ADVANCES IN FOOD AND NUTRITION RESEARCH, VOL. 42

DESIGN OF EMULSIFICATION PEPTIDES

DAVID SHEEHAN AND KATHLEEN CAREY

Department of Biochemistry
University College
Cork, Ireland

SIOBHAN O'SULLIVAN

TEAGASC Dairy Products Research Centre
Moorepark, Fermoy, Co.
Cork, Ireland

I. INTRODUCTION

The potential of newly designed peptides for emulsification is described in this chapter. Criteria important in their design, synthesis, and testing are stressed. We emphasize at the outset that there are limitations to this approach at present due to the difficulties in accurately predicting the behavior of a peptide in solution. For instance, it is known that peptide α-helices are more prone to fraying at their termini than are those of proteins (Lyu *et al.*, 1991). As will be clear from Section II, structural factors responsible for peptide folding in aqueous solution are still the subject of active

1043-4526/98 $25.00

research. However, we believe that the general approach outlined here provides an interesting model for the study of emulsification in the laboratory.

Food preparations often contain complex mixtures of water-soluble and oil-soluble components. A good example of this is milk, which contains fat droplets suspended in an aqueous medium. In the absence of emulsifiers, these components may partition out into separate phases. Emulsion instability may also result in creaming or fat separation (Halling, 1981; Kachholz and Schlingmann, 1987; Parker, 1988; Das and Kinsella, 1990). These effects are important in the manufacture and storage of food products. Caseins and albumins are important contributors to the functional characteristics of foods, especially emulsification (Macritchie, 1978). Proteins contain hydrophilic and hydrophobic side chains, and thus can sometimes display "amphiphilicity," that is, the ability to expose hydrophilic and hydrophobic faces in a single molecule. Indeed, this property is fundamental to protein structure since the presence of hydrophilic side chains on the protein surface and of hydrophobic residues in the protein interior is essential in protein solubility in acqueous solution and also important in protein folding (Creighton, 1992).

Studies on the emulsification properties of proteins in food systems in the past have, of necessity, often been somewhat empirical. This is due to the high degree of structural heterogeneity in the food and protein preparations used. Even in cases where quantitative amounts of a pure protein are available, they may have quite complex structures since they may consist of several domains or, like β-casein, may be extensively disordered. Attempts have been made to correlate emulsification propensity in proteins with their physiocochemical characteristics such as hydrophobicity (Hague and Kito, 1983; Nakai, 1983), solubility (Wang and Kinsella, 1976; McWatters and Holmes, 1979a,b), water–oil activity index (Elizalde *et al.,* 1988), and stability to heat (Foley and O'Connell, 1990). However, these studies have not been conclusive and it has been suggested that different factors may be important in different proteins (Klemaszewski *et al.,* 1992).

An alternative approach is the preparation of small peptides from the native protein and the study of their emulsification properties. Such an approach has, for instance, been used with peptides of αS1-casein (Shimizu *et al.,* 1983, 1984). It was found that αS1-casein (1–23) gave particularly good emulsification especially in conjunction with other casein peptides (Shimizu *et al.,* 1986; Kaminogawa *et al.,* 1987). Saito *et al.* (1993) studied emulsification by a trypsin-derived hydrolysate of bovine serum albumin (BSA) that gave better emulsification than BSA alone. These workers found that a 24-kDa peptide (corresponding to residues 377–582) was

preferentially adsorbed onto the oil globule surface. However, this peptide alone gave inferior emulsification compared to the whole hydrolysate, suggesting a role for other small peptides in conjunction with this 24-kDa peptide. These studies suggest that peptides are capable of emulsification although binding to oil globules alone does not completely explain this phenomenon. Synergies between peptides appear to be important in such systems for emulsification. Modeling the complex interactions between peptides in such mixtures is likely to be very difficult, however.

Due to developments in peptide synthesis technology it has now become feasible to synthesize peptides at will (Taylor and Kaiser, 1987; DeGrado, 1988). Moreover, thanks to improvements in computer modeling, it has also become possible to design novel peptides, the structure of which could be optimized for any particular property. For example, peptides have been designed to possess modified hormonal (Taylor and Kaiser, 1987), catalytic (Hahn *et al.,* 1990; Atassi and Manshouri, 1993), and antimicrobial (Blondelle and Houghten, 1992) activities. Thus it is now possible to design peptides with altered amino acid sequences that might be optimized for emulsification properties. Such an approach has been suggested by Bloomberg and his co-workers (Enser *et al.,* 1990; Bloomberg, 1991). The study of related families of such peptides offers a novel experimental model system for the study of emulsification (Saito *et al.,* 1995). A further relevance of this research, which is beyond the scope of the present chapter, is in the preparation of pharmaceutical formulations for delivery of drugs (Shimizu and Nakane, 1995).

II. SECONDARY STRUCTURE OF PEPTIDES

The major criterion used to distinguish between the terms "protein" and "peptide" is the presence of extensive order in the structure of the former (Creighton, 1984). Important exceptions to this, however, are the β-caseins, which are the most important protein emulsifiers. Due to the presence of Pro and lack of Cys residues these proteins adopt disordered structures in solution, and their adsorption and surface-activity properties have been extensively investigated (Dalgleish and Leaver, 1991; Clässon *et al.,* 1995; Leerwakers *et al.,* 1996).

Peptides often adopt a range of conformations when free in aqueous solution (i.e., they are often extensively disordered). However, peptides displaying surface-activity properties such as melittin from bee venom (Knoppel *et al.,* 1979; Inagaki *et al.,* 1989; Kaiser and Kezdy, 1987) and hemolytic toxins (Moellby, 1983) often take up ordered α-helical structures at membrane–water interfaces. A major structural factor in these phenom-

ena is the position of hydrophobic and hydrophilic residues in the peptide sequence. Because α-helices display a high degree of periodicity with 3.6 residues per turn resulting in five complete turns every 18 residues, it is possible for a polypeptide to present highly hydrophilic and hydrophobic "faces" to the surrounding milieu (Taylor and Kaiser, 1987). In proteins, there is a tendency to prefer acidic residues at the N-terminus and basic residues at the C-terminus of α-helices (Chou and Fasman, 1974; Blagdon and Goodman, 1975). The former residues are thought to be more important than the latter as contributors to stability (Dasgupta and Bell, 1993). It has been suggested that this requirement for polar residues at helix termini creates a tendency for apolar residues within the helix, which may explain the amphiphilicity frequently found in such secondary structures (DeGrado *et al.,* 1981; Eisenberg *et al.,* 1982). This phenomenon is known as "helix capping" and it has also been demonstrated in peptides (Forood *et al.,* 1993; see below). Amphiphilic faces will naturally tend to aggregate together (hydrophilic with hydrophilic and vice versa). This can result in the assembly to complex structures either in solution or on contact with lipid bilayers (Terwilliger and Eisenberg, 1982a,b; Vogel and Jähnig, 1986; Smith and Clark, 1992).

Melittin is known to adopt a tetrameric and helical structure at high pH, ionic strength, and peptide concentration (Talbot *et al.,* 1979; Bello *et al.,* 1982; Terwilliger and Eisenberg, 1982b; Goto and Hagihara, 1992). Hydrophobic faces of such multimeric assemblies may interact with phospholipid bilayers thus, for example, facilitating pore formation in cell membranes. This is the chemical basis of both hemolysis (Perez-Paya *et al.,* 1994) and antimicrobial peptide activity (Blondelle and Houghten, 1992).

Although it is known that most peptides display little secondary structure when free in aqueous, solution, it is possible to promote α-helix formation experimentally by exposing the peptide to weakly polar alcohols. Methanol and 2,2-dichloroethanol promote helix formation but 2,2,2-trifluoroethanol (TFE) is the most widely used solvent in this regard. The precise basis of this effect is unclear but is thought to be related to its weak basicity, which strengthens hydrogen bonds. It should be noted that while TFE often produces a secondary structure in peptides similar to those found in corresponding parts of the intact protein structure, this is not always the case (Sönnichsen *et al.,* 1992). TFE titration is thought to be a valuable indicator of helical propensity in synthetic peptides, however (Jasanoff and Fersht, 1994).

Comparisons of amino acid sequences of proteins of known secondary structure have allowed the identification of common features that may represent helix stability determinants (Presta and Rose, 1988) and the proposal of helix propensity scales for amino acids (Horovitz *et al.,* 1992;

Blaber *et al.*, 1993). An alternative approach (similar to the one described in the present work) is to prepare peptides with altered stuctural features and then to compare their stabilities experimentally.

The theoretical treatment of helix formation in peptides was first proposed by Zimm and Bragg (1959). These workers treated polypeptides as polymers of generic monomers and predicted that short peptides will not form helices in aqueous solution. While this is true for many situations, there are examples of peptides that do seem to form extensive secondary structure. It has been shown that the 13-residue C-peptide of RNase A shows helicity (Bierzynski *et al.*, 1982; Brown and Klee, 1971), which can be increased by suitable substitutions (Shoemaker *et al.*, 1982). From these studies, it has been concluded that side-chain interactions (which are not included in the Zimm–Bragg equation) play a role in α-helix stabilization. Much work has consequently been carried out on side-chain-specific effects such as helix capping and salt bridge formation (Bodkin and Goodfellow, 1995).

A number of empirical factors have been identified as being of importance of helix stability in peptides. The role of salt bridges/ion pairs was one of the first of these to be investigated (Marqusee and Baldwin, 1987). A "host–guest" approach studied the helical propensity of a Glu/Lys homopolymer (Lyu *et al.*, 1990). This indicated that Ala, Leu, Met, and Gln (in that order) strongly favored helix stabilization. The precise order of this differed from both previous guest polymer studies (Scheraga, 1978) and the helical propensities of residues in globular proteins (Chou and Fasman, 1974) although these residues are all strong helix formers in both of these systems. The agreement with later work on α-helix propensity in T4 lysozyme is much better, however (Blaber *et al.*, 1993, 1994). With the exception of Pro and Gly (which strongly destabilize) and Ala (which strongly stabilizes), these latter workers found a close correlation between α-helix propensity and residue buried surface area for 17 other amino acids. That is, they concluded that hydrophobic stabilization of α-helices may be a major side-chain-associated factor in stabilization of α-helices in small proteins. Forood *et al.* (1993), meanwhile, investigated the effect of helix capping by introducing residues at the termini of a helical peptide. As with globular proteins, Asp, Asn, Glu, Gln, and Ala stabilized at the N-terminus while Arg, Lys, and Ala stabilized at the C-terminus (Doig *et al.*, 1993).

Ala is known to be the strongest α-helix forming residue while Gly is the strongest helix breaker. A study using synthetic peptides (Chakrabatty *et al.*, 1991) concluded that Ala is 100 times more likely to form α-helix than Gly. Ala–Gly substitutions in the middle of the peptide were much more disruptive of helix than those near the termini, indicating a strong position-dependent effect (Fairman *et al.*, 1991). A study on Barnase (Ser-

rano *et al.*, 1992) generally confirmed these conclusions with the further observation that stability differences in this system could not be due to fraying at the helix termini. Interestingly, this study also found a strong correlation between solvent-accessible hydrophobic surface area and stability. This finding is similar to that later reported for T4 lysozyme and discussed above (Blaber *et al.*, 1993, 1994). However, it should be noted that Monte Carlo simulations using a rigid (Creamer and Rose, 1992) or flexible (Creamer and Rose, 1994) polypeptide backbone have suggested that it is loss of side-chain conformational entropy that correlates best with α-helix propensity scales rather than burial of accessible surface area.

Studies have also been performed using peptide models on β-sheet formation in aqueous solution (Zhang *et al.*, 1993; Forood *et al.*, 1995). Peptides with alternating hydrophilic and hydrophobic residues display an especially strong tendency to form β-sheet. Using a host–guest approach in a zinc-finger peptide it was possible to produce a propensity scale for β-sheet formation that agreed well with scales derived from statistical analysis of β-sheet in proteins of known structure (Kim and Berg, 1993). Another β-sheet propensity scale was proposed by Minor and Kim (1994) using the IgG-binding domain of Protein G. While there appears to be general agreement between these scales, it is noteworthy that the absolute $\Delta\Delta G$ values found in the latter system were an order of magnitude higher than those obtained for the zinc-finger host. By varying the environment (Baumrak *et al.*, 1994) or small chemical substituents (Taylor *et al.*, 1993) of peptides it is sometimes possible to cause them to interconvert between α-helix and β-sheet. It has been pointed out that β-sheet-producing residues such as Ile, Val, and Thr are frequently found in transmembrane α-helices and this has been attributed to the hydrophobicity of the bilayer (Li and Deber, 1992; Li *et al.*, 1995; Deber and Li, 1995).

It has been noted that Leu is α-helix stabilizing while Ile and Val are weakly destabilizing, perhaps due to branching adjacent to the α-carbon. Lyu *et al.* (1991) synthesized a series of peptides containing "unnatural" (i.e., nonbiologically occurring) aliphatic side chains in derivatives of Ala, Val, Leu, and Ile. These side chains contained two to four carbons. It was concluded from this study that restriction in side-chain conformational freedom is a major factor in α-helix stabilization. β-Branching has been shown to destabilize α-helices in aqueous solution (Padmanabhan *et al.*, 1990; Wojcik *et al.*, 1990; Lyu *et al.*, 1991). This is an environment-dependent affect, however, as it does not occur under hydrophobic conditions (Li and Deber, 1992). The role of hydrophobicity has been tested by varying the length of aliphatic side chains of test residues in a model peptide and comparing these to β-branched nonpolar residues. The aliphatic side chains

all promoted helicity, presumably due to a decrease in conformational entropy (Padmanabhan and Baldwin, 1991).

An interesting extension of this general approach (i.e., the use of residues occurring infrequently or not at all in biology) is the use of highly constrained side chains. These are often powerful promoters of α-helix formation (Balaram, 1992) and can be routinely introduced into peptides by genetic (Noren *et al.*, 1989) or chemical methods (Schnolzer and Kent, 1992). An example is the α,α-dialkylated glycine, α-aminobutyric acid, which occurs naturally in fungal membrane proteins (Karle *et al.*, 1991). Due to the introduction of van der Waals clashes, structurally favored conformational space is limited to helical regions (Karle and Balaram, 1990), which forces the peptide into an α-helix.

In visualizing secondary structures of peptides, therefore, it is important to recognize that, unlike proteins, they are highly flexible molecules capable of adopting a range of conformations in solution. The present chapter describes an approach to rational design of peptides with emulsification properties that takes advantage of the fact that amphiphilicity may be actively designed into such peptides. The overall approach is similar to that of Enser *et al.* (1990), who pioneered this type of design, and is summarized in Fig. 1. Briefly, naturally amphiphilic peptides and proteins are used as the starting point for the introduction of specific changes in sequence to increase the amphiphilicity of the final structure. The best candidate peptides may be synthesized by solid-phase methods (see Section IV) and the actual structure of the peptide may be investigated in solution. Studies on other properties such as toxicity and solubility may be performed. Last, the behavior of the peptide in a model emulsification system may be determined. This approach allows interpretation of differences in emulsification behavior due to alteration in the peptide primary structure.

III. MODELING OF PEPTIDE STRUCTURES

A. GRAPHICAL REPRESENTATIONS OF AMPHIPHILICITY

"Edmundson wheels" provide a simple and convenient means of identifying and visualizing amphiphilicity in a protein or peptide. This involves viewing along the axis of the helix with a residue protruding from a circle every 100°. Examples of this type of representation are shown in Fig. 2. For amphipathic peptides such as apolipoprotein and melittin, hydrophilic residues are seen to cluster to one side of the wheel and hydrophobic residues to the other, thus giving the wheel polar and nonpolar "faces."

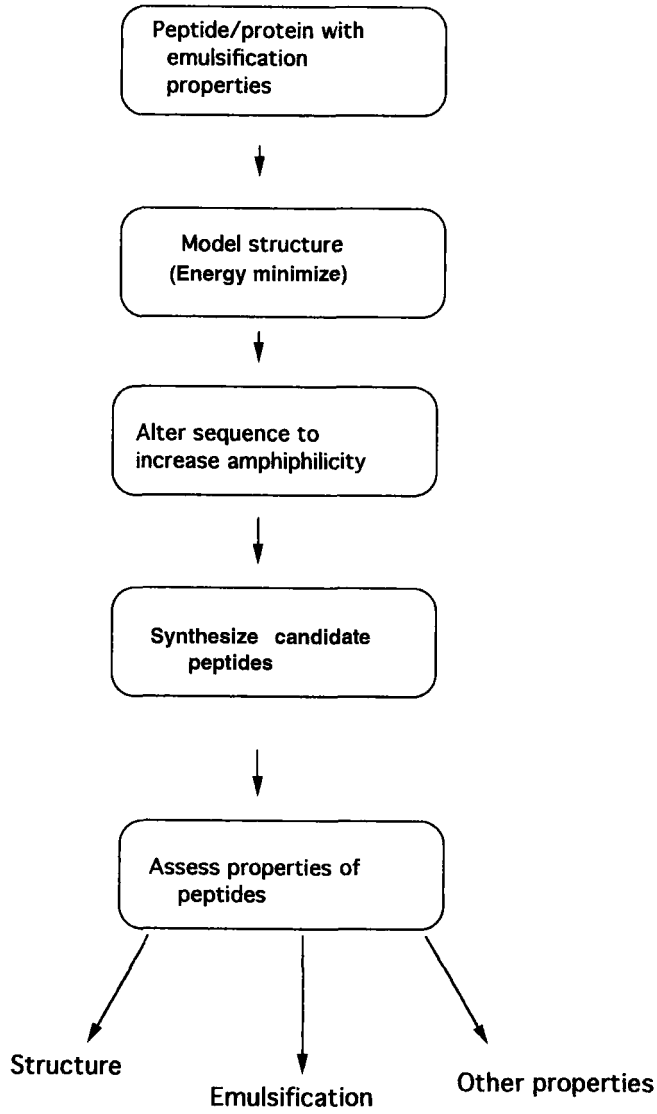

FIG. 1. Design of amphiphilic peptides.

Various workers have proposed classifications of amino acids as hydrophilic or hydrophobic. Hopp and Woods (1981) classified Leu, Ile, Val, Tyr, Phe, Trp, Pro, and Met as hydrophobic and Asp, Arg, Lys, and Glu as hydrophilic residues. In addition, Gln, Asn, Thr, Ser, and Gly are re-

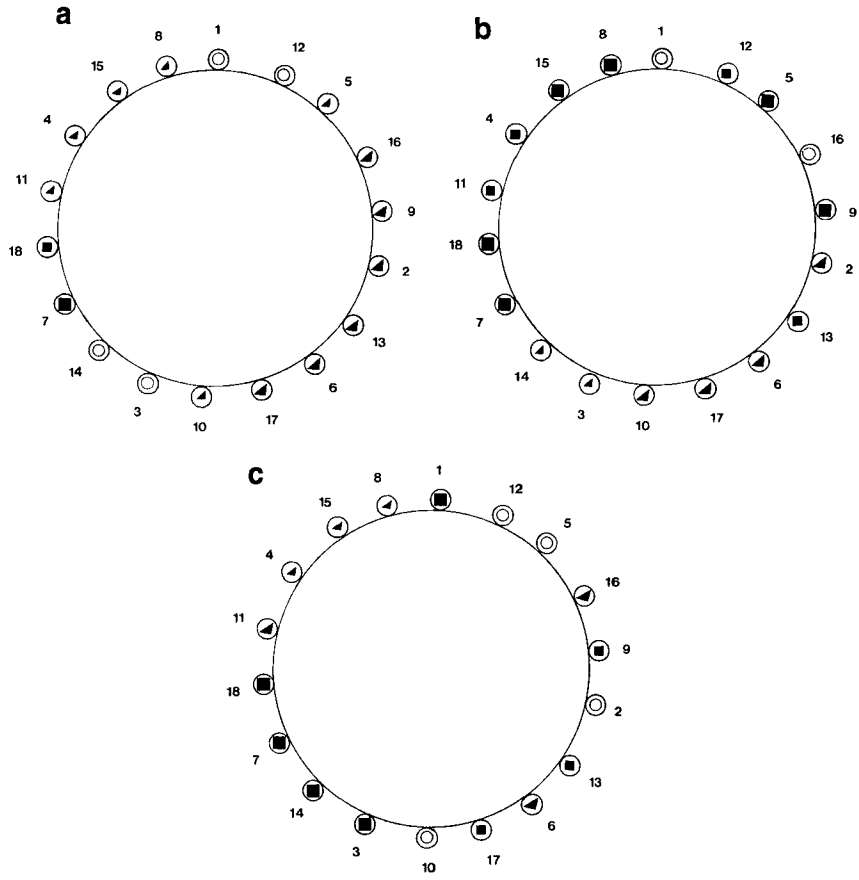

FIG. 2. Graphical representation of amphiphilicity–Edmundson wheels. (a) The first 18 residues of melittin, (b) apolipoprotein IV (181–198), (c) the first 18 residues of αS1-casein (1–23). Strongly hydrophilic (■), weakly hydrophilic (■), neutral (○), weakly hydrophobic (▲), and strongly hydrophobic (▲) residues are shown as classified by Hopp and Woods (1981).

garded as moderately hydrophilic while Cys, His, and Ala are moderately hydrophobic. The extent of hydrophilicity/hydrophobicity in a peptide/protein is therefore directly dependent on amino acid sequence. For helices, Eisenberg *et al.* (1982) quantitated a hydrophobic moment μ_H as

$$\mu_H = \frac{\left|\sum_{i=1}^{N} H_i\right|}{N}, \tag{1}$$

where N is the number of residues in the helix and H_i is the signed hydrophobicity associated with each side chain. By combining a hydrophobicity scale (e.g., that of Wolfenden *et al.* (1981) with the helix wheel concept of Edmundson, the hydrophobic moment can be visually expressed (Fig. 3). Using this type of approach, it has been possible to plot μ_H against net hydrophobicity H and to demonstrate that transmembrane helices, helices from proteins, and helices that seek interfaces between aqueous and nonpolar phases cluster in different regions of such a plot, thus suggesting a correlation between hydrophobic moment and protein function (Eisenberg *et al.*, 1982).

The best-studied amphipathic structures are those of melittin (Terwilliger and Eisenberg, 1982b), its analogs (DeGrado *et al.*, 1981; Blondelle and Houghten, 1992; Wade *et al.*, 1992; King *et al.*, 1994), apolipoproteins (Segrest *et al.*, 1994; Butchko *et al.*, 1995), their analogs (Anantharamaiah, 1986), and antimicrobial peptides (Zhong *et al.*, 1995; Merrifield *et al.*, 1995). In the present chapter, these molecules are taken as suitable starting points for the design of emulsification peptides. In addition, αS1-casein (1–23) has also been reported as possessing emulsification properties (Shimizu *et al.*, 1984, 1986). This molecule was also used in the design process described here.

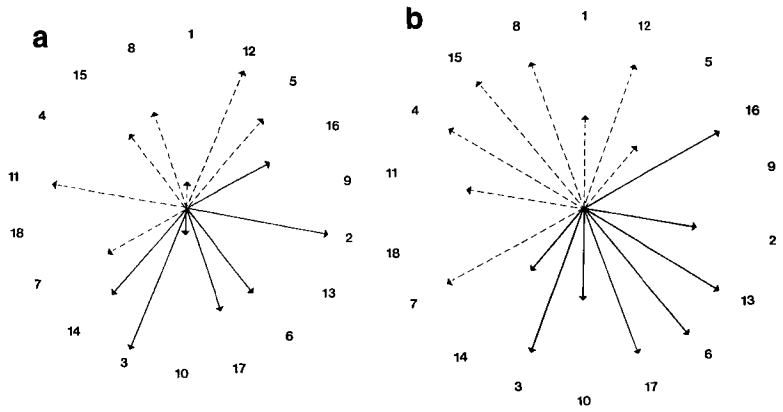

FIG. 3. Hydrophobic moment of (a) lipoprotein IV (181–198) and (b) model peptide 5. The contribution of each residue to hydrophobicity is represented as vectors of solid lines and to hydrophilicity as dashed lines (Eisenberg *et al.*, 1982). Hydrophobicity values determined by Wolfenden *et al.* (1981) were used. The magnitude of the vectors in b are greater, reflecting a larger hydrophobic moment. There is a clear "partitioning" between hydrophobic and hydrophilic faces in these helices.

B. COMPUTER-AIDED DESIGN

Although graphical representation of peptides is useful for simple visualization of amphiphilicity, the wide availability of molecular graphics programs and of computer workstations has allowed more realistic representations of structure (Jameson, 1989; Lesk, 1991; Olsen and Goodsell, 1992; Sun and Cohen, 1993). For example, while the amphiphilicity of melittin is adequately visualized by the representation shown in Fig. 2, this peptide is known to possess a "kink" in its sequence that links two α-helices. This has been observed both in crystals by X-ray crystallography (Terwilliger and Eisenberg, 1982a) and, when bound to micelles, by two-dimensional NMR (Inagaki *et al.,* 1989). To represent complexities such as this, more sophisticated graphics are required.

Modeling systems generally produce a structure using three basic components (Jameson, 1989). First, the structure is represented as an "energy field" incorporating covalent geometry, electrostatic interactions, van der Waals forces, and hydrogen bonding. This is established with the aid of parameter tables that create mathematical representations of atoms and their possible interactions, such as chemical bonds and nonbond effects. The second component is molecular mechanics, which calculates a local energy minimum for a given structure. Such calculations are not completely accurate with present computing facilities due to the number and complexicity of calculations required. However, by making appropriate simplifications to bond angles and lengths used, a structure that closely approximates the true one can be calculated. The third component is molecular dynamics (Hermans, 1993), which allows the peptide to be moved in space, thus eliminating steric conflicts and allowing calculation of a global energy minimum (an "energy-minimized" structure).

It is important to realize that structures predicted by this technology are limited in their accuracy by a number of considerations. The protein folding problem (i.e., how one-dimensional information such as an amino acid sequence can dictate a three-dimensional structure such as a folded protein) is still not solved (Lattman and Rose, 1993; Munoz and Serrano, 1994). Moreover, algorithms used to calculate energy minima are based, ultimately, on the comparatively small number (approximately 1000) of proteins for which crystal structures are available (Sutcliffe *et al.,* 1987; Ponder and Richards, 1987). These structures include few examples of membrane-bound proteins and may well represent a comparatively small subset of proteins that happen to crystallize fairly readily. The algorithms used also contain simplifications and approximations that may introduce inaccuracies into the final structure. When modeling peptides, in particular, it should also be borne in mind that peptide α-helices are generally longer than

protein α-helices and tend to be frayed at their termini (Lyu *et al.,* 1991). Also, because of their greater flexibility in solution, peptides are capable of taking up a variety of conformations. All of these caveats suggest that care should be taken in interpretation of structures designed by computer graphics. The technique is useful as a guide to design, however, and to the rational introduction of novel features into peptides. As we discuss later (see Sections IV and V), it is necessary to study the actual structure of peptides in aqueous solution by techniques such as circular dichroism and two-dimensional NMR to verify the success or otherwise of the design (Bradley *et al.,* 1990; Fezoui *et al.,* 1995).

A number of structural features (often, ones found in globular protein structures) have been designed *de novo* into synthetic peptides using the approach outlined above (Baglia *et al.,* 1992; Fezoui *et al.,* 1994, 1995; Kuroda, 1995). Since amphipathic helices occur as structural building blocks in globular proteins or are often found to have surface-active effects, a number of workers have designed such structures either *de novo* or by homology with peptides such as melittin (DeGrado *et al.,* 1981; Moser, 1992; Blondelle and Houghten, 1992; Zhou *et al.,* 1993; Perez-Paya *et al.,* 1994; Epand *et al.,* 1995). Enser *et al.* (1990) applied this rationale to the design of emulsification peptides. Briefly, it is expected that amphipathic α-helices are likely to display surface-activity properties. By maximizing the amphiphilicity of a peptide, it was hoped to generate a potent emulsifier. This approach was extended by Carey *et al.* (1994) who designed two novel peptides and demonstrated that they were effective emulsifiers. In the following section, the strategy used in the design of these and other peptides is described.

C. DESIGN OF EMULSIFICATION PEPTIDES

Naturally occurring and candidate synthetic peptides were modeled using INSIGHT II (Biosym Technologies) on a personal Iris 4D workstation (Model 891) from Silicon Graphics. The program was used in conjunction with the molecular graphics/dynamics package DISCOVER and required IRIX version 3.2 or higher incorporating the UNIX background. This facilitated three-dimensional modeling of peptides and the visualization of hydropathies. The structures were energy-minimized using a maximum criterion of 0.001 kCal/A and the steepest descents algorithm, which required no cross terms and no morse terms. The number of iterations and the time necessary for modeling varied for each peptide.

The first candidate structure is that of melittin shown in Fig. 4. This energy-minimized structure clearly shows the "kink" that is seen in the region of residues 10–14 in structures derived from both X-ray crystallogra-

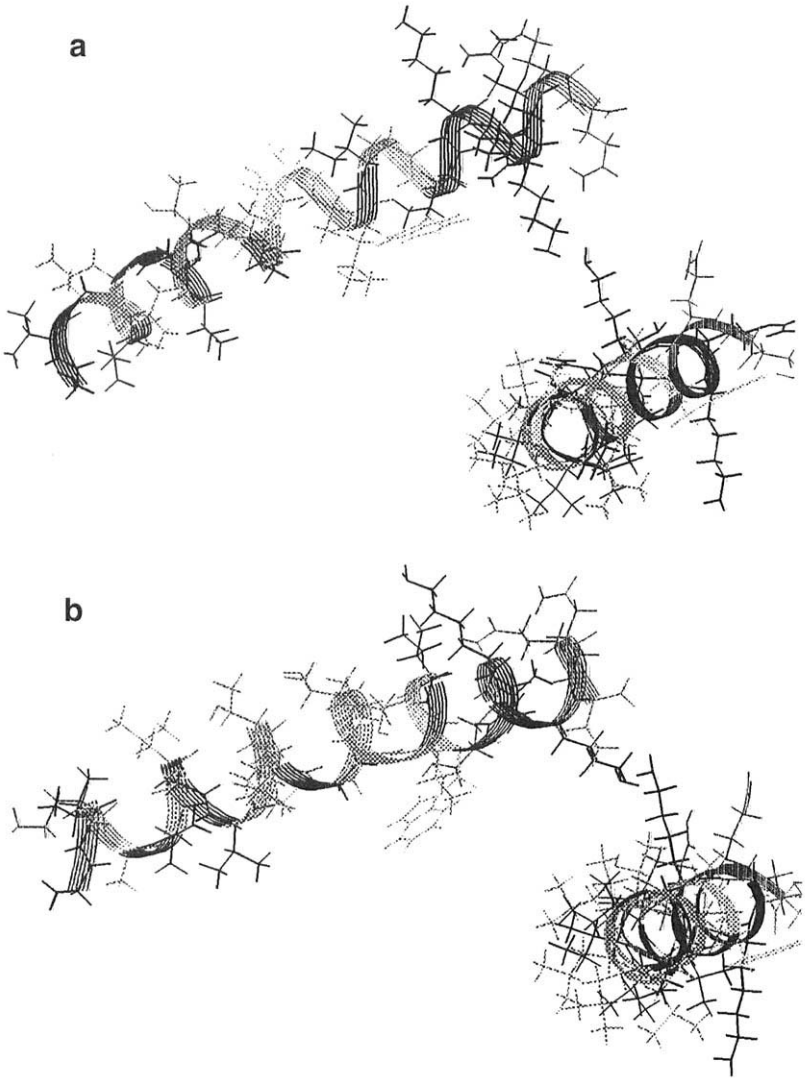

FIG. 4. Energy-minimized structure of melittin and model peptides derived therefrom. The structures were generated by DISCOVER and are shown as "side-on" (i.e., perpendicular to the helix axis) and "end-on" (along the helix axis) views. (a) Melittin, (b) model peptide 1, (c) model peptide 2, (d) model peptide 3, and (e) model peptide 4. Amino acid sequences are detailed in Table I.

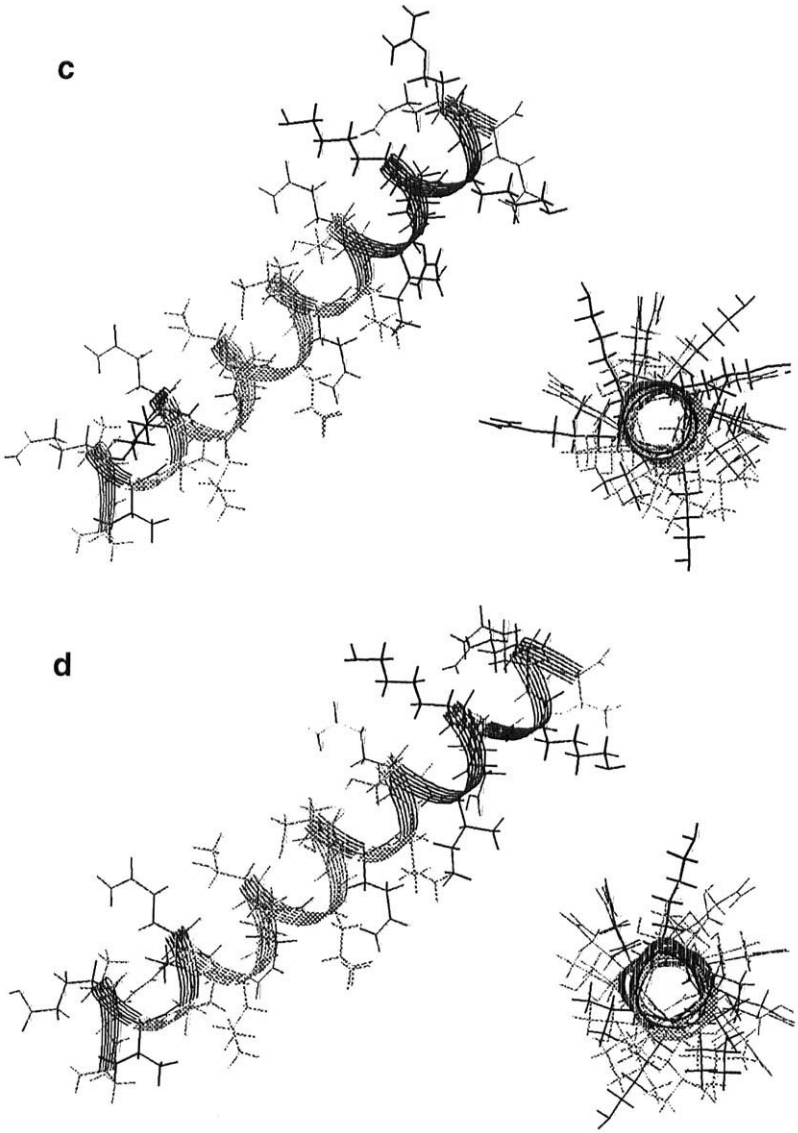

FIG. 4 Continued

phy and two-dimensional NMR studies (Terwilliger and Eisenberg, 1982a; Inagaki *et al.*, 1989). This feature of melittin's structure (which is thought to be essential for the peptide's toxicity; Dempsey *et al.*, 1991; Dempsey, 1992) was mentioned in Section IIIB. A number of considerations governed

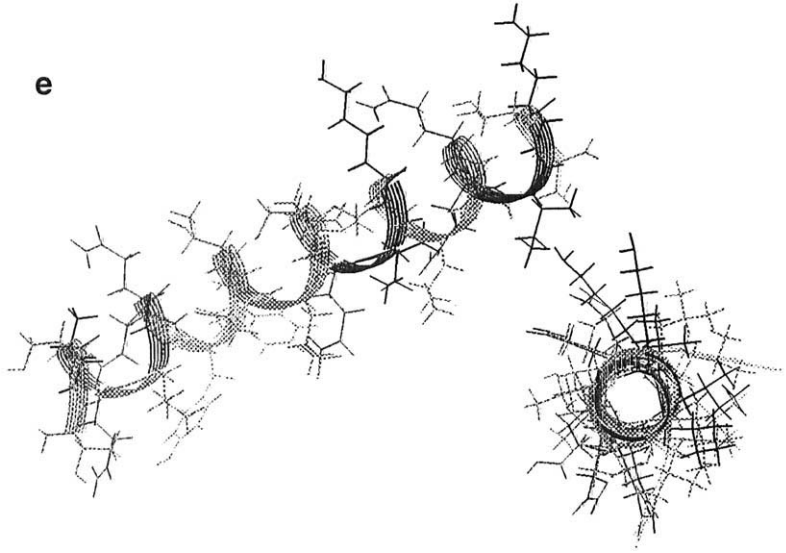

FIG. 4 Continued

the alterations made to this sequence. These will now be described for melittin but similar considerations governed the engineering of the other candidate sequences (see below). First, as melittin is highly cytotoxic, it was desired to abolish this toxicity. Second, changes were made, where possible, to introduce strongly helix-forming residues into the sequence and, conversely, to remove helix "breakers." Third, strongly hydrophilic/hydrophobic residues (e.g., Leu/Lys) were introduced in place of more neutral or weakly hydrophilic/hydrophobic residues (e.g., Met/Gly).

Beginning with the sequence for melittin shown in Table I, model peptide 1 was energy-minimized. This peptide contains alterations at six positions; ^{3}Gly → Glu, ^{7}Lys → Gly, ^{14}Pro → Thr, ^{22}Arg → Glu, ^{24}Arg → Gln, and ^{26}Gln → Thr. These had the effect of removing helix-breaking residues such as Gly and Pro from a number of positions and, as shown in Fig. 4, the modeled energy-minimized structure is somewhat more amphipathic than the parent. This structure still had a discernible kink, however, so further alterations were made to remove it. Model peptide 2 was generated by altering ^{1}Gly → Ser, ^{2}Ile → Thr, ^{3}Gly → Gln, ^{4}Ala → Val, ^{5}Val → Thr, ^{6}Leu → Gln, ^{8}Val → Leu, ^{9}Leu → Thr, ^{11}Thr → Gln, ^{12}Gly → Leu, ^{13}Leu → Thr, ^{14}Pro → Ser, ^{15}Ala → Gln, ^{17}Ile → Gln, ^{18}Ser → Thr, ^{19}Trp → Ile, and ^{20}Ile → Val. These alterations generated a strongly amphipathic structure. It was felt that there still remained some "overlap" between the hydrophilic and hydrophobic faces in this structure, however.

TABLE I

AMINO ACID SEQUENCES OF CANDIDATE AND TEST PEPTIDES

Peptide	Sequence																								
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Melittin	Gly	Ile	Gly	Ala	Val	Leu	Lys	Val	Leu	Thr	Thr	Gly	Leu	Pro	Ala	Leu	Ile	Ser	Trp	Ile	Lys	Arg	Lys	Arg	Gln	Gln
Model peptide 1	Gly	Ile	Glu	Ala	Val	Leu	Gly	Val	Leu	Thr	Thr	Gly	Leu	Thr	Ala	Leu	Ile	Ser	Trp	Ile	Lys	Glu	Lys	Gln	Gln	Thr
Model peptide 2	Ser	Thr	Gln	Val	Thr	Gln	Lys	Leu	Thr	Thr	Gln	Leu	Thr	Ser	Gln	Leu	Gln	Thr	Ile	Val	Lys	Arg	Lys	Arg	Gln	Gln
Model peptide 3	Ser	Thr	Glu	Val	Thr	Gln	Gln	Leu	Thr	Thr	Gln	Leu	Thr	Ser	Gln	Leu	Gln	Thr	Ile	Val	Lys	Glu	Lys	Gln	Gln	Arg
Model peptide 4	Thr	Val	Ser	Gln	Leu	Gln	Glu	Tyr	Trp	Thr	Thr	Leu	Leu	Ser	Gln	Ile	Lys	Thr	Leu	Leu	Gln	Gln	Ile	Lys	Thr	Ser
Apolipoprotein IV (181–198)	Pro	Phe	Ala	Asn	Glu	Leu	Lys	Glu	Lys	Phe	Asn	Gln	Asn	Met	Glu	Gly	Leu	Lys								
Model peptide 5	Thr	Phe	Leu	Gln	Asp	Leu	Lys	Glu	Lys	Val	Gln	Gln	Leu	Thr	Glu	Ala	Leu	Lys								
αS1-casein (1–23)	Arg	Pro	Lys	His	Pro	Ile	Lys	His	Gln	Gly	Leu	Pro	Gln	Glu	Val	Leu	Asn	Glu	Asn	Leu	Leu	Arg	Phe			
Model peptide 6	Arg	Ile	Val	His	Thr	Val	Ile	Thr	Trp	Ala	Leu	Glu	Leu	Ile	Gln	Glu	Leu	Leu	Glu	Gln	Leu	Thr	Thr			

Accordingly, further alterations were introduced into model peptide 2 to generate model peptide 3: ^{7}Lys → Gln, ^{22}Arg → Gln, ^{24}Arg → Gln, and ^{26}Gln → Arg. Again, it was felt that there was some overlap between the two faces and this prompted a further iteration of design. Model peptide 4 retains only three residues at the same positions (10, 11, and 13) as melittin. The alterations made were: ^{1}Gly → Thr, ^{2}Ile → Val, ^{3}Gly → Ser, ^{4}Ala → Gln, ^{5}Val → Leu, ^{6}Leu → Gln, ^{7}Lys → Glu, ^{8}Val → Tyr, ^{9}Leu → Trp, ^{12}Gly → Leu, ^{14}Pro → Ser, ^{15}Ala → Gln, ^{16}Leu → Ile, ^{17}Ile → Lys, ^{18}Ser → Thr, ^{19}Trp → Leu, ^{20}Ile → Leu, ^{21}Lys → Gln, ^{22}Arg → Gln, ^{23}Lys → Ile, ^{24}Arg → Lys, ^{25}Gln → Thr, and ^{26}Gln → Ser. This was the most amphipathic model structure and it was decided to proceed with synthesis of this peptide and to assess it as a potential emulsifier (see Sections IV and V).

This approach allows for modeling a number of peptides and optimizing aspects of their structures for α-helix formation and amphiphilicity. It has been pointed out that one of the great advantages of computer-aided molecular design is the possibility of relatively quickly assessing a variety of structures before committing valuable resources to synthesis (Sun and Cohen, 1993).

The structure obtained for an amphipathic sequence from apolipoprotein IV (181–198) is shown in Fig. 5. The amino acid sequence of this peptide was altered to enhance amphiphilicity. Apart from ^{4}Asn → Gln, ^{5}Glu → Asp, and ^{11}Asn → Gln, which are conservative mutations, six alterations were made to generate model peptide 5: ^{1}Pro → Thr, ^{3}Ala → Leu, ^{10}Phe → Val, ^{13}Asn → Leu, ^{14}Met → Thr, and ^{16}Gly → Ala. These changes had the effect of polarizing hydrophobic/hydrophilic residues on opposite faces of the structure. In addition, the introduction of Leu and Val (strongly hydrophobic) would be expected to make the peptide even more hydrophobic on its hydrophobic face. This is illustrated in Fig. 5. Since Leu is such a strong helix former, it would be expected to help the structure form a helix more readily than the parent peptide.

The third peptide selected for modeling was αS1-casein (1–23), which had previously been reported as capable of emulsification (Shimizu *et al.*, 1983, 1984, 1986). The energy-minimized structure obtained for this peptide is shown in Fig. 5. It is clear from the "end-on" view of this structure that this peptide is not as amphipathic as that of melittin (Fig. 4) or apolipoprotein IV (181–198; Fig. 5). While there are generally hydrophobic and hydrophilic faces, there are clearly residues present on the "wrong" side of the helix. For example, ^{1}Arg and ^{3}Lys appear to be on the more hydrophobic side of the structure. Of the 23 residues, 19 were altered in several iterations to generate the highly polarized model peptide 6. The following substitutions were made: ^{2}Pro → Ile, ^{3}Lys → Val, ^{5}Pro → Thr, ^{6}Ile → Val,

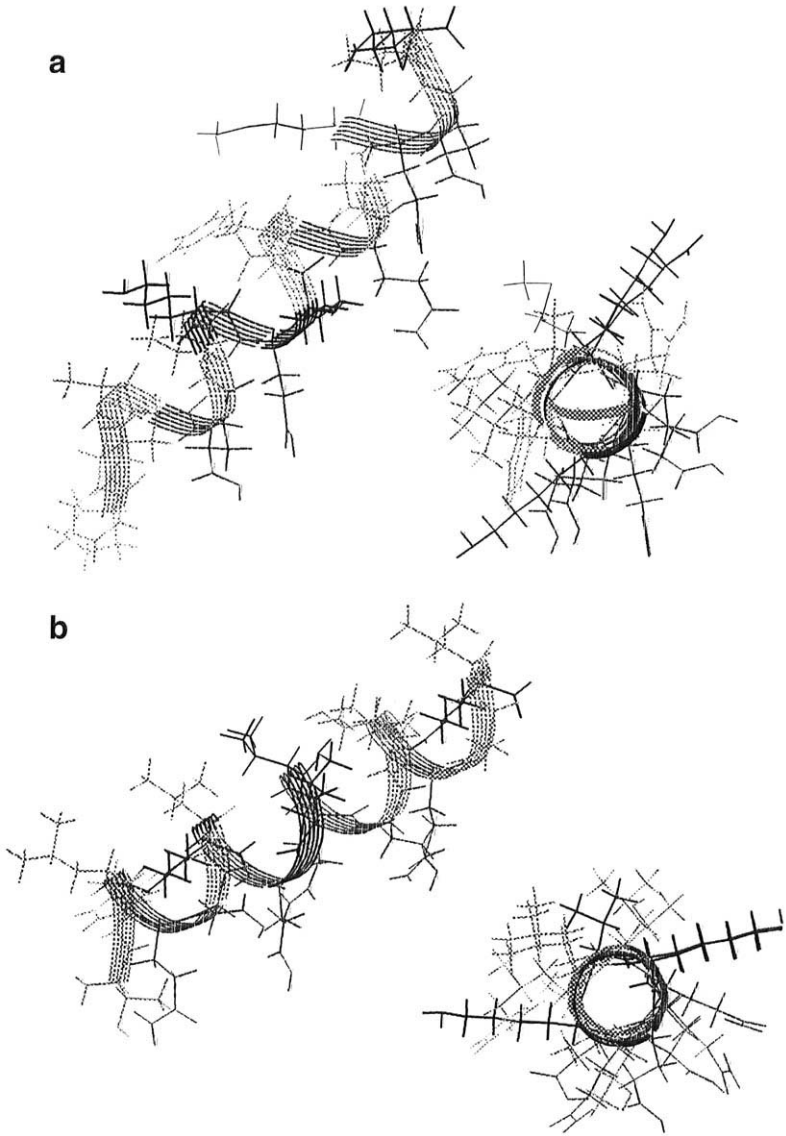

FIG. 5. Energy-minimized structures of apolipoprotein IV (181–198), αS1-casein (1–23), and model peptides derived therefrom. (a) Apolipoprotein IV (181–198), (b) model peptide 5, (c) αS1-casein (1–23), and (d) model peptide 6. Amino acid sequences are given in Table I.

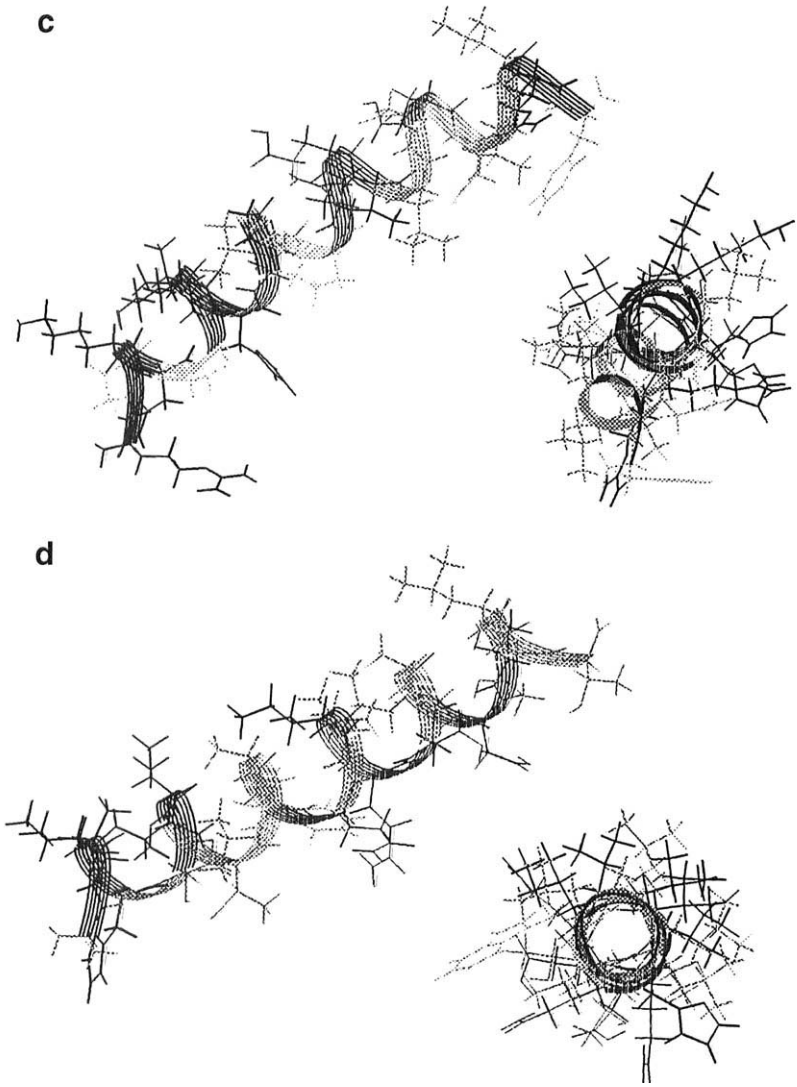

FIG. 5 Continued

^{7}Lys → Ile, ^{8}His → Thr, ^{9}Gln → Trp, ^{10}Gly → Ala, ^{12}Pro → Glu, ^{13}Gln → Leu, ^{14}Glu → Ile, ^{15}Val → Gln, ^{16}Leu → Glu, ^{17}Asn → Leu, ^{18}Glu → Leu, ^{19}Asn → Glu, ^{20}Leu → Gln, and ^{23}Phe → Thr. Model peptides 4, 5, and 6 now appeared to have the desired properties of amphiphilicity and were selected for synthesis and further study.

IV. SYNTHESIS OF DESIGNED PEPTIDES

Solid-phase peptide synthesis was originally developed by Merrified (1963) and allows the rapid production of peptides of high purity and defined sequence (Stewart and Young, 1984; Barany *et al.,* 1987; Fields and Noble, 1990). Because the peptide chain is attached to a resin, the technique lends itself to achieving high-efficiency coupling. Unreacted reagents and by-products of the reaction are conveniently flushed away from the resin. This facilitates high repetitive yields and the synthesis of peptides of maximum lengths 20–30 residues, although longer syntheses have been reported (Ramage *et al.,* 1989). By linking individually prepared peptides together, it is possible to prepare synthetic enzymes, such as RNase, that display enzymatic activity (Gutte and Merrifield, 1969). The basic approach is to establish a system in which reactivity of amino acid substituents is tightly controlled. The first amino acid is attached to a functional group (Stewart and Young, 1984; Barany *et al.,* 1987) on the surface of an insoluble resin (the solid phase, e.g., polystyrene or polyamide). This is achieved by using a chemically activated form of the C-terminal residue. Frequently, an activated ester of the residue (such as a pentafluorophenyl ester) or a symmetric or mixed anhydride are used. Undesired side reaction between pairs of the C-terminal derivative that would result in the unwanted formation of a dipeptide is prevented by the presence of a "blocking" group of its $-NH_2$ substituent. "Directionality" of the synthesis is therefore ensured by chemical activation of one group (–COOH) and masking of the other ($-NH_2$). Although Merrifield originally used *t*-butyloxycarbonyl as an $-NH_2$ blocker, the most commonly used blocking group at present is 9-fluorenylmethoxycarbonyl (F-moc). This may be removed by exposure to trifluoroacetic acid (TFA). The F-moc group and the TFA are washed from the resin and the second residue (i.e., that in the penultimate C-terminal position of the desired sequence) is then added. The activated –COOH group of this residue can now react with the deprotected $-NH_2$ group of the C-terminal residue in a reaction catalyzed by dicyclohexylcarbodiimide or similar reagent. This results in an anchored dipeptide that is now amenable to deprotection and, by repetitive cycles of coupling and deprotection, may be extended to the desired length and sequence. At the end of the synthesis, the peptide is cleaved from the resin by treatment with hydrofluoric acid and recovered.

Note that residues with reactive side chains (i.e., Glu, Lys, Ser) need to have these groups protected during synthesis to prevent unwanted reaction (e.g., with ε-NH_2 of Lys, thiol group of Cys, hydroxy groups of Ser/Thr, etc.; Meienhofer, 1985; Patek, 1993). These may be removed by treatment with hydrofluoric acid and other reagents after synthesis is complete

(Meienhofer, 1985). It is clear that, since the coupling and deprotection steps require quite different chemical conditions, the chemistry of this synthesis lends itself readily to automation. The model peptides designed in Section IIIC were synthesized in an Applied Biosystems Model 431A synthesizer with F-moc (model peptide 5) and Fast-moc (peptides 4 and 6; Fields and Noble, 1990; Applied Biosystems, 1990) amino blocking groups, respectively.

Two possible problems often arise with solid-phase synthesis of peptides. The first is the formation of deletion peptides due to a synthetic cycle not reacting to completion. This arises from incomplete coupling of a sterically hindered residue where the next residue added to the chain is small and couples efficiently. This can occasionally introduce variability into the coupling efficiency of each cycle of synthesis. The final peptide product may therefore be contaminated with significant amounts of one or more peptides lacking a single residue. This deletion peptide would therefore need to be removed after synthesis and recovery. Incomplete coupling is sometimes overcome by recoupling the difficult residue in a different solvent or by using a different coupling reaction. This phenomenon is sometimes difficult to predict as difficulties in coupling are occasionally sequence-specific. A second problem that may introduce heterogeneity into the peptide preparation is incomplete deblocking. It has been estimated that the efficiency of deblocking is as low as 93% and this is one of the factors that limits the length of peptides with which solid-phase synthesis is possible (Meienhofer, 1985; Patek, 1993).

In order to detect these problems, monitoring of the synthesis is necessary. For example, ninhydrin tests may be carried out to quantitate unreacted $-NH_2$ groups. The F-moc substituent may be conveniently measured spectrophotometrically at 300 nm and gives a useful measure of extent of deblocking and for comparing different cycles in the same synthesis. After synthesis is complete, it is necessary to establish the purity of the peptide by high-resolution techniques such as high-performance liquid chromatography (HPLC), mass spectrometry (MS), or capillary electrophoresis. MS is especially useful for detecting incomplete deblocking and deletion peptides because of its ability to resolve very small mass differences. It is therefore often possible to specify which coupling cycle did not go to completion or which blocking group was not completely removed from such an analysis and thus to facilitate repeat of the synthesis with greater success. For purposes of illustration, HPLC and MS analysis of model peptide 6 are shown in Fig. 6. This demonstrates a single peptide present on HPLC analysis with a mass of 2324 (estimated mass: 2323). The synthesized peptides were also sequenced by N-terminal microsequencing (Applied Biosystems Model 477A) as an aid to assessing the success of synthesis. This is

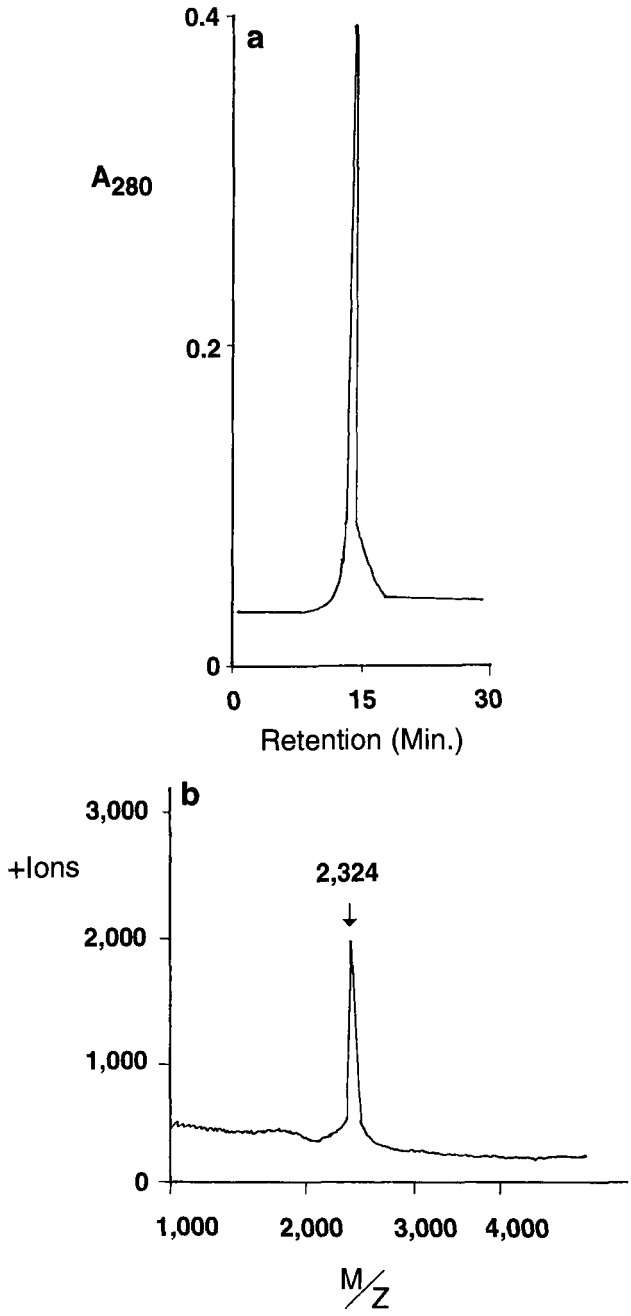

FIG. 6. Analysis of model peptide 6. After peptide synthesis it is essential to characterize the purity of the product. (a) Reversed-phase HPLC on C-8 resin. Buffer A: 0.1% trifluoroacetic acid; buffer B: 70% acetonitrile, 0.065% trifluoroacetic acid. A gradient of 5–100% B over 30 min was used and peptide was detected at 280 nm. (b) Time of flight MS analysis. The estimated mass based on amino acid sequence is 2323.

not as useful a criterion as HPLC and MS as the peptide may often be lost from the sequencer when it has been reduced to tetra- or tripeptide length. However, since N-terminal sequencing functions in the opposite "direction" to Merrifield peptide synthesis, it can give useful information on the later cycles of synthesis (which couple the earlier residues sequenced). Small amounts of incomplete deblocking or coupling are not as easy to detect by this technique alone as, for example, by MS, so it is essential that as many high-resolution analytical techniques as possible be applied to the peptides to ensure the investigator of successful synthesis before proceeding to test their secondary structure or performance as emulsifiers (Bradley *et al.*, 1990).

V. TESTING OF PEPTIDE EMULSIFICATION PROPERTIES

Foods are complex biochemical systems that contain lipid and water as well as other nutrients such as proteins, vitamins, and carbohydrates. Water and lipid do not intermix because of their polar and apolar natures, respectively (Tanford, 1980). Emulsifiers are frequently present or are added to food preparations to forestall phase separation (Friberg, 1976). Contact between lipid and water is thermodynamically disfavored but the presence of emulsifiers can allow them to function long enough to give useful shelf-lives (Das and Kinsella, 1990). Formation of an emulsion requires a large increase of the interfacial surface of emulsified droplets. This makes the ΔG of formation of an emulsion positive (Das and Kinsella, 1990). However, the time required for emulsion breakdown (and phase separation) may be much longer than the shelf-life of the product. Emulsion stability is influenced by the net balance between attractive (van der Waals and London forces) and repulsive (electrostatic, hydration) forces (Parker, 1988; Dickinson and Stainsby, 1988).

A wide variety of chemical structures possess emulsification properties. For example, the phospholipid molecule, lecithin, and proteins such as BSA. Molecules such as gum guar play a role in emulsification by acting as thickeners resulting in emulsion stabilization. A common feature observed, in cases where detailed structural data are available for emulsifiers, is the presence of distinct nonpolar hydrophobic and polar hydrophilic regions in the structure (i.e., the molecules are amphipathic; Bourrel and Schlechter, 1988; Kachholz and Schlingmann, 1987). However, the molecule may possess a range of charge states being positively or negatively charged or zwitterionic. When mixed in a model system, oil and water separate into two distinct phases with an oil–water interface between them. Emulsifiers lower the surface tension at this interface because of their surface-activity

properties (Fisher and Parker, 1988). These properties are directly consequential on the amphipathic structure of the emulsifier (Lang and Wagner, 1987). In forming an emulsion, then, the emulsifier may migrate to the oil–water interface and facilitate intermixing of the phases by stabilizing small droplets of oil (in an oil in water emulsion) or water (in the case of a water in oil emulsion).

A number of experimental methods may be used to study emulsions (Das and Kinsella, 1990). One of the most important measurements is droplet size. This requires a means of microscopically measuring droplets and determining the range of droplets in the emulsion. Interfacial surface tension may be measured in a tensionemeter (Tornberg, 1978).

In the present work, detailed measurements of this type were not carried out. A simple assay was used to determine emulsion stability (Carey *et al.*, 1994). Briefly, emulsifier was added to 10 ml of 1:1 rapeseed oil:20 m*M* sodium acetate/acetic acid buffer, pH 4.38, and homogenized. The time taken for the phases to separate was noted. Blank emulsions generally separated completely in approximately 2 hr at 6°C while emulsions prepared with known emulsifiers such as lecithin and BSA or an emulsion stabilizer such as gum guar took several days to separate (Fig. 7). Emulsion stabilities with melittin and the three model peptides designed in this work are shown in Fig. 8. These indicate that model peptide 4 is particularly effective by this criterion. This peptide stabilizes approximately an order of magnitude better than the emulsifier/stabilizers shown in Fig. 7 (i.e., 0.2 mg/ml model peptide 4 gives similar emulsion stability to 2 mg/ml BSA). Model peptides 5 and 6, though less effective than model peptide 4, were found to be as effective as conventional emulsifier/stabilisers.

Note that model peptide 4 is also significantly more effective (by approximately 10-fold) than its parent compound, melittin. Eleven of melittin's 26 residues are located near the N-terminus (giving a relatively large hydrophobic surface area here, which is thought to be important for attacking biological membranes). In model peptide 4, this number was reduced to 8, covering less than a third of the available surface. Moreover, because changes were deliberately introduced to create more clear-cut polarity between the hydrophilic and hydrophobic faces, it was possible to achieve an increase in surface activity while introducing greater net water solubility into the peptide.

Circular dichroism (CD) measurements were carried out to assess the actual secondary structure of the model peptides. Model peptide 6 was found to be only sparingly soluble in aqueous buffers and, accordingly, did not give a very good spectrum. Because of the amphipathic nature of emulsifiers, it is sometimes difficult to strike a balance between functional activity and water solubility. However, it was possible to determine the secondary structure of model peptides 4 and 5 by this method. The spectra

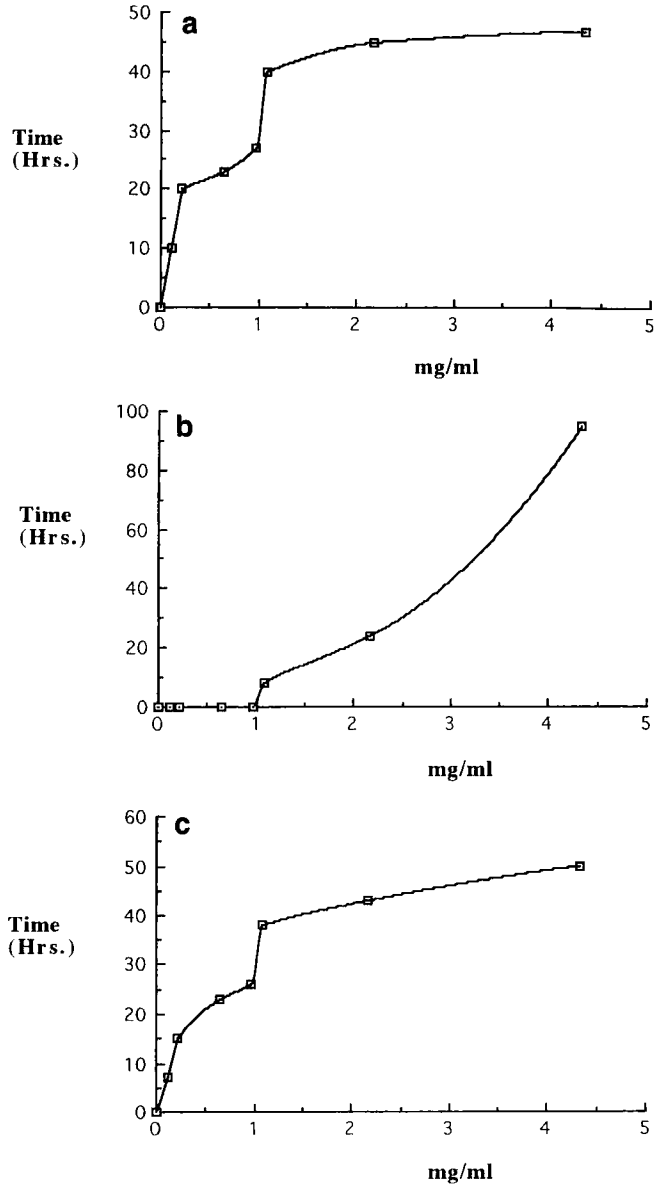

FIG. 7. Emulsification assay. The time required for oil–water separation was determined for a range of concentrations of the following known emulsifiers: (a) BSA, (b) gum guar, and (c) lecithin.

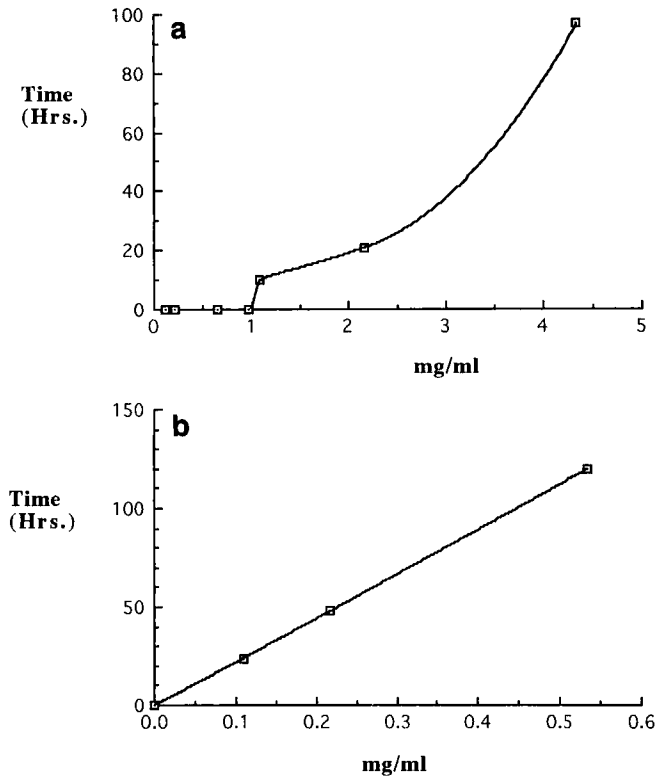

FIG. 8. Emulsification by amphipathic peptides. The time required for phase separation in a system identical to that of Fig. 7 was measured for a range of concentrations of (a) melittin, (b) model peptide 4, (c) model peptide 5, and (d) model peptide 6.

obtained are shown in Fig. 9 and the data are tabulated in Table II. Model peptides 4 and 5 form little ordered structure in aqueous buffer. However, the addition of TFE induces the formation of 90–100% order in the structure. Interestingly, only approximately 33–45% of this structure is α-helical while some 60% is β-sheet. This was unexpected and suggests that the peptides are capable of existing in a variety of conformations and, presumably, are capable of interconverting between them. Despite their different emulsification stabilities (Fig. 8), model peptide 5 consistently displayed slightly higher order than model peptide 4. When CD measurements of α-helix were made in an oil/water system, this observation still held true. This suggests that the two peptides do not assume the same structures in solution as predicted from their energy-minimized structures shown in Figs. 4 and 5 (Carey *et al.*, 1994). This is probably due to the shortcomings of the modeling approach used, which were highlighted earlier. Although

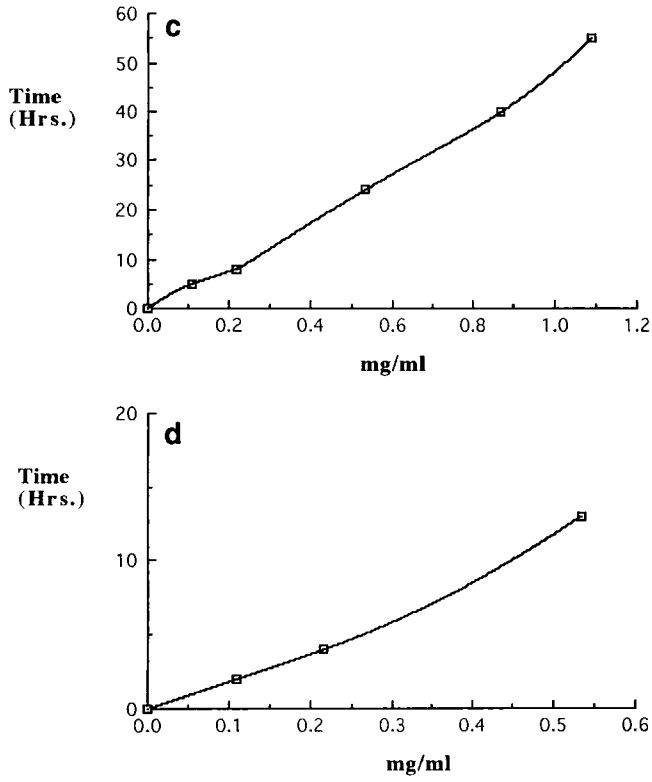

FIG. 8 Continued

more detailed investigations of emulsion stability were not performed on these peptides, we have found with other melittin-derived model peptides that droplets in the size range 1–10 μm are obtained with Coulter counter measurements (Ebeling *et al.* 1997).

Using naturally amphipathic structures as starting points, it has been possible to design peptides with enhanced emulsification stability by maximizing the amphipathic nature of the structure. These peptide emulsifiers were as good as or better than melittin, one of the most surface-active peptides known.

VI. FUTURE DIRECTIONS

A number of exciting developments suggest that the approach described in this paper could be fruitfully pursued in the future. The availability of

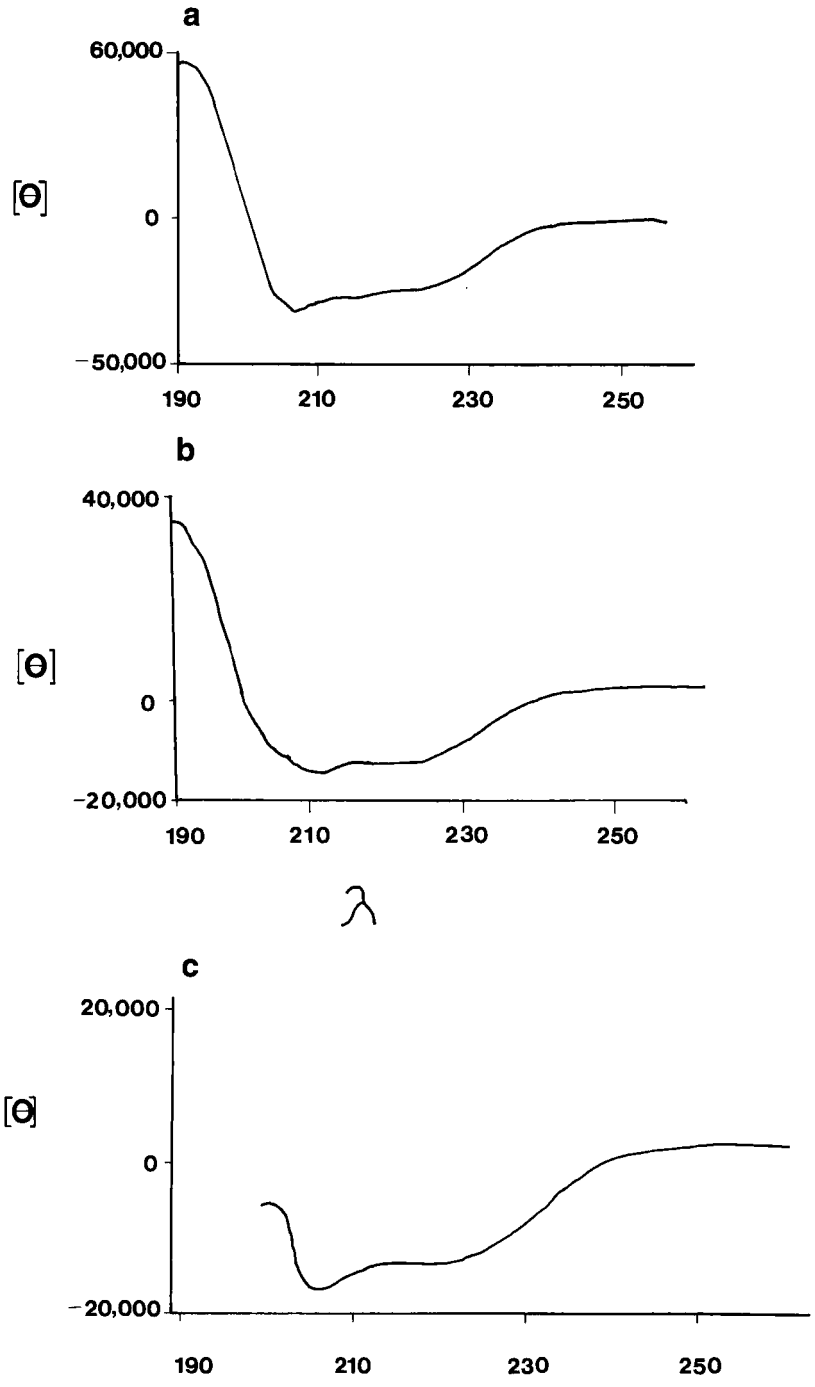
a
60,000
0
−50,000
[Θ]
190
210
230
250
b
40,000
0
−20,000
[Θ]
190
210
230
250
λ
c
20,000
0
−20,000
[Θ]
190
210
230
250

TABLE II
CIRCULAR DICHROISM OF MODEL PEPTIDES

Peptide	β-Sheet (%)	α-Helix (%)	Random coil (%)
Model peptide 4			
20 m*M* sodium acetate, pH 4.8	53	13 (14)	33
Buffer: 50% TFE	58	33 (32)	9
Buffer: Oil	—	(11)	—
Model peptide 5			
20 m*M* sodium acetate, pH 4.8	51	5 (0)	44
Buffer: 50% TFE	55	45 (49)	0
Buffer: Oil	—	(27)	—

faster and more powerful molecular modeling systems together with greater knowledge of the behavior of model peptides in solution suggest the possibility of improved design of peptides. The commercial availability of blocked, conformationally constrained residues suitable for peptide synthesis (Balaram, 1992) underlines the possibility of generating families of peptides with an even greater propensity to form α-helix or β-sheet structures than natural amino acids. A number of novel chemical options are also now available for peptide design. One of the more interesting of these is the concept of joining helices together into bundles by covalently linking their termini. The introduction of disulfide bridges may be achieved by the judicious introduction of Cys residues (Jackson *et al.*, 1991; Rivett *et al.*, 1996). An alternative is the use of "branching" residues such as ornithine and lysine at helix termini. These may be acylated at non-α amino groups, thus providing amide linkages between different helices (Hahn *et al.*, 1990). We are presently extending the work described here with studies of melittin analogs containing Cys residues. It is possible to generate peptide dimers and oligomers in this way and we have found that this affects surface activity (unpublished observations).

Although CD and MS measurements give adequate structural data, it would be of interest to determine the structure with two-dimensional NMR measurements in solution. A necessary prerequisite of such a study would be the assessment of the effects of variables such as pH and salt concentration on emulsification. These variables have been found to affect the struc-

FIG. 9. Circular dichroism spectra (50% TFE) of (a) model peptide 4, (b) model peptide 5, and (c) model peptide 6. Because of the limited solubility of peptide 6 in aqueous buffers, the spectrum was poor and it was not possible to determine secondary structure accurately. Secondary structures derived for the other two model peptides are tabulated in Table II.

tures of melittin (Bello *et al.*, 1982) and models derived therefrom (Daggett *et al.*, 1991; Moser, 1992). It should also be noted that surface activity alone, while important, is not the only property desired in a food emulsifier and we have not yet carried out studies on the more precise measures of emulsifier performance such as emulsification activity index, emulsification capacity, and emulsion stability (Das and Kinsella, 1990). In view of the impressive performance of model peptide 4 in the present study, this molecule is a tempting target for such studies.

ACKNOWLEDGMENTS

We are grateful to Professor C. Daly of the National Food Biotechnology Centre (NFBC), University College Cork, for his encouragement of this work and also to Mrs. Aine Healy of the NFBC for her expert help in peptide synthesis and analysis. Circular dichroism measurements were carried out by our collaborators, Dr. N. C. Price and Ms. S. M. Kelly, University of Stirling. Silicon Graphics equipment was a generous gift from Schering-Plough Corp. Innishannon, Co., Cork. DS is grateful to the Spanish government for a sabbatical fellowship at the Consejo Superior de Investigaciones Cientificas, Centro de Investigacion Y Desenvolupament (CSIC, CID), Barcelona, which facilitated preparation of the manuscript. We are also grateful to Dr. D. Wilcock (CSIC, CID) for critically reading the manuscript.

REFERENCES

Anantharamaiah, G. M. 1996. Synthetic peptide analogs of apolipoproteins. *Meth. Enzymol.* **128,** 626–668.

Applied Biosystems. 1990. FastMoc™ chemistry: HBTU activation in peptide synthesis on the model 431A. *User Bulletin No. 33,* **Nov.** 1–5.

Atassi, M. Z., and Manshouri, T. 1993. Design of peptide enzymes (pepzymes): Surface-simulation synthetic peptides that mimic the chymotrypsin and trypsin active sites exhibit the activity and specificity of the respective enzyme. *Proc. Natl. Acad. Sci. USA* **90,** 2828–8286.

Baglia, F. A., Bradford, A. J., and Walsh, P. N. 1992. Fine mapping of the high molecular weight Kininogen binding site on blood coagulation factor IX through the use of rationally designed synthetic analogues. *J. Biol. Chem.* **267,** 4247–4252.

Balaram, P. 1992. Non-standard amino acids in peptide design and protein engineering. *Curr. Opin. Struct. Biol.* **2,** 845–851.

Barany, G., Kneib-Cordonier, N., and Mullen, D. G. 1987. Solid phase peptide synthesis: A silver anniversary report. *Int. J. Pept. Prot. Res.* **30,** 705–739.

Baumrak, V., Huo, D., Dukor, R. K., Keiderling, T. A., Leievre, D., and Brack, A. 1994. Conformational study of sequential lys and leu based polymers and oligomers using vibrational and electronic circular dichroism spectra. *Biopolymers* **34,** 1115–1121.

Bello, J., Bello, H. R., and Granados, E. 1982. Conformation and aggregation of melittin: Dependence on pH and concentration. *Biochemistry* **21,** 461–465.

Bierzynski, A., Kim, P. S., and Baldwin, R. L. 1982. A salt bridge stabilizes the helix formed by isolated C-peptide of RNA-ase. *Proc. Natl. Acad. Sci. USA* **79,** 2470–2474.

Blaber, M., Zhang, X.-J., Lidstrom, J. D., Pepoit, S. D., Baase, W. A., and Matthews, B. M. 1994. Determination of alpha-helix propensity within the context of a folded protein. *J. Mol. Biol.* **235,** 600–624.

Blaber, M., Zhang, X.-J., and Matthews, B. M. 1993. Structural basis of amino acid alpha-helix propensity. *Science* **260,** 1637–1640.

Blagdon, D. E., and Goodman, M. 1975. Mechanisms of protein and peptide helix initiation. *Biopolymers* **14,** 241–245.

Blondelle, S. E., and Houghten, R. A. 1992. Design of model amphipathic peptides having potent antimicrobial activities. *Biochemistry* **31,** 12,688–12,694.

Bloomberg, G. 1991. Designing proteins as emulsifiers. *Food Market. Technol.* **5,** 14–15.

Bodkin, M. T., and Goodfellow, J. M. 1995. Competing interactions contributing to alpha-helical stability in aqueous solution. *Prot. Sci.* **4,** 603–612.

Boguski, M. S., Elshourbagy, N., Taylor, J. M., and Gordon, J. I. 1985. Rat apolipoprotein A IV contains thirteen tandem repetitions of a 22-amino acid segment with amphipathic helical potential. *Proc. Natl. Acad. Sci. USA* **81,** 5021–5025.

Bourrel, M., and Schlechter, R. S. 1988. The R-ratio. *In* "Microemulsions and Related Systems," pp. 1–27. Marcel Dekker, New York.

Bradley, E. K., Thomason, J. F., Cohen, F. E., Kosen, P. A., and Kuntz, I. D. 1990. Studies of synthetic helical peptides using circular dichroism and nuclear magnetic resonance. *J. Mol. Biol.* **215,** 607–622.

Brown, J. E., and Klee, W. A. 1971. Helix-coil transition of the isolated amino terminus of ribonuclease. *Biochemistry* **10,** 470–476.

Butchko, G. W., Rozek, A., Zhoug, Q., and Cushley, R. J. 1995. Sequence-specific ^{1}H NMR assignments and secondary structure of a lipid-associating peptide from human apolipoprotein C-I: An NMR study of an amphiphilic helix motif. *Pept. Res.* **8,** 86–94.

Carey, K. B., Kelly, S. M., Price, N. C., O'Sullivan, D. J., and Sheehan, D. 1994. Two amphiphilic, synthetic peptides display strong emulsification properties. *Food Chem.* **50,** 83–85.

Chakrabatty, A., Schellman, J. A., and Baldwin, R. L. 1991. Large differences in the helix propensities of alanine and glycine. *Nature* **351,** 586–588.

Chou, P. Y., and Fasman, G. P. 1974. Prediction of protein conformation. *Biochemistry* **13,** 222–245.

Clässen, P. M., Blomberg, E., Froeberg, J. C., Nylander, T., and Arrebant, T. 1995. Protein interactions at solid surfaces. *Adv. Colloid Interface. Sci.* **57,** 161–227.

Creamer, T. P., and Rose, G. D. 1992. Side-chain entropy opposes alpha helix formation but rationalises experimentally determined helix-forming propensities. *Proc. Natl. Acad. Sci. USA* **89,** 5937–5941.

Creamer, T. P., and Rose, G. D. 1994. Alpha-helix forming propensities in peptides and proteins. *Prot. Struct. Funct. Genet.* **19,** 85–97.

Creighton, T. E. 1984. "Proteins, Structures and Molecular Properties." W. H. Freeman, London.

Creighton, E. 1992. Folding and binding. *Curr. Opin. Struct. Biol.* **2,** 1–5.

Daggett, V., Kollman, P. A., and Kuntz, I. D. 1991. Molecular dynamics simulations of small peptides: Dependence on dielectric model and pH. *Biopolymers* **31,** 285–304.

Daggett, V., and Levitt, M. 1992. Molecular dynamics simulation of helix denaturation. *J. Mol. Biol.* **223,** 1121–1138.

Dalgleish, D. G., and Leaver, J. 1991. The possible conformations of milk proteins adsorbed on oil/water interfaces. *J. Colloid Interface Sci.* **141,** 288–294.

Das, K. P., and Kinsella, J. E. 1990. Stability of food emulsions: Physicochemical role of protein and nonprotein emulsifiers. *Adv. Food Nutr. Res.* **34,** 81–201.

Dasgupta, S., and Bell, J. A. 1993. Design of helix ends. Amino acid preferences, hydrogen bonding and electrostatic interactions. *Int. J. Pept. Prot. Res.* **41,** 499–511.

Deber, E. M., and Li, S. C. 1995. Peptides in membranes: Helicity and hydrophobicity. *Biopolymers* **37,** 295–318.

DeGrado, W. F. 1988. Design of peptides and proteins. *Adv. Prot. Chem.* **39,** 51–124.

DeGrado, W. F., Kezdy, F. T., and Kaizer, E. T. 1981. Design synthesis and characterization of a cytotoxic peptide with melittin-like characteristics. *J. Am. Chem. Soc.* **103,** 679–681.

Dempsey, C. E. 1992. Quantitation of the effects of an internal proline residue on individual hydrogen bond stabilities in an α-helix: pH-dependent amide exchange in melitin and [Ala-14] melittin. *Biochemistry* **31,** 4705–4712.

Dempsey, C. E., Bazzo, R., Harvey, T. S., Syperek, I., Boheim, G., and Campbell, I. D. 1991. Contribution of proline-14 to the structure and actions of melittin. *FEBS Lett.* **281,** 240–244.

Dickinson, E., and Stainsby, G. 1988. Emulsion stability. *In* "Advances in Food Emulsions and Foams" (E. Dickinson and G. Stainsby, eds.), pp. 1–44. Elsevier, Amsterdam.

Doig, A. J., Chakrabartty, A., and Baldwin, R. L. 1993. Helix N-cap propensities in peptides parallel those found in proteins. *Proc. Natl. Acad. Sci. USA* **90,** 11,332–11,336.

Ebeling, S. C., Kelly, S. M., O'Kennedy, B. T., Price, N. C., and Sheehan, D. (1997). Surface activity properties of cysteine-substituted C-terminal melittin analogues. *Biochemie* **79,** 503–508.

Eisenberg, D., Weiss, B. M., and Terwilliger, C. 1982. The helical hydrophobic moment: A measure of the amphiphilicity of a helix. *Nature* **299,** 371–374.

Elizalde, B. E., DeKanterewicz, R. J., Pilosof, A. M. R., and Bartholomai, G. B. 1988. Physicochemical properties of food proteins related to their ability to stabilize oil-in-water emulsions. *J. Food Sci.* **53,** 845–855.

Enser, M., Bloomberg, G. B., Brock, C. J., and Clark, D. C. 1990. *De novo* design and structure-activity relationships of peptide emulsifiers and foaming agents. *Int. J. Biol. Macromol.* **12,** 118–124.

Epand, R. M., Shai, Y., Segrest, J. P., and Anantharamaiak, G. M. 1995. Mechanisms for the modulation of membrane bilayer properties by amphipathic helical peptides. *Biopolymers* **37,** 319–338.

Fairman, R., Armstrong, K. M., Shoemaker, K. R., York, E. J., Stewart, J. M., and Baldwin, R. L. 1991. Position effect on apparent helical propensities in the C-peptide helix. *J. Mol. Biol.* **221,** 1395–1401.

Fezoui, Y., Weaver, D. L., and Osterhout, J. J. 1994. *De novo* and structural characterisation of an alpha-helical hairpin peptide: A model system for the study of protein folding intermediates. *Proc. Natl. Acad. Sci. USA* **91,** 3675–3679.

Fezoui, Y., Weaver, D. L., and Osterhout, J. J. 1995. Strategies and rationales for the *de novo* design of a helical hairpin peptide. *Prot. Sci.* **4,** 286–295.

Fields, G. B., and Noble, R. L. 1990. Solid phase peptide synthesis utilizing 9-fluorenylmethoxycarbonyl amino acids. *Int. J. Pept. Prot. Res.* **35,** 161–214.

Fisher, L. R., and Parker, N. S. 1988. Effect of surfactants on the interactions between emulsion droplets. *In* "Advances in Food Emulsions and Foams" (E. Dickinson and G. Stainsby eds.), pp. 45–90. Elsevier, Amsterdam.

Foley, J., and O'Connell, C. 1990. Comparative emulsifying properties of sodium caseinate and whey protein isolate in 18% oil in aqueous systems. *J. Dairy Res.* **57,** 377–391.

Forood, B., Feliciano, E. J., and Nambiar, K. P. 1993. Stabilisation of alpha-helical structures in short peptides via end capping. *Proc. Natl. Acad. Sci. USA* **90,** 838–842.

Forood, B., Perez-Paya, E., Houghten, R. A., and Blondelle, S. E. 1995. Formation of an extremely stable polyalanine β-sheet macromolecule. *Biochem. Biophys. Res. Commun.* **211,** 7–13.

Friberg, S. E. 1976. Emulsion stability. *In* "Food Emulsions" (S. E. Friberg, ed.), pp. 1–37. Marcel Dekker, New York.

Goto, Y., and Hagihara, Y. 1992. Mechanism of the conformational transition of melittin. *Biochemistry* **31,** 732–738.

Gutte, B., and Merrifield, R. B. 1969. The total synthesis of an enzyme with ribonuclease A activity. *J. Am. Chem. Soc.* **91,** 501–502.

Habermann, E. 1972. Bee and wasp venoms. *Science* **177,** 314–322.

Hague, Z., and Kito, M. 1983. Lipophilization of alpha S-1 casein: 1. Palmitoyl protein. *J. Agric. Food Chem.* **32,** 1225–1236.

Hahn, K. W., Wieslaw, A., Klis, A., and Stewart, J. M. 1990. Design and synthesis of a peptide having chymotrypsin-like esterase activity. *Science* **248,** 1544–1547.

Halling, P. J. 1981. Protein-stabilised foams and emulsions. *CRC Crit. Rev. Food Sci. Nutr.* **13,** 155–120.

Hermans, J. 1993. Molecular dynamics simulations of helix and turn propensities in model peptides. *Curr. Opin. Struct. Biol.* **3,** 270–276.

Hopp, T. P., and Woods, K. R. 1981. Prediction of protein antigenic determinants from amino acid sequences. *Proc. Natl. Acad. Sci. USA* **78,** 3824–3828.

Horovitz, A., Matthews, J. M., and Fersht, A. R. 1992. Alpha-helix stability in proteins. II. Factors that influence stability at an internal position. *J. Mol. Biol.* **227,** 560–568.

Inagaki, F., Shimado, I., Kawaguchi, K., Hirano, M. M., Terasawa, I., Ikura, T., and Go, N. 1989. Structure of melittin bound to perdeuterated dodecylphosphocholine micelles as studied by two dimensional NMR and distance geometry calculations. *Biochemistry* **28,** 5985–5991.

Jackson, D. Y., King, D. S., Chmielewski, J., Singh, S., and Schultz, P. G. 1991. General approach to the synthesis of short alpha-helical peptides. *J. Am. Chem. Soc.* **113,** 9391–9392.

Jameson, B. A. 1989. Modelling in peptide design. *Nature* **341,** 465–466.

Jasanoff, A., and Fersht, A. R. 1994. Quantitative determination of helical propensities from trifluoroethanol titration curves. *Biochemistry* **33,** 2129–2135.

Kachholz, T., and Schlingmann, M. 1987. Possible food and agricultural application of microbial surfactants. *In* "Biosurfactants and Biotechnology" (N. Kosaric, W. L. Cairns, and N. C. C. Grey, eds.), pp. 183–210. Marcel Dekker, New York.

Kaiser, E. T., and Kezdy, F. J. 1987. Peptides with affinity for membranes. *Annu. Rev. Biophys. Biophys. Chem.* **16,** 561–581.

Kaminogowa, S., Shimizu, M., Ametani, A., Lee, S. W., and Yamauchi, K. 1987. Proteolysis in structural analysis of alpha S1-casein adsorbed onto oil surfaces of emulsions and improvement of the emulsifying properties of protein. *J. Am. Chem. Soc.* **64,** 1688–1691.

Karle, I. L., and Balaram, P. 1990. Structural characterizations of alpha helical peptide molecules containing α-aminobutyric acid. *Biochemistry* **29,** 6747–6756.

Karle, I. L., Flipper-Andersen, J. L., Agarwalla, S., and Balaram, P. 1991. Crystal structure of zervamicin, a membrane ion-channel peptide: Implications for gating mechanism. *Proc. Natl. Acad. Sci. USA* **88,** 5307–5311.

Kim, C. A., and Berg, J. M. 1993. Thermodynamic β-sheet propensities measured using a zinc-finger host peptide. *Nature* **362,** 267–270.

King, T. P., Wade, D., Coscia, M. R., Mitchell, S., Kochoumian, L., and Merrifield, B. 1994. Structure and immunogenic relevance of melittin, its transposed analogues and D-melittin. *J. Immunol.* **153,** 1124–1131.

Klemaszewski, J. L., Das, K. P., and Kinsella, J. E. 1992. Formation and coalescence stability of emulsions stabilized by different milk proteins. *J. Food Sci.* **57,** 366–372.

Knoppel, E., Eisenberg, D., and Wickner, W. 1979. Interaction of melittin, a preprotein model, with detergents. *Biochemistry* **18,** 4177–4181.

Kuroda, Y. 1995. A strategy for the *de novo* design of helical proteins with stable folds. *Prot. Eng.* **8,** 286–295.

Lang, S., and Wagner, F. 1987. Structure and properties of biosurfactants. *In* "Biosurfactants and Biotechnology" (N. Kosaric, W. L. Cairns, and N. C. C. Grey, eds.), pp. 21–45. Marcel Dekker, New York.

Lattman, E. E., and Rose, G. D. 1993. Protein folding—What's the question? *Proc. Natl. Acad. Sci. USA* **90,** 439–441.

Leerwakers, F. A. M., Atkinson, P. J., Dickinson, E., and Horne, D. S. 1996. Self-consistent-field modelling of adsorbed β-casein: Effects of pH and ionic strength on surface coverage and density profile. *J. Colloid Interface Sci.* **178,** 681–693.

Lesk, A. M. 1991. "Protein Architecture." IRL Press, Oxford.

Li, S. C., and Deber, C. K. 1992. Glycine and β-branched residues support and modulate peptide helicity in membrane environments. *FEBS Lett.* **311,** 217–220.

Li, S. C., Kim, P. K., and Deber, M. 1995. Manipulation of peptide conformation by fine-tuning of the environment and/or primary sequence. *Biopolymers* **35,** 667–675.

Lyu, P. C., Liff, M. I., Marky, L. A., and Kallenbach, N. R. 1990. Side chain contributions to the stability of alpha-helical structure in peptides. *Science* **250,** 669–673.

Lyu, P. C., Sherman, J. C., Chen, A., and Kallenbach, N. R. 1991. Alpha-helix stabilisation by natural and unnatural amino acids with alkyl side-chains. *Proc. Natl. Acad. Sci. USA* **88,** 5317–5320.

Lyu, P. C., Wemmer, D. E., Zhou, H. Z., Pinker, R. J., and Kallenbach, N. R. 1993. Capping interactions in isolated α-helices: Position-dependent substitution effects and structure of a serine-capped peptide helix. *Biochemistry* **32,** 421–425.

Macritchie, F. 1978. Proteins at interfaces. *Adv. Prot. Chem.* **39,** 51–124.

Marqusee, S., and Baldwin, R. L. 1987. Helix stabilisation by Glu$^-$ · · · · · Lys$^+$ salt bridges in short peptides of *de novo* design. *Proc. Nat. Acad. Sci. USA* **84,** 8898–8902.

McWatters, K. H., and Holmes, M. R. 1979a. Salt concentration, pH and flour concentration effects on nitrogen solubility and emulsifying properties of peanut flour. *J. Food Sci.* **44,** 765–785.

McWatters, K. H., and Holmes, M. R. 1979b. Influence of pH and salt concentration on nitrogen solubility and emulsification properties of soy flour. *J. Food Sci.* **44,** 765–780.

Mercier, J.-C., Groscloude, F., and Ribadeau-Dumas, B. 1971. Structure primaire de la caseine αS1-bovine. Sequence complete. *Eur. J. Biochem.* **23,** 41–51.

Meienhofer, J. 1985. Protected amino acids in peptide synthesis. *In* "Chemistry and Biochemistry of the Amino Acids" (G. C. Barrett, ed.), pp. 297–337. Chapman and Hall, London.

Merrifield, R. B. 1963. Solid phase synthesis 1: The synthesis of a tetrapeptide. *J. Am. Chem. Soc.* **85,** 2149–2154.

Merrifield, R. B., Juvvadi, P., Andreu, D., Ubach, J., Boman, A., and Boman, H. G. 1995. Retro and retroenantio analogs of cecropin-melittin hybrids. *Proc. Natl. Acad. Sci. USA* **92,** 3449–3453.

Minor, D. L., and Kim, P. S. 1994. Measurement of the β-sheet-forming propensities of amino acids. *Nature* **367,** 660–663.

Möllby, R. 1983. Isolation and properties of membrane damaging toxins. In "Staphylococci and Staphylococcal Infections" (C. S. F. Easmon and C. Adlam, eds.), Vol. 2, pp. 619–669.

Moser, R. 1992. Design, synthesis and structure of an amphipathic peptide with pH-inducible haemolytic activity. *Prot. Eng.* **5,** 323–331.

Munoz, V., and Serrano, L. 1994. Elucidating the folding problem of helical peptides using empirical parameters. *Nature. Struct. Biol.* **1,** 399–409.

Nakai, S. C. 1983. Structure-function relationships of food proteins with an emphasis on the importance of protein hydrophobicity. *J. Agric. Food Chem.* **31,** 676–682.

Noren, C. J., Anthony-Cahill, S. J., Griffith, M. C., and Schultz, P. G. 1989. A general method for incorporation of unnatural amino acids into proteins. *Science* **244,** 182–188.

Olsen, A. J., and Goodsell, D. S. 1992. Macromolecular graphics. *Curr. Opin. Struct. Biol.* **2,** 193–201.

Padmanabhan, S., and Baldwin, R. L. 1991. Straight-chain-non-polar amino acids are good helix-formers in water. *J. Mol. Biol.* **219,** 135–137.

Padmanabhan, S., Marqusee, S., Ridgeway, T., Laue, T. M., and Baldwin, R. L. 1990. Relative helix forming tendencies of non-polar amino acids. *Nature* **344,** 268–270.

Parker, N. S. 1988. Properties and functions of stabilizing agents in food emulsions. *CRC Crit. Rev. Food Sci. Nutr.* **25,** 285–296.

Patek, M. 1993. Multistep deprotection for peptide chemistry. *Int. J. Pept. Prot. Res.* **42,** 97–117.

Perez-Paya, E., Houghten, R. A., and Blondelle, S. E. 1994. Determination of the secondary structure of selected melittin analogues with different haemolytic activities. *Biochem. J.* **299,** 587–591.

Ponder, J. W., and Richards, F. M. 1987. Tertiary templates for proteins: Use of packing criteria in the enumeration of allowed sequences for different structural classes. *J. Mol. Biol.* **193,** 775–791.

Presta, L. G., and Rose, G. R. 1988. Helix signals in proteins. *Science* **240,** 1632–1641.

Provencher, S. W., and Glöckner, J. 1981. Estimation of globular protein secondary structure from circular dichroism. *Biochemistry* **20,** 33–37.

Ramage, R., Green, J., and Ogunjobi, O. M. 1989. Solid phase peptide synthesis of ubiquitin. *Tetrahedron Lett.* **30,** 2149–2152.

Rivett, D. E., Kirkpatrick, A., Hewish, D. R., Reilly, W., and Werkmeister, J. A. 1996. Dimerization of truncated melittin analogues results in cytolytic peptides. *Biochem. J.* **316,** 525–529.

Rose, G. D., and Creamer, T. P. 1994. Protein folding: Predicting predicting. *Prot. Struct. Funct. Genet.* **19,** 1–13.

Saito, M., Chikuni, K., Monma, M., and Shimizu, M. 1993. Emulsifying and oil-binding properties of bovine serum albumin and its enzymatic hydrolyzate. *Biosci. Biotechnol. Biochem.* **57,** 952–956.

Saito, M., Ogasawara, M., Chikuni, K., and Shimizu, M. 1995. Synthesis of a peptide emulsifier with an amphiphilic structure. *Biosci. Biotechnol. Biochem.* **59,** 388–392.

Scheraga, H. A. 1978. Use of random copolymers to determine the helix-coil stability constants of the naturally occurring amino acids. *Pure Appl. Chem.* **50,** 315–324.

Schnolzer, M., and Kent, S. B. H. 1992. Constructing proteins by dovetailing unprotected synthetic peptides: Backbone engineered HIV protease. *Science* **256,** 221–225.

Scholz, J. M., and Baldwin, R. L. 1992. The mechanism of alpha-helix formation by peptides. *Annu. Rev. Biophys. Biomol. Struct.* **21,** 95–118.

Segrest, J. P., Garber, D. W., Brouillette, C. G., Harvey, S. C., and Anantharamaiah, G. M. 1994. The amphipathic alpha helix: A multifunctional structural motif in plasma apolipoproteins. *Adv. Prot. Chem.* **45,** 303–369.

Serrano, L., Neira, J. L., Sancho, J., and Fersht, A. R. 1992. Effects of alanine versus glycine in alpha-helices on protein stability. *Nature* **356,** 453–455.

Shimizu, M., Lee, S. W., Kaminogawa, S., and Yamauchi, K. 1984. Emulsifying properties of an N-terminal peptide obtained from the peptic hydrolyzate of αS1-casein. *J. Food Sci.* **49,** 1117–1120.

Shimizu, M., Lee, S. W., Kaminogawa, S., and Yamauchi, K. 1986. Functional properties of a peptide of 23 residues purified from the peptic hydrolyzate of αS1 casein: Changes in the emulsifying activity during purification of the peptide. *J. Food Sci.* **51,** 1248–1252.

Shimizu, M., and Nakane, Y. 1995. Encapsulation of biologically active proteins in a multiple emulsion. *Biosci. Biotechnol. Biochem.* **59,** 492–496.

Shimizu, M., Takahashi, T., Kaminogawa, S., and Yamauchi, K. 1983. Adsorption onto an oil surface and emulsifying properties of bovine αS1-casein in relation to its molecular structure. *J. Agric. Food Chem.* **31,** 1214–1218.

Shoemaker, K. R., Kim, P. S., and Baldwin, R. L. 1982. Nature of the charged-group effect on the stability of the C-peptide helix. *Proc. Natl. Acad. Sci. USA* **82,** 2349–2353.

Shoemaker, K. R., Kim, P. S., York, E. J., Stewart, J. M., and Baldwin, R. L. 1987. Tests of the helix dipole model for stabilisation of alpha-helices. *Nature* **326,** 563–567.

Siegel, J. B., Steinemetz, W. E., and Long, G. L. 1980. A computer-assisted model for estimating protein secondary structure from circular dichroism spectra. Comparison of animal lactate dehydrogenase. *Anal. Biochem.* **104,** 160–167.

Smith, L. J., and Clark, D. C. 1992. Measurement of the secondary structure of adsorbed protein by circular dichroism. 1. Measurements of the helix content of adsorbed melittin. *Biochim. Biophys. Acta* **1121,** 111–118.

Sönnichsen, F. D., Van Eyk, J. E., Hodges, R. S., and Sykes, B. D. 1992. Effect of trifluoroethanol on protein secondary structure: An NMR and circular dichroism study using a synthetic actin peptide. *Biochemistry* **31,** 8790–8798.

Stewart, J. M., and Young, J. D. 1984. The chemistry of solid phase peptide synthesis. *In* "Solid Phase Peptide Synthesis," pp. 1–85. Pierce Chemical Co., Rockford, IL.

Sun, E., and Cohen, F. E. 1993. Computer-assisted drug discovery—A review. *Gene* **137,** 127–132.

Sutcliffe, M. J., Hayes, F. R., and Blundell, T. L. 1987. Knowledge-based modelling of homologous proteins. Part II: Rules for the conformations of substituted side chains. *Prot. Eng.* **1,** 385–392.

Talbot, J. C., Dufourq, J., de Bony, J., Faucon, J. F., and Lussan, C. 1979. Conformation change and self-association of monomeric melittin. *FEBS Lett.* **102,** 191–193.

Tanford, C. 1980. "The Hydrophobic Effect." Wiley, New York.

Taylor, J. W., and Kaiser, E. T. 1987. Structure-function analysis of proteins through the design, synthesis and study of peptide models. *Meth. Enzymol.* **154,** 473–498.

Taylor, J. W., Shih, I. L., Lees, A. M., and Lees, R. S. 1993. Surface-induced conformational switching in amphiphilic peptide segments of apolipoproteins B and E and model peptides. *Int. J. Pept. Prot. Res.* **41,** 536–547.

Terwilliger, T. C., and Eisenberg, D. 1982a. The structure of melittin. 1. Structure determination and partial refinement. *J. Biol. Chem.* **257,** 6010–6015.

Terwilliger, T. C., and Eisenberg, D. 1982. The structure of melittin. 2. *J. Biol. Chem.* **257,** 6016–6022.

Tornberg, E. 1978. Functional characterisation of protein-stabilized emulsions: Emulsifying behavior of proteins in a valve homogenizer. *J. Sci. Food Agric.* **29,** 867–879.

Vogel, H., and Jaehnig, F. 1986. The structure of melittin in membranes. *Biophys. J.* **50,** 573–582.

Wade, D., Andreu, D., Mitchell, S. A., Silviera, A. M. V., Boman, A., Boman, H. G., and Merrifield, R. B. 1992. Antibacterial peptides designed as analogues or hybrids of cecropins and melittin. *Int. J. Pept. Prot. Res.* **40,** 429–436.

Wang, J. C., and Kinsella, J. E. 1976. Functional properties of novel proteins: Alfalfa leaf protein. *J. Food Sci.* **41,** 268–292.

Wojcik, J., Altman, K. H., and Scheraga, H. A. 1990. Helix-coil stability constants for naturally-occuring amino acids in water. XXIV. Half-cystine parameters from poly(hydroxybutylglutamine)-co-S-methylthio-L-cysteine. *Biopolymers* **30,** 121–134.

Wolfenden, R., Andersson, L., Cullis, P. M., and Southgate, C. C. 1981. Affinities of amino acid side chains for solvent water. *Biochemistry* **20,** 849–855.

Zhang, S., Holmes, T., Lockshin, C., and Rich, A. 1993. Spontaneous assembly of a self-complementary oligopeptide to form a stable macroscopic membrane. *Proc. Natl. Acad. Sci. USA* **90,** 3334–3338.

Zhong, L., Putman, R. J., Johnson, W. C., Jr., and Rao, A. G. 1995. Design and synthesis of amphipathic antimicrobial peptides. *Int. J. Pept. Prot. Res.* **45,** 337–347.

Zhou, N. E., Kay, C. M., Sykes, B. D., and Hodges, R. S. 1993. A single-stranded amphipathic alpha-helix in aqueous solution: Design, structural characterisation and its application for determining α-helix propensities of amino acids. *Biochemistry* **32,** 6190–6197.

Zimm, B. H., and Bragg, J. K. 1959. Theory of the phase transition between helix and random coil in polypeptide chains. *J. Chem. Phys.* **31,** 526–535.