Volume 298, number 2,3, 233–236 FEBS 10752 February 1992

Design and synthesis of an α-helical peptide containing periodic proline residues

Eiichi Kitakuni, Tokio Horiuchi, Yasushi Oda, Motohisa Oobatake, Haruki Nakamura and Toshiki Tanaka

Protein Engineering Research Institute, 6-2-3, Furuedai, Suita, Osaka 565, Japan

Received 6 January 1992

A thirty-residue peptide (PERI COIL-1) has been designed with a new type of α-helical structure, which is capable of folding into an amphiphilic helix bending at 4 periodic prolines in the sequence. Two such helices should form a dimer by supercoiling about one another in an antiparallel direction in the design. With this arrangement, close packing between them is maintained through the hydrophobic interaction pattern called 'leucine zipper'. PERI COIL-1 has been obtained by solid-phase peptide synthesis, and characterized by circular dichroic spectroscopy, sedimentation equilibrium experiments and NMR. The result of the analyses shows that it preferentially forms a helical tetramer in aqueous solution.

α-Helical structure; Amphiphilic helix; Proline; Leucine zipper; Protein design

1. INTRODUCTION

The two-stranded coiled coil protein, tropomyosin, has regularity in its sequence, containing an identical heptapeptide unit that is repeated 40 times. The structural hypothesis for tropomyosin has been presented by a minimalist approach based on the sequence regularity, and tested with model peptides. There, polymers of a heptapeptide paradigm for the repeated sequence are proposed to be a model for the two-stranded coiled coil protein [1]. The basic feature for a helical coiled coil structure is a fully periodic interaction pattern at the helix–helix interface, such as the close packing of leucine side chains ('leucine zipper') [2,3]. These side-chain packing interactions between the helices determine the interhelical geometry within the helical coiled coil. In the case of tropomyosin, the effective interdigitation of the side chains is maintained by coiling of the right-handed helices about one another with a slight left-handed superhelical twist.

Proline is known as a helix breaker, and therefore a unique residue within an α-helix. It is observed in the middle portion of helices in, for instance, citrate synthetase, alcohol dehydrogenase, and cytochrome peroxidase. In these cases, the proline produces a bend in the helix [4]. Some natural proteins, such as nucleolin, and NADH dehydrogenase, contain prolines at every seventh position in their primary sequences. But, any structures coded by these sequences remain unknown at present, and no secondary structure has yet been predicted for them.

In the present study, we show a thirty-residue peptide named 'PERI COIL-1', a new type of α-helical structure. Four prolines were placed at every seventh position in the sequence so that the structure should be kinked at these sites. Twelve leucines were positioned in the sequence to help packing among the helices, which should form a dimerical coiled coil structure. The design concept of PERI COIL-1 should provide us with a new direction for the design of an α-helix which is based on 2 features, namely an amphiphilic helix and bends produced by prolines. This design is entirely artificial.

2. MATERIALS AND METHODS

PERI COIL-1 was synthesized by standard protocols on a HMP resin for an α-COO^- group on the C-terminus in an Applied Biosystems peptide synthesizer, Model 430A using 9-fluorenylmethoxy carbonyl (Fmoc) chemistry. After cleavage from the resin by trifluoroacetic acid and water (95:5), the crude peptide was purified by preparative reversed-phase HPLC on an Aquapore Prep-10 (C-8, 300 Å pore size, Applied Biosystems) with 0.1% trifluoroacetic acid in a water/acetonitrile gradient. The amino acid sequence was confirmed by automated Edman degradation on an Applied Biosystems Model 477A.

CD spectra were recorded at 20°C on a J-600 spectropolarimeter (Japan Spectroscopic) with an 1-mm path-length cuvette. The peptide was dissolved in 50 mM citrate-phosphate buffer (pH 2.0–9.0), or 50 mM borate buffer (pH 9.0–11.0), or with 50% (v/v) trifluoroethanol (TFE), at a concentration ranging from 0.015 to 0.6 mg/l (5–200 μM). The peptide concentration was determined by quantitative amino acid analysis of a stock solution. CD spectra are presented as a plot of the mean residue ellipicity per residue ([θ], deg × cm^2/dmol).

Sedimentation equilibrium runs were performed in an analytical ultracentrifuge (Beckman Spinco Model E) equipped with Schrieren

Correspondence address: E. Kitakuni, Protein Engineering Research Institute, 6-2-3, Furuedai, Suita, Osaka 565, Japan. Fax: (81) (6) 872 8210.

and interference optical systems. Samples of 0.1–0.6% (w/v) were used at 25 and 37.5°C in 50 mM citrate-phosphate buffer (pH 7.0) with a double-sector cell (pathlength of 12 mm) and with an AnG rotor at 21,740 rpm. Apparent molecular weight was calculated from log fr (fringe) vs. r^2 plots [5].

For measurement of NMR spectra, the peptide was dissolved in 50% (v/v) TFE/^2H_2O at a concentration of 6 mg/ml (about 2 mM) at pH 2.5. ^1H NMR spectra were recorded at 25°C on a Bruker AM600 spectrometer. Phase-sensitive NOESY [6] spectrum was recorded with a mixing time of 300 ms. Phase-sensitive DQF-COSY [7] spectrum was also recorded to identify amino acid spin systems. Chemical shifts are expressed in ppm relative to 2,2-dimethyl-2-silapentane-5-sulfonate (DSS).

3. RESULTS AND DISCUSSION

The amino acid sequence of PERI COIL-1 is based on repeating the hepta peptide paradigm. Prolines are positioned in each heptad so that the helix has periodic bends at them. These bends should produce supercoiling in the helical structure. The paradigm of different length was examined on a progress of the design. The pitch of the supercoiling helix depends on the proline spacing so closely that a different paradigm will diversify the structure. For example, the structure for the hexapeptide paradigm becomes elongated, and the structures for paradigms above the octapeptide paradigm become widespread. Thus, the heptapeptide paradigm derived from the tropomyosin motif seems reasonable for a supercoiling helix.

The non-proline amino acids in the sequence are selected based on intrinsic conformational preferences that most strongly favor helix formation [8,9]. Leucine was chosen as an apolar aliphatic residue, glutamic acid and lysine as charged residues, and alanine as a default residue. The arrangement of amino acids in the heptad is based on amphiphilic orientation, and related to position-specific amino acid appearance in some helices kinked at prolines in natural proteins listed in the Brookheaven protein data bank. It was found that hydrophobic residues, such as Leu and Ile, often appear at the −2,+1 positions when the position for Pro is 0. This suggests that these positions in the helix should be available for packing interactions. These positions in the heptad are occupied by leucines, where they can provide an apolar face for helix–helix interaction. Glutamic acid on the opposite face can provide water-solubility to the structure, and the remaining positions are occupied by alanines. Leu-Ala-Glu-Ala-Leu-Ala-Pro is designed as the typical heptad, and it is repeated three times in the sequence (LAEALAP-LAEALAP-LAEALAP). Proline produces a bend of about 30° in the helix axis, and disrupts the pattern of α-helical hydrogen bonding. The change in geometry around Pro should cause the interhelical interaction to weaken to some extent. Therefore, two alanines in the middle heptad are replaced by two leucines for close packing. In addition, two leucines are placed close to the terminals to propagate helical geometry to the whole structure. Two lysines and two glutamic acids are in the C-terminus and the N-terminus respectively, which should stabilize the helical structure opposite to the

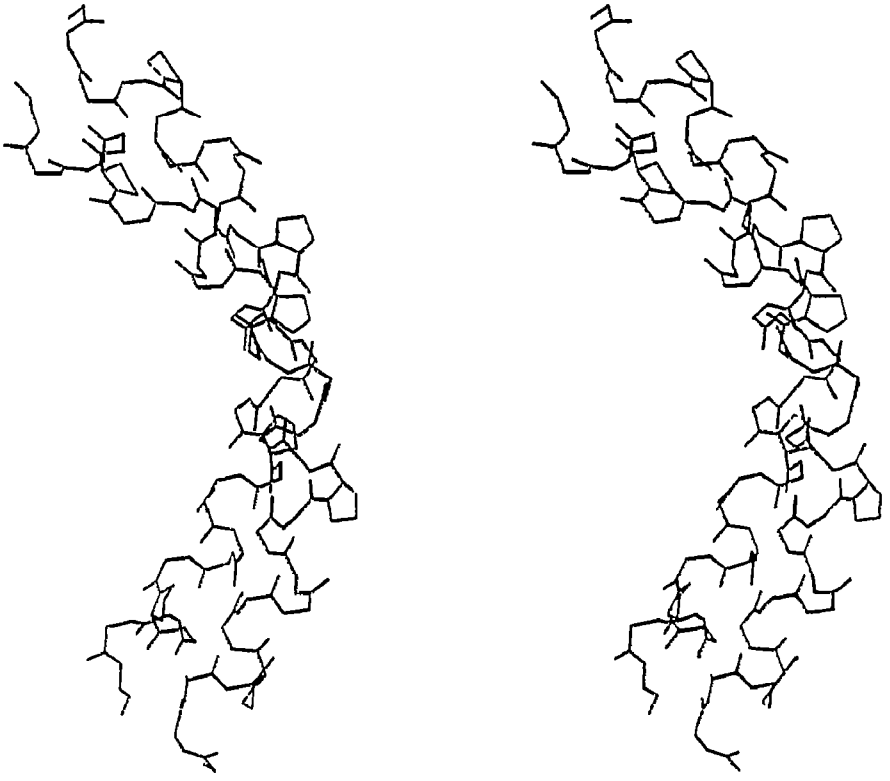

Fig. 1. Stereo view of model structure of PERI COIL-1 designed with a new type of α-helical structure.

helix dipole moment [10]. Furthermore, these residues should form a Glu^--Lys^+ salt bridge linkage between the terminals when the helices run in an antiparallel direction. The sequence of PERI COIL-1 is

EELLPLAEALAPLLEALLPLAEALAPLLKK

and the model structure is shown in Fig. 1.

Characteristics of PERI COIL-1. PERI COIL-1 contains a large number of hydrophobic residues (~70%), however, it is easily soluble in aqueous solution even at high concentration (~2 mM, at pH 7.0). This suggests that it forms a water-soluble structure by effective packing. The peptide shows a circular dichroic spectrum having negative maxima at 208 and 222 nm at pH 2.0–7.0 and room temperature (~20°C); these are characteristic of a helical conformation [11]. The ellipicity value at 222 nm of the peptide at a concentration of 30 μM is about 21,000 at pH 3.5, and 12,000 at pH 7.0, showing that the helical content is calculated to be about 50% and 30%, respectively [11–13]. In the presence of 50% (v/v) TFE as a helix-supporter, the helical content is increased to about 60%, even at pH 7.0 (Fig. 2). The dependence of helical content on the concentration was tested in aqueous solution at pH 7.0 (Fig. 3). The peptide shows an increase in helical content up to ~70 μM. These observations suggest that the peptides effectively form helical aggregates stabilized by hydrophobic interaction and protonation of polar residues. However, the effect of salt-bridge formation on stability was not clearly observed, and so we cannot indicate the orientation of the helices.

The apparent molecular weight of the peptide was determined by sedimentation equilibrium experiments

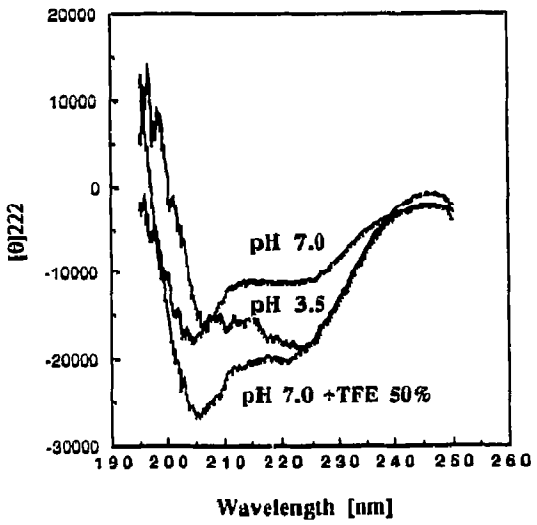

Fig. 2. The circular dichroic spectra of PERI COIL-1 dissolved in 50 mM sodium citrate-phosphate buffer at pH 3.5, 7.0, or in the presence of TFE 50% (v/v) at pH 7.0, and 20°C. A peptide concentration of 30 μM was used.

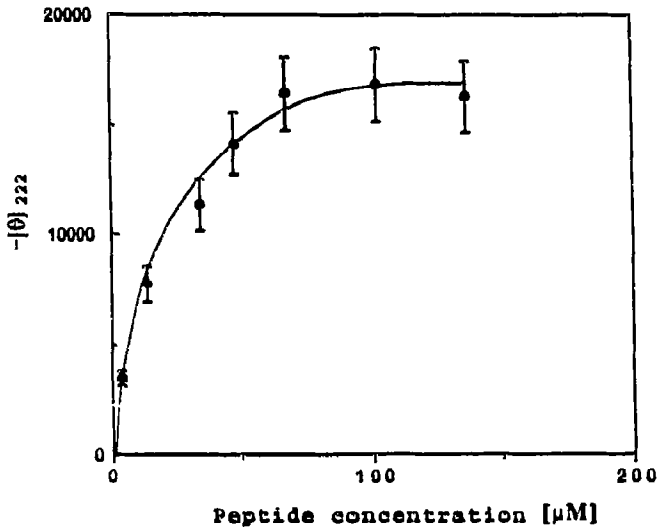

Fig. 3. A test for the dependence of helicity on peptide concentration at pH 7.0 and 20°C. The peptide was dissolved in 50 mM sodium citrate-phosphate buffer.

at pH 7.0. It is distributed around M_w 16,000 at 20°C and 14,000 at 37.5°C, consistent with M_w 12,000 determined by size-exclusion chromatography using Sephadex G-75 at pH 7.0 and 20°C. These sizes are approximately 4–5 times larger than the value based on amino acid composition of the monomer (M_w 3,160). PERI COIL-1 seems to preferentially form tetrameric species in the population. It is possible that some leucine side chains remain available for interhelical contact, and are involved in forming such an unexpectedly large aggregate. According to this speculation, the desired conformation is expected to be obtained by decreasing the number of leucines responsible for the tetrameric formation. However, we cannot exclude the hypothesis that the peptides form the helical tetramer in a non-specific manner, and in a way different from the design.

The conformational analysis of PERI COIL-1 was carried out in the presence of TFE 50% (v/v) with NOESY experiments, since the spectrum in aqueous solution shows band broadening. Under these conditions, the peptide forms an α-helix which does not form an aggregate. Standard sequential assignment procedures [14] were used to obtain specific assignments. The identified sequential NOE connectivities are summarized in Fig. 4 along with a representative region of the NOESY spectrum. In addition to these connectivities, the NOESY spectrum also shows numerous short distances between amino acid residues in position i and i+3 [$C^{\alpha}H(i) \rightarrow NH(i+3)$ and $C^{\alpha}H(i) \rightarrow C^{\beta}H(i+3)$]. Although some of the NOE cross peaks could not be determined due to overlapping resonances, these through-space connectivities provide strong evidence for the presence of an α-helix extending from residue 4 to residue 28. Moreover, the connectivities to $C^{\delta}H$ of each proline from NH of the previous residue are clearly

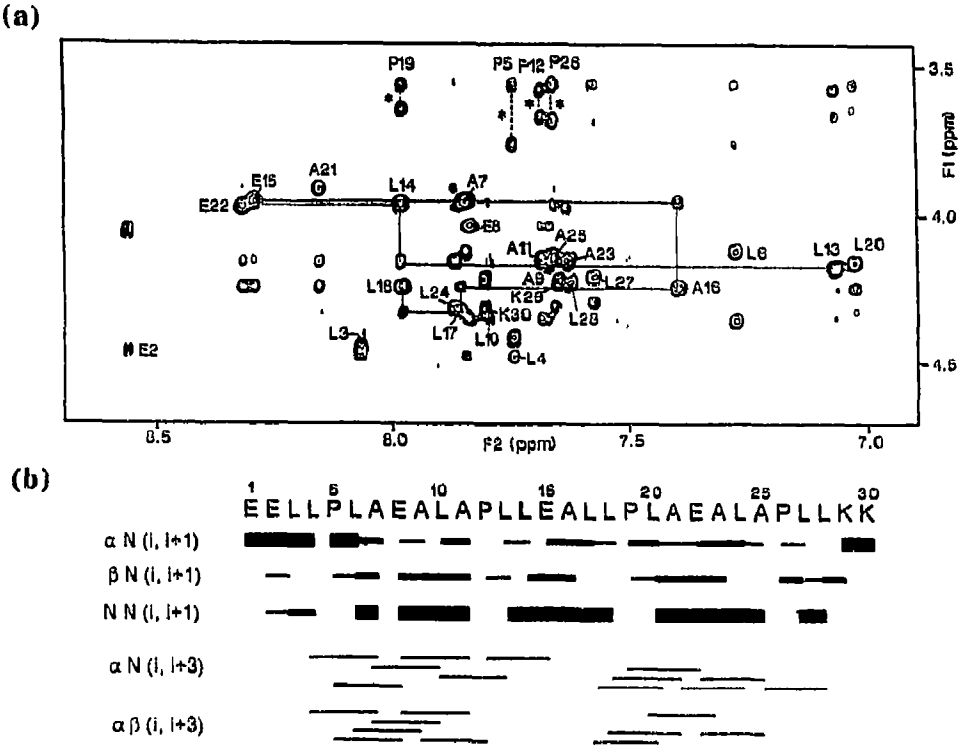

Fig. 4. (a) Fingerprint region of NOESY spectrum of PERI COIL-1 in 50% (v/v) TFE-d3/H_2O with a mixing time of 300 ms. The sequential connectivities from Leu^{13}-Leu^{18} through αCH-NH cross-peaks are shown by a continuous line. The intra residue cross-peaks are labeled. The asterisks denote the cross-peaks between δCH of the proline and NH of the previous residue. (b) Summary of NMR data for PERI COIL-1. NOE connectivities are classified into three groups (strong —, medium —, and weak ---), according to the number of contours of the cross-peaks.

observed (Fig. 4a). This also indicates that prolines are in the *trans* configuration [14]. These observations show that the peptide forms a proline-bending helix in 50% TFE.

Consequently, PERI COIL-1 is considered to form a helical structure stabilized by intermolecular interaction, and to form mainly tetramers in aqueous solution. However, it is not obvious whether the prolines produce bends in the structure in aqueous solution as in 50% TFE.

Acknowledgements: We thank Mr. Shinichi Okada (Kyoto Univ.) for support of sedimentation equilibrium experiments, Ms. Masami Kikuchi for assistance in peptide synthesis, Dr. Timothy J.P. Hubbard for helpful advice on the design, and Dr. Yutaka Kuroda for stimulating discussion.

REFERENCES

[1] Lau, S.Y.M., Taneja, A.K. and Hodges, R.S. (1984) J. Biol. Chem. 259, 13253–13261.
[2] O'Shea, E.K., Rutkowski, R. and Kim, P.S. (1989) Science 243, 538–542.
[3] O'Shea, E.K., Rutkowski, R., Stafford III, W.F. and Kim, P.S. (1989) Science 245, 646–648.
[4] Barlow, D.J. and Thornton, J.M. (1988) J. Mol. Biol. 201, 601–619.
[5] van Holde, K.E. and Baldwin, R.L. (1958) J. Phys. Chem. 62, p. 734.
[6] Jeener, J., Meier, B.H., Bachmann, P. and Ernst, R.R. (1979) J. Chem. Phys. 71, 4546–4553.
[7] Rance, M., Sørensen, O.W., Bodenhausen, G., Wagner, G., Ernst, R.R. and Wüthrich, K. (1983) Biochem. Biophys. Res. Commun. 117, 479–485.
[8] O'Neil, K.T. and DeGrado, W.F. (1990) Science 250, 646–651.
[9] Lyu, P.C., Life, M.I., Marky, L.A. and Kallenbach, N.R. (1990) Science 250, 669–673.
[10] Fairman, R., Shoemaker, K.R., York, E.J., Stewart, J.M. and Baldwin, R.L. (1989) Proteins 5, 1–7.
[11] Holzwarth, G. and Doty, P.J. (1965) J. Am. Chem. Soc. 87, 218–228.
[12] Greenfield, N. and Fasman, G.D. (1969) Biochemistry 8, 4108–4116.
[13] Fillipi, B., Borin, G., Moretto, V. and Marchiori, F. (1978) Biopolymers 17, 2545–2559.
[14] Wüthrich, K. (1986) NMR of Proteins and Nucleic Acids, Wiley-Interscience, New York.