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Design and Characterization of Peptides With Amphiphilic β-Strand Structures

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To extend our studies on peptides and proteins with amphiphilic secondary structures, a series of peptides designed to form amphiphilic β-strand structures was designed, synthesized, and characterized by circular dichroism and infrared spectroscopy. Amphiphilic β-strand conformations may be likely to appear in a variety of surface-active proteins, including apolipoprotein B and fibronectin. In a β-strand conformation, the synthetic peptides will possess a hydrophobic face composed of valine side chains and a hydrophilic face composed of alternating acidic (glutamic acid) and basic (ornithine or lysine) residues. The peptides studied had a variety of chain lengths (5, 9, and 13 residues), and had the amino groups either free or protected with the trifluoroacetyl group. While the peptides did not possess a high potential for β-sheet formation based on the Chou Fasman parameters, they possessed significant β-sheet content, with up to 90% β-sheet calculated for the 13-residue protected peptide. The driving force for β-sheet formation is the potential amphiphilicity of this conformation. The β-strand conformation of the 13-residue deprotected peptide was stable in 50% trifluoroethanol, 6 M guanidine hydrochloride, and octanol. The peptides are strongly self-associating in water, which would reduce the unfavorable contacts of the hydrophobic residues with water. It is clear that small peptides can be designed to form stable β-strand conformations.

Key words: self-association, infrared spectroscopy, Merrifield solid-phase peptide synthesis, circular dichroism, β-sheets, amphiphilic β-strand peptides

The importance of amphiphilic secondary structure in biologically active peptides and proteins has become evident in recent years. Peptides may possess a primary sequence that will cause a segregation of hydrophobic and hydrophilic residues when a secondary structure is induced. A structure of this type is ideally suited to interact with amphiphilic surfaces such as cell membranes or lipoproteins. In this laboratory, we have successfully designed and characterized peptide models of apolipoprotein A-I [1], the cytotoxin melittin [2], and peptide hormones including β-endorphin [3,4] and calcitonin [5,6], which possess potential amphiphilic α-helical segments. We have

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recently extended our studies of amphiphilic secondary structures to include peptides designed to form amphiphilic β-strand conformations.

The peptide backbone in a β-sheet conformation is almost completely extended, with hydrogen bonds formed between amide groups of neighboring chains [7]. The amino acid side chains project alternately above and below the plane of the peptide backbone. A peptide with alternating hydrophobic and hydrophilic residues will therefore be amphiphilic when in this conformation, having one face hydrophobic and the other hydrophilic. Peptides of this type could form a stable β-sheet structure in aqueous solution through the interaction of their hydrophobic faces. This interaction would remove the hydrophobic residues from unfavorable contacts with water. Another stabilizing force would be hydrogen bonding between the backbone amides of different strands. The interstrand nature of the stabilizing forces for these peptides means that these molecules should be highly self-associating. The stability of this amphiphilic β-sheet structure in aqueous solution has been illustrated by several studies in which polymers with alternating hydrophobic and hydrophilic residues possessed β-sheet structures, while random polymers of the same composition existed as α-helices [8,9]. The amphiphilic β-strand conformation could also be expected to be stable at a membrane-water interface, since the hydrophobic side chains could interact with the hydrophobic portion of the membrane while the hydrophilic residues interact with the aqueous environment.

We are not characterizing the properties of peptides capable of forming amphiphilic β-strand structures as functions of chain length, charge, and environment. The design of one such peptide has been described briefly before [10], but we will now describe the features of the class. The peptides that we have synthesized (Fig. 1) have sequences of repeating units of alternating hydrophobic and hydrophilic residues. Acidic and basic residues were also alternated on the hydrophilic face to minimize charge repulsion. The number of repeat units was varied from one to three, enabling us to determine the minimum chain length necessary to form an ordered structure. Merrifield solid-phase peptide synthesis methods were utilized to provide a more homogeneous product than is obtainable with polymerization methods. We synthe-

Name	Sequence
TFA β-13	(Val-Glu-Val-(TFA)Orn)$_3$-Val
β-13	(Val-Glu-Val-Orn)$_3$-Val
TFA β-9	(Val-Glu-Val-(TFA)Orn)$_2$-Val
β-9	(Val-Glu-Val-Orn)$_2$-Val
TFA β-5	(Val-Glu-Val-(TFA)Lys)$_1$-Val
β-5	(Val-Glu-Val-Lys)$_1$-Val

Fig. 1. Sequences of β-strand peptides.

sized the peptides with the basic groups protected with the trifluoroacetyl (TFA) group, which is not labile in liquid HF, and the acidic groups were protected with benzyl groups, which were removed. The peptide recovered from HF cleavage will therefore possess only negatively charged side chain functional groups and a free N-terminus. Treatment with 1 M piperidine will remove the TFA protecting groups and thus allow us to study the effect of different charge balances on these peptides. Previously, we have reported the potential usefulness of one of these peptides, β-13, as a model for the properties of apolipoprotein B [10]. We will now report some of the physical properties determined for solutions of peptides of varying length designed to form amphiphilic β-strands.

MATERIALS AND METHODS

Peptide Synthesis

Boc-protected amino acids were purchased from Peninsula Laboratories. These were as follows: L-glutamic acid γ-benzyl ester, L-valine, and N^{ϵ}-2-chlorobenzyloxycarbonyl-L-lysine. Ornithine was purchased from Aldrich. S-Ethyl trifluoroacetate and di-*t*-butyl dicarbonate were from Pierce. Merrifield resin, 1% crosslinked, 0.67 mmol/g chloride was from United States Biochemicals. Piperidine was purchased from Aldrich and was distilled from KOH before use.

N-δ-trifluoroacetylornithine was prepared using the method of Calvin [11]. This amino acid was protected using di-*t*-butyl dicarbonate [12]. Peptide couplings were performed using the symmetric anhydride method [13] as previously described [14]. The synthesis of peptides TFA β-13 and TFA β9 was performed in the following manner. Merrifield resin esterified [15] with 0.28 mmol/g of BocVal (1.0 mmol total amino acid) was used to initiate the synthesis. After the ninth coupling of the resin was removed from the synthesizer and dried overnight in vacuo. The resin was weighted and divided into two portions. One-third was set aside, comprising TFA β-9, and the remaining two-thirds was carried on through four more cycles to complete the synthesis of TFA β-13. TFA β-5 was synthesized on a 0.5-mmol scale.

High Pressure Liquid Chromatography

High pressure liquid chromatography (HPLC) was performed utilizing the following systems: a Waters 660 gradient controller with two 6000 series pumps and Perkin-Elmer LC-75 variable wavelength detector; an IBM LC/9533 ternary gradient chromatograph with Perkin-Elmer LC-75 detector; or a Beckman 450 controller, 114M pumps, and Beckman 164 variable wavelength detector. The organic eluant used for reversed-phase chromatography was acetonitrile. Aqueous buffer was either 0.1 M sodium phosphate/0.1 M sodium perchlorate, pH 6.8 (Buffer A), or 0.1% phosphoric acid/0.1 M sodium perchlorate, pH adjusted to 2.5 (Buffer B).

HPLC gel filtration was performed using a TSK-125 column fitted with a guard column purchased from BioRad. The buffer employed was 0.02 M sodium phosphate, pH 6.8, with 0.16 M sodium chloride. The flow rate used to calibrate the column was 0.5 ml/min using a Waters 6000 pump. Proteins used to calibrate the column were thyroglobulin, ovalbumin, myoglobin, cytochrome c, melittin, and bacitracin. Molecular weights of peptides were estimated from plots of log molecular weight vs elution volume.

Peptide Purification

After HF cleavage from the resin and removal of the benzyl protecting groups, the peptides were extracted from their respective peptide-resin mixtures using freshly prepared 50 mM ammonium bicarbonate solution and lyophilized. The TFA groups were removed from the side chain amines by treatment with 1 M piperidine in water. Purification of the penta peptides was achieved using a 2 × 60-cm column of bioGel P-2 equilibrated with 50 mM NH_4HCO_3. Following hydrolysis in trifluoracetic acid/HCl [16], the peptides gave the expected amino acid composition (all results are normalized to Orn or Lys) as follows: for TFA β-5: Val (3) 3.0, Glu (1) 1.1, Lys (1) 1.0; and for β-5: Val (3) 3.0, Glu (1) 1.0, Lys (1) 1.0. The nonapeptides were purified using semipreparative reversed-phase HPLC using acetonitrile gradients in aqueous phases of either 0.1% H_3PO_4/0.1 M $NaClO_4$ (pH 2.5) for β-9; or 0.1 M sodium phosphate/0.1 M sodium perchlorate (pH 6.8) for TFA β-9. The higher pH buffer was required to insure solubility of the acidic peptide. Amino acid analysis produced acceptable results for both peptides as follows: TFA β-9: Val (5) 4.9, Glu (2) 2.2, Orn (2) 2.0; and β-9: Val (5) 5.1, Glu (2) 2.1, Orn (2) 2.0. The purification of TFA β-13 was more difficult. Attempts to purify this peptide were complicated by its extreme tendency to self-associate. Formation of the N-terminal-N^{α}-phthalyl derivative [17] was necessary for the purification of this peptide using reversed-phase HPLC in Buffer A. The phthalyl protecting group was removed with acetic acid [17]. Amino acid analysis showed the following for TFA β-13: Val (7) 6.7, Glu (3) 2.9, Orn (3) 3.0. The TFA groups were removed with 1 M piperidine to yield peptide β-13. Attempts to elute this peptide from a reversed-phase column were unsuccessful. The peptide was therefore used without further purification after the removal of the protecting groups from the purified TFA β-13. Amino acid analysis revealed the expected composition: Val (7) 6.8, Glu (3), 3.2 Orn (3) 3.0.

Circular Dichroism Spectroscopy

Circular dichroism (CD) spectra were recorded on a Cary 60 spectropolarimeter calibrated with *d*-10-camphorsulfonic acid. Guanidinium hydrochloride was Sequanal grade from Pierce. For studies involving the effect of added 2,2,2-trifluoroethanol (TFE), NaCl, or guanidine hydrochloride (GnHCl), concentrated peptide stock solutions were prepared and diluted to yield the desired concentration of additive in the studies. To dissolve TFA β-13 in octanol, it was found that suspending a lyophilized powder of the peptide in 2 ml of octanol followed by the addition of 20 μl of 0.1 M HCl produced an optically clear solution.

Estimation of the β-sheet content in a sample composed solely of β-sheet and random coil conformations using a single wavelength method can be given as follows:

$$\%\beta\text{-sheet} = \frac{\theta(\text{rc}) - \theta(\text{obs})}{\theta(\text{rc}) - \theta(\beta\text{-sheet})} \times 100,$$

where rc = random coil, and obs = observed ellipticity. Using the values given by Greenfield and Fasman for poly (L-lysine) at 217 nm [18], this becomes:

$$\%\beta\text{-sheet} = \frac{4{,}600 - \theta(\text{obs})}{4{,}600 + 18{,}400} \times 100.$$

Infrared Spectroscopy

Infrared spectra were recorded on a Perkin-Elmer ratio-recording IR model 1420. Samples were prepared by evaporation of aqueous peptide solutions onto the surface of a CaF_2 plate. The instrument was calibrated with a sample of polystyrene.

RESULTS

The presence of β-sheet structure in these peptides was assessed by the use of circular dichroism spectroscopy of aqueous peptide solutions and infrared (IR) spectroscopy of peptide films. To examine the relationship between the length of the peptide and the amount of β-sheet found in aqueous solution, the CD spectra of the TFA-protected 5−, 9−, and 13−residue peptides were recorded at high concentration (10^{-4}-10^{-3}M). As seen in Figure 2, the peptides, as predicted, possessed spectra indicative of mixtures of random coil and β-sheet conformations. The value of θ_{217} changes from −3,000 to −6,000 to −16,700 deg cm^2/dmol upon increasing the chain length from 5 to 9 to 13, respectively. These values are consistent with a β-sheet content of 30% for the pentapeptide, 45% for the nonapeptide, and a remarkable 90% for TFA β-13. It is clear that even small peptides can possess a high degree of β-sheet character and that the stability of the β-sheet increases dramatically upon lengthening the peptide from 9 to 13 residues.

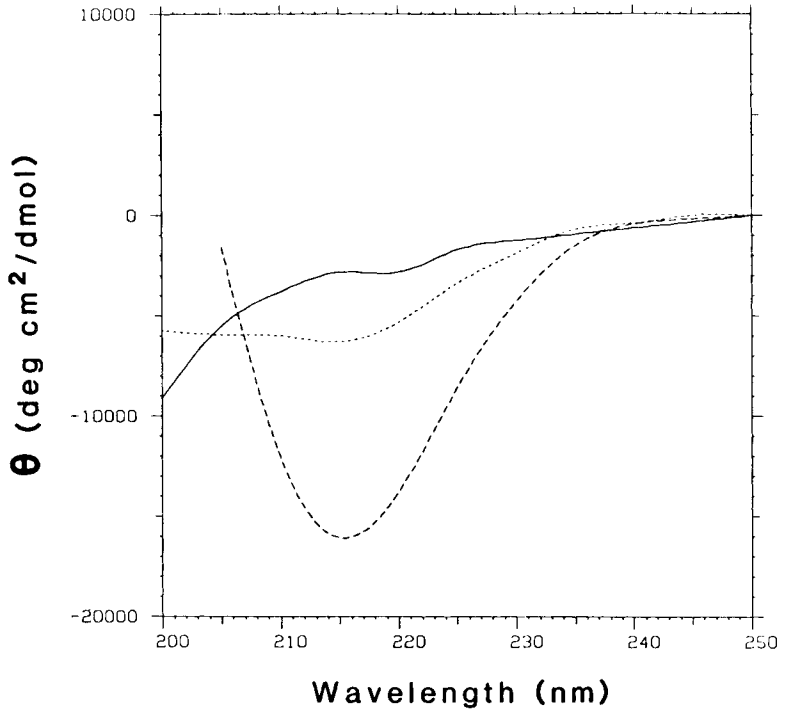

Fig. 2. CD spectra of TFA β-5, TFA β-9, and TFA β-13. All spectra recorded in 0.16 M KCl/0.02 M phosphate (pH 7.0). Concentrations of peptides were 1×10^{-3} M for TFA β-5 (————), 5.8×10^{-4} M for TFA β-9 (·····), and 2.2×10^{-4} M for TFA β-13 (— — —).

Infrared spectroscopy of peptide films supports the finding that these peptides form β-sheet structures. The most characteristic absorbance for the β-sheet conformation is the strong Amide I absorbance near 1,630 cm^{-1} [19]. As seen in Table I, TFA β-5, TFA β-13, and β-13 all exhibit the Amide I absorbance near 1,630 cm^{-1}. The antiparallel orientation of the β-sheet also produces a weak absorbance near 1,685 cm^{-1}. All three peptides possess this peak and therefore appear to be in the antiparallel β-sheet conformation in the solid state. Strong hydrogen bonding is predicted to shift the N-H stretch and the Amide I band to lower frequencies and the Amide II band to a higher frequency [20]. The typical values observed for antiparallel β-sheets are as follows: N-H stretch, 3,280–3,300 cm^{-1}; Amide I, 1,632 cm^{-1}; and Amide II, 1,530 cm^{-1}. TFA β-13 appears to be very strongly hydrogen bonded, with all three values shifted in the expected direction by at least 7 cm^{-1}.

We next investigated the effect of changing the charge borne by the peptides on their solution conformation. Initially we predicted that the protected peptides would possess less stable β-sheet structures than would the corresponding deprotected peptides, since removal of the trifluoroacetyl protecting groups should provide favorable electrostatic interactions. The results shown in Figure 3, however, indicate that this is not the case. In 0.16 M KCl with 0.02 M phosphate buffer, pH 7.0, the protected and deprotected pentapeptides yield virtually identical CD spectra. This demonstrates that there is little additional stability provided by the electrostatic interaction under these conditions. A comparison of the tridecapeptides reveals a more surprising result. The deprotected peptide actually possesses an estimated 65% β-sheet structure, 25% less than the protected peptide. Since β-strands should be highly aggregating systems, we investigated whether the two peptides also differed in their states of self-association, reflecting the differing amounts of β-sheet content.

We investigated the state of oligomerization of the peptides by measuring the concentration dependence of the CD spectra. By analogy with the results obtained from the study of amphiphilic α-helical peptides [21,22], it was likely that we would observe an increased amount of structure as the concentration of the peptide was increased. Since higher salt concentrations would lead to more β-sheet formation [8,23], we decreased the salt concentration to 0.10 M KCl/0.02 M sodium phosphate in order to observe the entire transition. A series of CD spectra of the β-13 peptide recorded at different concentrations demonstrated that there is a strong concentration dependence (Fig. 4). The spectra are all representative of mixtures of β-sheet and random coil conformations, with the β-sheet content increasing with concentration. A plot of the mean residual ellipticity at 217 nm versus the logarithm of the peptide concentration is given in Figure 4. The data points lie along a steep sigmoid curve indicative of a highly cooperative transition. A good fit to the data was obtained by

TABLE I. Infrared Absorption Bands of Model Amphiphilic β-Strand Peptides

	Frequency (cm^{-1}) of peptide group vibrations		
Peptide	N-H stretch	Amide I	Amide II
TFA β-5	3,280	1,695 1,632	1,550
TFA β-13	3,270	1,690 1,625	1,550
β-13	3,280	1,695 1,632	1,550

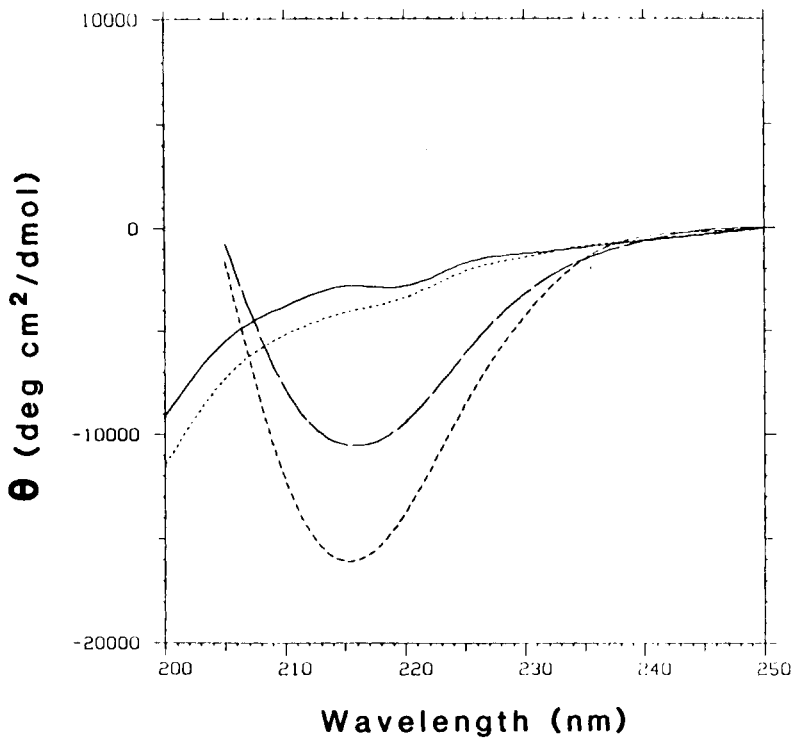

Fig. 3. CD spectra of TFA β-5, β-5, TFA β-13, and β-13, illustrating the effect of deprotection. Spectra recorded in 0.16 M KCl/0.02 M phosphate (pH 7.0). Concentrations of peptides were 1.0×10^{-3} M, both TFA β-5 (————) and β-5 (·····), 2.2×10^{-4} M for TFA β-13 (—— ——), and 3.0×10^{-4} M for β-13 (————).

assuming a cooperative monomer-octamer equilibrium. The theoretical curve plotted in Figure 5 was derived using the following values: θ(monomer) = −6,200 deg cm^2/dmol, θ(octamer) = −10,450 deg cm^2/dmol, and $K_d = 4 \times 10^{-42}$ M^7. From the dissociation constant, a value for $\Delta G°$ of −6.5 kcal/mol for the association can be calculated.

Results obtained by HPLC gel filtration of peptide β-13 on a calibrated column supported this analysis since a species corresponding to the size of an octamer was detected. The protected peptide, however, had no observable concentration dependency of θ_{217} over the range from 5×10^{-7} M to 5×10^{-4} M, remaining at −13,500 ± 200 deg cm^2/dmol. This result could indicate that the peptide either did not associate in this concentration range, self-associated with no change in its CD spectrum, or that it was already highly associated at the lowest concentration tested. Gel filtration analysis supported the third explanation. A significant portion of the injected peptide TFA β-13 elutes at the void volume of both the TSK 125 and TSK 250 gel filtration columns, indicating a species whose molecular weight is in excess of 300,000 daltons. Clearly this peptide is very strongly self-associating and forms structures many times larger than the octamers formed by β-13. It appears that the smaller ellipticity observed for the deprotected peptide β-13 is reflected by its lower

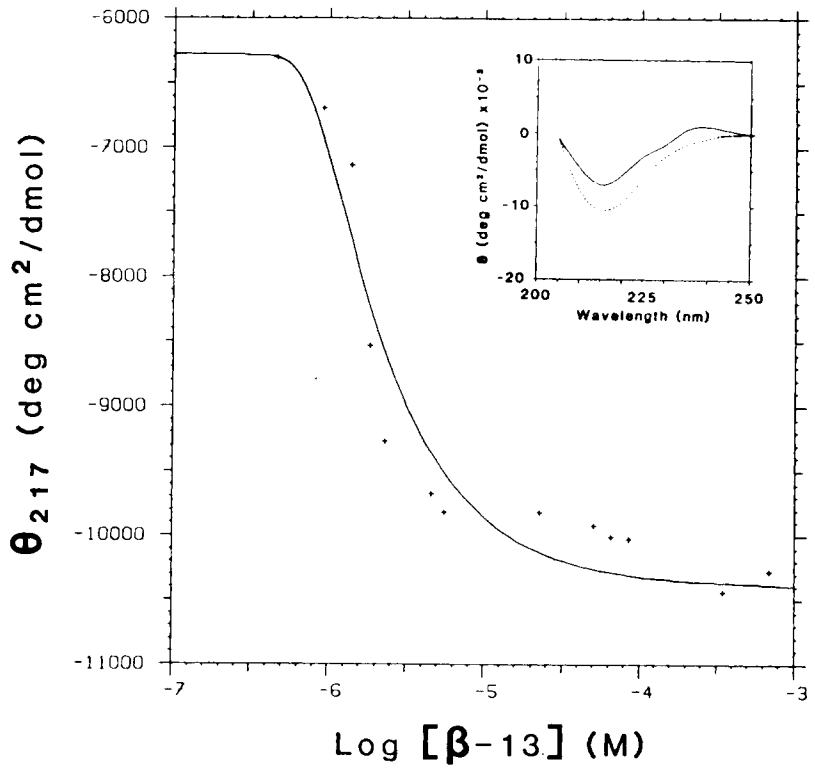

Fig. 4. Plot of the mean residual ellipticity at 217 nm vs the logarithm of the concentration of β-13. Spectra recorded in 0.10 M KCl/0.02 M phosphate (pH 7.0). The theoretical curve is plotted using the values of θ(monomer) = −6,280 deg cm^2/dmol, θ(octamer) = −10,400 deg cm^2/dmol, and $K_d = 4.2 \times 10^{-42}$ M^7. Inset: Effect of concentration on the CD spectrum of β-13. The spectra were recorded in 0.10 M KCl/0.02 M KCl at the following concentrations: 9.2×10^{-7} M (————), 1.84×10^{-6} M (·····), and 3.6×10^{-5} M (— — —).

degree of self-association and that the octameric form of β-13 has less β-sheet content than the larger aggregate formed by TFA β-13.

It is clear that the amount of β-sheet structure of the peptides can be modulated in some cases by varying the concentration of peptide, but other methods for inducing or disrupting the β-sheets are available. Previous work on polymeric β-sheet-forming peptides indicated to us that increasing concentrations of salt should increase the amount of β-sheet content in our peptides [8,23] by increasing the strength of the hydrophobic interactions. TFA β-9 was studied since it seemed most likely to undergo a pronounced transition in the accessible regions of salt concentration. In Figure 5 the spectra obtained at six concentrations of NaCl are represented. The spectra show a consistent trend toward a deeper minimum at 217 nm, indicating an increased β-sheet content, as the salt concentration is raised. We estimate a β-sheet content of 55% for the sample incubated in 1 M NaCl as opposed to only 25% for the same peptide in water. A plot of $-\theta_{217}$ versus NaCl concentration (Fig. 5) reveals an initial rapid increase in β-sheet content up to 0.3 M NaCl, followed by a slower increase that appears to continue beyond 1 M NaCl.

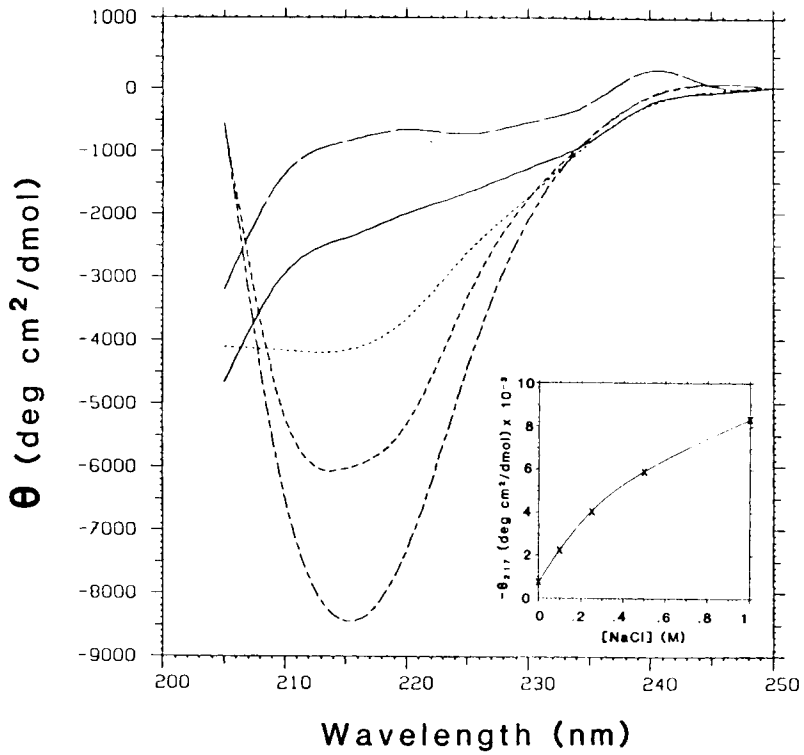

Fig. 5. Effect of increasing sodium chloride concentration on the CD spectrum of TFA β-9. The concentration of TFA β-9 is 6.0×10^{-4} M. The solvent is 0.02 M sodium phosphate (pH 7.0) to which aliquots of a concentrated sodium chloride stock solution have been added to yield solutions of the salt concentration indicated. [NaCl] (M): 0 (—— ——), 0.1 (————), 0.25 (·····), 0.50 (— — —), 1.0 (— — —). Inset: Graph of $-\theta_{217}$ vs sodium chloride concentration.

Modifying the solution in which a peptide is dissolved by the addition of alcohols typically induces α-helical structure at the expense of β-sheet structure [9,24]. Figure 6 shows the results of adding 50% 2,2,2-trifluoroethanol to solutions of TFA β-5 and TFA β-13. These two peptides were chosen to illustrate extremes of behavior. TFA β-5 shows a slight decrease in ellipticity at 217 nm from −1,700 to −4,000, corresponding to an increase in β-sheet structure from 25% to 37%. TFA β-13, however, exhibits a nearly identical spectrum before and after the addition of TFE. While TFE appears to induce structure in a peptide of relatively random structure, it fails to alter the strong β-sheet structure formed by TFA β-13.

The stability of the β-sheet structure of TFA β-13 in the presence of 50% TFE prompted further studies with this peptide in even higher concentrations of organic solvent. Specifically, we wished to examine the CD spectrum of this peptide in hydrophobic solvent to see if it could maintain its β-sheet structure in the absence of hydrophobic interactions between the peptide side chains. A report by Seno and colleagues [25] indicated that polylysine and polyornithine possessed β-sheet structures in reversed micelles formed by bis (2-ethylhexyl) sodium sulfosuccinate in octane. We thought that TFA β-13 might be soluble in a nonpolar solvent if the free carboxylic acid groups were protonated. It should be possible for the peptide to form

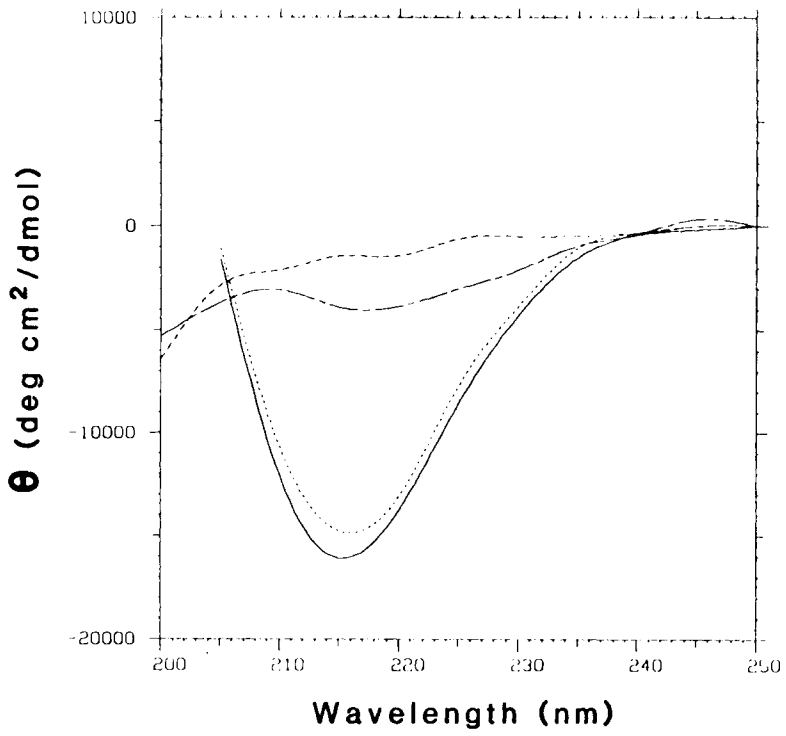

Fig. 6. CD spectra of TFA β-5 and TFA β-13 in aqueous buffer and 50% (v/v) TFE. TFA β-5 in 0.16 M KCl/0.02 M phosphate (pH 7.0) (———), or the same buffer with 50% TFE (— — —) (5×10^{-4} M). TFA β-13 in aqueous buffer (·····) or 50% TFE (————) (2.2×10^{-4} M).

a structure similar to a reverse micelle by presenting the hydrophobic face to the solvent. We chose octanol as the organic solvent since it is capable of hydrogen bonding to any exposed peptide functional groups yet is quite hydrophobic. Simply partitioning the peptide between octanol and water phases failed. If a basic aqueous solution of the peptide was acidified slowly and vortexed to mix the aqueous and organic layers, the result was a fluffy layer of precipitated peptide at the interface. We were successful in dissolving the peptide in octanol by avoiding the initial dissolution in water. The peptide was lyophilized from a solution of ammonium bicarbonate and then suspended in neat octanol. A small aliquot of 0.1 N HCl (aq) was added to the organic solvent producing an optically clear solution whose CD spectrum is presented in Figure 7 along with a spectrum of TFA β-13 in water for comparison. The curve observed with the peptide in octanol has a smaller value of θ_{217} (−11,000 deg cm^2/dmol) and the position of the minimum in ellipticity is red-shifted to 219 nm relative to the peptide in aqueous solution. It appears that the peptide is able to dissolve in organic solvent so long as the carboxyl groups are not fully charged. It also appears that the peptide maintains an ordered structure under these conditions.

We also investigated the effect on the CD spectrum of TFA β-13 upon addition of increasing amounts of the powerful denaturant, guanidinium hydrochloride (GnHCl) [26]. In Figure 8, a plot of $-\theta_{217}$ versus concentration of GnHCl illustrates the

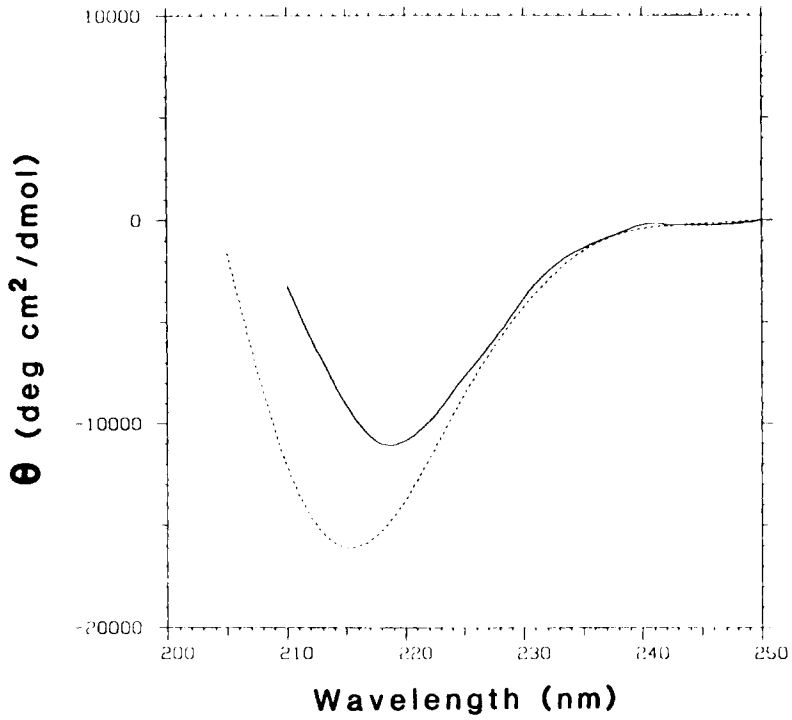

Fig. 7. CD spectra of TFA β-13 dissolved in octanol or in aqueous buffer. TFA β-13 [6.2 × 10^{-5} M] dissolved in octanol (conditions in text) (————), and in aqueous buffer (from Fig. 1) (---------).

stability of the β-sheet structure formed by this peptide. Concentrations up to 2 M GnHCl produce little effect on the CD spectrum. A broad transition to a less ordered structure takes place over a range from 2 M to 6 M GnHCl; but the denaturation never becomes complete. Even at 6 M GnHCl the ellipticity at 217 nm is still −10,000 deg cm^2/dmol, indicative of 60% β-sheet structure. Analysis of these data by a two-state denaturation model that assumes an equilibrium between the native and the partially denatured states [26], yields a transition midpoint of 3.5 M GnHCl and a ΔG° of 3.0 kcal/mol. A theoretical curve generated from this analysis is illustrated in Figure 8. We conclude that the structure assumed by the peptide is only partially reduced by concentrtaions of guanidine as high as 6 M. The native state is found to be 3.0 kcal/mol more stable than the partially denatured state. Unfortunately, we cannot estimate the stability of the native state relative to the totally denatured state. Thus, the β-sheet structure of TFA β-13 is not reduced by dilution to 5 × 10^{-7} M or addition of 50% TFE, and not eliminated by the use of even 6 M GnHCl.

DISCUSSION

The results cited in this article reveal that we have successfully designed peptides with amphiphilic β-strand structures. In designing the model β-strand peptides, we chose not to use the empirical predictive parameters developed by Chou and Fasman [27]. On theoretical grounds we felt that the method used by these authors is

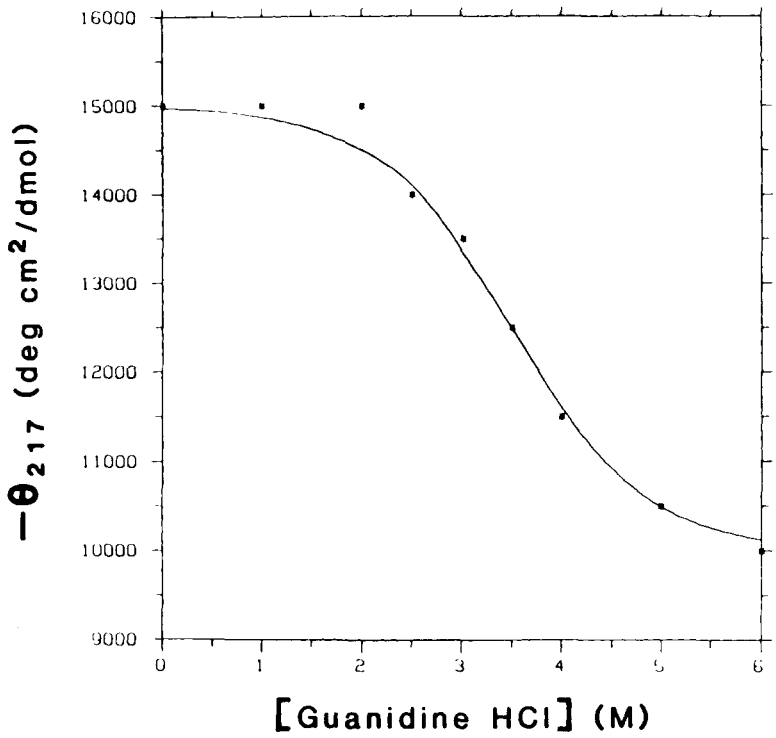

Fig. 8. Plot of $-\theta_{217}$ for TFA β-13 versus guanidine hydrochloride concentration. Solvent is 0.02 M sodium phosphate (pH 8.0) with guanidine hydrochloride at the concentrations indicated. [TFA β-13] = 6.0×10^{-5} M.

unsuitable for application in a system involving water-soluble β-sheets. Since protein β-sheets are typically found in the interior of proteins [28], a method relying on crystal structures of globular proteins as a data base will underestimate the potential for hydrophilic amino acids to participate in β-sheet structures. Thus, Glu, Asp, and Lys are all rated as very poor β-sheet formers. Experimentally, it has been demonstrated that polymers possessing alternating hydrophobic and hydrophilic residues tend to form β-sheet structures in aqueous solution. Even poly (Leu-Glu), which possesses a "β-sheet breaker" residue, Glu, at half the sites forms a β-sheet in aqueous salt solution [29]. Thus, the potential for forming an amphiphilic β-sheet structure outweighs the tendency of the individual amino acids to form α-helical conformations.

To provide a context for these results, we will compare the behavior of the peptides reported here with those of other peptides designed to form amphiphilic secondary structures. For purposes of discussion, comparison will be made with two model amphiphilic α-helical peptides: a model of apolipoprotein A-I (model A-I) [1] and a model melittin peptide [2] (Fig. 9). Model A-I is 22 residues long and contains one-third hydrophobic residues. Model melittin is 26 residues long and two-thirds of the residues are hydrophobic. These peptides were chosen for comparison since they are designed to possess essentially only one kind of conformation; that is, they do not

A

Pro-Lys-Leu-Glu-Glu-Leu-Lys-Glu-Lys-Leu-Lys-

Glu-Leu-Leu-Glu-Lys-Leu-Lys-Glu-Lys-Leu-Ala

B

Leu-Leu-Gln-Ser-Leu-Leu-Ser-Leu-Leu-Gln-Ser-Leu-Leu-Ser-

Leu-Leu-Leu-Gln-Trp-Leu-Lys-Arg-Lys-Arg-Gln-Gln-$CONH_2$

Fig. 9. Sequences of amphiphilic α-helical peptides. A) Model A-I. B) Model melittin.

possess spacer regions or disulfides. Also, model A-I possesses a lesser degree of hydrophobic character than β-13 and the melittin model a greater degree.

An examination of the Chou Fasman parameters [27] for the model β-13, model A-I, and model melittin reveals that all three peptides are predicted to α-helical with average P_α values ranging from 1.26 for model A-I, to 1.19 for β-13, to 1.09 for model melittin. Whereas models A-I and melittin are indeed α-helical, β-13 is strongly β-sheet-forming owing to its potential for amphiphilicity when in this conformation. The results presented demonstrate quite clearly that the amphiphilicity of the β-strand conformation is the dominant force in determining the structure of the β-strand model peptides.

When properly designed, small peptides are capable of forming a large amount of β-sheet even when the peptides are much smaller than the model amphiphilic α-helices. Model A-I possesses 30% α-helical structure when it is monomeric, which increases to 60% when it tetramarizes. Model melittin is similar, with 35% α-helicity in its monomeric form and 69% when it forms a tetramer. By comparison, we estimate 45% β-sheet structure for monomeric β-13 and up to 90% for self-associated TFA β-13. The infrared spectrum of the peptides clearly indicates the ability of TFA β-5 to form β-sheet structures in the solid state. Since the α-helical peptides are both larger than 20 residues, it is clear that much smaller peptides will suffice to give ordered structures when designing model amphiphilic β-sheet peptides.

The self-association behavior of the β-sheet models is quite remarkable also. The amphiphilic α-helical peptides typically self-associate to form small oligomeric structures such as the tetramers formed by model A-I and model melittin. Poly (Tyr-Lys) forms large aggregates whose apparent average molecule weight increases from 11,000 to 120,000 when β-sheet structure is induced by the addition of the salt [23]. It is unclear whether this large molecular weight reflects the presence of discrete oligomers or is merely a result of more or less random aggregation. The initial polydispersity of the samples would complicate a study of this question. From the concentration dependence of the CD spectrum and from HPLC gel filtration, it appears that β-13 forms an octameric species under the conditions employed in this study. This apparent degree of self-association of β-13 is quite large relative to that of the α-helical peptides. Our inability to detect a more highly associated species for

β-13 is also surprising, since a flat β-sheet would be capable of extending itself indefinitely by addition of monomers to the edges. This seems to be the case for TFA β-13, which appears to form large aggregates. While β-13 clearly might exhibit this behavior under a different set of experimental conditions, the predominance of the apparent octameric species in β-13 suggests that the structure formed is a closed one with no free edges for further polymerization. A structure similar to a β-barrel appears to be likely [30]. It is notable that this octamer would have a molecular weight in excess of 10,000, the size of a small protein.

The induction of structure by NaCl addition parallels that seen with the polymeric β-sheets. For poly (Leu-Lys) [29] the transition from random to β-sheet is complete at 0.1 M NaCl at pH 3.0. The TFA β-9 peptide exhibits a rapid increase in β-sheet content on increasing the salt concentration to 0.3 M and then increases at roughly half this rate up to the highest salt concentration measured (1 M). This behavior is quite reasonable, since nucleation of β-sheet regions by addition of salt should lead to a cooperative increase in the structure of previously random regions. Further salt increases should serve mainly to stabilize β-sheet conformations in peptides that are already partially organized.

Organic solvents, like TFE, tend to increase the α-helicity of peptides and destroy the β-sheet structure [9,24]. TFE promotes the formaton of hydrogen-bonded secondary structures by reducing the dielectric constant of the solvent relative to water, thereby destabilizing random coil conformations. This structure-promoting effect of TFE is clearly seen upon its addition to solutions of the α-helical model peptides and to TFA β-5. Model A-I increases in helicity from 30% to 60% upon addition of TFE, and TFA β-5 has an increase from 25% to 35% β-sheet structure. TFE also is expected to reduce the stability of the amphiphilic β-sheets by reducing the strength of the hydrophobic interactions, which are important for stabilizing this type of structure. The polymeric peptides poly (Leu-Lys) and poly (Leu-Orn) are 50% converted from β-sheet to a α-helical conformations upon addition of TFE to a final concentration of 15% [24]. In contrast, the addition of 50% TFE had no effect on the highly ordered TFA β-13. Perhaps the stability is due to strong hydrogen-bonding interactions between the carboxyl groups and the trifluoroacetylated amines that would stabilize the β-sheet conformation. Additionally, close contacts between the hydrophobic groups that preclude solvation might exist. Poly (Val-Lys) is reported to exist as a bilayer in the solid state with Val-Val contacts occurring in the middle of this bilayer [31]. TFA β-13 is already so highly structured before the addition of TFE that there is little driving force for a change in conformation, either by formation of additional β-sheet structure or by formation of an internally hydrogen-bonded α-helix (as in the case of poly [Leu-Lys]).

While it has been shown that polylysine and polyornithine will form a β-sheet structure in reversed micelles [25], TFA β-13 appears to be capable of forming its own reversed micelle. It is possible to envision a structure in octanol consisting of the hydrophobic side chains projecting into the solvent and the hydrophilic side chains projecting into the interior of the self-associated structure. A structure of this type could be formed by trans-membrane proteins. An x-ray scattering study of the major gap-junction protein connexon suggests that it may contain up to 80% β-sheet structure [32].

The severe conditions necessary for the partial denaturation of TFA β-13 demonstrates further the stability of this peptide's secondary structure. Even at high

GnHCl concentrations, the peptide remains predominantly in the β-sheet conformation. By comparison, the amphiphilic α-helical conformation of apolipoprotein A-II (apo A-II), a major component of human high density lipoproteins (HDL), is much less stable. Apo A-II is half denatured at a GnHCl concentration of only 0.63 M, with a $\Delta G°$ of only 1.02 kcal/mol [33]. Therefore, it appears that the conformational stability of TFA β-13's β-sheet structure is greater than that of a naturally occurring amphiphilic α-helical protein. The effect of GnHCl on apo B has also been described [34]. The transition occurs over a broad range in concentratioan that is similar to that seen in this work for TFA β-13. Apo B does appear to become more denatured, however, with a final value of θ_{222} of $-2{,}000$ deg cm^2/dmol. TFA β-13 exhibits a midpoint in its partial denaturation curve at 3.5 M GnHCl, the same concentration as the midpoint for the total denaturation of apo B.

The design of peptides that possess a desired secondary structure has been possible through the use of amphiphilicity. In the future, peptides possessing a predicted tertiary structure may be designed through the combination of small α-helical and β-strand structural units. Several repeated motifs in protein supersecondary structure have been noted, such as the β-α-β packing structure [28]. By a suitable choice of sequence, one might be able to design a peptide with such a structure by creating the potential for formation of a hydrophobic core and hydrophilic exterior upon folding.

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REFERENCES

1. Fukushima D, Kupferberg JP, Yokoyama S, Kroon DJ, Kaiser ET, Kezdy FJ: J Am Chem Soc 101:3703, 1979.
2. DeGrado WF, Kezdy FJ, Kaiser ET: J Am Chem Soc 103:679, 1981.
3. Taylor JW, Osterman DG, Miller RJ, Kaiser ET: J Am Chem Soc 103:6965, 1981.
4. Blanc JP, Kaiser ET: J Biol chem 259:9549, 1984.
5. Moe GR, Miller RJ, Kaiser ET: J Am Chem Soc 105:4100, 1983.
6. Moe GR, Kaiser ET: Biochemistry, 1985, (in press).
7. Pauling L, Corey RB: Proc Natl Acad Sci USA 37:729, 1951.
8. Siepke G, Arfmann H-A, Wagner KC: Biopolymers 13:1621, 1974.
9. Brack A, Spach G: J Am Chem Soc 103:6319, 1981.
10. Osterman DG, Mora R, Kezdy FJ, Kaiser ET, Meredith SC: J Am Chem Soc 106:6845, 1984.
11. Schallenberg EE, Calvin M: J Am Chem Soc 77:2779, 1955.
12. Moroder, L, Hallet A, Wunsch E, Keller O, Wersin G: Hoppe Seylers Z Physiol Chem 357:1651, 1976.
13. Hagenmaier H, Frank M: Hoppe Seylers Z Physiol Chem 353:1973, 1972.
14. Kroon DJ, Kaiser ET: J Org Chem 43:2107, 1978.
15. Horiki K, Igano K, Inouye K: Chem Lett 1978:165, 1978.
16. Tsudita A, Scheffler JJ: Proc Jpn Acad (Series B) 58:1, 1982.
17. Pechere J-F, Bertrand R: Methods Enzymol 1977:149, 1977.
18. Greenfield N, Fasman GD: Biochemistry 8:4108, 1969.
19. Miyazawa T, Blout ER: J Am Chem Soc 83:712, 1961.
20. Cantor CR, Schimmel PR: "Biophysical Chemistry Part II." San Francisco: W.H. Freeman and Company, 1980, pp 468-471.

21. Kaiser ET, Kezdy FJ: Proc Natl Acad Sci USA 80:1137, 1983.
22. Kaiser ET, Kezdy FJ: Science 223:249, 1984.
23. St. Pierre S, Ingwall RT, Verlander MS, Goodman M: Biopolymers 17:1837, 1978.
24. Ebert G, Kuroyangi Y: Int J Biol Macromol 5:106, 1983.
25. Seno M, Noritomi H, Kuroyangi Y, Iwamoto K, Ebert G: Colloid Polymer Sci 262:727, 1984.
26. Ghelis C, Yon J: "Protein Folding." New York: Academic Press, 1982, pp 307–317.
27. Chou PY, Fasman GD: Adv Enzymol 47:45, 1978.
28. Richardson J: Adv Protein Chem 34:168, 1981.
29. Brack A, Caille A: Int J Pep Protein Res 11:128, 1978.
30. Salemme FR: Prog Biophys Mol Biol 42:95, 1983.
31. Brack A, Orgel LE: Nature 256:383, 1975.
32. Phillips WC, Casper DLD, Saper R, Goodenough DA: Biophys J 47:252a, 1985.
33. Reynolds JA: J Biol Chem 251:6013, 1976.
34. Steele JCH Jr, Reynolds JA: J Biol Chem 254:1633, 1979.