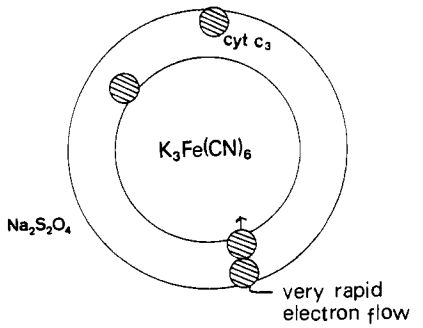

Figure 3. Schematic representation of electron channel in c_3-functionalized liposome. Size of the present c_3 is 33- × 39- × 34-Å,[12] and the thickness of lecithin double layer is 40–50 Å.[15-18]

more efficiently than through isolated c_3. This mechanism is in a good agreement with the observed high electric conductance of the solid c_3 layer, indicating that the intermolecular electron transfer between c_3 is extremely efficient.

Inefficiency of the electron transport through an isolated cytochrome c_3 molecule was compatible with the slow electron transport through the corresponding cytochrome c[14] membrane, $Fe^{III}(i)\cdot Lip^- c$, where an electron was transported only through a first-order kinetic process with cytochrome c (see Figure 2). Therefore, we may draw a conclusion that the "electron channel" formation by the self-aggregation on the artificial membrane is an unique and interesting characteristic of cytochrome c_3 (see Figure 3).[15-18]

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Design and Synthesis of a Model Peptide with β-Endorphin-Like Properties

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We wish to report the synthesis and the characterization of the biological and physical properties of peptide **1** (Figure 1), a model for β-endorphin (Figure 2). On the basis of studies of peptide models of apolipoprotein A-I and melittin, the suggestion has been made that amphiphilic α-helical segments might be important for the biological activities of a variety of peptides which interact with lipid or membrane surfaces.[1] An α-helical structure in the C-terminal region of β-endorphin has previously been postulated to play a role in the receptor binding and opiate activities[2] and resistance to proteolysis[3] of this molecule. We propose here that the β-endorphin molecule consists of the [Met^5]enkephalin region at the N terminus, a hydrophilic "spacer" region from residues 6 through 12, and an amphiphilic helical region between the helix breaker residues[4] Pro^{13} and Gly^{30}. The latter region corresponds either to an amphiphilic α helix with a hydrophobic domain which

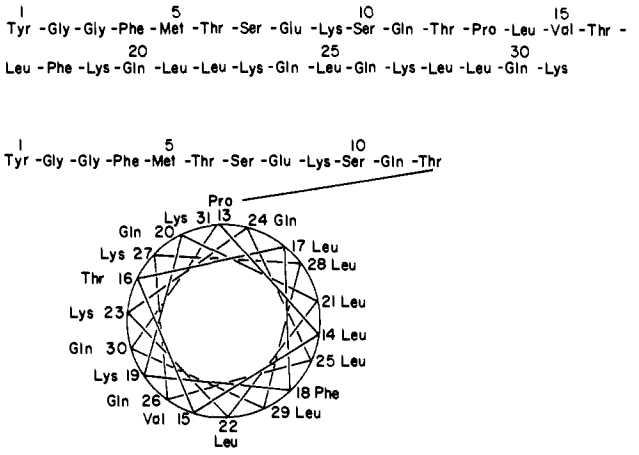

Figure 1. Peptide **1**: amino acid sequence (top) and representation of amphiphilic α-helical segment (bottom) on an Edmundson helical wheel.[12]

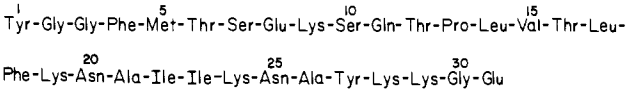

Figure 2. Amino acid sequence of human β-endorphin.

twists around the length of the helix or an amphiphilic π helix,[5] having a hydrophobic domain running straight along the length of the helix.

Because principles for the design of an amphiphilic π helix have not yet been elucidated, we began our development of models for β-endorphin with the synthesis of peptide **1**. As shown in Figure 1, peptide **1** contains a sequence of 31 amino acids having the potential to form an amphiphilic α helix from residues 14 through 31, with a hydrophobic domain running straight along the length of the helix. Peptide **1** has the same amino acid sequence as β-endorphin from residues 1 through 19, which includes the [Met^5]enkephalin region, but has no sequence homology and minimal amino acid residue homology to β-endorphin from residues 20 through 31. In the amphiphilic α or π helix we postulate for β-endorphin residues 14–29, hydrophobic residues cover approximately half of the helix surface, and the hydrophilic residues are either neutral or basic. The peptide **1** sequence from residues 20 through 31 was chosen to reproduce these general characteristics, employing leucines as hydrophobic residues, glutamines as neutral hydrophilic residues, and lysines as basic hydrophilic residues.

Peptide **1** was synthesized by the Merrifield solid-phase method.[6] Cleavage of the peptide from the polymeric support and deprotection was carried out by reaction with anhydrous HF in the presence of anisole at 0 °C. Following extraction of the peptide from its mixture with the resin employing 20% (v/v) aqueous acetic acid and lyophilization, gel filtration was performed on Sephadex G-15 with 0.2 M acetic acid as the eluant. The combined peptide-containing fractions were lyophilized, treated with 10% (w/v) aqueous dithiothreitol solution (0.02 M sodium phosphate buffer, pH 6.6), and then purified by ion exchange chromatography (0.05 M sodium borate buffer, pH 9.0, with a linear gradient of 0–0.25 M NaCl) on CM Sephadex C-25. After lyophilization and desalting, further purification was achieved by partition chromatography on Sephadex G-25 using the solvent system 1-butanol/1-propanol/pyridine/0.2 M aqueous acetic acid (40/19/1/60) followed by lyophilization and gel permeation chromatography (Sephadex G-10, 0.2 M aqueous acetic acid). The yield of pure peptide **1** was 10% on the basis of the crude peptide obtained after the initial Sephadex G-15 gel filtration.

The purified peptide had the expected amino acid composition within experimental error and showed single spots upon TLC in

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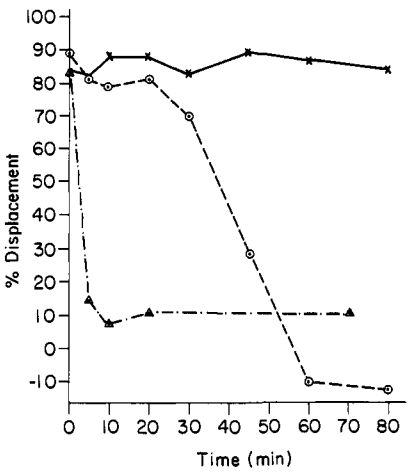

Figure 3. Relative resistance of peptide **1** (×), β-endorphin (○), and [Met⁵]enkephalin (△) toward degradation by proteolytic enzymes endogenous to rat brain. 1.0×10^{-5} M peptide solution in portions of a suspension of whole rat brain homogenate in 110 mL of 0.05 M Tris–HCl, pH 7.4, were incubated at 37 °C. The opiate receptor binding activities of these solutions after 100-fold dilution were then determined as a function of the time of incubation by the method described in the text.

several solvent systems. HPLC of peptide **1** on a Waters μ-Bondapak CN (reverse phase) column, using acetonitrile/0.25 M triethylammonium phosphate buffer, pH 3.0 (31/69), as the eluting solvent, showed one major symmetrical peak ($K' = 7.6$) corresponding to 98% of the components absorbing at 210 or 270 nm.

The circular dichroism (CD) spectra of peptide **1** solutions in 0.02 M sodium phosphate buffer, pH 7.4, containing 0.16 M KCl showed minima at 210 and 222 nm, indicative of α-helical structure. The mean residue ellipticity, θ, at 222 nm showed a concentration dependency consistent with a cooperative monomer–trimer equilibrium with $K_{diss} = 4.1 \times 10^{-12}$ M² (at 222 nm, $\theta_{monomer} = -8400$ deg cm²/dmol and $\theta_{trimer} = 16\,500$ deg cm²/dmol). From these data we calculated the helical content of **1** to be 50% for the trimer and 29% for the monomer.[7] In the presence of 50% trifluoroethanol, a structure promoting solvent, the CD spectra of peptide **1** and β-endorphin are very similar and indicative of α-helical structure. At 222 nm, the molar ellipticity for peptide **1** is −15 400 deg cm²/dmol, corresponding to 47% α helix and does not vary with concentration. For 1.3×10^{-5} M β-endorphin under the same conditions, $[\theta]_{222} = -12\,200$ deg cm²/dmol, which corresponds to 39% α helix,[7] in close agreement with the calculations of other workers[3] employing a similar method.[8] By the method of Bothwell et al.,[9] using a Beckman Spinco Airfuge, the apparent molecular weight of 4.0×10^{-5} M peptide **1** was determined to be 11 400, corresponding to a trimeric structure. In contrast, β-endorphin was found to be monomeric at this concentration.

The resistance of peptide **1** to proteolytic enzymes endogenous to rat brain was compared to that of β-endorphin and [Met⁵]-enkephalin. Equal concentrations (1.0×10^{-5} M) of peptide **1**, β-endorphin, or [Met⁵]enkephalin were incubated at 37 °C with a rat brain homogenate at pH 7.4. After various incubation periods, the aliquots were removed and assayed for opiate receptor binding activity. After 80 min, the incubation mixtures containing peptide **1** still retained full binding activity. β-Endorphin lost all receptor binding activity after 60 min, and [Met⁵]enkephalin was completely degraded in less than 5 min. These results, shown in Figure 3, clearly demonstrate that at the concentrations used peptide **1** has a resistance to enzymatic degradation even greater than that of β-endorphin. However, peptide **1** is primarily in the aggregated form under the conditions of the enzymatic degradation experiments, and therefore we are not comparing the stability of monomeric peptide **1** to monomeric β-endorphin. A different experimental approach than the one used will be necessary to make such a comparison and such experiments are under way.

Table I. Binding of Peptides to Opiate Receptors in Guinea Pig Brain Whole Membrane Preparations

	concentrations for 50% inhibition of specific binding by radioactive ligands, nM	
	[³H][D-Ala², D-Leu⁵]enkephalin (δ-receptor ligand)[a]	[³H]dihydromorphine (μ-receptor ligand)[a]
β-endorphin	35	230
peptide 1	16	80

[a] The abilities of peptide 1 and β-endorphin to inhibit the specific binding of the [³H][D-Ala²,D-Leu⁵]enkephalin (4.7×10^{-10} M) or [³H]dihydromorphine (2.9×10^{-10} M) to guinea pig whole membrane preparations were compared as previously described.[10] At these ligand concentrations, [³H][D-Ala²,D-Leu⁵]enkephalin selectively labels δ receptors and [³H]dihydromorphine selectively labels μ receptors.[11]

The affinities of peptide **1** for different opiate receptors are compared to those of β-endorphin in competitive binding studies,[10] using the δ-receptor ligand [³H][D-Ala²,D-Leu⁵]enkephalin or the μ-receptor ligand [³H]dihydromorphine.[11] The IC_{50} values, shown in Table I, indicate that the opiate receptor binding affinities of peptide **1** are very similar to those of β-endorphin, peptide **1** being slightly more potent in both assays. In the case of the binding study with the μ receptors, at the higher concentrations of peptide **1** which were employed, some trimerization of the peptide would be expected to occur. While this could affect the IC_{50} value, the effect is expected to be rather small.

The following properties, characteristic of β-endorphin, are reproduced by peptide **1**: (i) considerable α-helical structure in 50% aqueous trifluoroethanol; (ii) a high potency for displacing both δ- and μ-receptor ligands; (iii) strong resistance toward proteolytic enzymes, peptide **1** being more stable than β-endorphin. The principal respect in which peptide **1** differs from β-endorphin is in its tendency to aggregate in aqueous solution forming trimers with increased α-helical character in the concentration range 2×10^{-7} to 7×10^{-5} M. This is typical behavior for peptides containing structural units of the type shown in Figure 1.

Our results demonstrate that the C-terminal region from residues 20 through 31 (and, presumably, from residues 14 through 31) has a primarily structural role which can be simulated by a nonhomologous sequence of high α-helix forming ability and with the proper hydrophobic–hydrophilic balance. The close similarity to β-endorphin of the receptor binding affinities of peptide **1** (Table I), which conserves the basicity, lipophilicity, and potential amphiphilicity of β-endorphin using a nonhomologous sequence of amino acids from residues 20 through 31, strongly suggests that all of these properties are important in the receptor-bound conformation of both peptides. We are currently engaged in the synthesis and study of new analogues of β-endorphin, designed with similar considerations but having even less homology to the natural sequence. These studies should elucidate further the structural requirements of β-endorphin for its binding to receptors and resistance to enzymatic degradation.

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