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Origin of the positive 225–230 nm circular dichroism band in proteins

Its application to conformational analysis

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The 225–230 nm circular dichroism band found in many disulfide-containing proteins and peptides is sensitive to environmental changes. This band is assigned to the disulfide bond, the conformation of which influences both the intensity and λ_{max} of the band. This property can be used to monitor subtle conformation changes observed in many polypeptides. Examples using the α-neurotoxins of elapid venoms and neurohypophyseal hormones are discussed.

1. Introduction

The study of peptide-receptor interactions has been the focus of considerable activity over the past two decades. The binding of hormones to receptors and the interaction between toxins and acceptor proteins have been extensively studied [1–3]. As the subtleties associated with such interactions are gradually elucidated, the successful design of synthetic peptide agonists and antagonists has become a reality. The nicotinic acetylcholine receptor has been studied with a wide range of high-affinity ligands including elapid α-neurotoxins [4]. During our studies with this class of protein, we have observed an equilibrium between two conformational states of these antagonists, which is associated with the intensity of a 225–230 nm CD band [5,6]. The conformation change is sensitive to the dielectric constant of the medium and as this class of neurotoxin has evolved with the ability to undergo a well-defined conformational change, it has been argued that this may be essential for their extremely high affinity for the acetylcholine receptor [7]. The magnitude of the 228 nm CD band of these neurotoxins has proved to be an excellent probe for monitoring this conformational change [5,6].

More recently, we have studied a similar CD band which is found in the spectra of the hormones oxytocin and vasopressin [8,9]. Like the α-neurotoxins, this band is sensitive to temperature and chemical modification and is apparently associated with a conformational change. Oxytocin is believed to adopt two conformations which are differentiated by the chirality of the disulfide bond [10]. Both oxytocin conformations apparently bind to the receptor, but each conformer favors a different receptor conformation; the left-handed disulfide-containing oxytocin binds to the receptor in an active form, whereas the right-handed disulfide-containing oxytocin binds to an inactive form. In view of the possible critical dependence of the oxytocin-receptor interaction on the conformation of the hormone, we decided to investigate whether the 225–230 nm CD band could provide

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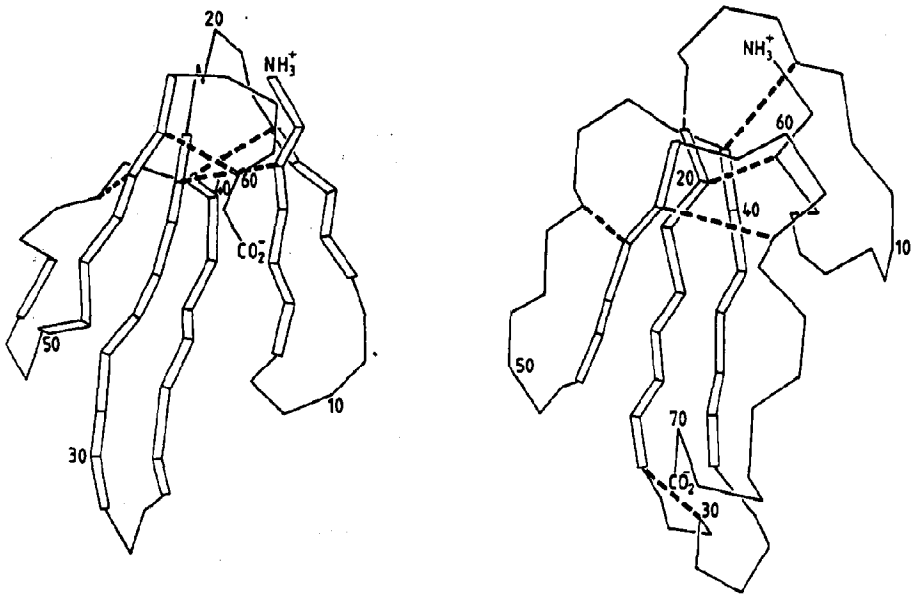
any information on the conformation of neurohypophyseal hormones and their analogs.

2. Elapid α-neurotoxins

Elapid snakes (including cobras, mambas and kraits) all possess polypeptide toxins (60–74 amino acid residues) in their venom, which are capable of binding to nicotinic acetylcholine receptors with high affinity (10^{-10}–10^{-11} M) [11]. Over 90 different sequences have been reported and the entire group is homologous, falling into two major classes: the short neurotoxins (60–62 amino acid residues) and the long neurotoxins (65–74 amino acid residues) [7] (fig. 1). The structures of several of the toxins have been elucidated in detail using X-ray crystallography and ^{1}H-NMR [3,12–14]. The backbone consists of extensive antiparallel β-sheets linked together by a knot of four disulfide bonds (fig. 1). The reactive site of the toxins is located on the second and third loops and possesses a similar chemical character to d-tubocurare [7,15], namely, two basic side chains, Arg-33/33 and Lys-47/49 in a conserved hydrophobic environment provided by Trp-29/25, hydrophobic residues 37/37, 45/52 and 52/54 and aromatic side chain 32/29. The CD spectra of these neurotoxins are typical of proteins containing a mixture of β-sheets and random coils. In addition, they all possess a positive CD band at 228 nm and the 260–320 nm region is dominated by contributions from tryptophan and disulfide bands (fig. 2). When heated, the CD spectrum over the region 220–320 nm changes quite markedly, without a corresponding large change in the 185–220 nm region (fig. 2).

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                    10        20        30        40        50        60        70
Erabutoxin-b        RICFNHQSSQPQTTKTCPSGESSCYHKQWSDFRGTIIERGCGCPTVKPGIKLSCCESEVCNN
α-cobratoxin        IRCFITPDITSKDCPNGHVCYTKTWCDAFCSIRGKRVDLGCAATCPTVKTGVDIQCCSTDNCNPFPTRKRP
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Fig. 1. Backbone structures and sequences of a short α-neurotoxin (erabutoxin) [12] and a long α-neurotoxin (α-cobratoxin) [13].

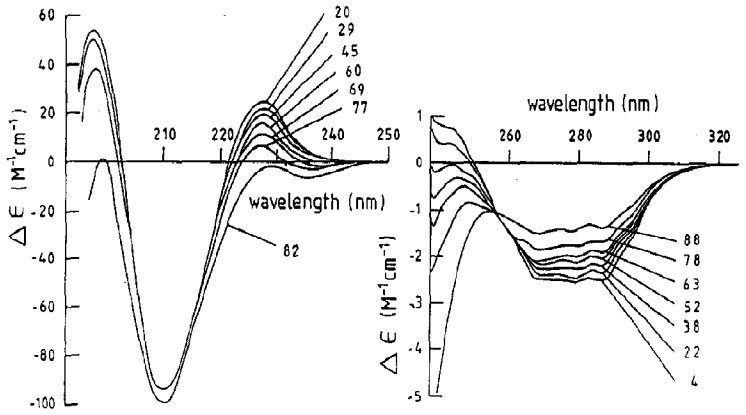

Fig. 2. Variation of the CD spectrum of α-cobratoxin with temperature. 1.2 mg cm^{-3} of toxin was dissolved in water and the pH was adjusted to 8.0. 185–260 nm (0.02 cm path length) and 240–330 nm (1 cm path length).

Again, this is observed with all α-neurotoxins and an isosbestic point is always observed in the region of 260 nm. The existence of the isosbestic point indicates the presence of a well-defined structural change between two sets of toxin conformations.

Parallel variable-temperature CD and ^{1}H-NMR studies with α-cobratoxin have identified residues located on loops 2 and 3 as being associated with the temperature-induced conformation change. These are half-cystine-20 and -41, Lys-24, Thr-25, Trp-26, Leu-39, Val-52 and Ile-54. Significantly, this region includes a large proportion of the reactive site of α-cobratoxin (fig. 3). The spectral change at 228 nm is not observed with α-neurotoxin analogs which are unable to interact with the nicotinic receptor, such as cardiotoxins [5,7] and 'Angusticeps type' toxins [7]. Consequently, the spectral change appears to be monitoring an essential property of the high-affinity cholinergic antagonists. Similar changes in CD spectra can be induced by the presence of nonaqueous solvents [7,16] or chemical derivatization [7].

The dominant chromophore responsible for the 228 nm band in α-neurotoxins has recently been demonstrated to be disulfide functions [17], and not tyrosine residues as previously thought. This is in agreement with the ^{1}H-NMR studies, which detect changes in the C_α protons of half-cystine-20 and -41, between 0 and 50 °C [6]. In contrast, no changes were observed in Tyr-21 proton resonances. Thus, the temperature-sensitive element of toxin structure encompasses both the disulfide core and the essential Trp-29 residue (fig. 3). The residues are connected by side chain interactions along the triple-stranded β-sheet, through which

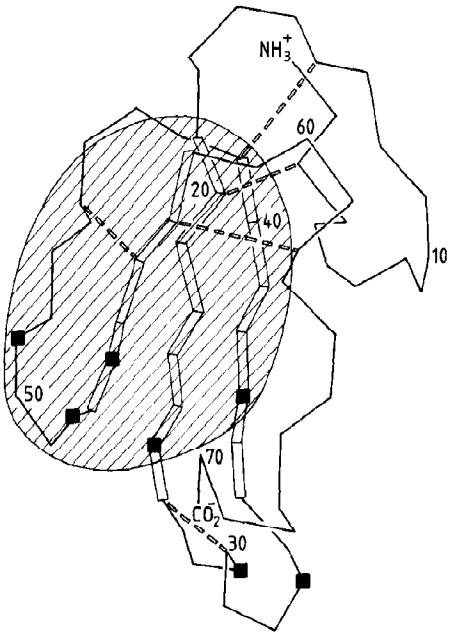

Fig. 3. Backbone structure of α-cobratoxin. (■) residues postulated as forming part of the interactive site with the nicotinic receptor [7]; residues which sense the conformational change associated with the 228 nm CD band [6] are indicated by the hatched area.

allosteric effects can be transmitted. This region is extremely sensitive to changes not only in temperature but also in solvent and to chemical modification [7]. It is likely that the changes in environment presented to the toxin on interaction with the nicotinic receptor will also trigger this conformational change.

3. Neurohypophyseal hormones

Although oxytocin, vasopressin and their analogs possess intramolecular hydrogen bonds in nonaqueous media and in the crystallised state [10,18,19], in water the structure is more flexible [20,21]. The CD of both molecules in the 185–220

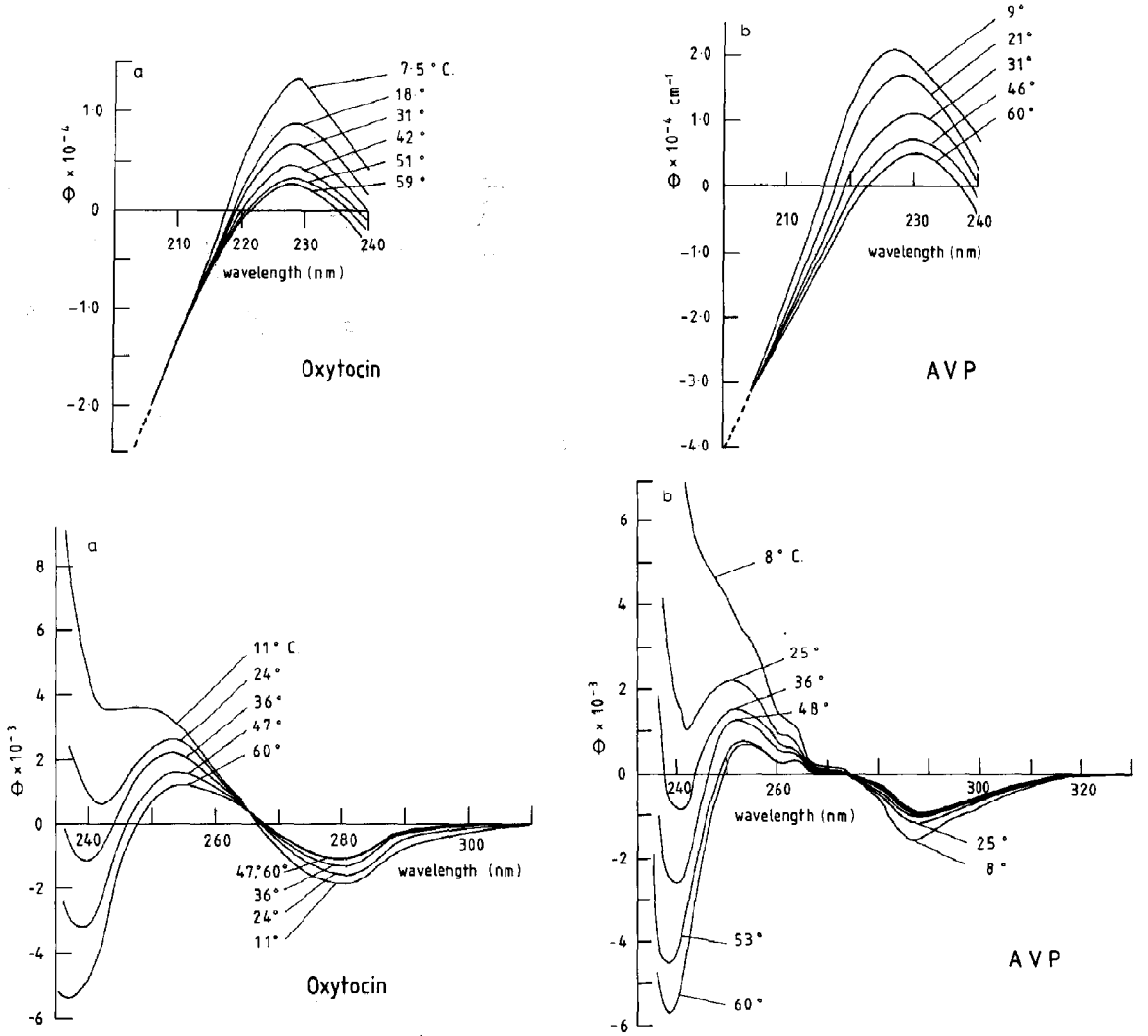

Fig. 4. (a) variation of the CD spectra of oxytocin with temperature. (b) Variation of the CD spectrum of vasopressin with temperature. 1 mg cm^{-3} of the peptides were dissolved in water and the pH adjusted to 8.0. 185–260 nm (0.02 cm path length) and 240–330 nm (1 cm path length).

nm region is typical of a random coil [8,9] (fig. 4). The spectra also possess a positive 225–230 nm band. Although Tyr-2 probably makes a contribution to this band, the disulfide bond would also be predicted to make a contribution in this region [17]. This assignment is supported by the findings that the following tyrosine-free peptides all possess a strong positive CD in the 220–230 nm region; Cys-Lys-Ala-Gly-Gly-Cys, 228 nm [22];

Boc-Cys-Ala-Aib-Gly-Cys-NHMe, 224 nm [23]

and [Pen^1,Leu^2]oxytocin,

220 nm [24]. The only common feature of these three peptides is the macrocyclic system which severely restricts the number of conformations possible for the disulfide structure. As with the

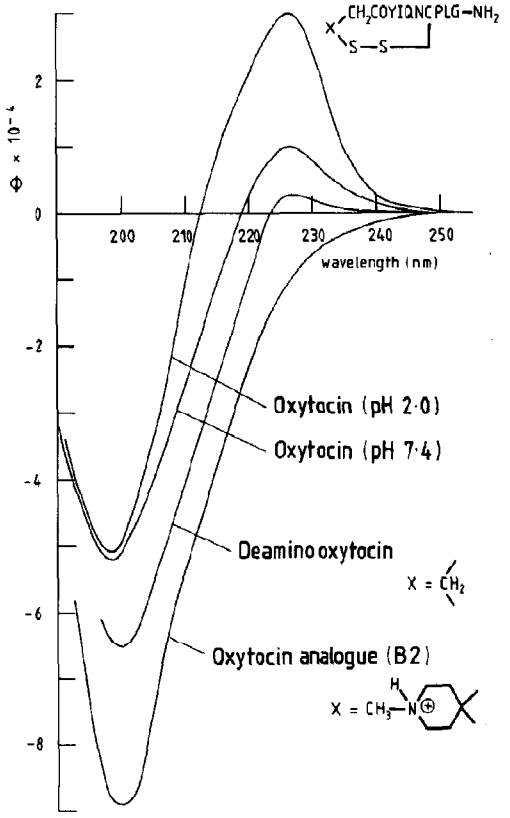

Fig. 5. CD spectra of oxytocin, deamino-oxytocin and oxytocin analog, B2. The peptides (1 mg ml^{-1}) were dissolved in Tris-HCl (10 mM, pH 7.4) unless otherwise stated. A 0.02 cm path length cell was used.

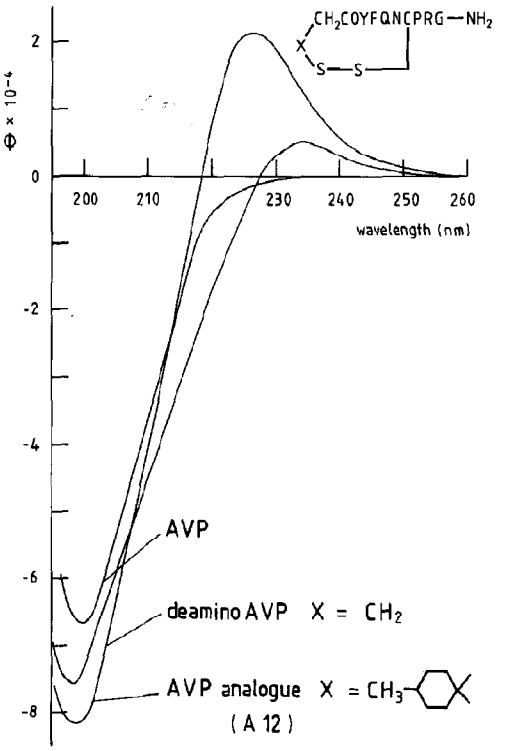

Fig. 6. CD spectra of vasopressin, deaminovasopressin and vasopressin analog, A12. The peptides (0.5–1 mg ml^{-1}) were dissolved in Tris-HCl (20 mM, pH 7.4). A 0.02 cm path length cell was used.

α-neurotoxins, the 228 nm band of both oxytocin [9] and vasopressin is highly temperature-sensitive (fig. 4), showing isosbestic points at 266 and 274 nm, respectively. As the disulfide bond is the only contributor to the CD spectra below 280 nm, the average conformation of this chromophore must differ in the average populations of the two conformational states. In contrast, the overall conformation of the peptide backbone is identical in the two conformational states as indicated by the ellipticity over the 190–210 nm range [9] (fig. 4).

The 228 nm band of oxytocin is extremely sensitive to pH, its amplitude increasing when the N-terminal amine function is protonated [8] (fig. 5), similar to a finding reported for both [Pen^1]oxytocin and [Pen^1,Leu^2]oxytocin [24]. In contrast, deamino-oxytocin possesses only a weak positive CD band at 228 nm (fig. 5) which is relatively insensitive to either protonation [8] or

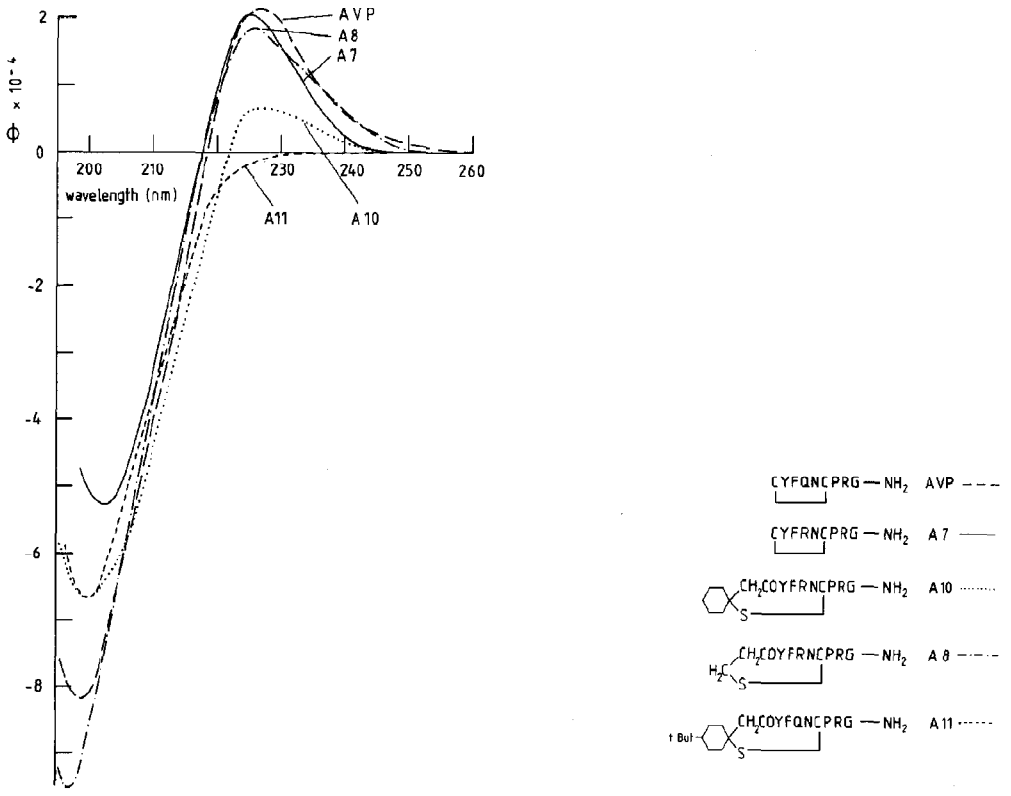

Fig. 7. CD spectra of vasopressin and vasopressin analogs, A7, A8, A10 and A11. The peptides (0.5–1.0 mg ml^{-1}) were dissolved in Tris-HCl (20 mM, pH 7.4). A 0.02 cm path length cell was used.

temperature [9]. An analog, B2, which is probably more rigid, lacks a positive CD band in this region (fig. 5). Two crystal structures of deamino-oxytocin have been reported [10]. They are closely related conformers but possess disulfide bridges of different chirality. It seems probable that in aqueous solution both exist. The CD in the 220–230 nm region is critically dependent on disulfide torsion angles [25]. If one of the conformers possesses a strong positive CD in the 225–230 nm region and the other a weaker band, then an explanation for the spectral differences between oxytocin and deamino-oxytocin becomes apparent. One of the disulfide torsion angles might be preferentially stabilised in oxytocin due to an intramolecular interaction between the protonated N-terminal function and an adjacent side chain, probably Tyr-2 [26,27]. Indeed, amino-tyrosine interactions have been shown to be favorable in proteins [28]. This interaction is not possible in deaminooxytocin and provides an explanation for the pH and temperature sensitivity of the oxytocin 228 nm band. That B2 lacks this band indicates that the disulfide bond possesses a different torsion angle from that favored by oxytocin.

A similar set of spectra are associated with vasopressin analogs (fig. 6). Thus, deamino-vasopressin possesses a weak positive band at 235 nm whereas vasopressin has a strong band at 227 nm. A more rigid vasopressin analog (A12) totally lacks this band. In fig. 7 the spectra of vasopressin analogs are compared with that of vasopressin. Some possess a positive 228 nm band (A7 and A8), some behave like deaminovasopressin (A10) and others totally lack the band (A11 and A12). It emerges that A7 and A8 behave as agonists and A10–A12 act as antagonists.

4. Conclusion

The disulfide chromophore is capable of producing a powerful CD in the 220–230 nm region. Because this signal is strongly dependent on the disulfide torsion angle, it has potential for the monitoring of small conformational changes. The CD of both oxytocin and vasopressin analogs in this region may prove of analytical use to the synthetic chemist.

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