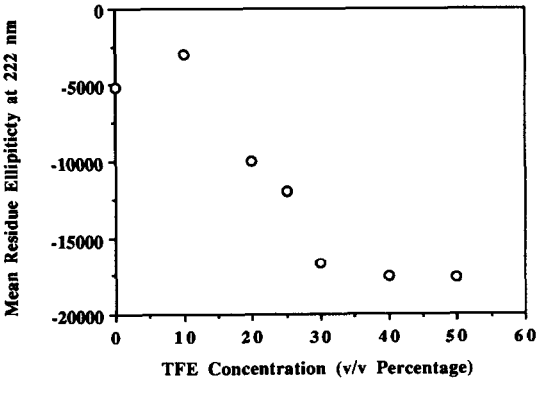

Figure 4
Effect of increased amounts of TFE upon the mean residue ellipticity measured at 222 nm.

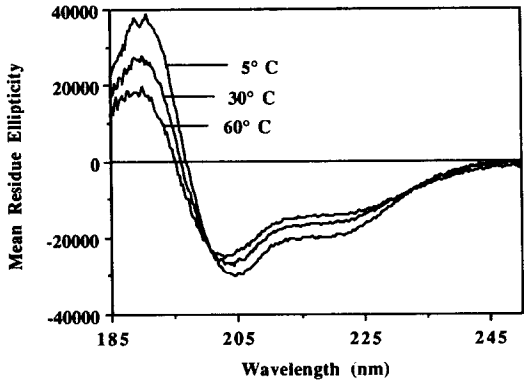

Figure 5
Effect of temperature upon CGRP(8–37) in 50% TFE (v/v).

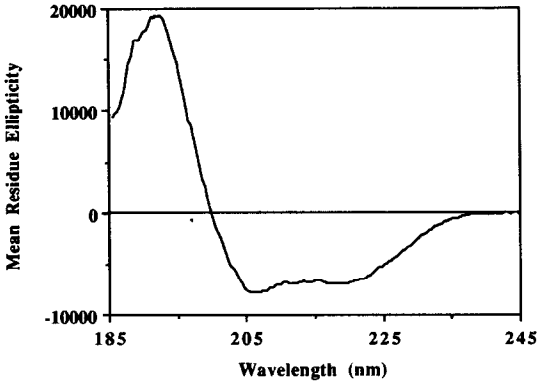

Figure 6
Difference spectrum of the far UV CD of CGRP(8–37) in 50% TFE (v/v) at 5 and 60°C.

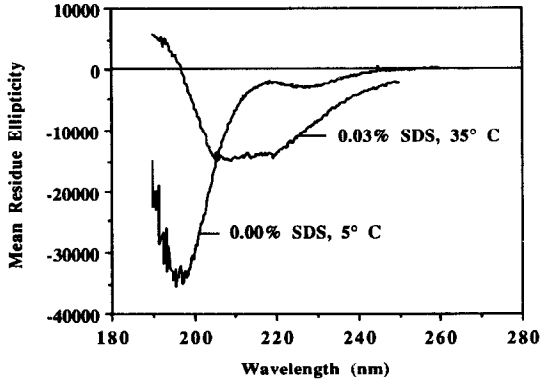

Figure 7
Far UV CD spectra of CGRP(8–37) in water at 5°C and in 0.03% SDS at 35°C.

action with anionic detergents can help stabilize these structures [10, 11, 12, 33]. Figure 7 shows that in the presence of 0.03% SDS, a significant increase in α-helix content is observed. A similar increase in helicity was observed for CGRP in the presence of SDS [10]. However, the helix is not as extensive as in samples placed in TFE. The positive band near 190 nm is only +5000 deg cm^2 $dmol^{-1}$ and the negative bands are blue-shifted relative to canonical values for an α helix, suggesting that a large portion of the peptide is still unordered. This may be due to the lack of charged residues in the 8–18 region, where a helix is most likely to form. Presumably, SDS is stabilizing an α-helix structure near the C-terminus where there are more positively charged groups.

Effect of metal binding on the secondary structure of CGRP(8–37)

It has been proposed that His-10 in CGRP may function in a similar fashion to the histidine on the B-chain of insulin, in that it may bind metal ions to produce a metal-stabilized aggregate [13]. Addition of zinc to CGRP(8–37) does not alter the far UV CD spectrum, even at a 10-fold molar excess of zinc. Interaction with zinc may occur, but it does not produce secondary structural changes of the type observed for insulin [34]. While the near UV CD spectrum might be more sensitive to changes induced by metal binding, CGRP(8–37) does not contain any tyrosines or tryptophans. Therefore, the intensity of the near UV CD is very small [12], and minor changes due to aggregation would be difficult to detect. Other techniques, such as nuclear magnetic resonance spectroscopy, may be better suited for detecting formation of a zinc–CGRP complex.

Conclusions

The conformation of CGRP(8–37) in aqueous solution appears to be significantly different from that of CGRP itself, especially in that the content of α-helix content of CGRP(8–37) is diminished relative to the intact hormone. However, upon interaction with detergents or nonaqueous solvents, the α-helix contents become similar (~60%). These findings suggest that the CGRP receptor does not necessarily recognize the solution conformation, but rather, recognizes a membrane-stabilized structure.

Acknowledgements — The authors wish to thank the University of Colorado and the Colorado Institute for Research in Biotechnology for funding. The authors also wish to thank Drs N. Sreerama and R.W. Woody of Colorado State University for access to programs used in calculating the secondary structure composition.

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[Received for review 23 June 1992; revised manuscript received 20 August 1992]