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Conformational behavior of ionic self-complementary peptides

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Abstract

Several de novo designed ionic peptides that are able to undergo conformational change under the influence of temperature and pH were studied. These peptides have two distinct surfaces with regular repeats of alternating hydrophilic and hydrophobic side chains. This permits extensive ionic and hydrophobic interactions resulting in the formation of stable β-sheet assemblies. The other defining characteristic of this type of peptide is a cluster of negatively charged aspartic or glutamic acid residues located toward the N-terminus and positively charged arginine or lysine residues located toward the C-terminus. This arrangement of charge balances the α-helical dipole moment (C $\rightarrow$ N), resulting in a strong tendency to form stable α-helices as well. Therefore, these peptides can form both stable α-helices and β-sheets. They are also able to undergo abrupt structural transformations between these structures induced by temperature and pH changes. The amino acid sequence of these peptides permits both stable β-sheet and α-helix formation, resulting in a balance between these two forms as governed by the environment. Some segments in proteins may also undergo conformational changes in response to environmental changes. Analyzing the plasticity and dynamics of this type of peptide may provide insight into amyloid formation. Since these peptides have dynamic secondary structure, they will serve to refine our general understanding of protein structure.

Keywords: α-helix; alternating ionic sequences; β-sheet; conformation plasticity; de novo design; temperature- and pH-induced structural change

One of the assumptions in studying protein structure has been that protein secondary structure is stable once formed, while tertiary structure is flexible to accommodate biological function. This assumption, central to the study of protein structure and function, is important for many reasons. An overwhelming number of examples support this assumption, in which drastic changes in tertiary structure can result from small changes in the environment, such as the receptor binding of a ligand or the catalytic action of enzymes. Although this approximation is widely accepted, accumulated experimental observations have revealed increasing examples of conformational changes in secondary structure leading to new functionality (Abel et al., 1996; Jardetzky, 1996; Minor & Kim, 1996; Prusiner, 1996; Tan & Richmond, 1998). These new findings challenge the current assumption that secondary structures are relatively static in proteins.

It is recognized that slight conformational changes of secondary structural units, α-helices and β-sheets, could have significant consequences in determining the overall protein structure and therefore its biological function. Alpha-helices and β-sheets are generally believed to be stable structural units after they have formed in folded proteins (Pauling, 1960; Schulz & Schirmer, 1979; Creighton, 1993). Recent analyses using high-speed computer simulations and using a variety of experimental approaches suggest that protein structures are dynamic and they can undergo structural changes to accommodate their biological function (Boutonnet et al., 1995; Cao et al., 1998; Kurzynski, 1998; Reddy et al., 1998; Yon et al., 1998). These include receptor-ligand interactions, enzyme catalysis and protein interactions with other molecules, including other proteins, nucleic acids, small molecules, cofactors, metal ions, neurotransmitters, and other substrates (Colson et al., 1998; Haouz et al., 1998; Rigney et al., 1998). This body of data suggests that the solved crystal structure of a protein may represent only a single frame of a movie image of proteins.

Here, we report several additional de novo designed ionic self-complementary oligopeptides that exhibit remarkable secondary structure plasticity and multifaceted behavior. These findings confirm our previous report that the DAR16-IV peptide with alternating hydrophobic and hydrophilic residues and distinct dipoles can form stable β-sheet and α-helix structures under the influence of temperature and pH (Zhang & Rich, 1997). They not only extend our early findings, but also demonstrate that this type of flexibility is not isolated to unique cases, but rather describes the conformational behavior of a class of peptides. These peptides have the ability to undergo secondary structural transitions from β-sheet to

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α-helix in response to changes in temperature or pH. Once in the α-helical form, these peptides return to β-sheet assemblies over extended time. The peptides that undergo this kind of conformational change have similar charge distributions. They are each composed of alternating hydrophobic and hydrophilic residues, with a clustering of negative charge toward the N-terminus, and a clustering of positive charge toward the C-terminus. These sequence characteristics provide these peptides with two distinct faces, one hydrophobic and the other hydrophilic when they exist in β-sheet form, and two distinct poles, one negatively charged and the other positively charged when they exist in helical form. Therefore, these sequences not only have propensity to form both α-helix and β-sheets, but also provide the peptides with interesting physical properties. It has previously been reported that these peptides, when in the β-sheet form, have the ability to form macroscopic lamellar matrices after the peptide solution is brought to physiological salt concentration (Zhang & Rich, 1997). This property is perhaps related to their primary sequence. They contain hydrophobic faces that are important to the formation of β-sheet assemblies (Rippon et al., 1973; Brack & Orgel, 1975; Osterman & Kaiser, 1981; Zhang et al., 1993; Xiong et al., 1995; Zhang & Rich, 1997; West et al., 1999), as well as a dipole charge distribution and helix capping consistent with α-helical stability (Blagdon & Goodman, 1975; Hol et al., 1981; Hol, 1985; Presta & Rose, 1988; Harper & Rose, 1993; Huyghues-Despointes et al., 1993; Parthasarathy et al., 1995; Aurora & Rose, 1998). It has also been reported that sequence dependence of secondary structure formation in host-guest peptides with either α-helix or β-sheet structure can be influenced by the supporting media (Mutter et al., 1985). Other types of peptide structural transition between α-helix and β-sheet have also been reported by Dado and Gellman (1993) through incorporating several regularly spaced methionines in an 18 residue peptide, YLKAMLEAMAKLMAKLMA. When these methionines are in the native state, i.e., lipophilic state, the peptide forms an amphiphilic α-helix. Upon oxidation of the methionine to methionine sulfone, it becomes more hydrophilic and the peptide transforms its structure into a stable β-sheet.

The structural properties of the self-complementary ionic oligopeptides in this study also resemble those of proteins and protein fragments that are involved in amyloid formation. Both these peptides and amyloid peptides can exist as stable α-helices or coils until changes in the environment induce the formation of β-sheet aggregates (Kirschner et al., 1987; Han et al., 1995; Harper et al., 1997; Harper & Lansbury, 1997; Kelly, 1997; Dobson, 1999). This conversion process is very slow in both cases, leading to the possibility that they are mechanistically similar or perhaps governed by similar thermodynamic and kinetic constraints (Huang et al., 1995).

Results

Correlation between peptide structure and sequence

The self-complementary ionic oligopeptides (Table 1) have many interesting sequence and structure properties. EAK12 a-d and the EAK16-IV series are derivatives from the original EAK16-II

Table 1. *Ionic self-complementary peptides and derivatives used in this study*[a]

Name	Sequence (N → C)	Structure	Direct conversion	Matrix formation
EAK12-a	+ + − − + + AKAKAEAEAKAK	r.c.[b]	No	No
EAK12-b	+ − − + + AKASAEAEAKAK	r.c.	No	No
EAK12-c	+ − − − + + AKAEAEAEAKAK	r.c.	No	No
EAK12-d	− − − − + + AEAEAEAEAKAK	α/β	Yes	Yes
DAR16-IV	− − − − + + + + n-ADADADADARARARAR	α/β	Yes	Yes
DAR16-IV*	− − − − + + + + n-DADADADARARARARA	α/β	Yes	Yes
DAR32-IV[c]	− − − − + + + + $(ADADADADARARARAR)_2$	α/β	No	Yes
RAD16-IV	+ + + + − − − − RARARARADADADADA	β	No	Yes
KAE16-IV	+ + + + − − − − KAKAKAKAEAEAEAEA	β	No	Yes
EAK16-IV	− − − − + + + + AEAEAEAEAKAKAKAK	β	Yes	Yes

[a]The peptides were made by solid-phase synthesis on either an Applied Biosystems Model 430A or Rainin Model PS3. N- and C-termini of the oligopeptides are acetylated and amidated, respectively. The peptides were purified by reversed-phase HPLC. The purity was determined by analytical HPLC. Composition was confirmed by mass spectroscopy and/or amino acid analysis as described previously. Direct conversion occurs when there is no detectable intermediate by CD spectroscopy. Matrix formation indicates whether or not the peptide solution forms macroscopic matrices upon exposure to saline conditions (Zhang et al., 1993, 1995). The number following each name indicates the chain length of the oligopeptides.

[b]r.c, random coil.

[c]DAR32-IV was purified in two distinct forms with reversed-phase HPLC, one α-helical, and the other β-sheet.

peptide, (AEAEAKAKAEAEAKAK), a repeated segment found in a yeast protein, Zuotin, which was initially characterized by its preferential binding to left-handed Z-DNA (Zhang et al., 1992). The DAR, RAD, KAE, and EAK series are de novo designed peptides created to systematically study the properties of ionic self-complementary oligopeptides and to develop a biological matrix scaffold for cell attachment in tissue engineering (Zhang et al., 1995; Zhang & Altman, 1999). Most of the peptides form hydrogels when exposed to physiological concentration of monovalent alkaline salts. Almost all of these peptides have alternating hydrophilic charged residues and hydrophobic alanine residues. The negatively charged residues are either glutamic acid (E) or aspartic acid (D) and positively charged residues are either lysine (K) and arginine (R). For example, EAK12-a is a direct truncation of the original EAK16-II peptide, in which a four-residue AEAE sequence has been removed from the N-terminal end, resulting in a peptide with a net positive charge near the N-terminus. EAK12-b is similar to EAK12-a except one of the glutamates has been replaced with serine (S), so that one negative charge is missing near the N-terminus. In the case of EAK12-c, the second positively charged lysine has been substituted by a negatively charged glutamate. The peptides EAK12-a, b, and c all have a random coil structure and cannot form visible matrices in the presence of salt. However, the situation is quite different when both positively charged lysines near the N-terminus of EAK12-d are substituted by two glutamates, thereby creating a cluster of negatively charged residues toward the N-terminus. This peptide can undergo a secondary structure transformation from β-sheet to α-helix with either temperature or pH.

Similarly, the peptides DAR16-IV, DAR16-IV*, and DAR32-IV all have a cluster of negatively charged residues toward the N-terminus. Both DAR16-IV and DAR16-IV* can also undergo secondary structure transformation from β-sheet to α-helix. It should be pointed out that the only difference between DAR16-IV and DAR16-IV* is a frameshift of the first residue. In DAR16-IV, the first amino acid is alanine, but in the DAR16-IV*, the first residue is aspartic acid (Table 1). There are no detectable differences between the two in terms of structural conversion, indicating that the terminal residues play a less critical role than charge distribution and helix capping in this case. On the other hand, DAR32-IV can exist as either a stable α-helix or a β-sheet but is unable to undergo a direct structural conversion. DAR32-IV was purified in two distinct forms with high-performance liquid chromatography (HPLC), one α-helical and the other β-sheet, indicating that even reversed-phase HPLC conditions were insufficient to rapidly interconvert the two forms. This is probably due to the increased peptide length, solubility, and the increased possibility of more complex intermolecular interactions. When positive and negative charge clusters are reversed, as in RAD16-IV, the peptide can no longer undergo secondary structure conversion. RAD16-IV, KAE16-IV, and EAK16-IV all form extremely stable β-sheet structures even at 90 °C and after extended incubation time (Zhang & Rich, 1997). It is interesting that DAR16-IV has a high structural plasticity but EAK16-IV does not, even though the amino acids residues E, A, and K have relatively high propensity to form helical structures (Cantor & Schimmel, 1980). It is likely that EAK16-IV forms unusually stable β-sheets and did not disassemble under the conditions used. It has also been observed that EAK peptides form more stable hydrogel matrices than that of the RAD series suggesting there may be structural differences between them (Zhang et al., 1995).

Structural transition with temperature

For both EAK12-d and DAR16-IV*, there exists a strong correlation between peptide structure and temperature as seen in the circular dichroism (CD). In both cases, an increase in temperature resulted an abrupt structural transition from β-sheet to α-helix. EAK12-d (Figs. 1, 2) exhibits a small loss in β-sheet content upon heating to 60 °C, as indicated by the ellipticity at 218 nm. In addition, the shift to 60 °C may result a change in β-backbone twist

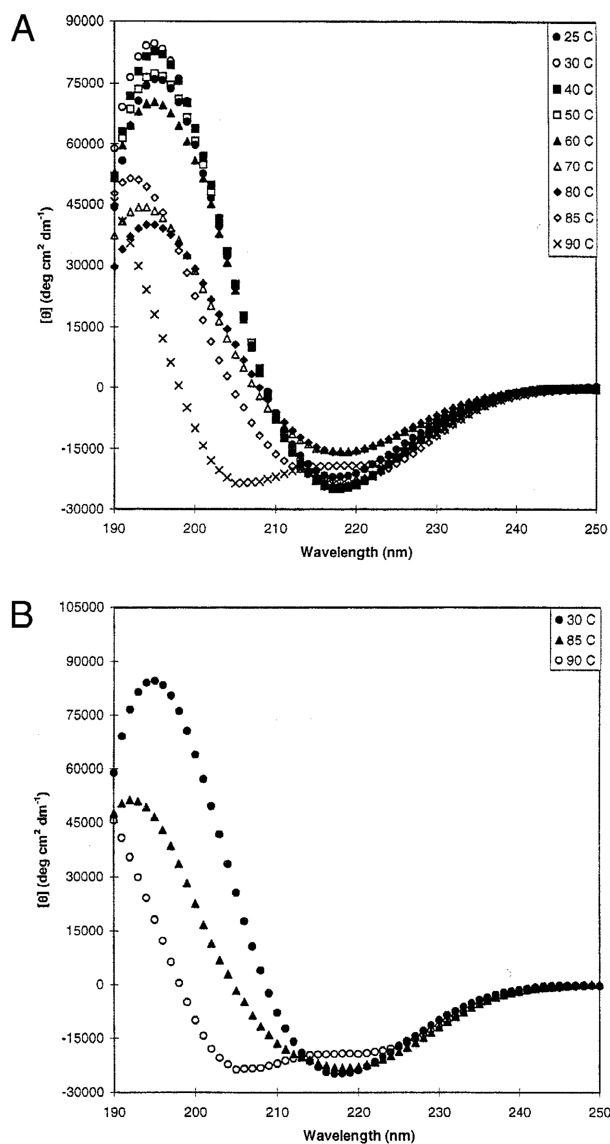

Fig. 1. Temperature effect on EAK12-d structural transitions. EAK12-d peptide solution was aliquoted into nine identical samples. The samples were incubated at indicated temperatures for 10 min and CD was measured at the same temperature. **A:** CD of EAK12-d from 25–90 °C. At 25 °C, it forms a stable β-sheet. This β-sheet structure is stable until 70 °C. It then underwent two distinctive transitions. During the first transition, the β-sheet content was not only reduced, but also changed its degree, which may be related to changes in right-handed twist (G. Fasman, pers. comm.). The second structural transition occurs drastically between 85–90 °C. Here, the β-sheet structure is converted to an α-helical structure. **B:** EAK12-d exhibits three distinctive structures at three different temperatures, 30, 85, and 90 °C. The isodichromic point at 213 nm.

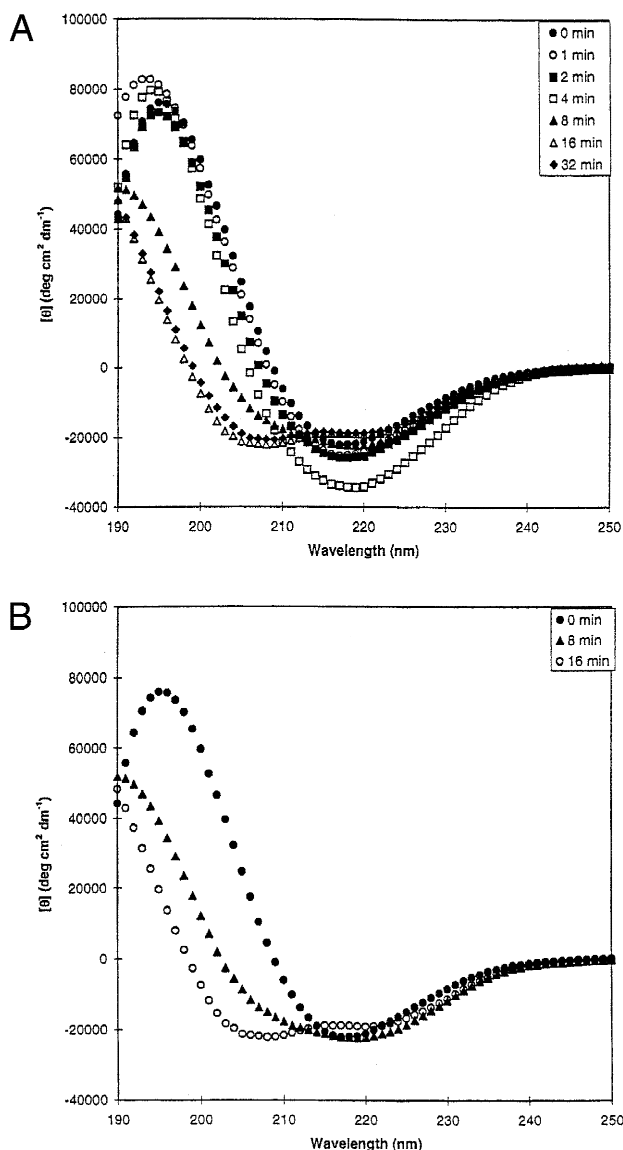

Fig. 2. Incubation at various time points induces EAK12-d structural transition. **A:** EAK12-d was incubated at 85 °C for 0, 1, 2, 4, 8, 16, and 32 min. It also underwent two phase transitions. Incubation from 0, 1, 2, and 4 min did not result in drastic structural changes. The first transition occurs at 8 min when the stable β-sheet became less stable and showed signs of structural change. After further incubation at 85 °C, it abruptly converted from a β-sheet to an α-helix by 16 min. Further incubation for 32 min did not additionally change the structure. **B:** EAK12-d exhibits three distinctive structures during incubation for different time periods. Note there is an imperfect isodichromic point at 213 nm.

as shown by a reduced ellipticity at 195 nm (G. Fasman, pers. comm.). At 70 °C, the beginnings of a structural conversion are observed as the 218 nm valley widens toward 210 nm. In addition, the ellipticity values at 195 nm begin to take on α-helical characteristics. Upon heating to 85 °C, a spectrum is obtained showing a profile between β-sheet and α-helix. There is still a considerable amount of sheet content, yet the widening of the valley toward 205 nm indicates increasing helical content. By 90 °C, the structural conversion is complete, and a characteristic helical curve is observed. Likewise, peptide DAR16-IV* undergoes a similar transformation (Fig. 3). The profiles in Figure 3B show two distinct spectra at 25 and 90 °C, typical β-sheet and α-helix at 25 and 90 °C, respectively. However, the CD profiles of DAR-IV* have noticeable differences from those of EAK12-d. First, DAR16-IV* begins and completes its structural transition at a lower temperature, 65–75 °C. In addition, the structural conversion seems to be much more abrupt in DAR16-IV*. It is interesting to note that the CD profile at 90 °C appears to have a higher helical content than the profile at 75 °C. These features are consistent with previously proposed structural properties of DAR16-IV (Zhang & Rich, 1997). Both EAK12-d and DAR16-IV* exhibit isodichromic points at 213

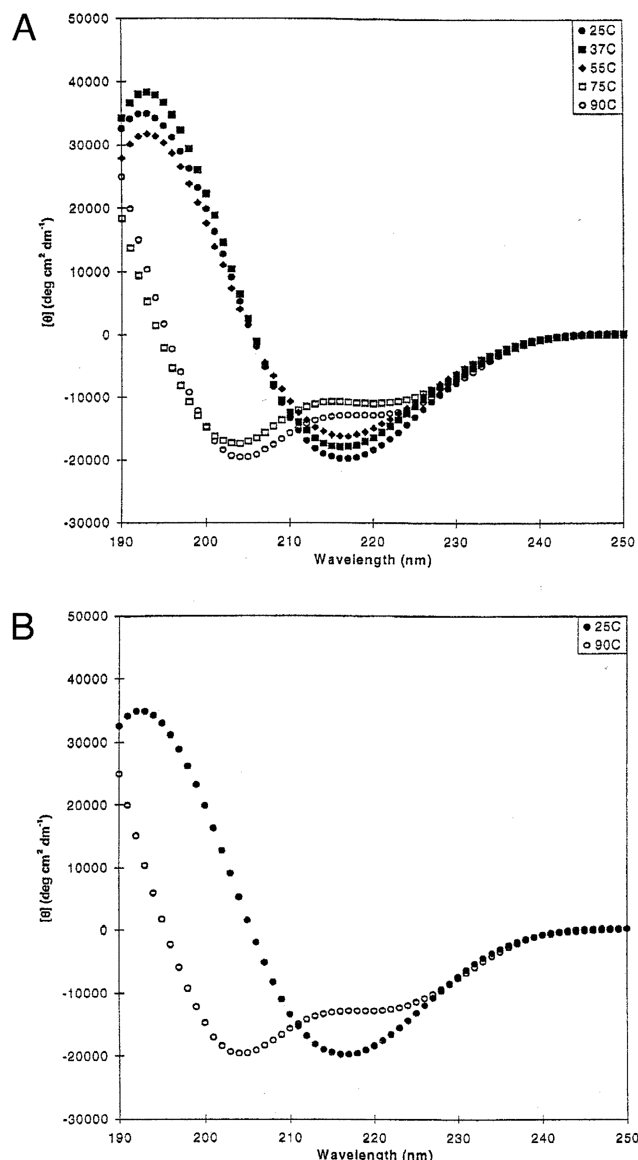

Fig. 3. Temperature effect on DAR16-IV* structural transition. DAR16-IV* was incubated at various temperatures for 10 min and measured at 25 °C. **A:** Structures of DAR16-IV* at from 25–90 °C. At 25 °C, it forms a stable β-sheet. This β-sheet structure is stable until 75 °C. Here, the β-sheet structure is abruptly converted to an α-helical structure with no detectable intermediate. The conversion in DAR16-IV* is much more abrupt than in EAK12-c, as only two distinct spectra forms are observed. **B:** DAR16-IV exhibits two distinctive spectra at two different temperatures, 25 and 90 °C.

and 211 nm, respectively, where the ellipticity is constant regardless of temperature. This was somewhat surprising since the classic work by Greenfield and Fasman showed that a two-state transition between the β-sheet and α-helix has an isodichromic point of 198 nm (Greenfield & Fasman, 1969). This may be partly due to the composition differences between their early observations and the observations reported here. Their substrate was poly-L-lysine, but the peptide composition here is more heterogeneous. Additional experiments are required to clarify the differences. The appearance of an isodichromic point suggests that the transition is direct between β-sheet and α-helix, without other detectable structures. Generally, the presence of an isodichromic point indicates that only two species exist in the pathway and that they interconvert directly. The lack of an isodichromic point would suggest an intermediate with a detectable lifetime and structure (Cantor & Schimmel, 1980).

Time dependence of structural transition

All chemical and enzymatic reactions are time dependent, and structural transitions are no exception. When EAK12-d was heated at 85 °C (Fig. 2), there was no considerable difference between heating for 1 or 2 min. There is a slight increase of ellipticity at 218 nm compared to the sample without heating. When it was heated for 4 min, there was a significant increase in ellipticity at 218 nm, from −22,000 to −35,000. Although the CD spectrum remains largely β-sheet, it showed a slightly atypical profile due to a shift toward far-ultraviolet (UV) wavelengths. A similar situation was observed for the previously reported DAR16-IV peptide (Zhang & Rich, 1997). After incubation for 8 min, the CD spectrum shifted further toward the far UV (blue shift). With further incubation for 16 min, the spectrum completely changed from a typical β-sheet to a characteristic helical profile. Additional incubation had no effect as indicated by a constant ellipticity at 222 nm. This observation is also consistent with the previously characterized DAR16-IV peptide.

Structural flexibility with pH change

EAK12-d can also undergo conformational changes as a function of pH. At pH 1–3, this peptide exhibited a typical β-sheet spectrum with minimum ellipticity at 218 nm and a maximum at 195 nm (Fig. 4). However, when the pH was changed from 1 to 3, there was a noticeable change in the peptide CD profile. Although they still formed β-sheets, the backbone twist may have changed as suggested by decreased ellipticity in the 195 nm region (G. Fasman, pers. comm.). When EAK12-d was incubated at pH 4, a very different spectrum was observed, consisting of a beta-type profile with greatly reduced β-sheet content and further altered backbone conformation. This profile suggests that there might exist a β-strand structural intermediate, or both sheet and helical structures may coexist. When EAK12-d was incubated at pH 5, it underwent a completely structural transition into a conformation with ~30% helical content. Additional changes from pH 6–10 did not result in a significant alteration of the peptide conformation suggesting that it has a stable structure (Fig. 4) within this range. Figure 4B summarizes the structural transitions of EAK12-d, and its ability to exist in three distinct states due to the influence of pH.

α-Helix to β-sheet reconversion with time

Some experiments have been conducted to study the α-helix to β-sheet reconversion process in DAR16-IV*. Overall, the reconversion process is very slow, and months are required to observe reemergence of the initial β-sheet content. After six months of incubation, only a small amount of increased sheet content was observed (not shown). One of the initial observations about the reconversion was that the rate seems to increase as a function of time. This leads to the possibility that the reconversion process is autocatalytic or cooperative, with the presence of β-sheet having the ability to recruit α-helices into an assembly. Similar findings have been reported previously that aggregated amyloid proteins

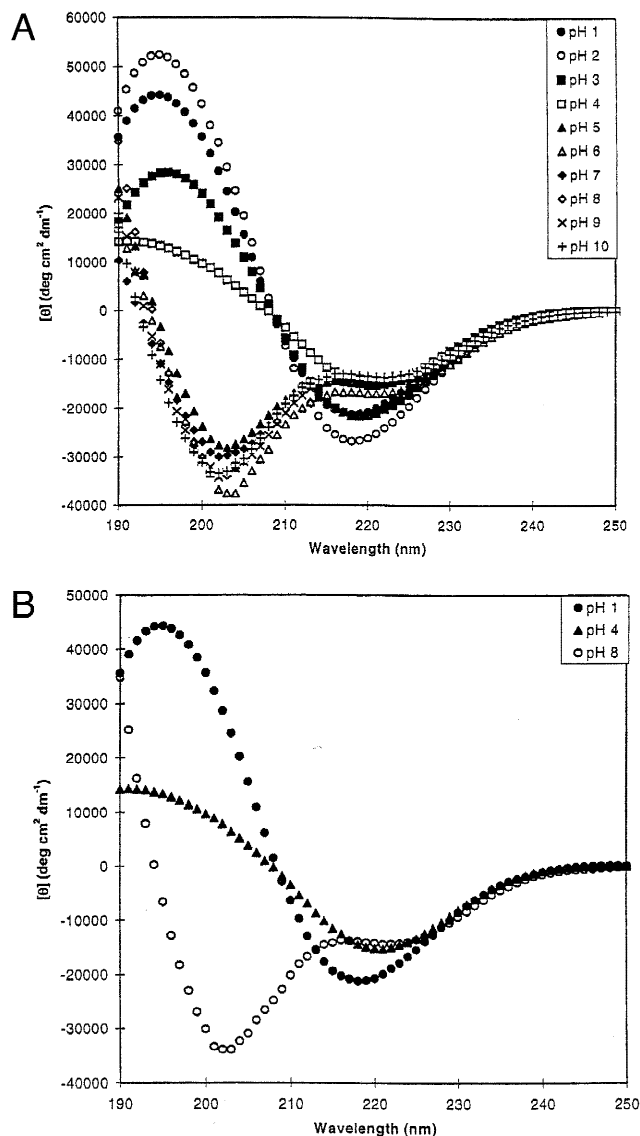

Fig. 4. pH effect on the EAK12-d structural transition. EAK12-d was incubated in solutions of various pH from 1–10. **A:** The structural properties suggested by the spectra of EAK12-d in different pH are variable. The β-sheet content is different at pH 1 and 2–3. EAK12-d at pH 2 has a high β-sheet content. The β-sheet content is essentially identical at pH 1 and 3. There exists a difference in the backbone twist among pH 1, 2, and 3 as shown in the 195 nm region. EAK12-d at pH 4 exhibits a very different spectrum. There seems to be an intermediate structure, with both helical and sheet character. When EAK12-d is incubated at pH 5–10, it exhibits an α-helical spectrum with about 30% helical content. There is an isodichromic point at 214 nm except at pH4. **B:** The spectra of EAK12-d suggest three different structures at pH 1, 4, and 8.

can recruit other properly folded amyloid proteins by inducing changes in secondary structure (Han et al., 1995; Wood et al., 1996; Harper & Lansbury, 1997; Harper et al., 1997, Takahashi et al., 1999).

Discussion

Comparison of structural transitions

Several of the ionic oligopeptides can undergo secondary structure transformations from β-sheet to α-helix and return back to β-sheet over a long period of time. However, they differ from one another in the ease with which the conversion takes place. For example, at the same concentration in water, DAR16-IV and DAR16-IV* underwent a structural transition at 60 °C, whereas EAK12-d did not undergo the transition until 85 °C, a significantly higher temperature. The higher temperature requirement for the EAK12-d structural transition suggests that the β-sheet form of this molecule is more stable. This higher temperature requirement for the transition also suggests why EAK16-IV did not change at all under the conditions tested. Although the time needed for conversion with both DAR16-IV and EAK12-d is similar, 1–4 min of heating did not result in significant deformation. In both cases, a noticeable transition took place by about 8 min and by the 16 min mark the transition is complete. Additional heating up to 32 min at the respective conversion temperature did not result in increased helical content with either peptide. There is no observable ellipticity increase at 222 nm after 16 min, the characteristic signature of helical content. To ascertain if the helix is a monomer or multimeric assembly, analytic ultracentrifugation experiments was carried out (not shown). The calculated molecular weight of acetylated EAK12-d is 1,260 Da, and the analytic ultracentrifugation experiment indicated that there was a molecule with a molecular weight near 1,330 Da, thus suggesting the EAK12-d formed a monomeric helix. This result is consistent with the previous observation of DAR16-IV, which is also a monomer under the same conditions examined (Zhang & Rich, 1997).

The role of pH changes in peptide structure

It is well known that pH changes have drastic effects on protein and peptide structures. Much of this work has been systematically carried out using polymeric peptides. For example, polylysine can undergo structural change from α-helix to β-sheet near pH 10 and higher when 95% of the lysine residues have become deprotonated (Applequist & Doty, 1962; Greenfield & Fasman, 1969). This conformational change is reversible when the pH is lowered allowing for reprotonation. In the same manner, the β-amyloid peptide [βA1–42] also undergoes conformational change as a function of pH. At pH 1.3 and 8.3, it has a helical structure, whereas at pH 5.4 it has a β-sheet structure (Barrow & Zagorski, 1991). EAK12-d has a calculated pI of 4.5 and a molecular weight of 1,260 Da. It is interesting to note that when it is fully protonated below pH 3, it has a β-sheet structure, similar to the case of polylysine mentioned above. The EAK12-d structural transition takes place at about pH 4, near its pI value, with an intermediate spectrum (Fig. 4B). When the pH is increased, the molecules become deprotonated, and the structure becomes more helical, again mirroring the situation observed for polylysine (Greenfield & Fasman, 1969). EAK12-d follows the same trend as the well-characterized polylysine, in which higher pH values increase the tendency to form an α-helix. Although EAK12-d and DAR16-IV can undergo conformational change in response to pH, their pH-induced structural transitions follow two different paths. In the case of DAR16-IV, at pH 1–2, it forms a helical structure, with a transition at pH 3 and a change to β-sheet at pH 5 and higher. EAK12-d forms a β-sheet at pH 1–3 with a transition at pH 4, changing to a more helical structure at pH 5 and above. Although both undergo pH dependent structural transitions, they are almost exactly reversed in their structures at a given pH. Again, this illustrates that the nature of the charge on the different amino acids plays more of a role than sequence alone in determining peptide structure, due to the correlation between pH and charge. Further studies will be needed to obtain a broad understanding the conformations of ionic peptides with other combinations of charged amino acids.

Proposed structure of β-sheet assembly

Monomeric peptides with an extended β-sheet backbone (β-strands) are inherently unstable and therefore must be stabilized on both faces to form an assembly. Self-complementary ionic peptides contain both a hydrophobic and hydrophilic face. Consequently, it is proposed that one face is stabilized by intermolecular ionic interaction, while the other is stabilized by intermolecular hydrophobic interaction in addition to conventional backbone hydrogen bonding. Due to the alternating hydrophilic and hydrophobic residues, it is possible for overlapping to occur between molecules creating a network complex. A proposed assembly structure of DAR16-IV* (Fig. 5C) schematically illustrates an explanation for the possibility of the previously observed physical properties of these peptides. These include strong resistance to proteases and denaturing agents due to unexposed side chains, β-sheet thermostability due to extensive ionic and hydrophobic bonding, and the formation of hydrogel matrices upon exposure to salt conditions when these assemblies are precipitated out of solution (Zhang et al., 1993, 1995; Zhang & Rich, 1997).

Proposed mechanism for β-sheet to α-helix structural conversion

Our experimental observations suggest that change in the twist of the β-sheet backbone (G. Fasman, pers. comm.) may take place a few minutes after raising the temperature (Fig. 1). This suggests that the β-sheet structure is becoming destabilized before there is any evidence of helical conversion. These observations lead to the proposal of two possible mechanisms for the structural changes, one involving a well-defined monomeric intermediate, and the other involving a complex intermediate. In the first mechanism, as the β-sheets in solution are heated, a monomeric unit of β-sheet, a β-strand, breaks away from the assembly (Fig. 6A). Once free from intermolecular forces, this beta strand can coil up into a helix due to intramolecular forces. The change in β-sheet backbone twist observed with CD spectroscopy might be associated with the β-sheet to β-strand conversion. This situation may not be uncommon in proteins. For example, interactions between other parts of the protein, or with other molecules during transport and catalysis, may cause one of the β-strands in a β-sheet to break away from the pack. In the second model, a segment of a peptide molecule begins to convert to helix while the other part of it is still bound to the β-sheet assembly (Fig. 6B). This may result an intermediate that is

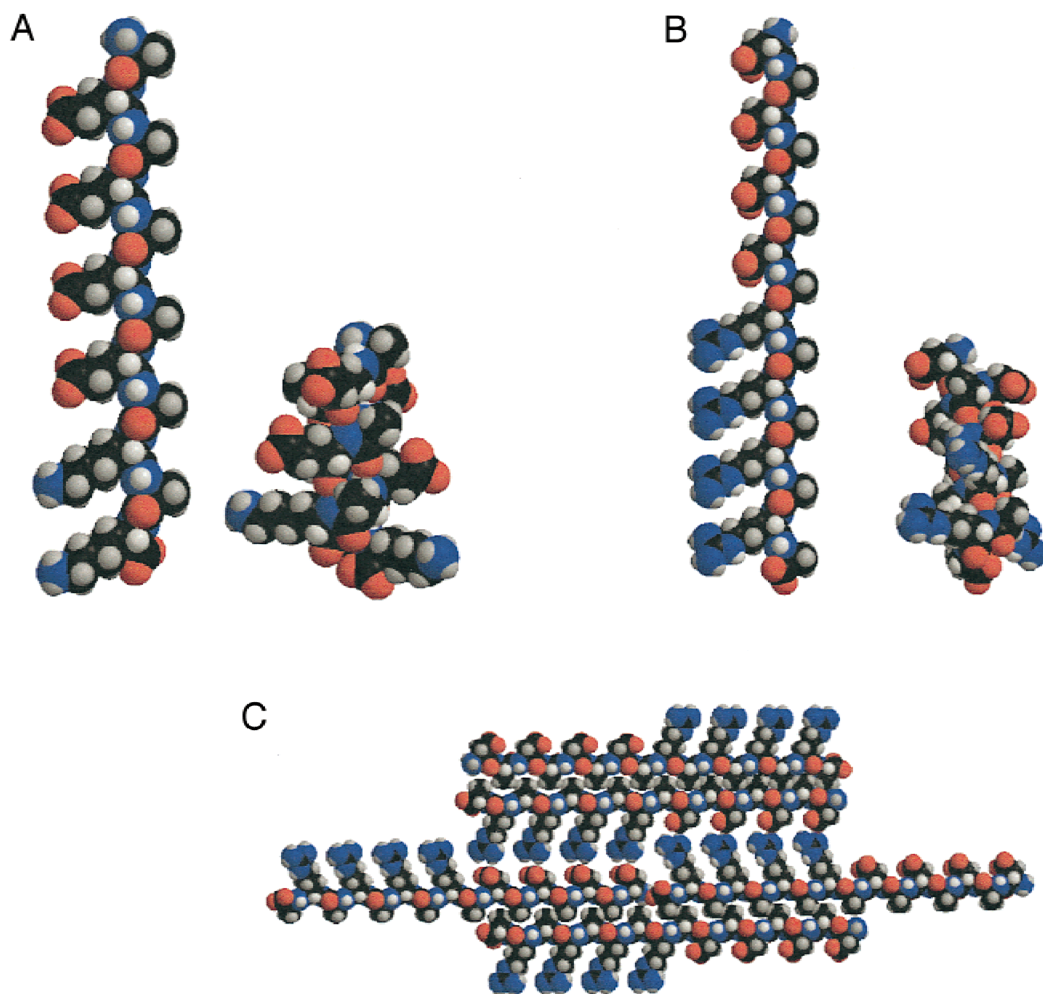

Fig. 5. Molecular models of self-complementary ionic peptides. **A:** EAK12-d in the extended β-strand and α-helix forms. **B:** DAR16-IV* in the β-sheet and α-helix forms. **C:** The proposed β-sheet assembly structure of DAR16-IV*. Note the two distinct faces, one hydrophobic and the other hydrophilic, as well as the extensive hydrophobic and ionic bonding. The conventional backbone hydrogen bonds between amino and carbonyl groups pointing toward the viewer (not shown). Molecular overlap is expected due to the highly repetitive nature of these peptide sequences on both hydrophobic and hydrophilic sides somewhat resemblance of repeated DNA sequences.

partially helix and partially extended, remaining complexed to the assembly. Eventually, the entire molecule becomes helical and separates from the assembly. In this case, the observed change in backbone twist may be accounted for by the peptide of mixed secondary structure. Although it is not possible at this point to determine which model is correct, the second model is energetically favorable because it does not involve a higher energy β-strand intermediate. Both mechanisms are reversible, and the reverse pathways can result in the reassembly of β-sheet over time.

Thermodynamics and kinetics of structural dynamics

Since it was observed that the α-helical peptides eventually return to the β-sheet form over extended periods of time, it can be assumed that the β-sheet is in a lower energy state. Even though the α-helix is stabilized by its dipole moment, hydrogen bonding, and solvation energy, the extensive hydrophobic and ionic bonding

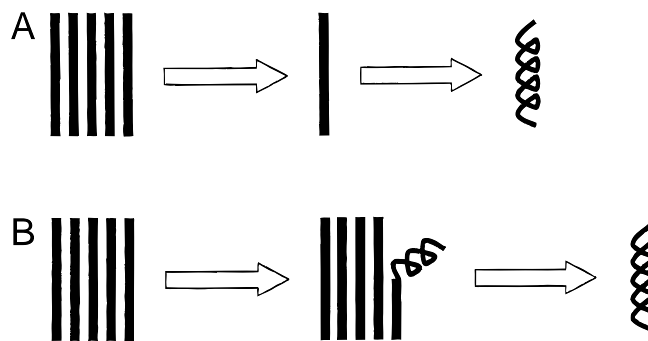

Fig. 6. Two possible scenarios for the β-sheet to α-helix conversion process. **A:** A monomeric β-strand leaves the β-sheet assembly and is then free to undergo intramolecular hydrogen bond formation to coil into a helix. **B:** A peptide molecule begins the helical conversion while still attached to the β-sheet assembly. Eventually, the molecule becomes completely helical and free from the assemblies.

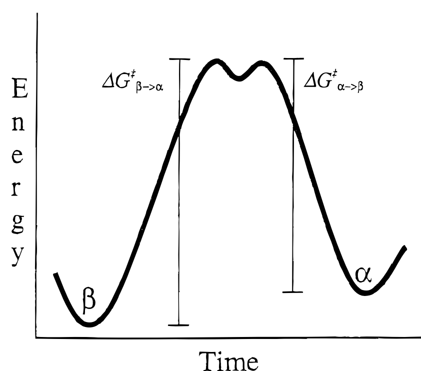

Fig. 7. Hypothetical schematic energy diagram for the β-sheet to α-helix structural conversion.

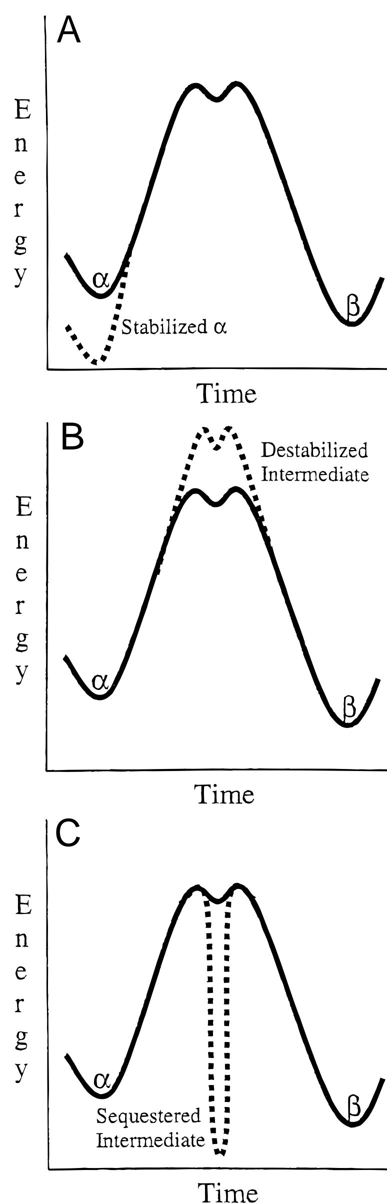

Fig. 8. Hypothetical diagram for three modes of inhibition of the α-helix to β-sheet amyloid structural transition. **A:** Stabilizing the α-helical form to increase the activation energy of transition. **B:** Destabilizing the structural intermediate to again increase the activation energy of transition. **C:** Sequestering the structural intermediate into a form that is more stable than the β-sheet assemblies, preventing it from continuing on the pathway.

makes the β-sheet formation more favorable. This suggests that the reason for the extended time periods in the α-helix to β-sheet reassembly is kinetic rather than thermodynamic. In other words, the kinetic pathway for the α-helix to β-sheet transition is energetically inaccessible, forcing the overall process to be extremely slow. This is possible because regardless of the mechanism, a large energy penalty must be paid to uncoil a stable α-helix structure. Therefore, the reassembly of the β-sheet occurs with a very low probability. We can propose a hypothetical energy diagram representing the two states of these oligopeptides, linked by a theoretical well-defined intermediate (Fig. 7). This hypothetical diagram provides a representation of the observed properties of these peptides. In both directions, there exists a large activation energy. The forward conversion from β-sheet to α-helix requires a large amount of thermal energy, while the reverse pathway of conversion from α-helix to β-sheet is kinetically inaccessible due to the large energy barrier that must be overcome without the aid of heat. This diagram also accounts for the possibility that excessively heating the α-helical structure, to the point where minimal helical character remains (above the activation energy for the α-helix to β-sheet transition), would allow formation of β-sheet structure upon cooling the system. In addition, this diagram also displays the structural dichotomy that exists in these ionic self-complementary oligopeptides. These peptides form very stable β-sheets as well as α-helices, but once one form is "selected," a very large amount of energy is required to overcome the stability of one state to change to the other.

Implications for inhibitors of amyloid formation

Another feature of this hypothetical energy diagram is that it may aid in the selection of target molecules when looking for inhibitors of the α-helix to β-sheet amyloid conversion. Overall, there are three general ways to inhibit this structural transition (Fig. 8). The first mode of inhibition is to lower the energy (stabilize) the α-helical form (Fig. 8A). This would increase the activation energy, thereby slowing down the rate of alpha to beta conversion. This is the preferred method found in biological systems to prevent amyloid formation in vivo. For example, chaperone proteins are known to promote correct protein conformation by binding nonspecifically to solvent exposed hydrophobic patches of unfolded, partially folded, or misfolded proteins in an effort to prevent aggregation and allow proteins to attain the correct structure. It has been shown that in yeast overexpression of chaperone proteins can strongly inhibit amyloid/prion formation in cells (DeBurman et al., 1997; Schirmer & Lindquist, 1997). Through genetics or drug action, it may be possible to regulate chaperone activity, making an increase in α-helix stability an efficient mode of amyloid inhibition. A second mechanism of amyloid inhibition would be to increase the energy of the transition state by destabilizing the structural intermediate (Fig. 8B). This also has the effect of increasing activation energy and slowing the rate of α-helix to β-sheet transition. Although the exact nature of the intermediate(s) is not clear at this point, there is a possibility that a drug may exist having the ability to interfere with the for-

mation of, or degrade intermediates on, the amyloid formation pathway. The third mode of α-helix to β-sheet amyloid inhibition is the "sequestering" of structural intermediates into a state that is more stable than the β-sheet amyloid product (Fig. 8C). Creating this highly stable intermediary complex removes the intermediate from the amyloid pathway and traps it in a form that prevents further aggregation. The idea is very similar to adding a chelating agent to a reaction that requires metal ions to proceed. Overall, these self-complementary ionic peptides may be a good model system for study of amyloid formation and may assist in understanding possible common mechanisms of formation. It may also be used to screen for possible inhibitors of this process. This mode of inhibition has already been demonstrated, for the addition of "decoy peptides" can substantially inhibit large amyloid formation (Blanchard et al., 1997). These decoy peptides are antagonistic for the aggregation regions in amyloid peptides and therefore trap amyloid peptides into more stable and soluble aggregates. The ultimate goal of this mode of inhibition would be to find either an antagonistic peptide or drug that binds strongly to the intermediate, forming a soluble low-order complex that is incapable of further aggregation. Natural processes could then degrade this complex in biological systems.

Other aspects of structural plasticity

α-Helices have a helical dipole moment along the backbone that possesses a directionality from C $\rightarrow$ N. Many α-helices have a preference for negatively charged residues located at the N-terminus and positively charged residues located at the C-terminus called helix capping (Blagdon & Goodman, 1975; Hol, 1985; Presta & Rose, 1988; Parthasarathy et al., 1995; Aurora & Rose, 1998). It has been postulated that the α-helix may be considered as an array of dipoles arranged in such a way in that they orient along the axis of the helix with the negative pole at the C-terminus and the positive pole at the N-terminus (Blagdon & Goodman, 1975). Therefore, it is presumed that amino acid sequences with negative charges at the N-terminus and positive charges at the C-terminus can enhance and stabilize the helical dipole moment, and in turn the helix itself. This phenomenon has been found to be statistically significant, and many helices in proteins agree with this prediction (Hol, 1985; Parthasarathy et al., 1995). It should be emphasized that not all helices have such a charge arrangement.

Protein secondary structures have recently been shown to be dynamic entities. A recent report showed that a segment of eight residues (QDMINKST) in a yeast Mat-α2 protein could adopt either an α-helix or a β-strand conformation in crystal structures resulting in different interactions (Tan & Richmond, 1998). Another example is the translational elongation factor EF-Tu in which a segment of six residues (PEEKAR) formed an α-helical structure in the GTP complex and changed to β-strand structure in the GDP complex (Abel et al., 1996). Wei et al. (1994) showed that a segment in one of the serine protease inhibitors was an α-helix in the native uncleaved form, but underwent a conformational change to a β-strand upon cleavage (Wei et al., 1994; Wright, 1996). Minor and Kim (1996) also reported an 11 residue segment in the G protein of an IgG binding domain that can fold into either an α-helix or a β-strand in the appropriate location of the protein. High resolution structures of prion proteins in different forms have been recently elucidated (Nguyen et al., 1995a, 1995b; Riek et al., 1996, 1998). α-Helix and β-sheet conformational changes in response to pH have also been observed in other proteins including β-amyloid (Barrow & Zagorski, 1991) and a voltage-dependent, anion-selective channel protein (VDAC) (Mannella, 1998). Furthermore, it has been found that a yeast protein, α-agglutinin, contains three distinct regions that have the ability to undergo structural transitions from β-sheet to α-helix in response to temperature or pH changes (M. Zhao, pers. comm., 1998 Protein Society Symposium Abstracts). The segment with the greatest propensity to undergo this conversion, region III, has the sequence YEYELENAKFFK. It contains a similar charge distribution, as well as alternating hydrophobic and hydrophilic residues as compared to the peptides studied here. Region III's response to changes in temperature or pH is similar to EAK12-d, with temperatures above 55 °C and with pH < 5 resulting in structural transition.

Systematic alterations of proteins through genetic manipulations and protein engineering have accelerated our understanding of protein structures and provided us with a great deal of detailed information about protein structures and their critical biological functions (Cunningham et al., 1990; Blundell, 1994; Shakhnovich, 1998). The rapid accumulation of protein sequences and structures deposited in the databases will likely provide us with critical information to improve and to refine our knowledge of protein structure, stability, plasticity, and complex conformational behavior.

Materials and methods

Peptide design and synthesis

The peptides EAK16-IV, KAE16-IV, DAR16-IV, RAD16-IV, and EAK12-a through EAK12-d were synthesized on an Applied Biosystems (Foster City, California) automated peptide synthesizer using t-Boc chemistry, and subsequently purified with reversed-phase HPLC at the Biopolymers Laboratory at the Massachusetts Institute of Technology as previously described (Zhang et al., 1994). The peptides DAR16-IV* and DAR32-IV were synthesized on a Rainin Model PS3 automated peptide synthesizer using F-moc chemistry and also purified with reversed-phase HPLC. All peptide products were verified with either amino acid analysis or mass spectroscopy or both. The peptides were dissolved in deionized water and stored at room temperature.

Circular dichroism analysis

All CD spectroscopy was performed on 20 μM solutions of EAK12-d in water or DAR16-IV* diluted from a 3 mM stock in water that was incubated at room temperature for at least one week. CD data were corrected by conversion to mean residue ellipticity to account for differences in molecular weight and concentration. The effect of temperature on peptide structure was determined by either taking CD scans at different temperatures from 25 to 90 °C, or by taking scans at sequential time intervals up to 32 min at a constant temperature of 85 °C. The effect of pH on peptide structure was determined by incubating the 20 μM peptide solutions from pH 1–12 overnight before measuring with CD. Initial experiments to analyze the α-helix to β-sheet reconversion process were conducted by taking 20 μM solutions of DAR16-IV*, heating them at 90 °C for 15 min, and then measuring them with CD on a weekly basis for several months.

Sedimentation equilibrium analysis

For determination of molecular weight, the EAK12-d oligopeptide was heated in water to 90 °C for 30 min and its α-helical structure

was confirmed by CD. Three different concentrations of the oligopeptide were then loaded into a hexa-cell chamber with three cells containing water as the references. The samples were then centrifuged at 50,000 rpm at 25 °C for 24 h to sedimentation equilibrium. Each sample was then measured several time independently. All points were collected and analyzed. In all cases, the samples showed an average molecular weight 1,330 Da that is close to the calculated monomeric molecular weight of 1,260 Da of EAK12-d. We therefore concluded the helical form of EAK12-d is a monomer in water in our experiment.

Database searching

A search of all current protein databases was performed using BLAST to identify sequences similar to any of the peptides that undergo structural transition. Although an exact match was not found, there exist many examples of protein segments with similar charge distributions, along with alternating hydrophobic and hydrophilic residues.

Molecular modeling and simulation

All molecular modeling and simulation were performed with the Quanta software package from Molecular Simulations Inc. (Burlington, Massachusetts) on SGI machines at the MIT Center for Biomedical Engineering computing cluster.

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References

Abel K, Yoder MD, Hilgenfeld R, Jurnak F. 1996. An alpha to beta conformational switch in EF-Tu. *Structure 4*:1153–1159.

Applequist J, Doty P. 1962. In: Stahmann MA, ed. *Polyamino acids, polypeptides and proteins*. Madison, Wisconsin: University of Wisconsin. pp 161–171.

Aurora R, Rose G. 1998. Helix capping. *Protein Sci 7*:21–38.

Barrow CJ, Zagorski MG. 1991. Solution structures of beta peptide and its constituent fragments: Relation to amyloid deposition. *Science 253*:179–182.

Blagdon DE, Goodman M. 1975. Mechanisms of protein and polypeptide helix initiation. *Biopolymers 14*:241–245.

Blanchard BJ, Konopka G, Russell M, Ingram VM. 1997. Mechanism and prevention of neurotoxicity caused by β-amyloid peptides: Relation to Alzheimer's disease. *Brain Res 776*:40–50.

Blundell TL. 1994. Problems and solutions in protein engineering—Towards rational design. *Trends Biotechnol 12*:145–148.

Boutonnet NS, Rooman MJ, Wodak SJ. 1995. Automatic analysis of protein conformational changes by multiple linkage clustering. *J Mol Biol 253*: 633–647.

Brack A, Orgel LE. 1975. Beta structures of alternating polypeptides and their possible prebiotic significance. *Nature 256*:383–387.

Cantor C, Schimmel P. 1980. *Biophysical chemistry*. San Francisco: W H. Freeman & Co.

Cao Y, Musah RA, Wilcox SK, Goodin DB, McRee DE. 1998. Protein conformer selection by ligand binding observed with crystallography. *Protein Sci 7*:72–78.

Colson AO, Perlman JH, Jinsi-Parimoo A, Nussenzveig DR, Osman R, Gershengorn MC. 1998. A hydrophobic cluster between transmembrane helices 5 and 6 constrains the thyrotropin-releasing hormone receptor in an inactive conformation. *Mol Pharmacol 54*:968–978.

Creighton TE. 1993. *Proteins: Structures and molecular principles*. 2nd ed. New York: WF Freeman & Co.

Cunningham BC, Henner DJ, Wells JA. 1990. Engineering human prolactin to bind to the human growth hormone receptor. *Science 247*:1461–1465.

Dado GP, Gellman SH. 1993. Redox control of secondary in a designed peptide. *J Am Chem Soc 115*:12609–12610.

DeBurman SK, Raymond GJ, Caughey B, Lindquist S. 1997. Chaperone-supervised conversion of prion protein to its protease-resistant form. *Proc Natl Acad Sci USA 94*:13938–13943.

Dobson C. 1999. Protein misfolding, evolution and disease. *Trend Biochem Sci 24*:329–332.

Greenfield N, Fasman GD. 1969. Computed circular dichroism spectra for the evaluation of protein conformation. *Biochemistry 8*:4108–4116.

Han H, Weinreb PH, Lansbury PT Jr. 1995. The core Alzheimer's peptide NAC forms amyloid fibrils which seed and are seeded by β-amyloid: Is NAC a common trigger or target in neurodegenerative disease? *Chem Biol 2*:163–169.

Haouz A, Glandieres JM, Zentz C, Pin S, Ramstein J, Tauc P, Brochon JC, Alpert B. 1998. Solvent effects on horse apomyoglobin dynamics. *Biochemistry 37*:3013–3019.

Harper ET, Rose GD. 1993. Helix stop signals in proteins and peptides: The capping box. *Biochemistry 32*:7605–7609.

Harper JD, Lieber CM, Lansbury PT Jr. 1997. Atomic force microscopic imaging of seeded fibril formation and fibril branching by the Alzheimer's disease amyloid-beta protein. *Chem Biol 4*:951–959.

Harper JD, Lansbury PT Jr. 1997. Models of amyloid seeding in Alzheimer's disease and scrapie: Mechanistic truths and physiological consequences of the time-dependent solubility of amyloid proteins. *Annu Rev Biochem 66*:385–407.

Hol WG. 1985. The role of the α-helix dipole in protein function and structure. *Prog Biophys Mol Biol 45*:149–195.

Hol WG, Halie LM, Sander C. 1981. Dipoles of the α-helix and β-sheet: Their role in protein folding. *Nature 294*:532–536.

Huang Z, Prusiner SB, Cohen FE. 1995. Scrapie prions: A three-dimensional model of an infectious fragment. *Fold Des 1*:13–19.

Huyghues-Despointes BM, Scholtz JM, Baldwin RL. 1993. Helical peptides with three pairs of Asp-Arg and Glu-Arg residues in different orientations and spacings. *Protein Sci 2*:80–85.

Jardetzky O. 1996. Protein dynamics and conformational transitions in allosteric proteins. *Prog Biophys Mol Biol 65*:171–219.

Kelly JW. 1997. Amyloid fibril formation and protein misassembly: A structural quest for insights into amyloid and prion diseases. *Structure 5*:595–600.

Kirschner DA, Inouye H, Duffy LK, Sinclair A, Lind M, Selkoe DJ. 1987. Synthetic peptide homologous to beta protein from Alzheimer disease forms amyloid-like fibrils in vitro. *Proc Natl Acad Sci USA 84*:6953–6957.

Kurzynski M. 1998. A synthetic picture of intramolecular dynamics of proteins. Towards a contemporary statistical theory of biochemical processes. *Prog Biophys Mol Biol 69*:23–82.

Mannella CA. 1998. Conformational changes in the mitochondrial channel protein, VDAC, and their functional implications. *J Struct Biol 121*:207–218.

Minor DL Jr, Kim PS. 1996. Context-dependent secondary structure formation of a designed protein sequence. *Nature 380*:730–734.

Mutter M, Maser F, Altmann KH, Toniolo C, Bonora GM. 1985 Sequence-dependence of secondary structure formation: Conformational studies of host-guest peptides in alpha-helix and beta-structure supporting media. *Biopolymers 24*:1057–1074.

Nguyen JT, Baldwin MA, Cohen FE, Prusiner SB. 1995a. Prion protein peptides induce α-helix to β-sheet conformational transitions. *Biochemistry 34*: 4186–4192.

Nguyen JT, Inouye H, Baldwin MA, Fletterick RJ, Cohen FE, Prusiner, SB Kirschner DA. 1995b. X-ray diffraction of scrapie prion rods and PrP peptides. *J Mol Biol 252*:412–422.

Osterman DG, Kaiser ET. 1981. Design and characterization of peptides with amphiphilic β-strand structures. *J Cell Biochem 29*:57–72.

Parthasarathy R, Chaturvedi S, Go K. 1995. Design of α-helical peptides: Their role in protein folding and molecular biology. *Prog Biophys Mol Biol 64*:1–54.

Pauling L. 1960. *The nature of chemical bond*, 3rd ed. New York: Cornell University Press.

Presta LG, Rose GD. 1988. Helix signals in proteins. *Science 240*:1632–1641.

Prusiner SB. 1996. Molecular biology and pathogenesis of prion diseases. *Trends Biochem Sci 21*:482–487.

Reddy VS, Giesing HA, Morton RT, Kumar A, Post CB, Brooks CL III, Johnson JE. 1998. Energetics of quasiquivalence: Computational analysis of protein-protein interactions in icosahedral viruses. *Biophys J 74*:546–558.

Riek R, Hornemann S, Wider G, Billeter M, Glockshuber R, Wüthrich K. 1996. NMR structure of the mouse prion protein domain PrP(121-321). *Nature 382*:180–182.

Riek R, Wider G, Billeter M, Hornemann S, Glockshuber R, Wüthrich K. 1998.

Prion protein NMR structure and familial human spongiform encephalopathies. *Proc Natl Acad Sci USA 95*:11667–11672.
Rigney E, Kojima M, Glithero A, Elliott T. 1998. A soluble major histocompatibility complex class I peptide-binding platform undergoes a conformational change in response to peptide epitopes. *J Biol Chem 273*:14200–14204.
Rippon WB, Chen HH, Walton AG. 1973. Spectroscopic characterization of poly(Glu-Ala). *J Mol Biol 75*:369–375.
Schirmer EC, Lindquist S. 1997. Interactions of the chaperon Hsp104 with yeast Sup35 and mammalian PrP. *Proc Natl Acad Sci USA 94*:13932–13937.
Schulz GE, Schirmer RH. 1979. *Principles of protein structure*. New York: Springer-Verlag.
Shakhnovich EI. 1998. Folding nucleus: Specific or multiple? Insights from lattice models and experiments. *Fold Des 3*:108–111.
Takahashi Y, Ueno A, Mihara H. 1999. Optimization of hydrophobic domains in peptides that undergo transformation from α-helix to β-fibril. *Bioorg Med Chem 7*:177–183.
Tan S, Richmond TJ. 1998. Crystal structure of the yeast MATalpha2/MCM1/DNA ternary complex. *Nature 391*:660–666.
Wei A, Rubin H, Cooperman BS, Christianson DW. 1994. Crystal structure of an uncleaved serpin reveals the conformation of an inhibitory reactive loop. *Nat Struct Biol 1*:251–258.
West MW, Wang W, Patterson J, Mancias JD, Beasley JR, Hecht MH. 1999. De novo amyloid proteins from designed combinatorial libraries. *Proc Natl Acad Sci USA 96*:11211–11216.
Wood SJ, Chan W, Wetzel R. 1996. Seeding of a beta fibril formation is inhibited by all three isotypes of apolipoprotein E. *Biochemistry 35*:12623–12628.
Wright HT. 1996. The structural puzzle of how serpin serine proteinase inhibitors work. *Bioessays 18*:453–464.
Xiong H, Buckwalter BL, Shieh HM, Hecht MH. 1995. Periodicity of polar and nonpolar amino acids is the major determinant of secondary structure in self-assembling oligomeric peptides. *Proc Natl Acad Sci USA 92*:6349–6353.
Yon JM, Perahia D, Ghelis C. 1998. Conformational dynamics and enzyme activity. *Biochimie 80*:33–42.
Zhang S, Altman M. 1999. Self-assembling peptide in polymer science and engineering. *Reactive and Functional Polymers 41*:91–102.
Zhang S, Holmes TC, DiPersio CM, Hynes RO, Su X, Rich A. 1995. Self-complementary oligopeptide matrices support mammalian cell attachment. *Biomaterials 16*:1385–1393.
Zhang S, Holmes T, Lockshin C, Rich A. 1993. Spontaneous assembly of a self-complementary oligopeptide to form a stable macroscopic membrane. *Proc Natl Acad Sci USA 90*:3334–3338.
Zhang S, Lockshin C, Cook R, Rich A. 1994. Unusually stable β-sheet formation in an ionic self-complementary oligopeptide. *Biopolymers 34*:664–672.
Zhang S, Lockshin C, Herbert A, Winter E, Rich A. 1992. Zuotin, a putative Z-DNA binding protein in *Saccharomyces cerevisiae*. *EMBO J 11*:3787–3796.
Zhang S, Rich A. 1997. Direct conversion of an oligopeptide from a β-sheet to an α-helix: A model for amyloid formation. *Proc Natl Acad Sci USA 94*: 23–28.