fluid was mapped onto that for the well-known spherical model.[33] This predicts $\beta = {}^1/_2$, in full agreement with our results. But it indicates nonclassical values for ν, δ, and other critical exponents that are yet to be measured.

Hafskjold and Stell[34] give an excellent review of theory for ionic fluids to 1982. But their treatment for the critical exponents was inconclusive and was based on considerations for simple fluids with a single type of particle, as was our recent discussion.[26] It now appears[31,35] that an electrolyte with its long-range-force pattern involving repulsive forces between like particles and attractive forces between unlike particles is fundamentally different and that arguments by analogy to simple fluids are not valid.

There are many opportunities for further research on ionic fluids. For example, measurements of properties related to the other critical exponents would be most welcome, as well as theoretical advances giving predictions over a range of temperature, etc., in the critical region.

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Determining the Functional Conformations of Biologically Active Peptides

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In recent years, the development of microscale protein sequencing and cloning technologies has provided a wealth of information on the amino acid sequences of polypeptides. However, knowledge of the chemical composition of a peptide is of little value in understanding its biological function, unless information concerning its active conformation is also available. In cases where the peptide chains are long enough, they fold into stable globular structures composed of helices, sheets, turns, and loops that can often be characterized in detail by X-ray crystallography and, increasingly, by NMR spectroscopy. This type of structural characterization usually provides an adequate basis for selecting analogues (mutants) that may be prepared by genetic engineering and expression, in order to understand and manipulate function in greater detail.

Unfortunately, many biologically active peptides do not maintain their functional conformations when isolated in a crystalline form or in aqueous solution. They do not fold into independently stable globular structures with a close-packed hydrophobic core, but depend instead upon extensive intermolecular interactions in order to define their active states. Most peptides consisting of fewer than 50 amino acid residues and having no more than one disulfide bridge fall into this category, including a wide variety of peptide hormones that are of great pharmacological and therapeutic interest. These peptides exist in aqueous solution in multiple, rapidly interchanging conformational states and can be expected to adopt different conformations in different environments. Determining the active conformation of such peptides has, therefore, been particularly difficult, since they are often large enough to have complex conformations that contain elements of secondary and even tertiary structure, and they cannot generally be studied directly in their functional environment. This Account describes three complementary approaches to this problem.

Study of Conformation Induction in Peptides at Model Interfaces

Since the biological activities of many flexible, multiconformational peptides are generally expressed upon binding to a cell surface receptor or other biological interface, their functional conformations are most likely to include structural elements that bind to these interfaces. The potential importance of this type of conformation induction at interfaces was introduced in a general form by Kaiser and Kezdy,[1] who suggested that amphiphilic secondary structures, having one surface comprising predominantly hydrophobic ele-

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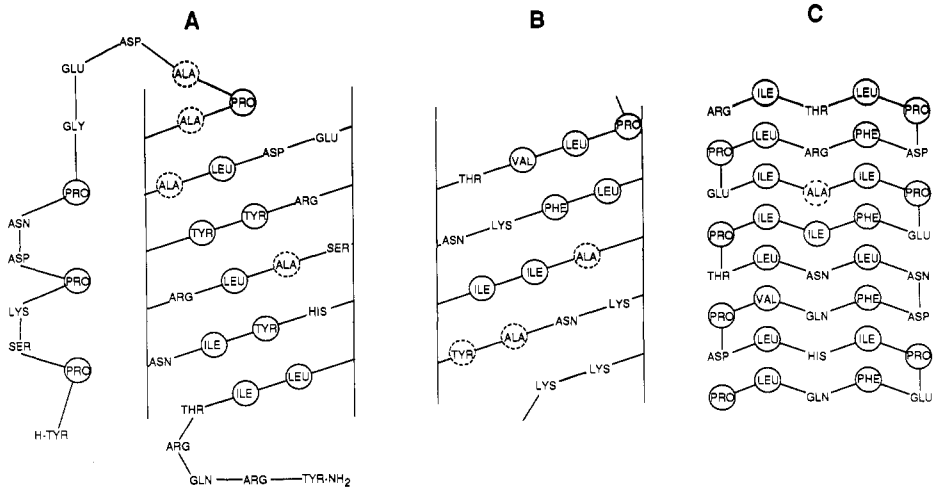

Figure 1. Amphiphilic structural domains in natural peptides. (A) Neuropeptide Y, with the positions of residues 13–32 on a helix surface schematically represented with a helical net diagram;[4] (B) β-endorphin residues 13–29 represented with a helical net diagram; (C) apolipoprotein B residues 2678–2717 in a schematic representation of the potential amphiphilic, antiparallel β-sheet conformation.[6] Hydrophobic residues are circled.

ments and the other predominantly hydrophilic, would be ideal components of the active conformations of flexible peptides. Since that time, a wide variety of biologically active peptides with the potential to form amphiphilic α-helical structures have been identified.[1,2] An example, illustrated in Figure 1A, is neuropeptide Y, which has the potential to form an amphiphilic α-helical segment in residues 13–32 that has a hydrophobic face covering just over half of the helix surface and lying nearly parallel to the helix axis. In this case, an amphiphilic polyproline II like helical conformation can also be postulated for residues 1–8, by comparison with the crystal structure of the homologous avian pancreatic polypeptide.[3] A second example is the opioid peptide β-endorphin, which has a potential amphiphilic α-helical structure in residues 13–29 where the hydrophobic residues form a single domain on the helix surface that curves around the helix in a clockwise direction along its length (Figure 1B).[1,2] Fewer peptides with the potential to form amphiphilic β strands and β sheets have been identified. These include the potential β-strand segments of luteinizing hormone-releasing hormone[1] and dynorphin A(1–17)[2,5] and the proline-rich five-residue repeats in serum apolipoprotein B,[6] which are hypothesized to form the strongly amphiphilic β-sheet structure illustrated in Figure 1C.

The potential importance of surface-induced structural elements has encouraged the development of several approaches to the characterization of peptide conformations at model interfaces. The peptide hormones calcitonin and parathyroid hormone(1–34) have been shown by CD spectropolarimetry to form water-soluble complexes with phospholipids with concomitant induction of amphiphilic α-helical structures within their sequences.[7] Peptide–lipid micelles are also accessible to detailed conformational analyses by NMR, and this approach has identified lipid-induced conformations in glucagon[8] and the enkephalins,[9] for example. Peptide interactions with small unilamellar phospholipid vesicles may also be studied by CD spectropolarimetry with minimal light scattering or absorption flattening artifacts,[10] and information on both the conformation *and* orientation of peptides incorporated into planar phospholipid bilayers deposited onto suitable solid substrates may be obtained from FTIR[11] or CD spectra.[12]

Another system that has been investigated extensively as a model for biological interfaces is the air–water interface.[13] Model peptides, designed to have idealized amphiphilic α-helical[14,15] and β-strand[15,16] structures, have been shown to form stable monolayers on buffer surfaces on a Langmuir trough. Furthermore, when this apparatus is used to monitor the surface pressure (π) generated by these monolayers as a function of their surface area (A), the resultant π–A isotherms are distinctly different for the two types of secondary structures, even when helix- and strand-forming model peptides that have identical amino acid compositions are compared:[15] the idealized amphiphilic α-helical model peptides generally form moderately compressible monolayers that collapse into the aqueous subphase at intermediate surface pressures, whereas the model β-strand peptides self-associate in the plane of the interface to form highly incompressible sheets that collapse at very high surface pressures. These differences may be described in a quantitative fashion by

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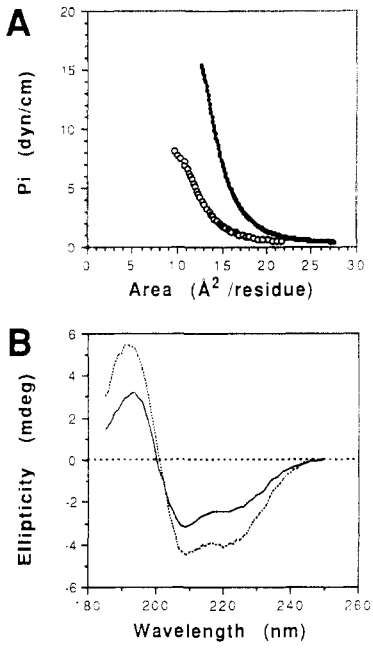

Figure 2. Physicochemical properties of amphiphilic peptides at interfaces. (A) Compression isotherms of β-endorphin (○) and neuropeptide Y (●) monolayers at the air–water interface; (B) CD spectra of β-endorphin (—) and neuropeptide Y (···) adsorbed from aqueous solution onto siliconized quartz slides.

fitting the compression isotherms to equations of state such as that described by Fukushima et al.,[14] $\pi[A - A_0(1 - \kappa\pi)] = nRT$. In this equation, the simple two-dimensional equation of state is modified to include a term describing the surface area occupied by the peptide molecules as a linear function of π that has a limiting value of A_0 at $\pi = 0$ and an associated compressibility constant, κ. This curve-fitting procedure gave values of $A_0 = 23$ Å^2/residue and $\kappa = 0.021$ cm/dyn for an idealized model amphiphilic α-helical peptide, and an apparent molecular weight corresponding closely to the calculated monomer value. In contrast, idealized model amphiphilic β-strand peptides usually have lower A_0 and κ values (typically, 16 Å^2/residue and 0.005 cm/dyn[16,17]) and high apparent molecular weights indicative of an extensively aggregated state.[15-17]

Analysis of the compression isotherms of several peptide hormones that have the potential to form amphiphilic α-helical structures indicates that these helical structures may be stabilized at the air–water interface and that other segments of the peptides lie in the aqueous subphase, unless they also contain significant hydrophobic or amphiphilic elements.[18] For example, analysis of the β-endorphin isotherm shown in Figure 2A gave values of $A_0 = 405$ Å^2/molecule, $\kappa = 0.039$ cm/dyn, and an apparent molecular weight corresponding to the dimer. When the A_0 value is divided by the number of residues in the proposed amphiphilic α-helical structure shown in Figure 1B, giving 24 Å^2/residue, this behavior corresponds well with that of the idealized model α helix described above. A similar analysis of the neuropeptide Y isotherm[3] in Figure 2A gave $A_0 = 551$ Å^2/molecule, or 28 Å^2/residue of the amphiphilic α helix (Figure 1A), and $\kappa = 0.013$ cm/dyn and also indicated an average molecular weight corresponding to the dimer. In this case, the higher value of A_0 calculated in terms of the α helix alone suggests that the amino-terminal amphiphilic polyproline II like helix may also occupy surface area at the air–water interface.

Studies of homopolymeric peptides by Stevens et al.[19] and Cornell[20] have indicated that spectroscopic information concerning the conformations of peptides at the air–water interface may be obtained by transferring their compressed monolayers onto suitable planar solid substrates. In CD experiments, peptides are transferred onto quartz slides dipped vertically through the compressed peptide monolayers. The slides are then placed in a custom-built holder in the path of the circularly polarized light. The number of slides required to give a measurable CD spectrum is readily calculated from the molar ellipticity values and surface areas reported for the transferred monolayers of model amphiphilic α-helical and β-sheet peptides.[15] For example, an α-helical peptide monolayer that occupies 20 Å^2/residue when it is transferred onto quartz slides at 15 dyn/cm is expected to have a mean residual molar ellipticity of −35 000 deg cm^2/dmol, or approximately −6 mdeg Å^2/residue, at 208 nm. This corresponds to an ellipticity at 208 nm of −0.3 mdeg for one monolayer of this helical peptide, so that at least two coated quartz slides (four peptide monolayers) would be required to obtain a spectrum with a reasonable signal-to-noise ratio. Similarly, a β-sheet peptide occupying 14 Å^2/residue at the same pressure and having a mean residual molar ellipticity of −18 000 deg cm^2/dmol at 216 nm will produce an ellipticity of approximately −0.2 mdeg/monolayer, so that at least three coated slides would be required to give a 1-mdeg signal.

The transfer of biologically active peptides from the air–water interface onto siliconized quartz slides is often less efficient than it is for idealized amphiphilic model peptides, and a measurable CD spectrum is not always obtained.[17] Because of these difficulties, we have explored the simpler approach of adsorbing peptides directly from aqueous solution onto coated quartz slides and find that this is frequently more successful.[5,21] For example, both β-endorphin and neuropeptide Y were efficiently adsorbed from buffered saline solution onto siliconized quartz slides within 5 min. After these slides were rinsed with distilled water to remove excess peptide solution and allowed to dry under ambient conditions, the CD spectra shown in Figure 2B were obtained. These spectra indicate that the surface-adsorbed conformations of the two peptides consist predominantly of α helices lying in the plane of the slides, as expected. Assuming that both peptides occupy surface areas of 12 Å^2/residue on the slides, corresponding to the areas of their collapsing monolayers at the air–water interface (Figure 2A), an ellipticity of −0.42 mdeg/monolayer of α-helical peptide is predicted. This is in reasonable agreement with the spectra in Figure 2B, which were measured by using four slides, or eight coated surfaces.

This adsorption method has also been used to characterize β-sheet and disordered surface-binding peptides

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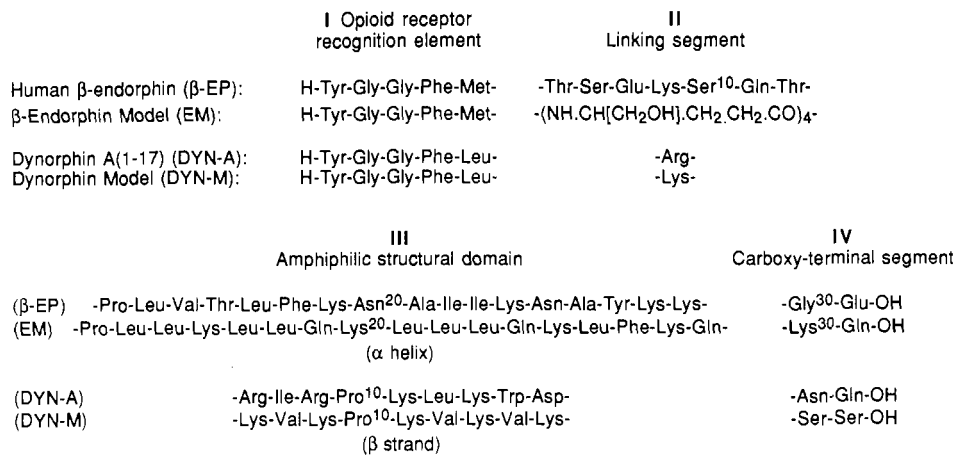

Figure 3. Comparison of the potential structural domains of β-endorphin and dynorphin A(1–17) and their minimally homologous models.

and distinguishes these from peptides that have a low surface-binding potential and do not bind to the siliconized slides.[17,21] It appears, therefore, to be a suitable addition to the current range of model systems that may be studied in order to identify peptide conformations of potential functional importance at biological interfaces.

Design and Study of Minimally Homologous Peptide Models

The identification of regions of a peptide sequence that have the potential to form amphiphilic secondary structures often allows the entire peptide chain to be considered in terms of a small number of hypothetical structural domains that have quite distinct characteristics.[2] This type of analysis is usually facilitated by consideration of data obtained from more traditional structure–activity studies involving single-residue deletion or substitution analogues. For example, both of the opioid peptides β-endorphin and dynorphin A(1–17) have regions of potential amphiphilic secondary structure. The amphiphilic α helix that might be formed by residues 13–29 of β-endorphin has already been described (Figure 1B), and the properties of this peptide at interfaces (Figure 2) indicate that this structural element is indeed likely to be induced on cell surfaces. On the basis of the simple observation that strongly hydrophilic, charged residues and relatively hydrophobic, apolar residues occupy alternate positions in the peptide chain, residues 7–15 of dynorphin A(1–17) are, in contrast, predicted to adopt an amphiphilic β-strand conformation on cell surfaces.[2,5] In this case, however, the proline residue in position 10 appears to introduce sufficient irregularity into the potential β-strand structure to prevent dynorphin A(1–17) from self-associating in the form of strongly amphiphilic β sheets at the planar model interfaces described above.[17] Nevertheless, we expect cell surfaces to have a less regular hydrophobic/hydrophilic boundary than these model interfaces and provide a stabilizing, complementary environment for this amphiphilic structure also.

Structure–activity studies of opioid peptides in general indicate that residues 1–5 have highly specific interactions with all opioid receptor subtypes.[2] In contrast, the residues carboxy-terminal to these appear to have few specific receptor contacts, and yet these residues are essential determinants of the receptor subtype selectivity and agonist activity of the opioid peptides. This allows us to propose that β-endorphin and dynorphin A(1–17) are both comprised of three functionally important structural elements (Figure 3): both have opioid receptor recognition sites in residues 1–5 that are connected, at their carboxy-terminal end, to different positively charged amphiphilic secondary structures via hydrophilic linking elements. The proposed hydrophilic linking elements are also distinctly different for the two peptides: the β-endorphin linker is seven residues long and overall neutral, whereas the dynorphin A(1–17) linker consists of a single basic residue.

In order to test the structural hypothesis described above for β-endorphin and dynorphin A(1–17), we have adopted the approach of studying minimally homologous synthetic peptide models.[1,2] In this approach, it is first assumed that there may be structural domains in biologically active peptides where the general characteristics of the functional conformation, such as its amphiphilic or ionic character, are of much greater importance than the specific amino acid residues in the peptide chain. Under these circumstances, if the proposed active structure is replaced by a minimally homologous, idealized model structure that is able to reproduce its essential physicochemical properties and biological function, then evidence in support of the structural hypothesis will have been obtained. In the present case, the selectivities of β-endorphin and dynorphin A(1–17) for the μ-, δ-, and κ-opioid receptor subtypes have been compared to those of the minimally homologous peptide models described in Figure 3.[5,22] The β-endorphin model peptide includes a nonnatural structure consisting of four residues of (*S*)-γ-(hydroxymethyl)-γ-aminobutyric acid in place of the native hydrophilic linker sequence[23] and a substituted sequence of natural amino acid residues chosen for their high helix-forming propensity[24] and designed to mimic the amphiphilic and cationic character of the proposed α-helical structure in β-endorphin residues 13–29. When these two peptides are compared, the endorphin model has only four sequence homologies with β-en-

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Table I
Binding of Peptide Models to Opioid Receptors in Guinea Pig Brain Membranes

peptide	IC_{50} ± SEM, nM		
	μ sites	δ sites	κ sites
β-endorphin	2.57 ± 0.53	2.64 ± 0.42	313 ± 69
endorphin model	1.60 ± 0.44	1.41 ± 0.35	106 ± 38
dynorphin A(1–17)	3.16 ± 0.622	13.1 ± 2.9	0.626 ± 0.189
dynorphin model	4.82 ± 1.11	39.1 ± 3.2	0.535 ± 0.044

[a] Receptors were selectively labeled with [^{3}H][D-Ala^2,Me-Phe^4,Gly^5]hydroxyenkephalin (μ sites), [^{3}H][D-Pen^{2,5}]enkephalin in the presence of excess [D-Ala^2,Me-Phe^4,Gly^5]hydroxyenkephalin (δ sites), and [^{3}H]bremazocine in the presence of excess [D-Ala^2,Me-Phe^4,Gly^5]hydroxyenkephalin and [D-Ala^2,D-Leu^5]enkephalin (κ sites), as described before.[22]

dorphin residues 6–31. The dynorphin model peptide includes Lys substituted for Arg, as the hydrophilic linker, and a sequence of alternating lysine and valine residues substituting for the proposed amphiphilic β-strand segment, the valine residues being chosen because of their expected preference for that conformation.[24] This model has only three sequence homologies with dynorphin A in residue positions 6–17. Both model peptides were designed to retain proline residues that were expected to be important and unique elements of the proposed active conformations.

The results of our assays of the competitive binding of these native and model opioid peptides to selectively radiolabeled μ-, δ-, and κ-opioid receptors in guinea pig brain membranes are summarized in Table I. These results indicate that the β-endorphin model is able to reproduce the potency of β-endorphin for binding to μ and δ receptors and its selective affinity for these receptors over κ receptors. Similarly, the dynorphin model is equipotent to dynorphin A(1–17) in the κ receptor assays and also successfully reproduces the selective affinity of this native peptide for κ receptors over μ and δ receptors. We conclude, therefore, that the model linker and amphiphilic secondary structure elements reproduce all of the structural and conformational characteristics of the corresponding elements in the native opioid peptides that determine the affinities and selectivities of these peptides for their receptors.

A recent model proposed by Schwyzer[25] attributes the relative affinities and selectivities of opioid peptides for μ, δ, and κ receptors to ionic interactions of the peptide ligands with the surrounding negatively charged phospholipid surface. In this model, the binding site on κ receptors for the opioid receptor recognition elements (residues 1–5) of the ligands is located deep in the membrane surface, so that cationic ligands such as dynorphin are selectively bound; the corresponding binding site on δ receptors is located in the aqueous phase well removed from the membrane surface and is postulated to have an associated positive charge, so that anionic ligands such as the enkephalins are selectively bound; and the corresponding site on μ receptors lies between these two extremes, but also in the polar, aqueous phase. In view of the surface-binding properties of amphiphilic peptide structures in general, the functional domains identified in β-endorphin and dynorphin A(1–17) by our model peptide studies may be related to Schwyzer's proposal as shown in Figure 4. In this schematic representation, the positively charged

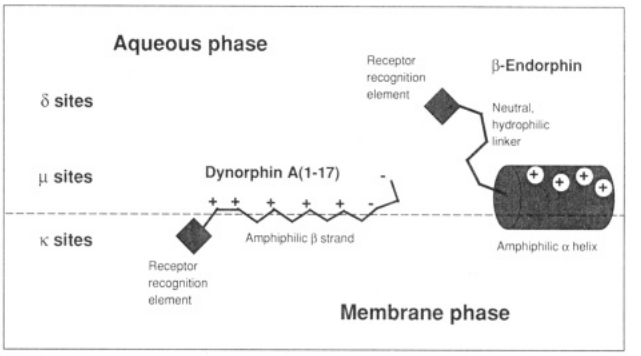

Figure 4. Opioid receptor binding of β-endorphin and dynorphin. The cationic amphiphilic structural domains bind to the hydrophilic/lipophilic interface of the anionic membrane surface (dashed line). The characteristics of the linking peptides connecting these amphiphilic structures to the receptor recognition elements at the amino termini determine the selectivities of these elements for their binding sites on μ, δ, and κ receptors, which are located at different levels relative to the interface.[25]

amphiphilic secondary structures are induced upon binding to the anionic cell surface interface. The relatively nonspecific binding of these structural elements to this interface may serve to facilitate location of opioid receptors on the membrane surface by limiting diffusion of the ligands to the plane of the interface. In addition, attachment of the receptor recognition element in residues 1–5 of dynorphin A(1–17) to the amphiphilic β strand via a short, basic linker element has the effect of concentrating the receptor recognition element at the level of the κ sites, so that the receptor affinities are ordered $\kappa > \mu > \delta$. In contrast, the amphiphilic α-helical structure in β-endorphin is separated from residues 1–5 by a lengthy, flexible peptide chain that has a neutral, hydrophilic character compatible with the environment of both the μ and the δ sites, but not with the relatively hydrophobic environment of the κ sites. The receptor affinities of β-endorphin are, therefore, ordered $\mu = \delta \gg \kappa$.

Synthesis of Peptides with Multiple Conformational Constraints

As might be expected, some flexible peptide hormones appear to have a functional requirement for specific residues in regions of their structures that form amphiphilic α helices at model interfaces. For these peptides, the approach of studying minimally homologous models is often inconclusive. For example, even a limited number (eight) of conservative substitutions for residues on the hydrophilic face of the potential amphiphilic α helix in neuropeptide Y reduced its potency in receptor binding assays 500-fold, although the physicochemical properties of this model were similar to those of the native peptide.[3] This example and others[2] illustrate the need for a different approach, where homology is initially maximized, in order to determine the functional role of structural segments that have been proposed for a flexible peptide. Even when the functional importance of a small number of discrete structural domains in a peptide is well established, as in the case of β-endorphin, the studies of minimally homologous models need to be extended in order to determine the spatial relationship between these domains. The most useful approach to both of these problems is likely to be the study of peptide analogues incorporating conformational constraints.

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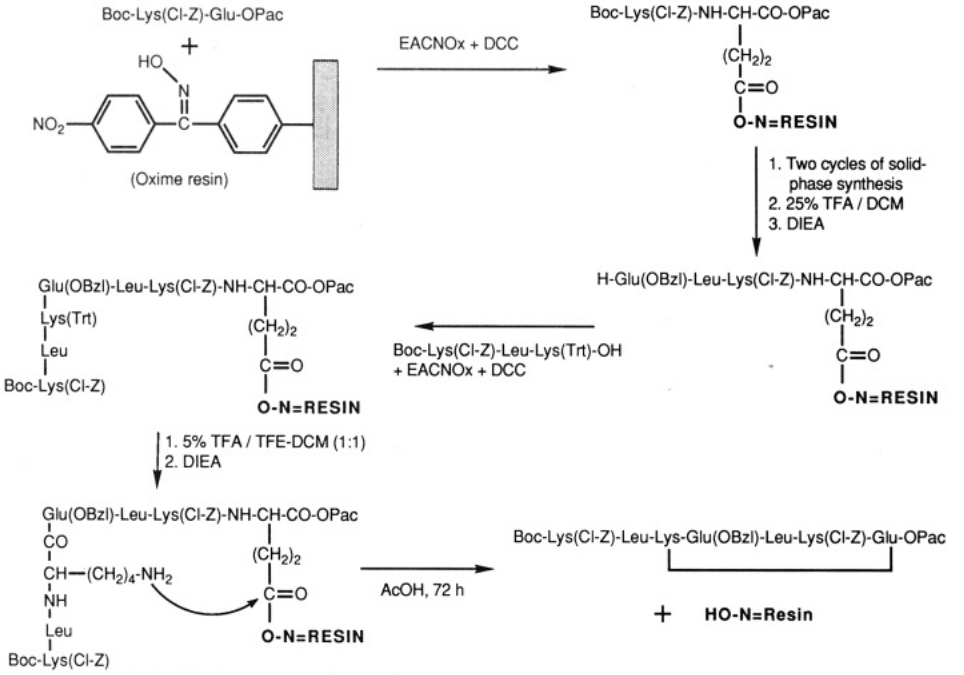

Figure 5. Synthesis of an amide-bridged, protected peptide segment using an oxime resin. The intrachain cyclization reaction that forms the amide bridge simultaneously cleaves the peptide from the resin. [Abbreviations: DCC, *N,N'*-dicyclohexylcarbodiimide; EACNOx, ethyl 2-(hydroximino)-2-cyanoacetate.]

The introduction of conformationally constrained, nonnatural amino acid residues, such as N^{α}-methylated residues, α-aminoisobutyric acid residues, or β-turn analogues, has been successfully applied to many small peptides, including the enkephalins, somatostatin, and a number of enzyme substrates and inhibitors.[26] These substituted structures can define the conformation of a few specific bonds, usually in the peptide backbone, to within quite narrow limits. When longer peptide chains are considered, the study of cyclic or side-chain-bridged peptides is initially preferred, as this provides a looser constraint on larger segments of the peptide. For this purpose, amide or disulfide bridges are usually employed, since these structures are easily incorporated into peptide syntheses. The longer the peptide, however, the more important an initial structural hypothesis becomes, and the more such bridges must be incorporated, in order to test that hypothesis. For example, the introduction of single disulfide bridges at random into the β-endorphin structure yielded no information concerning possible functional conformations of that hormone.[27] In contrast, Felix and colleagues have recently incorporated one or two pairs of side-chain-bridged aspartic acid and lysine residues into a growth hormone-releasing factor (GRF) analogue[28] in order to stabilize amphiphilic helical structures in this hormone whose functional importance had already been suggested by studies of a minimally homologous peptide model.[29] The helix-stabilizing effects of the lactam bridges in solution correlated with enhanced pharmacological potency of the analogues, providing additional evidence supporting the functional importance of these amphiphilic structures.

With the above goal in mind, we have developed an efficient approach to the chemical synthesis of peptides that contain multiple side chain to side chain amide linkages.[30] This approach is based on the use of an oxime resin[31] for the solid-phase synthesis of fully protected, cyclic peptide segments (Figure 5). The carboxy-terminal dipeptide of the desired segment is first attached to the oxime resin via the carboxylic acid side chain that will ultimately form the amide (lactam) bridge. The peptide segment is then built onto this dipeptide by standard solid-phase methods,[32] with temporary N^{α}-*tert*-butyloxycarbonyl (Boc) protection and conventional semipermanent side-chain-protecting groups. At the appropriate point, the amine side-chain component of the bridge, temporarily protected by the trityl group (Trt), is coupled to the amino-terminal end of the segment. Selective deprotection of this amine with dilute TFA then allows it to react with the oxime ester side-chain linkage to the resin. This reaction is catalyzed by acetic acid and occurs at intrachain sites with high frequency, resulting in cyclization and concomitant cleavage of the cyclic peptide segment from the resin in high yield (61%, in the example shown). Selective N^{α} deprotection from TFA and the use of orthogonal cleavage conditions (Zn/AcOH) for the C^{α}-protecting phenacyl (Pac) group, allow side-chain-bridged peptide segments of this type to be incorporated into peptide chains at any desired position, either by solution-phase condensation[33] or during a solid-phase

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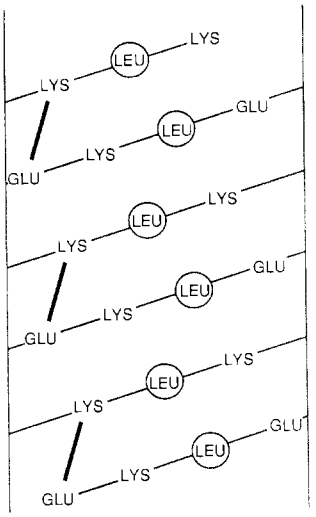

Figure 6. Helical net diagram[4] of an amphiphilic, α-helical peptide with three pairs of side-chain-bridged Lys^{i} and Glu^{i+4} residues. This peptide was synthesized by repeated condensation of the amide-bridged, protected heptapeptide segment that had been prepared by the oxime resin method (Figure 5). The amide bridges are indicated by thick diagonal lines, and hydrophobic residues are circled.

synthesis by the Merrifield method.[32]

The oxime resin approach has significant advantages over existing methods for the preparation of cyclic or amide-bridged peptides.[28,34,35] Because only intrachain cyclization results in cleavage from the oxime resin, the product obtained in solution is essentially free of polymeric contaminants; the dilute conditions required for intramolecular cyclization in solution are avoided, and the segment condensation approach allows much greater flexibility in the synthesis of multiply bridged peptides than the equivalent solid-phase methods. Combining segment condensations with standard solid-phase methods should also produce purer products than would normally result from the solid-phase method alone, since the cyclic protected segments can be purified prior to their incorporation into the peptide chain. This method is compatible with any sequence of natural amino acid residues in the intervening positions between the bridged residues. Furthermore, we have synthesized cyclic peptides with ring sizes incorporating up to 10 residues using the oxime resin, indicating that the number of intervening residues between the bridged side chains in such peptides may also be varied over a wide range.

In order to demonstrate the potential of this oxime method for the synthesis of peptides incorporating multiple amide bridges, we have condensed together three units of the bridged peptide segment shown in Figure 5.[30] The resultant 21-residue peptide was designed to form an amphiphilic α helix with the three lactam bridges on its hydrophilic side (Figure 6). CD spectra of this peptide in 50% TFE or adsorbed from aqueous solution onto siliconized slides indicate that it has a high content of α-helical structure, demonstrating that the multiple Lys^{i}, Glu^{i+4} side-chain amide linkages are compatible with induction of the α-helical conformation. However, in contrast to the Asp^{i}, Lys^{i+4} side-chain bridges incorporated into GRF by Felix and his colleagues,[28] these longer bridges do not seem to have any significant helix-stabilizing effect on this peptide sequence in aqueous solution.

Conclusion

There is increasing evidence that flexible peptides often have important functional conformations induced in them upon interaction with macromolecular surfaces.[7-12,25,26,28] The identification of surface-induced conformations in peptides bound to model interfaces, combined with studies of minimally homologous peptide models and analogues that incorporate multiple conformational constraints, should provide us with low-resolution pictures of the receptor-bound conformations of peptide hormones and other biologically active peptides. This information will, in turn, enable us to adopt a rational approach to the design of analogues that have useful pharmacological and, possibly, clinical properties, including enhanced potencies, altered receptor specificities, or increased antagonist character.

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Registry No. Neuropeptide Y, 82785-45-3; β-endorphin, 60617-12-1; dynorphin A, 88161-22-2.

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