Secondary Structure of the Designed Peptide Alpha-1 Determined by Nuclear Magnetic Resonance Spectroscopy

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Abstract: The solution structure of Alpha-1, a 12 amino acid designed peptide, has been investigated by one- and two-dimensional ^{1}H NMR spectroscopy. The peptide was designed as part of a project to investigate the formation of four-helix bundles. At high concentrations, the peptide forms an oligomer in which each peptide forms a regular helix, while at low concentrations the monomer is a random coil. These results are consistent with previous CD studies on the peptide.[1] The secondary structure in solution is the same for residues 1-9 as the recently determined crystal structure of Alpha-1[2] but differs for residues 10-12 which are extended in the crystal and helical in solution.

Introduction

An important approach to developing an understanding of protein folding[3,4] is the structural study of peptides which have been designed to fold into established protein secondary and tertiary structural motifs.[5-7] One example of such a structural motif is the four-helix bundle which occurs, for example, in the proteins cytochrome *c'* and myohemerythrin.[8] An initial approach, by Eisenberg and DeGrado and their co-workers, toward de novo synthesis of a four-helix bundle protein[9,10] was the synthesis of Alpha-1a, a 16 amino acid peptide (Gly-Lys-Leu-Glu-Glu-Leu-Leu-Lys-Lys-Leu-Leu-Glu-Glu-Leu-Lys-Gly), which was designed to self-associate to form an α-helical tetramer.[1] The amino acid sequence was chosen so that amphiphilic helices could form, which would drive the association of the peptides via interdigitation of the Leu side chains on the apolar face of each helix, with an antiparallel arrangement of neighboring helices in the tetramer.

A byproduct of the synthesis of Alpha-1a was the 12 amino acid peptide, Alpha-1 (acetyl-Glu-Leu-Leu-Lys-Lys-Leu-Leu-Glu-Glu-Leu-Lys-Gly-COOH). Initial CD studies on Alpha-1 and Alpha-1a indicated that both self-associated into helical oligomers in solution at pH 7.[1,9] The association of the shorter Alpha-1 peptide is weaker than for the 16mer, so, although it was concluded that both form tetramers, the number of the peptides in the oligomer has only been rigorously demonstrated for Alpha-1a. In addition, it was not clear whether Alpha-1 would form the same helical bundle structure designed for Alpha-1a but with staggered ends, a shortened bundle with a different registration of the apolar leucine residues, or some other helical oligomer.

Recently, the crystal structure of Alpha-1 was determined from crystals grown from acid (pH 3.4) ammonium sulfate solutions.[2] Although the crystal structure shows some features of the original design, including a mostly helical amphiphilic monomer unit, the overall structure is not a four α-helical bundle. Instead, a molecular crystal is formed which contains both tetramers and hexamers and in which the monomer units have an extended C-terminus. Concomitant with the crystal structure studies, we have investigated the solution structure of Alpha-1 by using one- and two-dimensional NMR techniques. Our results indicate that at high peptide concentration an oligomer forms in which the monomers are regular helices. These results are compared with the crystal structure of Alpha-1.

Materials and Methods

Alpha-1 (a gift from D. S. Eisenberg and W. F. DeGrado) was synthesized and purified essentially as previously described.[1] NMR samples were prepared by dissolving appropriate amounts of the peptide in 400 μL of buffer. Concentrations were calculated from the dry weight of the peptide. Samples were transferred to 5-mm NMR tubes, rinsed with H_2O to assure quantitative transfer, dried down in the NMR tube under a stream of $N_{2(g)}$, and redissolved in 400 μL of D_2O or 90%H_2O/10%D_2O. Sample conditions were 20 mM phosphate, pH 7.0 or 3.2 (meter reading in the NMR tube), and 150 mM NaCl unless otherwise indicated. Initially, sample conditions at pH 7.0 were chosen to approximate those at which CD studies on Alpha-1 had been done.[1] Subsequent spectra were obtained at low pH (3.2) in order to slow amide exchange so that more NOEs to amide protons could be observed and to partially simulate the crystallization conditions. Additional spectra were obtained on samples with added ammonium sulfate in order to more closely approximate the crystallization conditions.

All NMR spectra were obtained at 500 MHz on a General Electric GN500 spectrometer. Chemical shifts were obtained by reference to the chemical shift of water, which had been previously calibrated relative to 2,2-dimethyl-2-silapentane-5-sulfonate (DSS). All two-dimensional spectra (NOESY,[11,12] COSY,[13] DQF-COSY,[14] HOHAHA,[15] and ROESY[16,17]) were obtained in the phase-sensitive detection mode of States et al.,[18] by using standard pulse sequences. Spectra were acquired with 2048 complex points in t_2 and 256-512 complex FIDs in t_1 with 24-64 transients for each FID. For spectra acquired on the sample in H_2O, the carrier was centered on the H_2O resonance and the spectral width was 6024 Hz in both dimensions. For the sample in D_2O, the carrier was centered midway between the residual HDO signal and the methyl resonances and the spectral width was reduced to 2500 Hz in both dimensions. HOHAHA spectra were collected with mixing times of 100 and 120 ms for samples in H_2O and D_2O, respectively, and a MLEV17[17,19]

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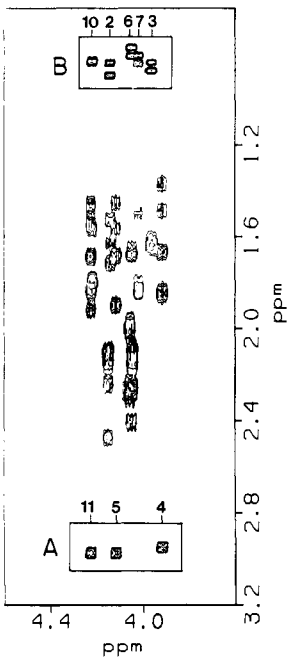

Figure 1. Portion of a HOHAHA spectrum of Alpha-1 (8 mM peptide) at 35 °C at pH 7.0 in D_2O showing the region of crosspeaks between the amide and the side-chain proton resonances. Boxed regions contain crosspeaks due to long-range coherence transfer between (A) the C_α and C_ϵ protons of lysine and (B) the C_α and methyl protons of leucine. The spectrum was zero-filled in t_1 to obtain a final matrix size of 2048 real points in both dimensions and processed with a 75° phase shifted sinebell in both dimensions.

pulse sequence was used to drive the coherence transfer. Continuous low-power irradiation of the H_2O signal was applied during the recycle time (and mixing time for NOESY) in all spectra in H_2O. Processing parameters are given in the figure captions.

Exchange of the amide proton resonances was monitored by drying down the 12 mM low pH sample from 90% H_2O. Immediately after redissolving the sample in D_2O, it was placed in the NMR spectrometer, and one-dimensional spectra were acquired at 20 °C every 5 min for approximately 3 h.

Results

Proton Resonance Assignments. Sequence specific proton resonance assignments were made by using the standard sequential assignment method.[20] Amino acid spin systems were identified by analysis of through bond connectivities in phase-sensitive COSY, DQF-COSY, and HOHAHA in H_2O and D_2O (not shown, except HOHAHA in D_2O). The long side-chain lysine and leucine residues were identified in the HOHAHA spectrum in D_2O shown in Figure 1. Coherence transfer was complete to all side-chain protons in the individual spin systems, resulting in direct observation of lysine CH_α to CH_ϵ connectivities (Figure 1, box A) and leucine CH_α to methyl connectivities (Figure 1, box B). Assignments of the other protons in these spin systems were obtained from analysis of a DQF-COSY in D_2O. The remaining crosspeaks not connected to these spin systems were assigned to the glutamate spin systems. The glycine proton assignments, and extension of the assignments to the amide proton resonances, were obtained from the fingerprint region of a COSY in H_2O. The spin system assignments were confirmed by RELAY and HOHAHA experiments in H_2O.

Sequential assignments of the identified spin systems were obtained from the NN (Figure 2A), αN (Figure 2B), and βN (not shown) NOE connectivities observed in a NOESY spectrum in H_2O at pH 7.0 and pH 3.2 (Figure 3) and comparison of these connectivities to the amino acid sequence. Observed sequential connectivities are summarized in Figure 4A for the peptide at pH 7.0 and Figure 4B at pH 3.2. The lower pH allowed additional

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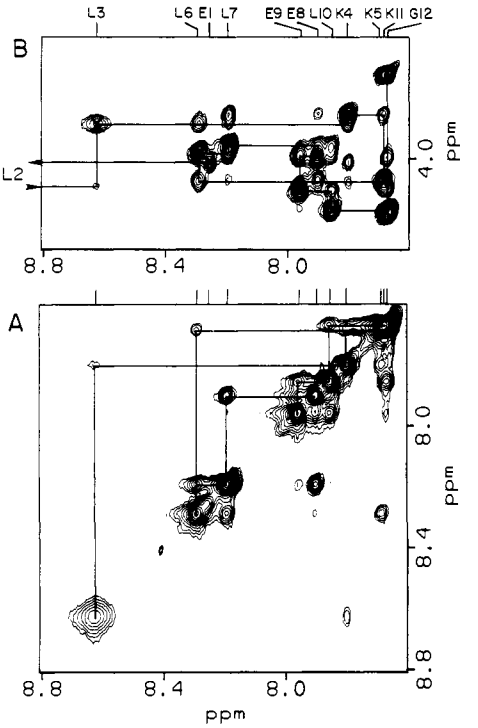

Figure 2. Portions of a NOESY spectrum (τ_m = 250 ms) of Alpha-1 (16 mM peptide) in 90% H_2O/10% D_2O at 35 °C at pH 7.0 showing (A) the amide resonances and amide–amide crosspeaks and (B) the crosspeaks between amide and C_α proton resonances. Assignments are indicated at the top of the spectrum, and sequential connectivities are indicated by solid lines. Crosspeaks which also occur in the COSY spectrum are indicated by x. Processing parameters were the same as in Figure 1.

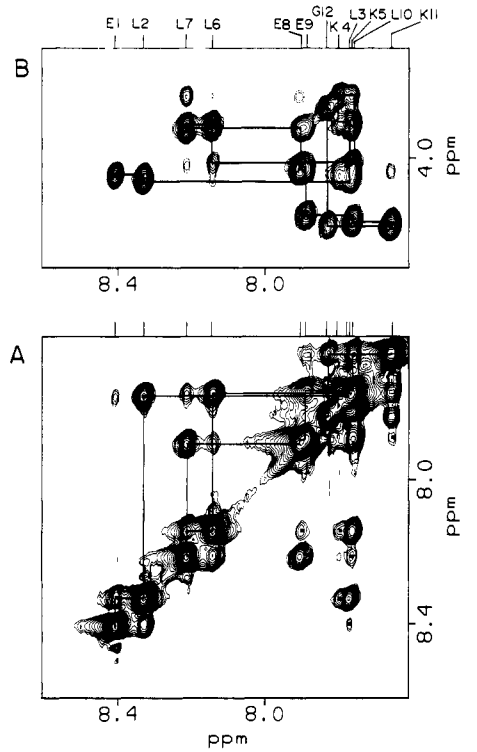

Figure 3. Portions of a NOESY spectrum (τ_m = 250 ms) of Alpha-1 (8 mM peptide) in 90% H_2O/10% D_2O at 35 °C at pH 3.2 showing (A) the amide resonances and amide–amide crosspeaks and (B) the crosspeaks between amide and C_α proton resonances. Assignments are indicated at the top of the spectrum, and sequential connectivities are indicated by solid lines. Crosspeaks which also occur in the COSY spectrum are indicated by x. Crosspeaks in (A) arising from $NN_{i,i+2}$ interactions are indicated by ■ (solid box).

Table I. Chemical Shifts of Alpha-1 Proton Resonances[a]

		NH	$C^{\alpha}H$	$C^{\beta}H$, $C^{\beta}H$	$C^{\gamma}H$, $C^{\gamma}H$	$C^{\delta}H$, $C^{\delta}H$	$C^{\epsilon}H$, $C^{\epsilon}M$	methyls
$-COCH_3$	a							2.02
	b							2.02
Glu-1	a	8.27	4.02	1.96, 2.00	2.24, 2.26			
	b	8.41	4.06	2.05, 2.05	2.46, 2.46			
Leu-2	a	9.37	4.10	1.69, 1.50	1.60			0.87, 0.81
	b	8.34	4.08	1.68, 1.48	1.60			0.89, 0.83
Leu-3	a	8.51	3.93	1.61, 1.61	1.61			0.85, 0.81
	b	7.78	3.91	1.69, 1.57	1.60			0.85, 0.82
Lys-4	a	7.81	3.89	1.81, 1.81	1.46, 1.34	1.63, 1.63	2.92, 2.92	
	b	7.81	3.85	1.83, 1.83	1.46, 1.33	1.65, 1.65	2.93, 2.93	
Lys-5	a	7.73	4.09	1.87, 1.87	1.54, 1.42	1.66, 1.66	2.95, 2.95	
	b	7.77	4.03	1.87, 1.87	1.55, 1.40	1.65, 1.65	2.97, 2.97	
Leu-6	a	8.25	4.02	1.64, 1.64	1.64			0.78, 0.75
	b	8.15	3.93	1.65, 1.67	1.60			0.74, 0.74
Leu-7	a	8.17	3.99	1.80, 1.47	1.75			0.82, 0.78
	b	8.23	3.93	1.83, 1.46	1.78			0.79, 0.79
Glu-8	a	7.93	4.02	2.06, 2.12	2.23, 2.38			
	b	7.92	4.05	2.14, 2.22	2.49, 2.61			
Glu-9	a	7.98	4.12	2.08, 2.13	2.22, 2.44			
	b	7.90	4.17	2.12, 2.22	2.53, 2.77			
Leu-10	a	7.87	4.19	1.77, 1.53	1.73			0.81, 0.81
	b	7.77	4.20	1.87, 1.85	1.8			0.78, 0.78
Lys-11	a	7.72	4.20	1.81, 1.89	1.52, 1.44	1.66, 1.66	2.95, 2.95	
	b	7.67	4.20	1.88, 1.88	1.50, 1.46	1.67, 1.67	2.97, 2.97	
Gly-12	a	7.69	3.72, 3.72					
	b	7.84	3.88, 3.88					

[a] Conditions: (a) 8 mM Alpha-1, 150 mM NaCl, 20 mM P, pH 7.0, 35° and (b) 8 mM Alpha-1, 150 mM NaCl, 20 mM P, pH 3.2, 35°.

connectivities from the more rapidly exchanging amides of the three N-terminal residues to be seen. The sequential and medium range connectivities observed indicate that under these conditions (8–16 mM peptide) Alpha-1 is completely helical, as discussed in detail below.

Once sequence specific resonance assignments were complete, all protons in the peptide were identified at their chemical shift positions. All of the protons in the peptide were assigned (Table I), but stereospecific assignment of protons in the amino acid side chains were not made. The complete resonance assignments at both pH 7.0 and pH 3.2 are listed in Table I.

Concentration Dependence of Alpha-1 NMR Spectra. The concentration dependence of the ^1H NMR spectra of Alpha-1 at pH 7.0 is shown in Figure 5 for the well-resolved amide resonances. Circular dichroism spectra of Alpha-1 at pH 7.0 in aqueous solution indicated that the peptide self-associates into helical aggregates as the concentration is raised. The molar ellipticity at 222 nm is dependent on approximately the fourth power of the concentration of Alpha-1, suggesting that it might form tetramers.[1] However, the monomer–oligomer equilibrium has a midpoint at about 5 mg/mL of peptide, indicating that it is thermodynamically rather weak. At pH 3.25, a similar concentration dependence is observed, with a midpoint at a slightly lower peptide concentration (2 mg/mL; DeGrado, W.; personal communication) although in both cases a monomer–hexamer equilibrium fits the data almost as well. The NMR spectra also indicate a concentration-dependent self-association of the peptide into helical oligomers. Spectra in Figure 5 are plotted relative to the intensity of the ϵ protons of the lysines, which occur as a group of unresolved resonances, i.e., this intensity is set to be the same for all spectra. As the concentration is decreased from 16 to 1 mM, the amide resonances become less well-resolved and their intensity greatly decreases relative to the nonexchangeable resonances. These results are consistent with a random coil monomer peptide conformation at low concentrations which is in equilibrium with a helical oligomer at higher concentrations.

Confirmation of the monomeric random coil state of the peptide at 2 mM peptide concentration was obtained from NOESY and ROESY spectra (not shown). Since monomer Alpha-1 has a molecular weight of 1480 g/mol, weak or no NOEs would be expected in a NOESY spectrum at 500 MHz for the monomer,[21] and this is what we observed. Because of decreased spectral dispersion for the monomer (low concentration) spectra, only partial assignments could be made from the ROESY. For the sample at low concentration, no $\alpha N_{i,i+3}$ or $\alpha N_{i,i+4}$ are observed in the N-terminal half of the molecule, but in the C-terminal half this is not clear due to spectral overlap. This indicates that part or all of *monomer* Alpha-1 is a random coil.

The pH Dependence of the Amide Chemical Shifts. The amide resonances of Leu 2 and Leu 3 shift downfield by 1 and 0.7 ppm, respectively, when the pH of the sample is raised from 3.2 to 7.0. Bundi and Wuthrich[22] showed that the deprotonation of the glutamate carboxylate group will cause a large downfield titration shift of the amide proton to which it is hydrogen bonded. The pK_as of the two leucine amide protons are 5.1, which is comparable to those reported for the carboxyl group of glutamate.[22,23] There are three glutamate residues in the peptide. At this point, based on the available NMR data, we cannot determine which glutamate side chain is hydrogen bonded to the leucine amides. Titrations of the three sets of glutamate γ-CH_2 protons were inconclusive (data not shown). A more detailed study of the pH dependence of the Alpha-1 spectra and structure is in progress.

Discussion

Secondary Structure of Alpha-1 in Oligomeric State. NOE connectivities observed between protons of neighboring and nonneighboring amino acid residues indicate that Alpha-1 is helical in solution.[20] Strong sequential connectivities between $NN_{i,i+1}$ and weaker sequential connectivities between $\alpha N_{i,i+1}$ were observed along most of the peptide chain at pH 7.0 (Figure 2) and extend along the entire peptide at pH 3.2 (Figures 3 and 4). The additional crosspeaks are observed at pH 3.2 due to a decrease in exchange rate of the amides at low pH. Some of the medium range NOE connectivities, which characterize α helix structures, are observed for $NN_{i,i+2}$ and $\alpha N_{i,i+3}$ (Figures 2 and 3) and are summarized in Figure 4 for pH 7.0 and 3.2. Due to the narrow spectral dispersion of the α helix and amide resonances, many of the medium range NOEs are ambiguous. In addition to the $NN_{i,i+2}$ and $\alpha N_{i,i+3}$ connectivities shown in Figure 4, there are several unambiguous $\alpha N_{i,i+4}$ crosspeaks (L2-L6, K4-L8, and L7-K11 at pH 3.2 and E1-K5 and K4-L8 at pH 7.0) and weak

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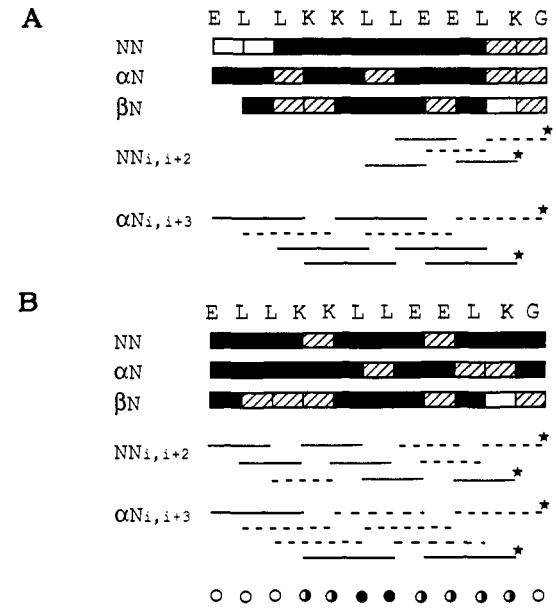

Figure 4. Schematic showing the sequential and medium-range NOEs observed for Alpha-1 at (A) pH 7.0 and (B) pH 3.2. Solid lines indicate unambiguous NOEs, dashed lines indicate ambiguous NOEs due to spectral overlap, and blank spaces indicate that an NOE was not observed. Starred (*) connectivities indicate distances which are greater than 5 Å in the crystal structure.[2] Relative exchange rates of amide protons at pH 3.2 are indicated at bottom of (B), as measured by their rates of disappearance after dissolving the sample in D_2O: [open circles], fast, amide resonance disappears in less than 5 min; [shaded circles], medium, resonance loses 50% of intensity after ≥80 min; [closed circles], slow, resonance loses 50% of intensity after ≥250 min.

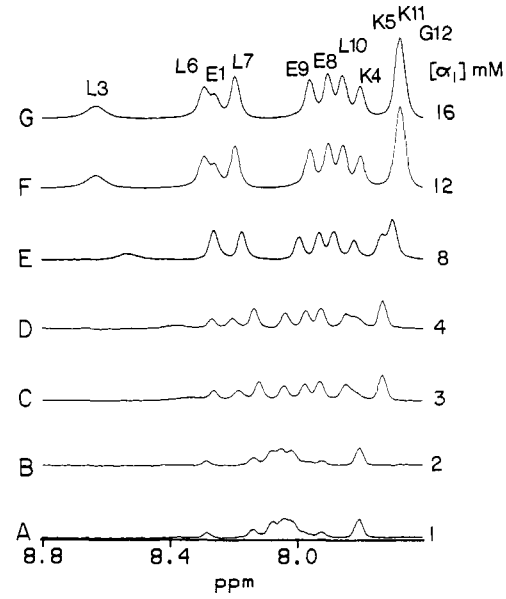

Figure 5. One-dimensional spectra of the amide resonances of Alpha-1 as a function of concentration in 20 mM phosphate, pH 7.0 (meter reading), 150 mM NaCl, 90% H_2O/10% D_2O: (A) 1, (B) 2, (C) 3, (D) 4, (E) 8, (F) 12, and (G) 16 mM. Spectra were obtained with a spectral width of 6024 Hz, 2048 complex points, 128 acquisitions, and a 2-recycle delay. The spectra were processed with a skewed (0.8, 1200) 75° phase-shifted squared sinebell.

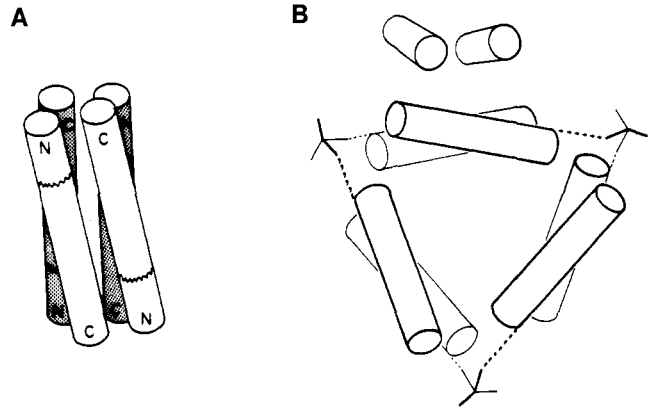

Figure 6. (A) Schematic of four-helix bundle consistent with the design of Alpha-1a. The jagged lines near the N-terminus indicate the shorter bundle with exposed C-terminal ends which might form for the 12 amino acid Alpha-1 studied here. Alternatively, a reregistration of the Alpha-1 helices could result in a four-helix bundle without exposed C-terminal ends. (B) Schematic illustrating the arrangement of Alpha-1 peptides in the crystal structure. The hexamer with antiparallel dimers and associated sulfate ions is shown viewed down a 3-fold axis. The four peptides at top illustrate the arrangement of the tetramer in the crystal structure (from Hill et al.[2]).

unambiguous $\alpha\beta_{i,i+3}$ crosspeaks (K4-L7 and L6-E9 at pH 3.2 and L6-E9 and L7-L10 at pH 7.0) characteristic of an α helix. However, there are also a few weak unambiguous $\alpha N_{i,i+2}$ crosspeaks (K4-L7 and L6-E9 at pH 3.2 and L2-K4 and K5-L7 at pH 7.0) in the middle of the peptide which are usually seen in a 3^{10} helix. (Most of the crosspeaks discussed above are seen even at 50 ms NOESY mixing time.) The spectral overlap makes it extremely difficult to distinguish the two types of helix based on NOE connectivities only. The data indicate that Alpha-1 forms a helix involving all 12 amino acid residues at both low and neutral pH when it is associated at high concentration. Although we cannot decisively determine the exact form of the helix, the NOEs observed clearly show that the peptide is helical throughout its entire length. There are no crosspeaks expected for a helical conformation which are unambiguously absent.

Qualitative exchange rates were obtained for the amide resonances by monitoring the disappearance of the amide proton resonances as a function of time after redissolving samples that had been dried down from H_2O solution in D_2O (Figure 4B). At pH 7, all of the amides disappeared in less than the five minutes it took to obtain the first time point. Exchange rates were therefore obtained on samples at pH 3.2 both with and without 1 M sulfate. The latter conditions were chosen to approximate the conditions in which the crystals were obtained. The amide of Lys-4 exchanges more slowly than those of residues 1–3, suggesting that it is hydrogen bonded, although it would not normally be hydrogen bonded in an α helix backbone. The Lys-4 amide proton might hydrogen bond to the carbonyl oxygen of the N-terminal acetyl in an α-helical interaction. The slow exchange of the amide protons 4–11 at low pH is additional evidence for helix formation along the entire peptide in the oligomeric state.

Monomer-to-Oligomer Equilibrium. Observation of NOESY crosspeaks for Alpha-1 at high concentration indicated that the peptide was forming an oligomer of some sort, since the monomer would be expected to have a correlation time which would result in weak or no NOEs at 500 MHz. Since only one set of resonances is observed at high concentration, this indicates that the peptide associates into a symmetric oligomer or that the monomer–oligomer exchange is fast or near fast exchange on the NMR time scale. In order to investigate the latter possibility, the concentration study summarized in Figure 5 was done. Only one set of resonances is observed throughout the concentration range studied, indicating that the monomer-to-oligomer exchange is at or near fast exchange on the NMR time scale. Therefore, no conclusions can be made about the symmetry of the peptides in the oligomer.

The line widths of amide resonances of Leu-2 and Leu-3 are very broad relative to the rest of the amide resonances at pH 7.0. It appears that the broadening is due to exchange between conformation, either monomer-to-oligomer exchange or some sort of intramonomer rearrangement. It does not appear to be due to exchange with solvent because the resonances sharpen as the temperature is raised (data not shown).

Comparison to Crystal Structure and Design. A model for the packing of Alpha-1a in a four-helix bundle is shown in Figure 6A.

Since the design of Alpha-1a was a 16 amino acid peptide, it has been proposed that the 12 residue Alpha-1 might have a different registration than the 16 residue peptide Alpha-1a in order to prevent exposure of the hydrophobic groups at the C-terminus.[2] The crystal structure of Alpha-1[2] differs significantly from the four α-helix bundle design (Figure 6A) in several ways. First, although the monomer unit is α-helical at the N-terminus, the three amino acids at the C-terminus form an extended structure. Second, a four-helix bundle with neighboring helices tilted 18° to each other does not form. Rather, a molecular crystal is observed which contains antiparallel helical dimers at a crossing angle of −31.5°, which are arranged at crossing angles of ~90° in hexamers and ~40° in tetramers, as illustrated in Figure 6B. The tetramers are in an arrangement reminiscent to that of melittin (crossing angle ~60°)[24] rather than four-helix bundle (crossing angles often ~20°) and the leucine side chains of neighboring antiparallel helices abut rather than interdigitate. The structure also contains one sulfate ion for every two monomers, which is hydrogen bonded to both side chains of Glu 9 and amides of Leu 2.

The secondary solution structure of Alpha-1 cannot be the same as that in the crystal structure. The Alpha-1 monomers in the oligomer are helices along their entire length at pH 7.0 and pH 3.2 (Figures 2–4). The $NN_{i,i+2}$ and $\alpha N_{i,i+3}$ crosspeaks observed between residues 8 and 12 indicate that the C-terminus is helical. NOEs which would not be observed in the crystal structure are starred in Figure 4. Further preliminary NMR data show that a fully α-helical structure is also formed at pH 3.2 in the presence of 1 M ammonium sulfate (data not shown). Thus, the extended conformation of the C-terminal tripeptide observed in the crystal structure may be a result of packing interactions specific to the crystalline environment. Observed NOEs which are indicative of α helix at the C-terminus of the peptide which would be missing if the peptide were extended at the C-terminus as in the crystal structure are starred in Figure 4. Although the secondary structure of the peptide is different in solution and the crystal for residues 10–12, the question remains whether fully α-helical monomer units could be accommodated in the tetramer or hexamer of the crystal structure. Examination of the crystal structure indicates that fully α-helical monomer units could be accommodated in the hexamer but would require some movement of the main chain atoms of the helices in the tetramer due to resulting steric clash of the carbonyl oxygens on residues 9 and 10 with those on adjacent helices (Hill, C. P.; Eisenberg, D., personal communication).

Unfortunately, because of the fast exchange between monomer and oligomer, we are not able to conclude anything about the symmetry or stoichiometry of the structure of the oligomer. We also did not observe any NOEs that could unambiguously be assigned to interpeptide interactions. Since most of the interpeptide NOEs expected for a four-helix bundle would involve crosspeaks between leucine methyls, which would be difficult to resolve, we cannot distinguish between interdigitation as predicted from the four-helix bundle model and the abutting of leucine side chains seen in the crystal structure. There is some evidence for an ordered arrangement of the leucine side chains in the oligomer, however. At high concentrations, all of the leucines except Leu−10 show two resolved methyl crosspeaks in the HOHAHA spectrum (Figure 1B), which indicates restricted rotation about the $C\beta$–$C\alpha$ bond. In contrast, for the random coil monomer structure (low concentration), only one leucine shows two resolved methyls (not shown).

Summary. The peptides synthesized to investigate folding in the four-helix bundle motif were originally designed with structure determination by crystallography in mind. Thus, Alpha-1 has a highly repeating sequence and a potentially symmetric oligomer structure which makes a complete three-dimensional structure determination in solution by NMR methods quite difficult. An added complication is the fast exchange between monomer and oligomer which precludes conclusions on the symmetry of the oligomer. Nevertheless, important information on the secondary structure and its relation to the crystal structure has been obtained. The peptide associates at high concentration to form an oligomer in which the monomer units are helical throughout the length of the peptide. This is the same as the secondary structure in the crystal for residues 1–9 but differs for residues 10–12 which are extended at the C-terminus in the crystal. It also indicates that if the solution oligomer is a tetramer, it must differ in tertiary structure from the tetramer observed in the crystal structure. Finally, it is noteworthy that, as illustrated here, a peptide as short as 12 amino acids can form a regular helix in solution when stabilized by self-association.[25]

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