trophys. J. Lett. **254**, L47 (1982); see also K. Davidson, *Mercury* **11**, 138 (1982).
24. In the CNO cycle, carbon, nitrogen, and oxygen nuclei serve as catalysts to convert hydrogen into helium. The relative abundance ratios of C, N, and O approach an equilibrium state in which ^{14}N is prevalent. See G. R. Caughlan and W. A. Fowler, *Astrophys. J.* **136**, 453 (1962); G. R. Cauglan, *ibid.* **141**, 688 (1965); C. de Jager (*1*, pp. 162–164).
25. C. D. Andriesse, B. D. Donn, R. Viotti, *Mon. Not. R. Astron. Soc.* **185**, 771 (1977).
26. D. van Blerkom, *Astrophys. J.* **221**, 186 (1978).
27. J. B. Hutchings, *ibid.* **203**, 438 (1976).
28. E. P. Hubble and A. Sandage, *ibid.* **118**, 353 (1953); R. M. Humphreys, *ibid.* **200**, 426 (1975) and **219**, 445 (1978); L. Rosino and A. Bianchini, *Astron. Astrophys.* **22**, 453 (1973); A. Bianchini and L. Rosino, *ibid.* **42**, 289 (1975).
29. R. M. Humphreys, C. Blaha, S. D'Odorico, T. R. Gull, P. Benvenuti, *Astrophys. J.*, in press.
30. T. Schmidt-Kaler and J. V. Feitzinger, in *The Most Massive Stars, ESO Workshop Proceedings*, S. D'Odorico, D. Baade, K. Kjär, Eds. (European Southern Observatory, Garching, West Germany, 1981), p. 105; and *Astrophys. Space Sci.* **41**, 357 (1976); J. V. Feitzinger, W. Schlosser, T. Schmidt-Kaler, C. Winkler, *Astron. Astrophys.* **84**, 50 (1980).
31. J. P. Cassinelli, J. S. Mathis, B. D. Savage, *Science* **212**, 1497 (1981).
32. B. D. Savage, E. L. Fitzpatrick, J. P. Cassinelli, D. C. Ebbets, *Astrophys. J.*, in press.
33. Visual observations [R. T. A. Innis, *Catalogue of Double Stars* (1927); C. E. Worley, unpublished results; Y. H. Chu and M. Wolfire, *Bull. Am. Astron. Soc.* **15**, 644 (1983)] reveal that R136a is double with a separation of 0.46 arc sec (≃ 0.1 pc). Recent speckle interferometric measurements by G. Weigelt [quoted in (*30, 32*)] are consistent with the visual data, but those by J. Meaburn, J. C. Hebden, B. L. Morgan, and H. Vine [*Mon. Not. R. Astron. Soc.* **200**, 1 (1982)] are not.
34. N. R. Walborn, *Astrophys. J.* **182**, 121 (1973).
35. H. Shapley and J. S. Paraskevolpoulos, *ibid.* **86**, 340 (1937); J. Melnick, in *Wolf-Rayet Stars, IAU Symposium 99*, C. W. H. de Loore and A. J. Willis, Eds. (Reidel, Dordrecht, 1982), p. 545.
36. D. C. Ebbets and P. S. Conti, *Astrophys. J.* **263**, 108 (1982); J. M. Vreux, M. Dennefeld, Y. Andrillat, *Astron. Astrophys.* **113**, L10 (1982).
37. P. S. Conti and P. Massey, *Astrophys. J.* **249**, 471 (1981); S. D'Odorico and M. Rosa, *ibid.* **248**, 1015 (1981); S. D'Odorico and P. Benvenuti, *Mon. Not. R. Astron. Soc.*, in press; P. Massey and P. S. Conti, *Astrophys. J.*, in press.
38. P. Massey and J. B. Hutchings, *Astrophys. J.*, in press.
39. C. de Loore, J. P. De Greve, H. Lamers, *Astron. Astrophys.* **61**, 251 (1977); C. Chiosi, E. Nasi, S. R. Sreenivasan, *ibid.* **63**, 103 (1978); R. Stothers and C.-W. Chin, *Astrophys. J.* **233**, 267 (1979); A. Maeder, *Astron. Astrophys.* **92**, 101 (1980); *ibid.* **99**, 97 (1981); *ibid.* **102**, 401 (1981).
40. R. Stothers and C.-W. Chin, *Astrophys. J.* **264**, 583 (1983).
41. A. Maeder, *Astron. Astrophys.* **120**, 113 (1983).
42. ———, *ibid.* **105**, 149 (1982).
43. E. Schatzman, *ibid.* **56**, 211 (1977).
44. R. B. Larson and S. Starrfield, *ibid.* **13**, 190 (1971); F. D. Kahn, *ibid.* **37**, 149 (1974). See also K. Davidson and M. Harwit [*Astrophys. J.* **148**, 443 (1967)], where the term "cocoon star" first appeared.
45. M. G. Wolfire and J. P. Cassinelli, *Bull. Am. Astron. Soc.* **15**, 644 (1983).
46. G. T. Bath, *Nature (London)* **282**, 274 (1979).
47. For comparison, the sun has the following parameters: $M_\odot = 2 \times 10^{33}$ g, $L_\odot = 4 \times 10^{26}$ W, $R_\odot = 7 \times 10^5$ km, and $T_\odot = 5800$ K.
48. Spectral type refers to the appearance of the absorption lines in a star's spectrum and is an indicator of the surface temperature. To some extent luminosity as well as temperature can be inferred from spectroscopy.
49. We are grateful to N. R. Walborn, P. Conti, and R. Kennicutt for many discussions about massive stars. In our work on this topic, we have been guest observers in the NASA-supported IUE program, as well as visiting astronomers at Kitt Peak National Observatory and Cerro Tololo Inter-American Observatory, which are operated by the Association of Universities for Research in Astronomy, Inc., under contract with the National Science Foundation. R.M.H.'s work is partially supported by an NSF grant.

Amphiphilic Secondary Structure: Design of Peptide Hormones

E. T. Kaiser and F. J. Kézdy

Summary. Peptide synthesis can be used for elucidating the roles of secondary structures in the specificity of hormones, antigens, and toxins. Intermediate sized peptides with these activities assume amphiphilic secondary structures in the presence of membranes. When models are designed to optimize the amphiphilicity of the secondary structure, stronger interactions can be observed with the synthetic peptides than with the naturally occurring analogs.

The rational design and construction of biologically active peptides and polypeptides is now an attainable goal through the applications of the tools of modern chemistry (*1*). Foremost among these tools is the technique of solid phase peptide synthesis (*2*). No longer is it necessary for a chemist interested in preparing a peptide 20 or 30 amino acids in length to spend a substantial portion of his career in its construction. Rather, through the judicious application of modern purification and analytical techniques such as high-performance liquid chromatography (HPLC), it is now possible for a graduate student in the course of his thesis studies to prepare as many as five to ten peptides of this size and to characterize their physical and biological properties. Because of these technological advances, it is feasible not only to propose structural hypotheses for the construction of biologically active peptides and polypeptides but also to test thoroughly the experimental aspects of these proposals.

Although we have increased our understanding of tertiary structure in recent years, we have not yet reached the point at which the folding of a peptide with a given amino acid sequence into a tertiary structure can be predicted with confidence. In contrast, a solid foundation has been laid for the prediction of amino acid sequences that form certain types of secondary structures. The recognition of the importance of these secondary structures has led us to a new approach to the design of biologically active peptides such as hormones.

A host of biologically important peptides composed of 10 to 50 amino acids are devoid of well-defined tertiary structure. As a rule, they lack disulfide bonds, they are linear (*3*), and their conformation depends entirely on their environment; they can assume completely different secondary structures in water, in a detergent micelle, or in trifluoroethanol or other organic solvents. Many of these peptides play very specific roles as hormones, cofactors, signals for membrane translocation, and the like. The high activity and the specificity of their action imply a well-defined structure traditionally associated with ligand-enzyme and ligand-receptor interactions, although their behavior in aqueous solution gave no evidence for any predominant structure that could be associated with their biological activity. Probing for the "active site" of many of these molecules by selective chemical modification gave different results from those seen in similar experiments with enzymes; it appeared that all parts of the molecule were essential for high activity, although in some cases a number of amino acids could be modified without a major change in activity. The requirement that most of the

E. T. Kaiser is a professor and head of the Laboratory of Bioorganic Chemistry and Biochemistry at Rockefeller University, New York 10021. F. J. Kézdy is a professor in the Department of Biochemistry at the University of Chicago, Chicago, Illinois 60637.

molecule—not only the part which comes into direct contact with the receptor—be present argues against the hypothesis that the biologically active conformation would be imposed on the peptide by the complementary tertiary structure of the macromolecule which is the target of the peptide; that is, the receptor or the antibody upon which the peptide acts. Indeed, most ligand-macromolecule interactions that have been studied are limited to the specific interaction of only a few amino acid residues.

Many peptide hormones act at membranes that represent a characteristic, anisotropic environment due to the amphiphilic nature of the dividing line between the aqueous solution and the lipid bilayer of the membrane. It is then possible that the amphiphilic environment could impose a secondary structure on the peptide. If such a membrane-directed secondary structure exists, it should be characterized by the amphiphilic distribution of the individual amino acid side chains. One face of the peptide should be occupied preferentially by lipophilic side chains (*4*), such as Ile, Leu, Val, Phe, Trp, Tyr, and Met, whereas all hydrophilic residues, such as Asp, Glu, Arg, and Lys should be located at the opposite face. The free energy contribution of locating the amino acid residues in their optimal microenvironment is considerable, yielding additional stability (as much as 1 to 3 kcal/mole) per amino acid residue. Thus, secondary structures which would only have marginal or no stability in an aqueous solution could very well become the particular conformation of the peptide once located in the membrane. Most important, secondary structures that are energetically too unfavorable to exist in aqueous solution could be easily generated in an amphiphilic environment, provided that full advantage is taken of the hydrophilic and lipophilic interactions of the amino acid side chains. Thus, one could hypothesize that the biologically active conformation of peptides acting in membranes could be any of the sterically allowed secondary structures, namely, right- and left-handed α-helix, β-pleated sheet, 3,10-helix, or π-helix.

The search for the possibility of the occurrence of amphiphilic secondary structures in peptides needs only very simple tools. Once the amino acid sequence is known, regular alternation of the lipophilic and hydrophilic residues indicates the possibility of an amphiphilic β-sheet. The possibility of amphiphilic α- or π-helices is detected by the construction of an "Edmundson wheel" (*5*) of the appropriate pitch and noting the segregation of the hydrophilic and lipophilic residues on opposite faces of the cylinder circumscribing the helix. Alternatively, the helix can be projected in a two-dimensional fashion, which then shows more distinctly the interactions among side chains along the axis of the helix as well as the occurrence of the hydrophilic and lipophilic domains. Figure 1, A to C, shows the application of such constructions to a peptide with a potential pleated sheet, an α-helix, and a π-helix. The prediction of the occurrence of possible amphiphilic structures can then be further affirmed by calculating the probability of secondary structures by one or another of the semiempirical methods, such as the Chou-Fasman method (*6*) and the calculation of the free energy changes associated with the transfer of lipophilic residues into a nonaqueous environment (*7*).

Once the possible occurrence of an amphiphilic secondary structure is established, numerous experimental approaches are needed to establish the existence of such a conformation. Indirect evidence can be derived from the fact that the peptide would bind readily to single bilayer phospholipid vesicles and would form stable monomolecular layers at the air-water interface (*8*). Also, the peptides should form readily "micellar structures," that is, small oligomers formed with a well-defined number of monomers, where the lipophilic domains are strongly interacting. The secondary structures of such assemblies can then be probed by conventional methods, that is, nuclear magnetic resonance spectroscopy, optical rotatory dispersion, or circular dichroism.

The ultimate confirmation of active secondary amphiphilic structures can come from the use of peptide synthesis. Indeed, in many instances one can postulate that most amino acids in the peptide assume only a structural role and their replacement by other amino acids of the same lipophilicity should not impair the biological activity of the peptide (*9*). On the contrary, one could imagine that the amino acid sequence of the biologically active peptide is dictated not only by structural considerations but also by considerations of biocompatibility, degradability, specificity, and lack of induction of immunoreaction. Thus, the naturally occurring peptide might be suboptimal as far as the stability of the amphiphilic structure is considered. One could then optimize the structural requirements at the expense of other considerations and obtain a synthetic peptide with a higher biological activity than the naturally occurring peptide.

A brief review of the early work in our laboratories which forms the basis of this

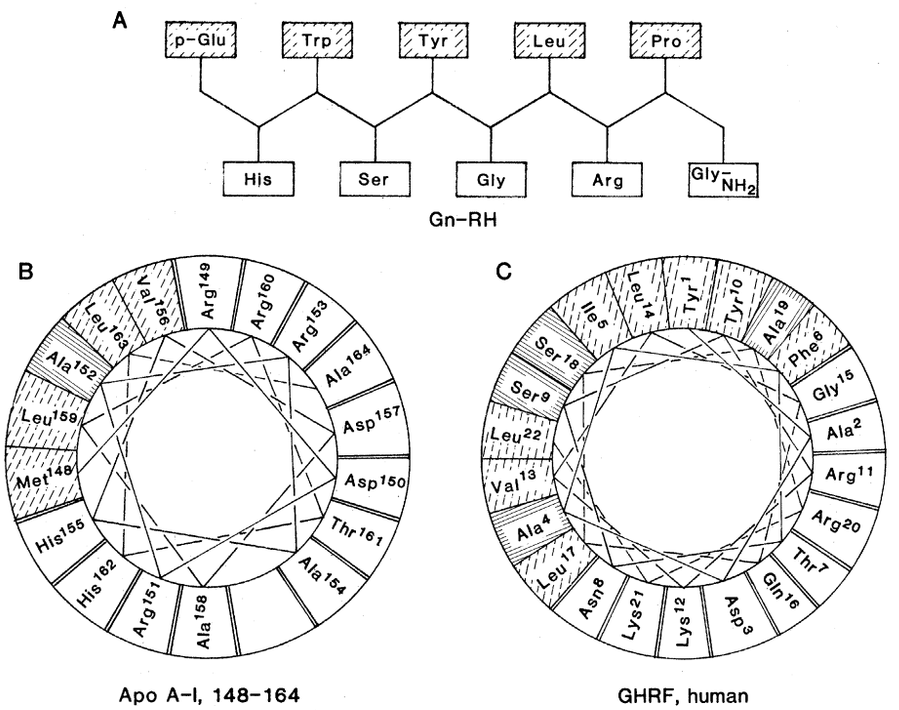


Fig. 1. Graphical method for searching for amphiphilic secondary structures. (A) β-Pleated sheet conformation of gonadotropin releasing hormone. (B) Axial projection of a potential α-helical conformation of residues 148 to 164 of apolipoprotein A-I. Residues in the white areas are hydrophilic. Those in the areas containing dashed lines are lipophilic. (C) Axial projection of a potential π-helical conformation of residues 1 to 22 of human growth hormone releasing factor. The segregation of the hydrophilic and lipophilic residues is shown.

approach seems in order. We began our work by searching for peptide systems where secondary structural features might dominate the biological activity. From the outset it was clear that such systems were likely to be found among the peptides that bind to phospholipids, membranes, or other amphiphilic surfaces. There appeared to be a good possibility that the peptides that bind to such surfaces, which have the characteristic of being hydrophobic on one face and hydrophilic on the other, might have complementary amphiphilic secondary structures.

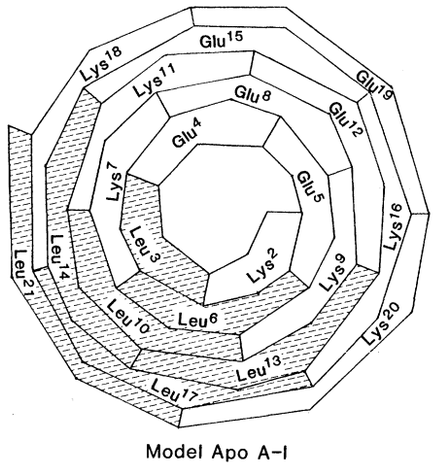


Fig. 2. Two-dimensional axial projection of the α-helical conformation of a model peptide designed to simulate the properties of apolipoprotein A-I.

Synthetic Studies on Apolipoproteins and Peptide Toxins

Significant clues to the importance of amphiphilic secondary structures to the properties of surface-active peptides and proteins came from work on apolipoproteins. Examination of the sequence of apolipoprotein A-I (apo A-I), the principal polypeptide constituent of high density lipoprotein (HDL) and of the molecular models of this polypeptide, led to the proposal (*10*) that there might be amphiphilic α-helical regions throughout a considerable portion of the apo A-I molecule. This suggestion was amplified by analyses made independently by Fitch (*11*) and McLachlan (*12*) in which it was suggested that the amphiphilic α-helical regions had a repeating character and that they might be on the order of 22 amino acids in length. According to this picture, the α-helical segments were punctuated at regular intervals by proline or glycine residues acting as helix breakers.

Model building of the amphiphilic α-helical structures revealed to us that they would provide a very reasonable mode for the interaction of surface active peptides like apo A-I with phospholipid vesicles and other amphiphilic surfaces. When a model of apo A-I was constructed we saw that the helices could be placed on the surface of an HDL particle in such a way that the axes of the helices were roughly tangential to the particle surface. The model showed that the hydrophobic faces of the helices could penetrate into the particle in the spaces between the phospholipid head groups, allowing these faces to come into contact with the long hydrophobic chains of the phospholipids. The helices then had their hydrophilic faces oriented toward the aqueous environment. Whereas this picture appeared to be attractive, an experimental test had to be performed to determine whether amphiphilic α-helices were induced in the apo A-I molecule in its binding to phospholipid in the HDL particle. At the point when we began, the physical chemical methodology for examining the secondary structures of peptides bound to membrane surfaces, while undergoing development, did not appear to us to provide the most incisive approach toward attacking the problem. Rather, it appeared to us that a more definitive picture might come from a synthetic organic study of the amphiphilic α-helix and its role in apolipoprotein binding to phospholipid surfaces.

If it is argued that a secondary structural feature like an amphiphilic α-helical region is crucial to the biological activity of a peptide such as apo A-I, then a fundamental prediction which emerges is that this activity ought to be simulated by a peptide with a radically different amino acid sequence but designed to have essentially the same secondary structure (*13*). To test this basic hypothesis, we designed a 22 amino acid peptide which represented an "idealized" version of the amphiphilic α-helical regions of apo A-I (Fig. 2). The peptide was constructed primarily of three types of amino acid residues with a high potential to participate in α-helical structures: Leu, a hydrophobic amino acid; Lys, a hydrophilic residue positively charged in the *p*H region near neutrality; and Glu, a negatively charged hydrophilic residue (*9*). The 22 amino acid model peptide was designed to be capable of forming an amphiphilic α-helix with a hydrophobic face covering about one-third of the surface and the hydrophilic face covering the remaining two-thirds. Most importantly, it was designed to have minimal homology to any of the amphiphilic α-helical regions of the naturally occurring protein. The synthetic model peptide was shown to mimic successfully the properties of apo A-I, a 243 amino acid peptide (*9, 14*). The model bound comparably to phospholipid surfaces, showed related α-helical character in water and water-trifluoroethanol solutions and showed similar aggregation behavior. Also, the model peptide showed appropriate biological activity, acting as a good activator of the enzyme lecithin:cholesterol acyltransferase (E.C. 2.3.1.43), an important function of apo A-I (*14*).

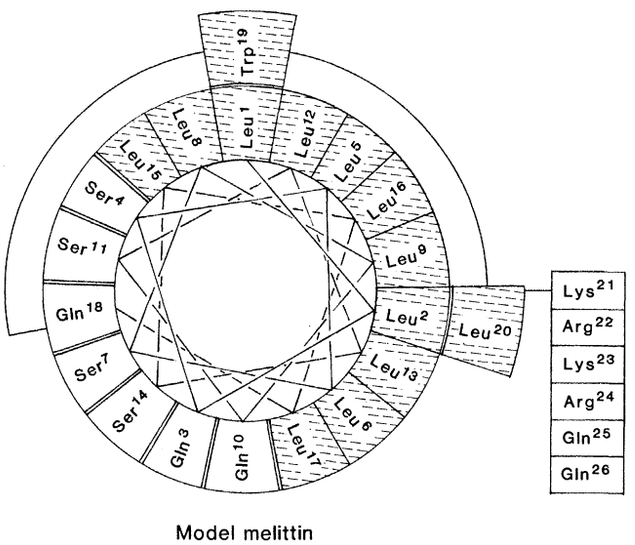
A peptide in which an amphiphilic secondary structural region was attached to a simple "active site" was the next most complex system examined (*15*). The bee venom toxin, melittin, an activator of the enzyme phospholipase A_2 and an effective hemolytic agent, contains in its major form a peptide segment about 20 amino acids in length, which has the potential to form an amphiphilic α-helix when bound to an appropriate surface. It contains a proline residue part way through the sequence, which should cause a kink in the helix. The helical region is attached to a hexapeptide segment which contains a cluster of positive charges (*16*). When the hexapeptide segment is removed from melittin, the remaining 20 amino acid segment, while capable of binding to erythrocytes, does not lyse them (*17*). Accordingly, a model was constructed in which the 20 amino acid segment starting at the amino terminus was designed to have relatively little homology to the melittin sequence itself but to have a comparable hydrophobic-hydrophilic balance and a somewhat accentuated potential for forming an α-helix (*15*). In this case, the helical portion was constructed with Leu residues as the hydrophobic groups, and because it appeared that the charge was not an important factor in the helix, Gln and Ser residues were chosen as the hydrophilic groups (Fig. 3). The hexapeptide portion attached at the COOH terminus to the 20 amino acid peptide had the same sequence as that in natural melittin. The 26 amino acid model peptide constructed in this fashion proved to be both a highly effective lytic agent and a satisfactory activator of phospholipase A_2 (*18*).

Amphiphilic Secondary Structures in Hormones

Our studies on apolipoproteins and toxins showed that the analysis of the structure of surface active peptides in terms of amphiphilic secondary structure yielded important new information. In

view of the limited number of possible secondary structures and, as suggested by others, because of the likelihood that various peptide hormones might have α-helical regions, the question arose whether or not the amphiphilic secondary features might play a major role in hormone structure (*15, 19*). When we considered the structures of peptide hormones and the vast amount of data on this topic, it seemed to us that these compounds might be classified into three general categories. First, there are systems such as the opioid peptide [Met^5]-enkephalin where the whole hormone consists of a relatively short sequence of amino acids, an "active site" containing specific amino acids required for eliciting biological activity. In a second category of hormones consisting of peptides where the structures are rather complex and where, as in the case of insulin, multiple disulfide bonds may be present, tertiary structure is very important. In a third category, however, the peptides either have no disulfide bridge or have only a single bridge, and quite frequently have a length in the vicinity of 10 to 50 amino acids. In this last category, it seemed quite likely that binding to the amphiphilic environment of a receptor might require the presence of an amphiphilic secondary structure in the peptide for the active site to be in the proper geometry to interact with the receptor. When we set out to search for such systems, it soon became rapidly obvious that there were many peptide hormones where sizable regions of the molecule could be forming amphiphilic secondary structures in appropriate environments (*19*).

The amphiphilic structural regions of most of the hormones that we have examined up to now are α-helical in nature. However, as will be discussed below, we have found systems where, we believe, the amphiphilic structures are π-helices or β-pleated sheets. The peptide hormones containing amphiphilic α-helical regions are treated first.



Model melittin

Fig. 3. Model melittin. Axial projection of the α-helical conformation of a model peptide designed to have melittin-like activity.

A

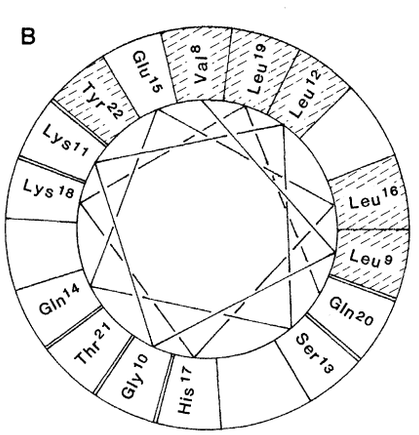
SCT-I

5
H_2N-Cys-Ser-Asn-Leu-Ser-Thr-Cys-Val-
10 15
Leu-Gly-Lys-Leu-Ser-Gln-Glu-Leu-His-
20 25
Lys-Leu-Gln-Thr-Tyr-Pro-Arg-Thr-Asn-
30
Thr-Gly-Ser-Gly-Thr-Pro-NH_2

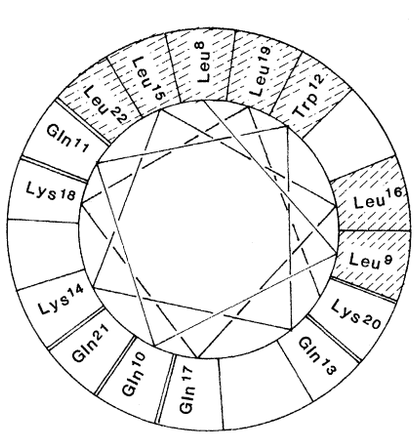
MCT-I

5
H_2N-Cys-Gly-Asn-Leu-Ser-Thr-Cys-Leu-
10 15
Leu-Gln-Gln-Trp-Gln-Lys-Leu-Leu-Gln-
20 25
Lys-Leu-Lys-Gln-Leu-Pro-Arg-Thr-Asn-
30
Thr-Gly-Ser-Gly-Thr-Pro-NH_2

B



SCT-I



MCT-I

Fig. 4 (A). Amino acid sequences of salmon calcitonin (SCT-I) and a model peptide designed to have calcitonin-like activity (MCT-I) (*20*). (B) Axial projections of the potential α-helical conformation of residues 8 to 22 in SCT-I and MCT-I.

Calcitonin

We have proposed that calcitonin, a peptide with potent hypocalcemic activity, consists of an "active site" amino terminal heptapeptide containing a disulfide loop between Cys^1 and Cys^7, an amphiphilic α-helical region from residues 8 through 22, and a hydrophilic COOH-terminal region from residues 23 through 32 (Fig. 4A for the sequence of salmon calcitonin, SCT-I) (*20*). To test this hypothesis, a 32 amino acid peptide, MCT-I (Fig. 4A), designed to retain the structural characteristics of the amphiphilic α-helical region, but having an amino acid sequence from residues 8 through 22 with minimal homology to any naturally occurring calcitonin, was synthesized. The axial projections of the amphiphilic α-helical regions of SCT-I and MCT-I (Fig. 4B) show that the residues are segregated into opposing hydrophilic and hydrophobic faces. The physical and biological properties of MCT-I were studied and compared to those of SCT-I. In aqueous solution, the model peptide MCT-I was slightly more α-helical than SCT-I, and in an amphiphilic environment—the air-water interface—it formed a somewhat more stable monolayer. Most important, MCT-I, while somewhat less effective than SCT-I, specifically binds to calcitonin receptors in rat brain particulate fractions and has potent hypocalcemic activity, as measured using a rat bioassay. Taken together, the results obtained provide strong evidence that the region from residues 8 to 22 of calcitonin has a primarily structural role, interacting in the amphiphilic

α-helical form with the amphiphilic environment of the calcitonin receptor (*20*).

We have recently undertaken work on a new calcitonin model, MCT-II (Fig. 5) (*21*). This model is basically similar to MCT-I in the region 8 to 22 except for the change of Trp^{12} to Leu and of Leu^{22} to Tyr. The amphiphilic α-helical segment in MCT-II is expected to be in closer structural analogy than that of MCT-I to the corresponding region in the very potent naturally occurring calcitonin, SCT-I. Although our experiments on MCT-II are not complete, it appears that in its ability to bind to calcitonin receptors and in its hypocalcemic activity it is approximately equivalent to the salmon calcitonin, despite the substantial nonhomology between the two peptides in the α-helical region. Our experiments thus demonstrate that the use of the secondary structural analysis of peptide hormones enables us to produce new peptide sequences that have activities comparable to the most effective naturally occurring peptide hormones both in vitro and in vivo.

Corticotropin Releasing Factor

Corticotropin releasing factor (CRF), a hormone produced by the hypothalamus, increases the rate of secretion of corticotropin by the pituitary gland. Recently, Vale *et al.* (*22*) determined the primary structure of ovine CRF (Fig. 6). The helical potential of CRF has been analyzed by the method of Chou and Fasman (*6*), indicating that two large sections of the molecule have very pronounced α-helical potential. Axial projection of the amino acid sequence of the two regions of high helical potential, using an Edmundson wheel, shows that the hydrophilic and hydrophobic residues are segregated on opposite sides of the cylindrical helix. The hydrophobic domain is nearly twice as large as the hydrophilic one.

We have examined (*23*) the tendency of CRF to assume an amphiphilic secondary structure when it is exposed to amphiphilic environments, such as the air-water interface and the surfaces of unilamellar phospholipid vesicles. In aqueous solution CRF exists predominantly as a random coil. When the peptide is at concentrations greater than $10^{-5}M$, it shows a tendency to self-aggregate with a concurrent slight increase in the apparent α-helical content as measured by circular dichroism. As expected from its potential amphiphilic structure, CRF binds readily to the surface of single bilayer egg phosphatidylcholine vesicles. This binding fits a simple Langmuir isotherm with the following parameters: dissociation constant $K_d = (1.3 \pm 0.6) \times 10^{-7}M$ and capacity at saturation $N = (1.1 \pm 0.1) \times 10^{-2}$ mole of peptide per mole of phospholipid. At the air-water interface CRF also forms an insoluble monolayer composed of monomers of the hormone with a molecular area which suggests the presence of a compact secondary structure. In addition, this compactness is indicated by the low compressibility of the monolayer. Indeed, the collapse pressure, 19.0 ± 0.1 dynes per centimeter, of the monolayer suggests that the amphiphilicity of CRF approximates that of the plasma apolipoproteins which, as we have seen, comprise a class of proteins of the most pronounced amphiphilic character. Thus, the binding of CRF to the cell membrane appears to be accompanied by the induction of an α-helical secondary structure and it is the predominantly helical form which is the biologically active conformation of the peptide (*23*). The design of models for this peptide hormone along the same lines as our earlier studies on the apolipoproteins, melittin, and calcitonin should help elucidate the structure of CRF further.

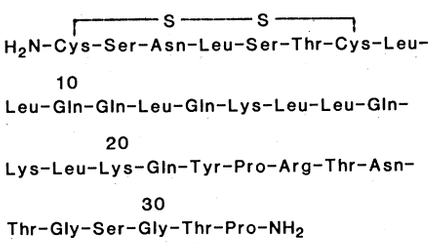


Fig. 5. Amino acid sequence of second model peptide designed to have high calcitonin-like activity (MCT-II) (*21*).

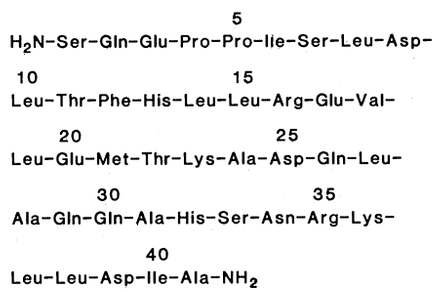


Fig. 6. Amino acid sequence of ovine CRF.

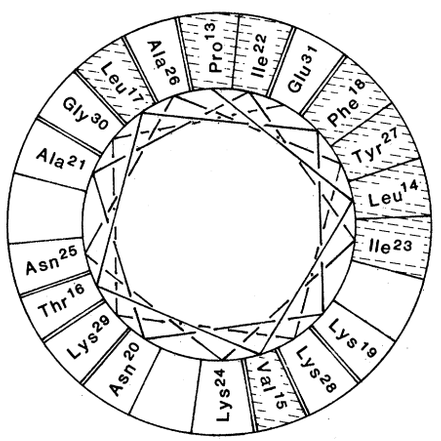


Fig. 7. Axial projection of a potential π-helical region of β-endorphin showing the segregation of the hydrophobic and hydrophilic residues.

β-Endorphin

In an approach similar to those for calcitonin and CRF, we have analyzed (*19*) the structure of β-endorphin, a 31 amino acid peptide hormone with potent opiate activities. Examination of the linear amino acid sequence of β-endorphin has led us to propose that three separate structural regions can be distinguished: a very specific opiate recognition site in residues 1 to 5 that is identical to [Met^5]enkephalin; a hydrophilic spacer region in residues 6 to 12 and a 16-residue sequence between Pro^{13} and Gly^{30}, capable of forming an amphiphilic α- or π-helix with half of its surface hydrophobic and the hydrophilic residues being neutral or basic. As in the cases of calcitonin (*20*) and CRF (*23*), the hydrophobic domain in the proposed α-helical region of β-endorphin is continuous. However, in contrast to calcitonin and CRF, where this domain lies straight along the length of the helix, in the α-helical form of β-endorphin the hydrophobic domain would twist along the length of the helix. As an alternative, the COOH-terminal region might be in a π-helical form and, in this case, the hydrophobic domain would lie straight along the length of the helix (Fig. 7) (*19*). At present, we are not sure which of these models for β-endorphin is the correct one, and since most of our earlier studies were with α-helices, we have until now concentrated our efforts primarily on testing α-helical models for the COOH-terminal region of the hormone.

Several model peptides have been synthesized and tested to evaluate the validity of the amphiphilic helical structural hypothesis for β-endorphin (*19, 24–26*). These models include a peptide in which, in addition to the construction of an amphiphilic helical region from residues 13 to 31 with a sequence having minimal homology to that of β-endorphin, a hydrophilic spacer region from residues 6 to 12 with minimal homology to the equivalent region of the natural hormone

was built (*24*). In a very stringent test for the structure of β-endorphin, we prepared and examined a peptide designed to be a "negative" model (*26*). The latter peptide retains almost all of the proposed features of β-endorphin, the only difference being that in an α- or π-helical conformation of the region 13 to 31, the amphiphilic character present in the previous models is minimized. Our results with this model, as well as with the earlier models which contained amphiphilic helices, provide evidence for the importance of an amphiphilic helical structure in β-endorphin residues 13 to 31, which determines the resistance to proteolysis of the natural hormone and which makes some contribution to the interactions of the hormone with the opiate receptors δ and μ. Indeed, the amphiphilicity of the COOH-terminal helical structural region was found to be essential for high opiate activity on the rat vas deferens (ε receptors), whereas no such structural requirement appears to be necessary for interaction with the opiate receptors on the guinea pig ileum (*26*).

Although our results do not allow us to choose between the α- or π-helical models for the COOH-terminal region of β-endorphin, our work illustrates the power of the structural analysis we have developed in conjunction with our synthetic approach in establishing the requirements for receptor binding and biological activity in pharmacologically complex situations like that encountered with β-endorphin.

Growth Hormone Releasing Factor

The π-helix is not a structure that is commonly encountered in globular proteins, probably because it is not compact and would be a metastable structure under the conditions where most x-ray crystal structure determinations are performed. Nevertheless, examination of molecular models of various peptide hormones leads us to propose the possibility that amphiphilic π-helices may play a significant role in the binding of these molecules to membrane surfaces. Although the π-helical structures may not be very stable under conditions where close packing is crucial, the proposal that such structures may be formed when peptide hormones bind to membrane surfaces is reasonable. Examination of the region 1 to 29 of human growth hormone releasing factor (hGRF) shows that this segment has an excellent potential to form an amphiphilic π-helix, as illustrated for the sequence 1 to 22 in

```
                                5
H2N-His-Ser-Gln-Gly-Thr-Phe-Thr-Ser-

      10                                15
Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Ser-Arg-

            20                                25
Arg-Leu-Gln-Glu-Phe-Leu-Gln-Trp-Ala-

Leu-Gln-Thr
```

Fig. 8. Amino acid sequence of glucagon analog.

Fig. 1C. We have shown that hGRF forms a very stable monolayer at the air-water interface (*27*). Furthermore, it binds tightly to unilamellar phosphatidylcholine vesicles. These observations are consistent with the hypothesis that hGRF forms an amphiphilic secondary structure, most likely a π-helix, in amphiphilic environments such as the air-water interface and phospholipid surfaces. Measurements of the physical properties of a synthetic peptide designed to have the structural features proposed for hGRF would allow us to test further the concept of the π-helix.

Glucagon

The binding of glucagon to hepatic plasma membrane receptors is the first event in the action of this hormone (*28*). Linkage of the occupied receptor to the catalytic entity of adenylyl cyclase results in elevation of the cytoplasmic cyclic adenosine monophosphate (AMP) and in the resulting modulation of cell metabolism (*29*). The binding of glucagon to hepatocytes and plasma membrane vesicles has been analyzed quantitatively in terms of two noninteracting receptor populations (*30*). We have been engaged in preparing and characterizing several models for glucagon, including one in which the COOH-terminal region is an amphiphilic α-helix in which the aromatic residues of the natural sequence have been preserved but the remainder of the helical region has been "idealized" (Fig. 8). When we employed rat hepatocytes, this model peptide was found to mimic (i) the activity of glucagon in binding to receptors, (ii) the elevation of cyclic AMP, and (iii) the inhibition of the incorporation of carbohydrate into glycogen (*31*). Binding of glucagon itself to the rat hepatocytes is consistent with the presence of two separate populations of hormone binding sites with the association constants of 57 p*M* and 41 n*M*. In contrast, we found that a single sigmoidal binding curve was obtained for the model peptide, extending over a concentration range of only two orders of magnitude. Even at a 50 μ*M* concentration of the analog only 50 percent inhibition of the binding of ^{125}I-labeled glucagon to hepatocytes was observed. Our data fit a scheme where the model peptide competes with the binding of ^{125}I-labeled glucagon to only one of the two receptor populations present on rat hepatocytes. In other words, the binding of the model peptide obeys a single thermodynamic equilibrium with the dissociation constant of 1.2 μ*M*. To establish which of the two glucagon receptor types was responsible for the binding of the model peptide, we studied the inhibition of ^{125}I-labeled glucagon binding by unlabeled glucagon in the presence of the model peptide at 10^{-5}*M*. Under such conditions, the binding curve for glucagon became a single sigmoidal one which extended over less than two orders of magnitude of competitor concentration. The dissociation constant thus obtained for the binding of glucagon was K_d = 46 n*M*, indicating that the hormone was interacting with its low affinity receptor. Thus, the use of the model peptide provides conclusive support for the hypothesis that there are two distinct, homogeneous populations of glucagon receptors in the rat hepatocytes.

The model peptide has been very useful in helping us to understand the interaction of glucagon with its receptors. These results, together with those previously obtained with β-endorphin, show the power of the structural approach we have adopted not only in testing the conformational requirements of a peptide to act as a hormone but also in permitting us to determine what parts of the molecule are crucial for binding to particular receptors in those cases where multiple receptors exist.

Conclusions

The results presented in this article provide a glimpse of new approaches that peptide synthesis offers for the elucidation of basic problems in biochemistry. Direct extension of the present methods of design should yield answers to a host of important questions. To cite a few: What is the relation between the extent of the hydrophobic domains and the biological activity? Is the size of the secondary structural domain critical or can one extend it without any interference with the receptor macromolecule? Can one replace the structural amphiphilic domain with a non–peptide moiety of comparable amphiphilic properties? Models designed with these questions in mind should also possibly yield new

classes of molecules with interesting biochemical and pharmacological properties.

Further extension of our studies should involve the design of hitherto unobserved secondary structures of peptides. Judicious use of the stabilizing forces provided by the medium should allow one to stabilize energetically unfavorable conformations of the peptide bond, such as dihedral bond angles and even cis-peptide bonds which, in turn, should generate novel secondary structures.

On the basis of the available data it appears that peptides interact with their receptor macromolecules by a mechanism which is similar to that of information-bearing molecules of singular tertiary structure. In particular, the number of functional groups interacting with the receptor must be small and most of the amino acid components serve a purely structural role, namely, the proper positioning of the ligand functions.

References and Notes

1. E. T. Kaiser and F. J. Kézdy, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 1137 (1983).
2. G. Barany and R. B. Merrifield, in *The Peptides*, E. Gross and J. Meienhofer, Eds. (Academic Press, New York, 1980), vol. 2, p. 3.
3. See, for example, T. Blundell and S. Wood, *Annu. Rev. Biochem.* **51**, 123 (1982).
4. Abbreviations for the amino acid residues are: Ala, alanine; Arg, arginine; Asn, asparagine; Asp, aspartic acid; Cys, cysteine; Gln, glutamine; Glu, glutamic acid; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine; Trp, tryptophan; Tyr, tyrosine; Val, valine.
5. M. Schiffer and A. B. Edmundson, *Biophys. J.* **7**, 121 (1967).
6. P. Y. Chou and G. D. Fasman, *Annu. Rev. Biochem.* **47**, 251 (1978).
7. C. Edelstein, F. J. Kézdy, A. M. Scanu, B. W. Shen, *J. Lipid, Res.* **20**, 143 (1979).
8. D. J. Kroon, J. P. Kupferberg, E. T. Kaiser, F. J. Kézdy, *J. Am. Chem. Soc.* **100**, 5975 (1978).
9. D. Fukushima *et al.*, *ibid.* **101**, 3703 (1979).
10. J. P. Segrest *et al.*, *FEBS Lett.* **38**, 247 (1974).
11. W. M. Fitch, *Genetics* **86**, 623 (1977).
12. A. D. McLachlan, *Nature (London)* **267**, 465 (1977).
13. D. Fukushima *et al.*, *Ann. N.Y. Acad. Sci.* **348**, 365 (1980).
14. S. Yokoyama, D. Fukushima, F. J. Kézdy, E. T. Kaiser, *J. Biol. Chem.* **255**, 7333 (1980).
15. W. F. DeGrado, F. J. Kézdy, E. T. Kaiser, *J. Am. Chem. Soc.* **103**, 679 (1981).
16. E. Habermann, *Science* **177**, 314 (1972).
17. E. Schröeder, K. Lübke, M. Lehmann, I. Beitz, *Experientia* **27**, 764 (1971).
18. W. F. DeGrado, G. F. Musso, M. Lieber, E. T. Kaiser, F. J. Kézdy, *Biophys. J.* **37**, 329 (1982).
19. J. W. Taylor, R. J. Miller, E. T. Kaiser, *J. Am. Chem. Soc.* **103**, 6965 (1981).
20. G. R. Moe, R. J. Miller, E. T. Kaiser, *ibid.* **105**, 4100 (1983).
21. G. R. Moe, in preparation.
22. W. Vale, J. Spiess, C. Rivier, J. Rivier, *Science* **213**, 1394 (1981).
23. S. H. Lau, J. Rivier, W. Vale, E. T. Kaiser, F. J. Kézdy, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 7070 (1983).
24. J. W. Taylor, R. J. Miller, E. T. Kaiser, *Mol. Pharmacol.* **22**, 657 (1982).
25. J. W. Taylor, R. J. Miller, E. T. Kaiser, *J. Biol. Chem.* **258**, 4464 (1983).
26. J. Blanc, R. J. Miller, E. T. Kaiser, *ibid.* **258**, 8277 (1983).
27. S. H. Lau, private communication.
28. V. Bonnevie-Nielsen, K. S. Polonsky, J. J. Jaspan, A. H. Rubenstein, T. W. Schwartz, H. S. Tager, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 3189 (1979), and references therein.
29. E. M. Ross and A. C. Gilman, *Annu. Rev. Biochem.* **49**, 533 (1980).
30. V. Bonnevie-Nielsen and H. S. Tager, *J. Biol. Chem.* **258**, 11313 (1983).
31. G. F. Musso, E. T. Kaiser, F. J. Kézdy, H. S. Tager, *Abstracts, Eighth American Peptide Symposium, Tuscon, Ariz., May, 1983.*

32. We thank our collaborators and faculty colleagues whose contributions are cited in this article and without whose enthusiastic work and helpful discussions this paper could not have been written. The work presented here was supported by Public Health Service Program Project HL-18577 and by a grant from The Dow Chemical Company Foundation.

Balzan Prize to Ernst Mayr

Stephen Jay Gould

In the public fanfare that accompanies the announcement of Nobel prizes, and in the exalted status conferred thereby upon recipients, we often forget that these premier awards are quite narrow in the scope of disciplines so honored. Entire fields are excluded. Thus, the second greatest revolution in the history of geology, the establishment of plate tectonic theory, has won no Nobel notoriety for its founders. The prizes pass over an entire style of scientific work, thus reinforcing a narrow and conventional stereotype about our shared enterprise.

The Nobel prizes focus on quantitative, nonhistorical, deductively oriented fields with their methodology of perturbation by experiment and establishment of repeatable chains of relatively simple cause and effect. An entire set of disciplines, different though equal in scope and status, but often subjected to ridicule because they do not follow this pathway of "hard" science, is thereby ignored: the historical sciences, treating immensely complex and nonrepeatable events (and therefore eschewing prediction while seeking explanation for what has happened) and using the methods of observation and comparison.

Evolutionary biology is a quintessential historical discipline. It has, since the mid-19th century, been continually in the forefront of science—both in its technical progress *in camera*, and in the public eye. Yet only once, when the definition of medicine was stretched to include the ethologists K. Lorenz, N. Tinbergen, and K. von Frisch, has an evolutionary biologist won a Nobel Prize.

Fortunately, the Balzan prizes, with their wider range, can rectify this situation and honor great historical scientists. In 1981, they cited a trio of geophysicists for work in plate tectonics: P. McKenzie, D. H. Matthews, and F. J. Vine. Last year, they honored the great plant physiologist K. Thimann. The Balzan prizes were first awarded in 1961 from a foundation established by Angela Lina Balzan to honor her father, Eugenio Balzan, former head of Italy's leading newspaper, *Corriere della Sera*. After designating two scientists (the ethologist K. von Frisch and the mathematician A. Kolmogorov), several scholars in the humanities, and leading fighters for "humanity, peace and brotherhood among peoples," the prizes were discontinued for 14 years and only reinstated in 1978. This year, and for the first prize designated in zoology, the Balzan Foundation has rightly selected our greatest living evolutionary biologist, Ernst Mayr.

Ernst Mayr was born and educated in Germany, receiving his Ph.D. in 1926 at the University of Berlin. Between 1928 and 1930, as an assistant at the Zoological Museum of the University of Berlin, he led three expeditions to study birds in various parts of New Guinea and the Solomon Islands. He emigrated to the United States in 1932, where he worked until 1953 as curator of birds at the American Museum of Natural History in New York. He then moved to Harvard as Alexander Agassiz Professor of Zoology at the Museum of Comparative Zoology, where he served as director from 1961 to 1970. As professor emeritus since 1975, Mayr has worked continuously and relentlessly at a pace that would exhaust most men half his age and

The author is Alexander Agassiz Professor of Biology at the Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts 02138.