STUDIES ON THE CONFORMATION OF β-ENDORPHIN AND ITS CONSTITUENT FRAGMENTS IN WATER AND TRIFLUOROETHANOL BY CD SPECTROSCOPY

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1. Introduction

Structure–function studies on β-LPH fragments have revealed that the in vitro opiate agonist activity is an exclusive property of fragments containing the complete structure of Met-enkephalin (LPH (61–65)) [1] at their NH_2-terminus and that the biological potency is a function of the chain length of the peptides [2–6]. Peptides intermediate between Met-enkephalin and LPH (61–91) (named β-endorphin by Li and Chung, [7]) were found to be less potent than Met-enkephalin in different in vitro bioassays [2–6]. Elongation of the peptide chain of LPH (61–79) by addition of the 12 COOH-terminal residues of β-LPH [4–6] to form β-endorphin caused a dramatic change in the biological properties of the molecule. Among all the fragments studied so far, β-endorphin is the most active in receptor binding [2,7] in guinea-pig ileum bioassays [3–6] and the least active in mouse vas deferens test [5,6]. In addition, β-endorphin exerts the most profound analgesic [2,4,8,9] and behavioral effects [10,11] when administered intraventricularly [2,4,8,10,11] or intravenously [9] to different animals.

From the above relationships the impression has been created that the COOH-terminal portion of the β-endorphin molecule may exert its biological effects by inducing an overall conformational change in the molecule [5,6]. To test this assumption, we have investigated the solution conformations of β-endorphin and its constituent fragments by CD spectroscopy, a method which has been useful to explore the conformation not only of large polypeptides and proteins [12,13] but also of small peptides containing 6–32 amino acid residues [14,15].

2. Experimental

Porcine β-endorphin, LPH-(61–79) and LPH-(80–91) were prepared as described previously [16,17]. TFE was of spectroscopic grade (Uvasol-Merck). CD Spectra were obtained on a Roussel-Jouan dichrograph mark III (Jobin-Yvon) at sensitivities of 10^{-6} ΔE/mm. Concentration of the samples ranged between 0.5–1.5 mg/ml. Path length of the cell used was 0.01 cm. CD is expressed in mean residue ellipticity units $[\theta]$(deg.cm^2/dmol). No concentration-dependence was observed for the CD spectra of the peptide fragments in the above concentration-range.

3. Results

CD Spectra of β-endorphin and its fragments, LPH-(61–79) and LPH-(80–91) in water* are given in fig.1.

Abbreviations: CD circular dichroism, LPH lipotropic hormone, TFE trifluoroethanol, HFIP hexafluoroisopropanol

*Similar spectra were obtained in 0.05 M ammonium acetate of pH 7.4

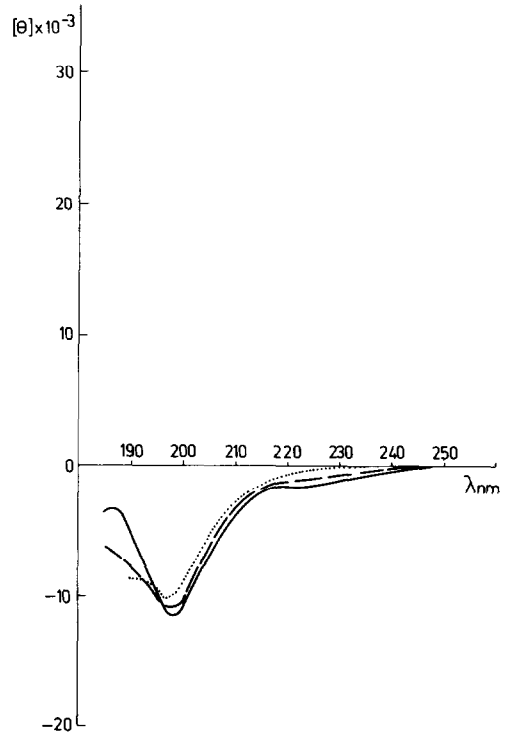

Fig.1. CD spectra of fragments of LPH in water. β-Endorphin (———), LPH-(61–79) (– – – –), LPH-(80–91) (. . . . . .).

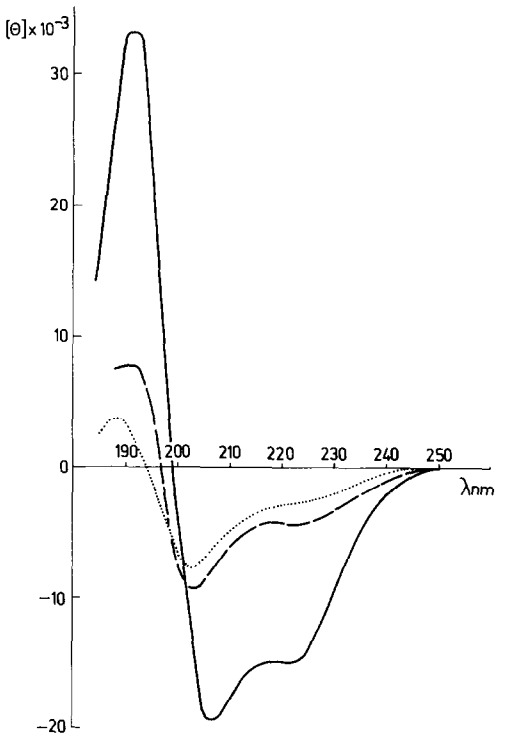

Fig.2. CD spectra of fragments of LPH in TFE. β-Endorphin (———), LPH-(61–79) (– – – –), LPH-(80–91) (. . . . . .).

These spectra, characterized by a strong negative band in the 195–198 nm region, can be attributed to a mostly unordered conformation of the above fragments. Denatured proteins and random polypeptides have similar CD spectra displaying a large negative CD band below 200 nm [13]. The spectrum of β-endorphin, however, differs somewhat from those of LPH-(61–79) and LPH-(80–91) (fig.1). In addition to the negative CD extremum at about 200 nm, a weak but definite CD extremum appears at 222 nm. The presence of this negative band can be regarded as a sign of some α-helix content in β-endorphin in water. Unordered polypeptides show a weak positive Cotton effect at about 220 nm in salt free aqueous solution [13] and aromatic side chain chromophores, too, have positive contribution to the CD in this wavelength range. The rather low negative ellipticity values in the CD spectrum of β-endorphin, below 195 nm, may also be attributed to a positive contribution of the helix content of the molecule.*

The assumption that β-endorphin has a tendency to form α-helix was supported by CD measurements in TFE. The CD spectrum of β-endorphin in pure TFE can be characterized by two negative bands, at 222 nm and 207 nm, and a positive one at 192 nm (fig.2). Cotton effects at the same wavelength and of about the same intensity have been observed in the CD spectra of proteins and polyamino acids of a high degree of α-helical conformation [18]. LPH-(61–79) and LPH-(80–91) in TFE also give CD spectra of

*The low negative ellipticity values below 195 nm cannot be due to the positive contribution of the aromatic chromophores alone because CD values of LPH-(61–79) which has a higher aromatic amino acid/amino acid ratio than β-endorphin (fig.1) are much more negative in this region of the spectrum

helical type (fig.2). The intensities of the extrema are, however, much lower than in the case of β-endorphin. This, together with the blue-shift of the second negative CD band, indicate a relatively low α-helix content of the smaller peptides.

Further investigation of the CD properties of the peptides in TFE/water mixtures were expected to give some information on the stability of the helical conformation in the fragments. The spectra presented in fig.3 show that β-endorphin contains a considerable amount of helical order even at relatively high water concentrations. CD spectra characteristic to a mostly unordered conformation have only been measured in solutions containing less than 30% TFE. In contrast, addition of about 20% water to the TFE solutions of LPH-(61–79) and LPH-(80–91) resulted in an almost complete disruption of the helical order.

The percentage of α-helix, β-structure and unordered conformation in the three β-LPH fragments – in water, TFE and TFE/water mixtures – were calculated from the CD spectra (figs 1–3) by the methods of Greenfield and Fasman [12] and of Rosenkranz and Scholtan [13] (table 1). In addition to the above procedures, we also used a third approach which was assumed to give more reliable percentages of α-helix for polypeptides in nonaqueous media. This latter procedure is based on the method of Greenfield and Fasman [12] with the modification that parameters from CD-values measured in HFIP were applied [20] (see the formula given in table 1)*. As to the reliability of our calculations, it is rather promising that all the three methods gave comparable values for the α-helix content of the peptides in different solvent systems (table 1).

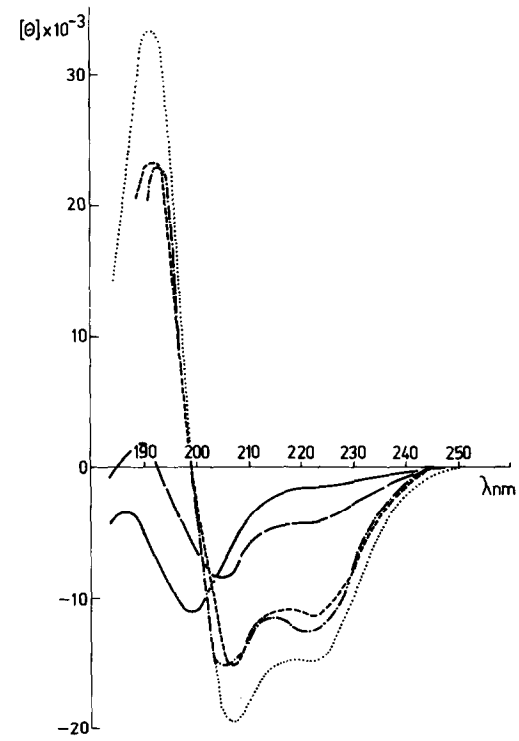

Fig.3. CD spectra of β-endorphin in mixtures of TFE/water. TFE (. . . . . .), TFE/water (60:40) (- - - - - - - -), TFE/water (40:60) (– · – · – · –), TFE/water (15:85) (– – – –), water (————).

4. Discussion

Our CD studies indicate β-endorphin to have a low α-helix content even in water (fig.1, table 1). With increasing TFE concentration a gradual increase of this α-helix content was observed (fig.3, table 1). TFE is known (see ref. [15] and references therein) to have a β-sheet and α-helix stabilizing effect by inducing formation of intramolecular hydrogen bonds and/or nonpolar interactions. It has also been suggested by Urry et al. [19] that TFE may mimic the natural lipophilic environment of the receptors in the membrane.

The predictive rules of Chou and Fasman [21] indicate that the most probable location of a helical region in β-endorphin is between Pro^{73} and Lys^{89}. Indeed, this particular 17 membered helical stretch may account for the 55–60% α-helix content of β-endorphin in pure TFE (fig.2, table 1). In contrast, LPH-(80–91), a dodecapeptide fragment derived from the same portion of β-endorphin shows unexpectedly low tendency to form α-helix even in pure TFE (fig.2, table 1). This seeming contradiction suggests

*HFIP and TFE have very similar solvent properties as demonstrated by the nearly identical CD spectra of poly-γ-methyl-L-glutamate in the two solvents (cf. ref. [18] and [20]). We think that elliplicity values of poly-γ-methyl-L-glutamate in HFIP for the α-helix and those of poly-γ-morpholinylethyl-L-glutamamide for the unordered conformation [20] can well be used for the evaluation of the α-helix content in TFE, too.

Table 1
Estimated percentages of α-helix (α) and unordered conformation (ρ) of β-endorphin and its fragments from CD curves by the methods of Greenfield and Fasman (method I and II, [12,20]) and Rosenkranz and Scholtan [13][a]

Fragments of LPH	Solvent TFE/water	Method I		Method II[b]		Method III	
		%α	%ρ	%α	%ρ	%α	%ρ
61–91	100% TFE	60	40	57	–[c]	55	70[d]
	60% TFE	40	60	43	–[c]	35	80[d]
	40% TFE	40	60	43	–[c]	35	80[d]
	30% TFE	30	70	39	–[c]	30	85[d]
	15% TFE	10	–[c]	26	–[c]	10	100[d]
	Water	< 5	–[c]	–	–	< 10	100
61–79	100% TFE	10	–[c]	25	–[c]	15	100[d]
	Water	0	–[c]	–	–	< 5	100
80–91	100% TFE	< 10	–[c]	25	–[c]	10	100[d]
	Water	0	–[c]	–	–	< 5	100

[a] β-Conformation in fragments of LPH could not be detected by CD spectroscopy
[b] Percentage of α-helix in TFE is estimated according to the following formula using parameters from CD spectra measured in HFIP [20]

$$\%\alpha = \frac{[\theta]_{211} - 5000}{-34\,000 - 5000} \times 100$$

$[\theta]_{211}$ is the measured ellipticity at the β-structure – unordered chain isoellipticity point
[c] Percentage of unordered conformation cannot be estimated by this method
[d] Percentage of unordered conformation is overestimated by this method in solvents containing TFE

that the α-helical structure of intact β-endorphin is stabilized by presumably nonpolar interactions between the helical COOH-terminal and nonhelical NH_2-terminal parts of the molecule. The partial resistance of β-endorphin to exopeptidase action [5,6] lends further support to the assumption that the NH_2-terminal hydrophobic portion of the molecule is involved in these interactions. Similar α-helix stabilizing interactions have been recognized earlier in lysozyme [22] and secretin [14].

Based on the above spectroscopic observations a well-defined steric structure can be proposed for β-endorphin, in an α-helix promoting environment, at least. The most characteristic features of this architecture are the helical conformation of the peptide chain between residues 73–89 and the spatial proximity of the helical stretch and the non-helical NH_2-terminal portion (residues 61–65) of the molecule. It may be recalled that this latter sequence is the active site of β-endorphin [1–7]. Since the membrane surface can be regarded as α-helix promoting environment [15,19] one may assume that the above proposed steric structure represents the biologically active architecture of β-endorphin. The extreme biological properties of β-endorphin [2,4–11] may be well explained on the basis of this proposal.

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