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Conformation of β-Endorphin Analogues in Cerebroside Sulfate Solution

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SUMMARY

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The conformation of synthetic β-endorphin fragments in cerebroside sulfate solutions was studied by circular dichroism. The lipid was solubilized by the inert nonionic surfactant cetylpoly(oxyethylene) ether to facilitate optical measurements. All peptides show an aperiodic conformation in water. Addition of cerebroside sulfate induces a partial helical structure for human peptides, β_h-endorphin(14–31), (17–31), and (1–5)–(16–31), and porcine peptides, β_p-endorphin(1–25) and (6–31), but α-endorphin, γ-endorphin, and β_h-endorphin(1–19), (6–17), (22–31), and (26–31) remain unordered in the lipid solution. Thus, the helical segment in β-endorphin is deduced to be in the middle region of the parent molecule, probably involving two to three helical turns of approximately eight to nine amino acid residues between residues 13 and 24. This helical segment may bring the active sites of the otherwise flexible polypeptide to a correct geometry in the lipid environment in order to express its biological activity.

INTRODUCTION

Naturally occurring polypeptides without disulfide bonds usually exist in an unordered form in aqueous solution (1, 2), but they should assume a rigid structure when interacting with a biological system appropriate to initiate a biological response. β-EP,[6] a newly discovered endogenous peptide which interacts with opiate receptors at the surface of synaptosomal membrane (3), has 31 amino acid residues (4). The sequence of human β_h-EP (5) is Tyr^{+}-Gly-Gly-Phe-Met^{5}-Thr-Ser-Glu^{-}-Lys^{+}-Ser^{10}-Gln-Thr-Pro-Leu-Val^{15}-Thr-Leu-Phe-Lys^{+}-Asn^{20}-Ala-Ile-Ile-Lys^{+}-Asn^{25}-Ala-Tyr-Lys^{+}-Lys^{+}-Gly^{30}-Glu^{2-} [β_p-EP has Val-23, His-27, and Gln-31 instead of Ile, Tyr, and Glu (6)]. β-EP is unordered in aqueous solution but assumes a partial helical conformation in the presence of acidic lipids of brain membrane (7). To elucidate the peptide segment that is crucial for the helix formation, we report herein the conformations of synthetic β-EP fragments in cerebroside sulfate solution as deduced from their CD spectra. The acidic lipid fulfills the requirement of electrostatic interaction and simulates a hydrophobic environment in the membrane that is conducive to the formation of a partial helical structure in the peptide hormone, which brings about a correct geometry necessary for its biological function.

MATERIALS AND METHODS

β-EP analogues were synthesized as described previously (8). β_h-EP(6–31) and β_h-EP(1–5)–(16–31) were gifts from Peninsula Laboratory (Belmont, Calif.). Cerebroside sulfate (M_r 894) was purchased from Supelco, Inc., Bellefonte, Pa. $C_{16}E_{13.5}$ (Kao Soap Company, Tokyo, Japan) was purified from a 1-butanol solution (9).

Appropriate amounts of the peptide, cerebroside sulfate, and the surfactant from stock solutions were mixed with the aid of a Vortex mixer. The molar ratio of cerebroside sulfate to $C_{16}E_{13.5}$ was kept at 1:5 and that of the peptide (residue) to cerebroside sulfate varied between 1:10 and 1:15 (in this range the conformational changes reached a maximum and the CD spectra were

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[6] The abbreviations used are: β-EP, β-endorphin (β_h for human and β_p for porcine); $C_{16}E_{13.5}$, cetylpoly(oxyethylene) ether; SDS, sodium dodecyl sulfate.

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independent of the lipid concentration) (7). Concentrations of the polypeptides were determined spectrophotometrically. Absorbances, $A^{1\%}_{1cm}$ at 276 nm were calculated from the respective amino acid compositions, assuming a molar absorbance of 1200 for tyrosine and 130 for phenylalanine. Two polypeptides without tyrosine residues were weighed and corrected for an assumed 10% moisture content. Table 1 lists the molecular weights, mean residue weights (M_0), and absorbances of β-EP and its analogues.

CD was measured with a Jasco J-500A spectropolarimeter with a data processor DP-500 attachment under nitrogen flush. The cell holder was a specially designed aluminum block with a circulating water jacket and its temperature was maintained at 25° with a Haake constant-temperature regulator. Fused silica cells of various path lengths were used to cover the wavelength range of 188 to 240 nm. The cells were calibrated with a sucrose solution of known optical rotation (National Bureau of Standards grade) on a Cary 60 spectropolarimeter. Both spectropolarimeters had been standardized with a *d*-10-camphorsulfonic acid solution (10).

The CD spectra of freshly prepared solutions were measured within 1 hr. Both the sample solutions and the blanks, which included the optically active lipids, were scanned with the data processor at least four times at 2 nm/min or eight times at 5 nm/min. The readings of the blanks were subtracted directly from those of the sample solutions on the data processor. The data were expressed in terms of mean residue ellipticity, from the equation: $[\theta] = (M_o/100)(\theta/lc)$, where θ is the ellipticity in degrees, l is the light path in decimeters, and c is the polypeptide concentration in grams per milliliter (11).

RESULTS

β-EP and all of its analogues have an aperiodic (or unordered) conformation in water or in $C_{16}E_{13.5}$ solution as judged from their CD spectra, which have a strong negative band near 198 nm (11). The addition of cerebroside sulfate induces a partial helical conformation in 6 of the 12 polypeptides studied. Figure 1 presents the CD spectra of the six polypeptides that lack an ordered conformation in lipid solution. These include three peptides from the *N*-terminus, β-EP(1–16), β-EP(1–17), and β-EP(1–19); two from the *C*-terminus, β_h-EP(22–31) and β_h-EP(26–31); and one peptide in between, β-EP(6–17). All except β_h-EP(26–31) show a 198-nm minimum that is typical of an unordered form. The difference in ellipticities among the polypeptides may reflect different degrees of flexibility of the peptide chains. β_h-EP(26–31) has two maxima at 225 and 207 nm due to the tyrosine residue at position 2 and a minimum that is shifted to 192 nm (for reasons unknown). The two maxima can no longer be detected when four nonaromatic residues are added to the hexapeptide as in β_h-EP(22–31).

TABLE 1

Molecular weights, mean residue weights, and absorbance of β-endorphin and its analogues

β-Endorphin and its analogues	Molecular wt (no. of residues)	Mean residue wt	$A^{1\%}_{1cm}$ at 276 nm
β-EP(1–16)[a]	1746 (16)	109	7.62
β-EP(1–17)[b]	1841 (17)	108	7.22
β-EP(1–19)	2135 (19)	112	6.84
β_p-EP(1–25)	2774 (25)	111	5.26
β_h-EP(1–31)	3465 (31)	112	7.70
β_p-EP(6–31)	2869 (26)	110	—
β_h-EP(14–31)	2050 (18)	114	6.49
β_h-EP(17–31)	1737 (15)	116	7.66
β_h-EP(22–31)	1164 (10)	116	10.31
β_h-EP(26–31)	695 (6)	116	17.27
β-EP(6–17)	1304 (12)	109	—
β_h-EP(1–5)-(16–31)	2394 (21)	114	11.10

[a] α-Endorphin.
[b] γ-Endorphin.

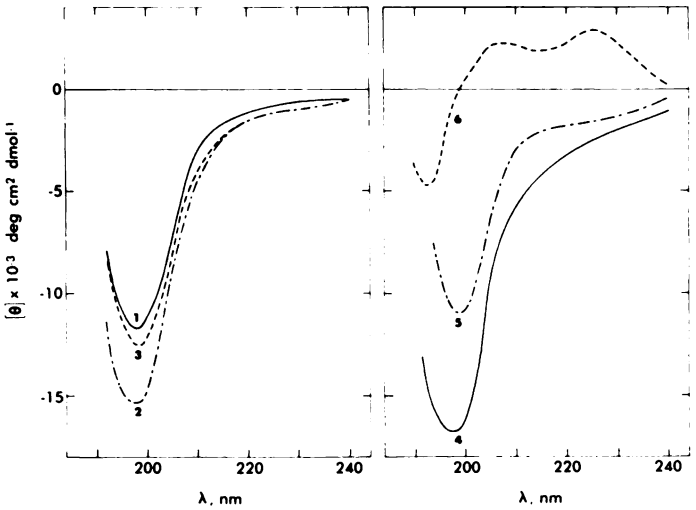

FIG. 1. *CD spectra of β-endorphin fragments in cerebroside sulfate solution at 25°*

Curves: *1*, 23.6 μM β-EP(1–16); *2*, 22.1 μM β-EP(1–17); *3*, 21.1 μM β-EP(1–19); *4*, 32.1 μM β-EP(6–17); *5*, 26.3 μM β_h-EP(22–31); *6*, 58.3 μM β-EP(26–31). Solutions represented by curves *1* and *2* contained 280 μM cerebroside sulfate and 1.6 mM $C_{16}E_{13.5}$. The remainder contained 320 μM cerebroside sulfate and 1.6 mM $C_{16}E_{13.5}$. The pH without adjustment ranged between 3.5 and 4.3.

The conformation of β_p-EP(1–25) and β_h-EP(1–5)-(16–31), which has 20 amino acid residues, differs markedly from that of the three *N*-terminal polypeptides with 16, 17, and 19 residues. In cerebroside sulfate solution, the CD spectra of these two polypeptides (Fig. 2, curves *1* and *2*) resemble the spectrum of β_h-endorphin (curve *3*). All show two negative bands at 222 and 208 nm and a stronger positive band near 192 nm, which are characteristics of a helical conformation (11). On the basis of CD magnitudes, approximately one-quarter or more of each polypeptide molecule assumes a helical conformation (for the method of CD analysis, see ref. 12).

The CD spectra of the three C-terminal polypeptides, β_h-EP(6–31), β_h-EP(14–31), and β_h-EP(17–31), also show the characteristic double minima and a maximum in the ultraviolet region (Fig. 3) and indicate the presence of a partial helical conformation. Removal of the *N*-terminal pentapeptide (Met-enkephalin) from β-EP actually increases the ellipticities by 10–15% (curve *1*; cf. Fig. 2, curve *3*), suggesting that the first 5 residues of β-EP are not involved in the formation of a helix, but the CD

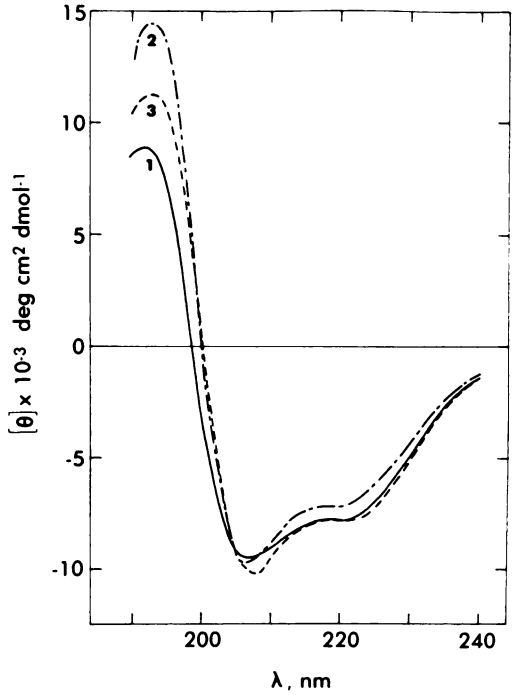

FIG. 2. *CD spectra of β-endorphin fragments at 25°*

Curves: *1*, 15.6 μM β_p-EP(1–25) in 200 μM cerebroside sulfate; *2*, 23.3 μM β_h-EP(1–5)-(16–31) in 280 μM cerebroside sulfate; *3*, 12 μM β_h-EP in 180 μM cerebroside sulfate. All solutions contained $C_{16}E_{13.5}$ (5 times the amount of cerebroside sulfate); pH 3.9–4.3.

magnitudes decrease upon removing the first 13 residues (curve *2*). β_h-EP(17–31) retains only a small amount of helix as is evidenced from its CD spectrum (curve *3*).

DISCUSSION

The first step of the complex formation of β-EP and its analogues with cerebroside sulfate probably involves the binding of positively charged lysine residues with the sulfate group. Additional cerebroside sulfate molecules can then cluster onto the peptide chain and extend the micelle-like amphiphiles in both directions. These amphiphile clusters provide a hydrophobic environment through interaction among their nonpolar tails, which, in turn, stabilizes any ordered structure of a naturally occurring peptide that might have otherwise been broken up because of the peptide backbone-water interactions. It has been postulated that the conformation of polypeptides in an amphiphile solution is related to the structure-forming potential of peptide segments as dictated by their amino acid sequences (1, 2). This hypothesis is applicable to many oligo- and polypeptides in surfactant solutions, mainly in SDS solution (1, 2, 13, 14) and has been extended to the study of a few proteins.[7] Correlation between the induced conformation and peptide sequence in lipid solutions is still not well understood. The helicity of many membrane proteins (15–17) and lipoproteins (18) is enhanced in the presence of lipids, but our CD results seem to indicate that lipids are less potent inducers for the helix than are anionic surfactants, in particular SDS. For instance, $[\theta]_{222}$ of β-EP is only −8,000 (Fig. 2, curve *3*; see also ref. 7) in cerebroside sulfate solution as compared with −12,000 in SDS solution (13). This may be related to differences in binding affinity, degree of hydrophobicity, and the bulkiness of the amphiphile tails (single-chain versus double-chain).

In the absence of X-ray crystallographic results, it has been fashionable to predict the secondary structure of globular proteins from their primary structure. We use the Chou-Fasman method (19, 20), which claims an 80% accuracy, although other empirical sequence-predictive methods can also be used with varying degrees of success (20). Each of the 20 amino acid residues is assigned a parameter for each ordered conformation that is based on probabilities of its occurrence in a particular conformation for globular proteins. A segment of a polypeptide chain is then predicted to have such a conformational potential as dictated by its average conformational parameter that exceeds a chosen limit. According to the Chou-Fasman empirical rules, β_h-EP may have two helical segments (residues 4–9 and 21–24) and a β-segment (residues 14–18). β_p-EP is expected to have approximately the same amount of structure-forming potentials as β_h-EP because the three replacements at positions 23, 27, and 31 virtually do not affect the average conformational parameters of the predicted segments. By the Chou-Fasman method, residues 13–19 can also have a helix-forming potential which overlaps the β-forming potential. This uncertainty makes it difficult to assign unequivocally the structure segments of a polypeptide chain. The exact number of amino acid residues in each segment is also uncertain and can be over- or underesti-

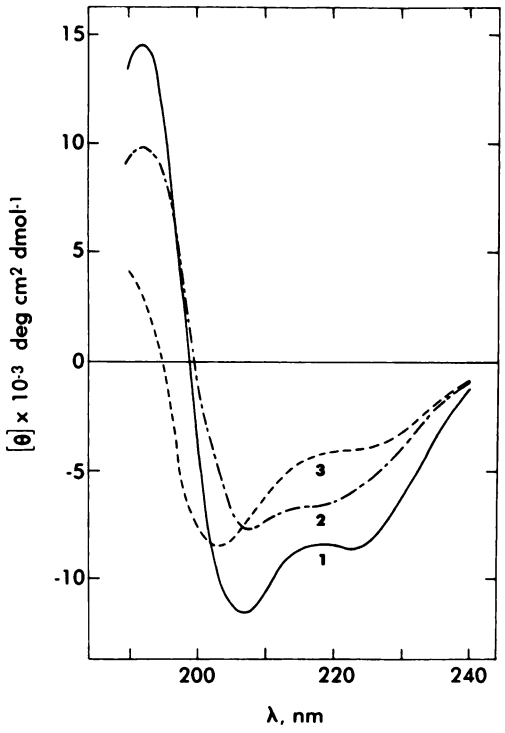

FIG. 3. *CD spectra of β-endorphin fragments at 25°*

Curves: *1*, 22.6 μM β_p-EP(6–31) in 340 μM cerebroside sulfate; *2*, 21.7 μM β_h-EP(14–31) in 320 μM cerebroside sulfate; *3*, 11.9 μM β_h-EP(17–31) in 180 μM cerebroside sulfate. All solutions contained $C_{16}E_{13.5}$ (5 times the amount of cerebroside sulfate); pH 3.8–4.2.

[7] C.-S. C. Wu and J. T. Yang, manuscript in preparation.

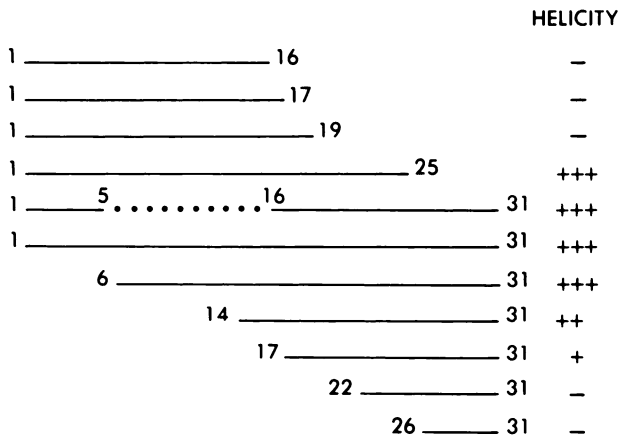

FIG. 4. *Relative helicities of β-endorphin analogues*

mated. Strictly speaking, the method is applicable only to compact, rigid proteins, which is not the case with β-EP. Despite these uncertainties, the sequence-predictive method can qualitatively complement the conclusions drawn from CD spectra (1, 2). By comparing the conformation of β-EP analogues, the helical segments in the parent molecule may be mapped more easily. Figure 4 summarizes the relative helicities of β-EP analogues in cerebroside sulfate solution [β-EP(6–17) is part of β-EP(1–17) and is therefore not listed]. The absence of any ordered structure for the first three *N*-terminal peptides and last two *C*-terminal peptides suggests that at least several residues at either terminus of β-EP do not form part of the helical segments. Because the CD magnitude of β-EP (Fig. 2, curve *3*) is slightly less than that of β-EP(6–31) (Fig. 3, curve *1*) the possibility is ruled out that the first five *N*-terminal residues (Met-enkephalin) are part of the helical segment; otherwise, the removal of this pentapeptide would have lowered the ellipticities of the helical conformation. Thus, the helical segment in β-EP(1–5)-(16–31) is expected to be located in the *C*-terminal 16 residues. This peptide with 21 residues should have a helicity close to that in β-EP(14–31) with 18 residues. Actually, the CD magnitudes (Fig. 2, curve *2*, versus Fig. 3, curve *2*) indicate a smaller helicity for β-EP(14–31). We attribute this difference mostly to the end effect which makes it difficult to initiate a helix from the *N*-terminus or terminate at the *C*-terminus.

Elongation of β-EP(1–19) to β-EP(1–25) converts the peptide from an unordered form into a partial helix, suggesting that residues 20–25 might be a strong helix former or that the addition of a few residues eliminates the end effect in β-EP(1–19). Likewise, the addition of five residues to the *N*-terminal side of β_h-EP(22–31) induces a trace of helix, inclusion of residues 14–16 enhances the helicity, and extension of the *N*-terminus to residue 6 further increases the helicity. Some of these differences may reflect the end effect. Since the *N*- and *C*-terminal peptides overlap, we suspect that the helical segment of β-EP may be located in the middle section of the molecule (probably between residues 10 and 25). By the Chou-Fasman method (19, 20) this span could be narrowed down to residues 13–24, but the actual initiating and terminating boundaries of the peptide in solution cannot be determined. On the basis of the CD spectra, β-EP could have about 25–30% helix, which amounts to eight or nine residues or two to three helical turns.

It remains to be explained why β-EP(1–19) does not form any helix in cerebroside sulfate solution, although the Chou-Fasman method predicts a helical segment for the *C*-terminal one-third of the peptide. It could be that, in addition to the end effect, the binding affinity of cerebroside sulfate to this peptide with one nonterminal positively charged lysine residue is too low to induce an ordered conformation. Indeed, this peptide, but not β-EP(22–31), in SDS solution does form a partial helix.[8]

Although the opiate receptor has been shown to exist on synaptic plasma membrane (3), its chemical nature has not yet been indentified. A model of the opiate analgesic receptor has been proposed on the basis of currently available information about the receptor (21). In this model, the receptor consists of a complex containing two topologically distinct sites, one with a high binding affinity for enkephalin and the other for alkaloids. β-Endorphin can interact with both sites, doing so at the enkephalin site through its *N*-terminal tyrosine and at the alkaloid site through a region near its *C*-terminus. The five lysine residues at positions 9, 19, 24, 28, and 29 are expected to interact with the acidic lipids on the membrane. The helical region in the middle of the peptide, probably including both Lys-19 and Lys-24, brings the β-EP molecule to a correct geometry that fits both sites of the receptor. Deletion of any segment or drastic substitution of the amino acid residues that disturb this stereospecificity is expected to destroy the biological activity of β-EP. Recent studies have shown that camel β_c-EP(6–31) or β_c-EP(20–31) can inhibit morphine- or β-EP-induced analgesia in the mouse tail-flick assay, whereas the *N*-terminal peptide β-EP(1–15) is ineffective (22). These results and the study reported herein raise the possibility that the *C*-terminal analogues, which are inactive or weakly analgesic, may compete with β-EP or morphine at the alkaloid site of the receptor. This binding may require a relatively rigid structure such as those shown here for several β-EP *C*-terminal analogues.

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