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Structural Determinants of the Unusual Helix Stability of a De Novo Engineered Vascular Endothelial Growth Factor (VEGF) Mimicking Peptide

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Dedicated to Professor C. Pedone on the occasion of his 70th birthday

Understanding how an amino acid sequence folds into a well organized three-dimensional structure remains a challenge. The interest in protein folding comes from the possibility to predict the protein structure from genome-derived sequence, design proteins with new fold and understand protein misfolding.[1] Peptide helix is a simple model system in which various contributions to helix formation can be dissected and understood qualitatively.[2,3] Many strategies have been pursued to design peptide helices[4–7] and notable results have been achieved even with very short sequences,[8] but mainly these methods rely on the use of nonnatural amino acids or introducing constraints.[8–13] In this paper, we report on the stability characterization, using CD, NMR and MD studies, of a designed, α-helical, 15-mer peptide (named QK), composed only of natural amino acids (sequence Ac-KLTWQELYQLKYKGI-NH_2), which activates the VEGF-dependent angiogenic response.[14] The QK peptide shows an unusual thermal stability, whose structural determinants have been determined. These results could have implication in the field of protein folding and in the design of helical structured scaffolds for the realization of peptides for applications in chemical biology.

As recently described, the NMR structure of QK in pure water presents a central helical sequence (residues 4–12), which corresponds to the VEGF N-terminal helix (residues 17–25), flanked by N- and C-capping regions.[14] The helical conformation of QK represents an important prerequisite for its biological activity, since the isolated peptide, corresponding to the helix region of VEGF, does not assume a helical conformation and does not have significant biological activity. Interestingly, QK represents one of the very few examples of bioactive helical designed peptides, composed of only natural amino acids. To gain an insight into the molecular determinants of QK helical propensity, we examined the effect of the temperature on the QK structure through NMR and CD analyses.

Primarily, the aggregation state of the peptide under conditions identical to those used in the NMR structure determination was confirmed by NMR DOSY experiments (see Supporting Information). The DOSY-derived diffusion coefficient value of $1.98\times10^{-10}\,m^2s^{-1}$ is consistent with a QK monomer state. QK structure variations upon temperature increase were followed by TOCSY experiments. In the 298–343 K range only small changes of the backbone chemical shifts were observed (Table 1 Supporting Information). The temperature dependences of Hα chemical shift deviations from the random coil values ($\Delta\delta$Hα) are reported in Figure 1a. Unusually, the chemical shift index (CSI) analysis indicates that at 343 K the peptide retains at least the 80% of the helix conformation at 298 K and the slight reduction occurs uniformly in 4–12 region (Figure 1a). The thermal behavior was also analyzed by CD spectroscopy which allowed

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Supporting information for this article is available on the WWW under http://www.chemistry.org or from the author: Peptide synthesis, circular dichroism, nmr spectroscopy and molecular dynamic simulations.

to extend the temperature range from 278 to 368 K. The analysis of the spectra and the dependence of 222 nm ellipticity with the temperature (Figure 1b), showed that the peptide loose, reversibly, part of its helical structure but neither at 368 K appears to assume a complete random coil conformation. The CD analysis, accordingly with the NMR results, indicates that QK retains 79 and 65% of its room temperature helix content at 343 and 368 K, respectively, as calculated from the ellipticity at 222 nm.[8]

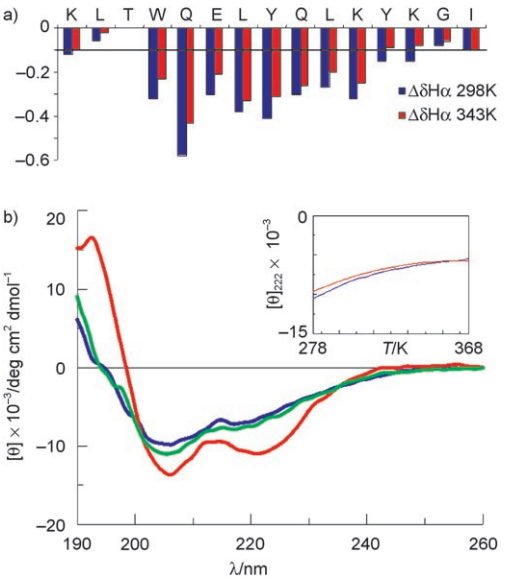

Figure 1. a) Hα chemical shift deviations from random-coil values (ΔδHα) of QK at 298 and 343 K. The continuous line represents the CSI threshold for amino acids in helical conformation. b) CD spectra of QK peptide at 298 K (——), 343 K (——) and 368 K (——). In the inset the unfolding (——) and refolding (——) curves are showed. [θ] is the molar ellipticity per residue.

This thermal stability is unusual for a short peptide and has been reported only for peptides with unnatural constrains.[8,11,13,15] To assess the determinants of the helix stabilization in solution, experimental structural data have been complemented with extensive all-atom molecular dynamics simulations in explicit water. Five QK structures of the NMR ensemble were used as starting structures for MD simulations which covered a total of 2.4 microseconds exploring different temperature conditions (300, 320, 340, 380 K). Moreover, to highlight the possible folding mechanism, four different simulations with lengths ranging from 50 to 100 ns, at 350 K, were run from a completely extended polypeptide structure. All the simulations starting from the helical structures showed a clear, unusual stability of the helix that is maintained at high temperatures (Figure 2a) for most of the simulation time, consistently with NMR observations. Cluster analysis[16] of the trajectories (Figure 2b), and the evaluation of stabilizing contacts, showed the presence of a network of contacts always involving the hydrophobic side chains of residues 7 and 10. The analysis of the folding simulations (Figure 3) showed a higher tendency for

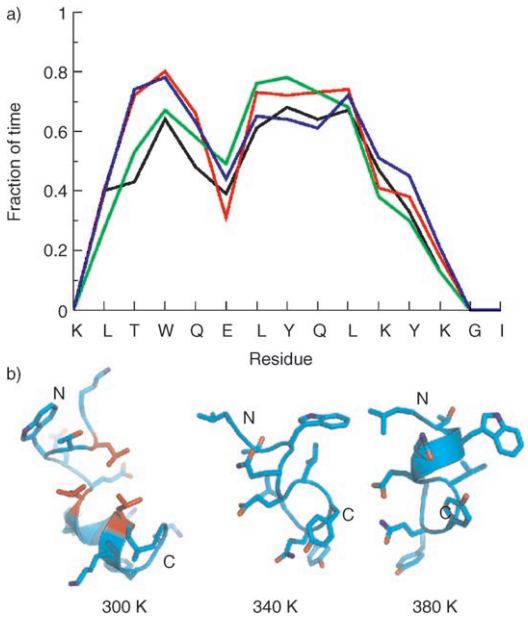

Figure 2. a) Percentage of time that each residue spends in helical conformation at 300 K (——), 320 K (——), 340 K, (——) and 380 K (——). b) Representative conformations of the main cluster obtained from the analysis of all the trajectories at different temperatures.

residues located at the N-terminal region to adopt a helical structure in the first events of the QK folding. This result indicates a relevant role of the N-capping in stabilizing and nucleating the nascent α-helical turn which, then, propagates towards the C-terminal region.

To confirm experimentally these contributions to QK helix stability, we designed three novel peptides: in particu-

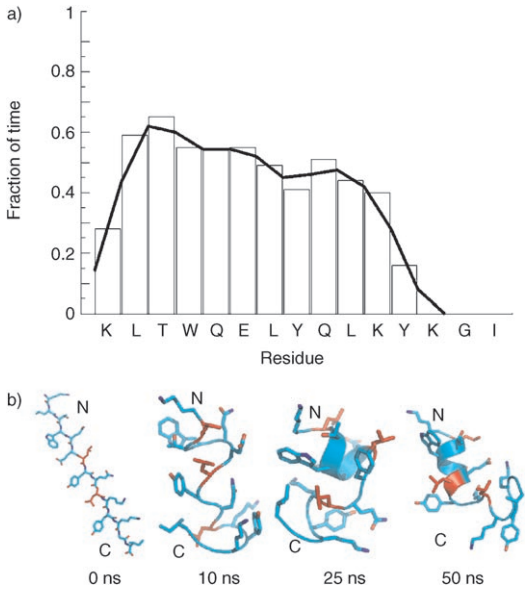

Figure 3. a) Percentage of helical conformation attained by each residue during the refolding process. b) Selected structures along the refolding trajectories. Leu7 and Leu10 are highlighted in red.

lar, Leu10 was replaced by an alanine, in QK10A, and N- and C-capping sequences were deleted, respectively, in QK4–15 and QK1–12. The CD spectra (Figure 4a) at 298 K confirmed the theoretical results, showing that QK1–12 entirely retains the helical content, whereas QK4–15 and QK10A loose about half of the QK helicity. Accordingly, NMR analysis of QKA10, based on $\Delta\delta H\alpha$ measurements (Figure 4b) showed at 298 K a 45 % helicity decrease with respect to QK, and furthermore indicated that at 343 K QK10A is predominantly in random conformation, thus lacking the QK unusual thermal stability.

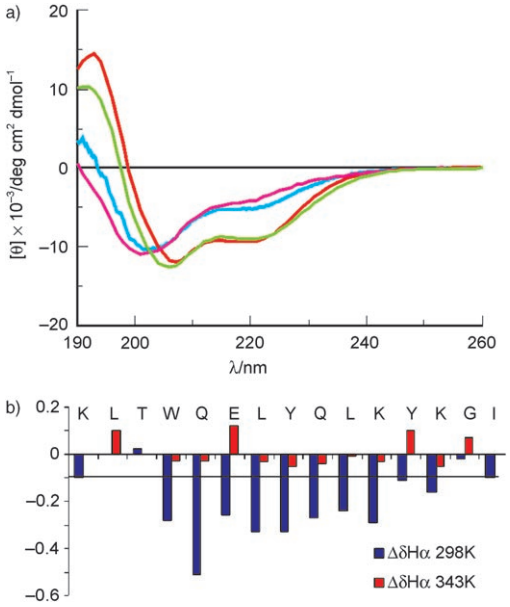

Figure 4. a) CD spectra of peptide QK (——), QK10A (——), QK4-15 (——) and QK1–12 (——) at 298 K. [θ] is the molar ellipticity per residue. b) Deviation of Hα chemical shifts of QK10 A mutant peptide from random-coil values ($\Delta\delta H\alpha$) at 298 K and 343 K. The continuous line represents the CSI threshold for amino acids in helical conformation.

In this communication we have reported the unusual thermal stability of a bioactive peptide which retains a high degree of helix structure at high temperature. We identified the N-capping region and a crucial hydrophobic interaction (Leu7-Leu10) as playing relevant roles in stabilizing the helical fold of QK. The remarkable QK helix stability and the elucidation of its structural determinants make this peptide a potential scaffold for the design of helical peptides with specific side chain arrangements.

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Keywords: helical structures · molecular dynamics · NMR spectroscopy · peptides · thermal stability

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