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HIGH-RESOLUTION, REAL-SPACE IMAGING OF CONFORMATIONAL STRUCTURES OF POLY-L-PROLINE HELIXES

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Abstract

In 1954, Edsall postulated that the imino-acid proline, which is a frequently found constituent of protein molecules, is a key determinant to the three-dimensional architecture of proteins. It not only should play a fundamental role in stabilizing helical structures of polypeptides, it should allow for sharp bends and even for a complete reversal of the direction of a helix looping back on itself. No direct evidence has yet been published to prove this prediction. Using scanning tunneling microscopy, we have presented high-resolution, real-space images of two conformations of poly-L-proline, where one structure clearly exhibits the predicted 180° back-folding behavior. The measured length, 1.89 nm, of the repeating unit cells agrees with available X-ray data for poly-L-proline I with *cis*-peptide bonds. We further observe aggregated poly-L-proline II, consisting of highly-ordered, periodically and parallel-linked *trans*-peptide chains which are 2.4 nm apart from each other. Stacking of these aggregates with their orientation rotated by 90° is also observed.

KEY WORDS: Scanning tunneling microscopy, helix structures, poly-L-proline, highly oriented pyrolytic graphite, amino acid

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Introduction

The three-dimensional conformation of protein molecules is a fundamental determinant of their specific biological activity. Protein molecules predominantly consisted of coiled, folded, staggered, pleated or aggregated polypeptide chains that are made up of sequentially ordered combinations of up to 20 different amino-acids. Nineteen of these amino-acids differ from each other in the chemical structure of their side chain[7]. Only in proline is the side chain covalently bonded to the nitrogen atom of the peptide group thus forming a cyclic ∝-imino-acid ring which imposes, due to the partially double-bounded character of the peptide link between the N and C^{α} atom of the backbone, severe steric constraints on the molecules, thereby limiting the number of conformations[13,14].

In the past few decades, the structure of many proteins was greatly elucidated by a series of mostly indirect approaches such as X-ray diffraction, infra-red absorption, Raman spectroscopy, electron microscopy, nuclear magnetic resonance, etc.[1-3,9,12,16]. The proposed structure is, however, often model-dependent[14]. Using scanning tunneling microscopy (STM), we present the first real-space, high-resolution images of two conformational structures of poly-L-proline[6,14]. With partially limited success, the technique of STM has already been applied to uncoated biological systems[8,10,11,15], and it has been demonstrated that electron transport is not a major problem for species of thickness less than a few hundred Angstroms[15].

The imino-acid proline consists of a five-sided pyrrolidine ring where the amino nitrogen atom has no amide hydrogen atom available for hydrogen bonding and, therefore, has a helical form that is entirely different from that of the ∝-helix. Early studies on solid-state poly-L-proline indicated the C^{α} atoms can be either in a *cis* or *trans* sterio-isomeric configuration which leads to two completely different conformational structures[5,16]. Poly-L-proline I forms a right-handed helix with *cis*-peptide bonds and poly-L-proline II consists of a left-handed helix with *trans*-peptide bonds. From C- and H-NMR experiments, there is evidence that mutarotation of form I into II (and v. v.) occurs when poly-L-proline is dissolved in appropriate solvents3[3,12].

Intimated by Pauling's early work[13], Edsall predicted that the presence of proline residues in the *cis* configuration can produce a sharp bend of a helix and virtually reverse the direction of its axis as it folds around this bend[6]. It is obvious that the experimental verification of this prediction is not only of fundamental importance to poly-L-proline conformational studies but to any study on the folding behavior of polypeptide chains where proline residues are present.

Experimental

The samples were prepared by dissolving poly-L-proline powder (Sigma Chemical Co.: molecular weight: 1000-10000) in a 0.1 Mol NaH_2PO_4 solvent at a cocentration of 5 w.-% and depositing small amounts of this solution onto a (0001)-oriented surface of freshly cleaved, highly-oriented pyrolytic graphite (HOPG). After air-drying for two days, the samples were put into the STM for the experiments.

The STM used for our studies on uncoated samples of poly-L-proline is home-designed and has a cylindrical symmetry. A piezo-electric ceramic tube of 1-inch length and 0.5-inch diameter was used to raster scan a probing tip across a sample surface which was mounted on a commercial inchworm (Burleigh Instruments). The tunneling current was maintained constant during scanning with a feedback circuit, while the voltages applied on the z-component of the scanner were recorded, representing the surface topography. The microscope suspended by springs for external vibration isolation is very rigid and routinely produces atomic-resolution images of HOPG (0001) surfaces. All images presented below were acquired with a tungsten wire of 1 mm diameter, chemically etched in a NaOH solution and with a raster rate of 1 Hz along the x-axis. Typical tunneling voltage and current we used were 0.1 V (tip positive) and 0.3 nA, respectively, which corresponds to a tunneling resistance of $3x10^8$ ohms.

Results

Fig. 1 shows a color-coded STM image where two distinct features, typically found in all studies and labeled A and B, respectively, can be identified. Type A represents aggregates of poly-L-proline of dimensions up to 50 nm x 50 nm. Aggregates of lateral dimensions much larger than 50 nm x 50 nm are also observed. But these large aggregates are mainly formed by partial-overlapping aggregates of type A stacked on top of each other. Type B represents coiled poly-L-proline molecules of total width of 5 nm and length of 40 nm.

Fig. 2 is a high resolution STM image of a type A aggregate. Ordered structures formed by parallel alignment of individual polypeptide chains are clearly seen. The distance between two neighboring strands is about 2.4 nm. The possible conformational structure of these chains is that of poly-L-proline II which has a left-handed helix with *trans*-peptide bonds and a repeating pseudo-hexagonal unit cell with c=0.936 nm and a=0.662 nm[5]. The lack of sharp bends further suggests the existence of *trans*-peptide bonds along the individual poly-L-proline II chains.

Figure 1: Low-resolution, color-coded STM image (scanning area: 280 nm x 230 nm) of poly-L-proline conformers on HOPG with light-green representing high protrusion. All images represent raw data except a linear subtraction of the background plane. At low magnification mode with a resolution of about 1 nm, we are able to search for interesting features in large areas of up to 3.0 μm x 3.0 μm. The two structures which are labeled A and B in Fig. 1, were also scanned in high-resolution mode and are shown in Figs. 2 and 3, respectively.

Figure 2: High-resolution STM image (scanning area: 49 nm x 31 nm) of an aggregate of type A (label A in Fig. 1). All STM images are recorded in constant current mode. Typical imaging parameters are 0.5 nA for the tunneling current, +50 mV for the tip bias voltage, and a scanning time per image (128 x 128 pixels) of about 1 minute. Individual atoms of HOPG can be easily resolved with the high magnification mode which, however, at present, limits the maximum scanning area to 150 nm x 150 nm.

Figure 3: (a) STM image (area: 14 nm x 40 nm) of a single-stranded poly-L-proline helix of type B (label B in Figure 1). The image is generated by appropriate overlapping of three high-resolution images (scanned area 14 nm x 14 nm). (b): Schematic sketch of the contours of the molecule. The coiled and back-folded behavior, evaluated from the poly-L-proline chain shown in (a), can be clearly identified.

Fig. 3a illustrates Type B proline with the STM operated at the high magnification mode. A twisted and single stranded poly-L-proline helix chain can be easily identified. The helical structure along the chain cannot be identified from the image. Nevertheless, the periodic structure can still be seen at the top part of Fig. 3a. The length of the repeating unit cell is 1.89 nm and the average width of the helix is 1.3 nm. Comparing these two values with available X-ray data[16] (pseudo-hexagonal unit cell with c = 1.90 nm and a = 0.905 nm), we conclude that this single stranded poly-L-proline is the conformational structure of poly-L-proline I which has a right-handed helix with *cis*-peptide bonds.

Several sharp bends can also be seen from the image. The helix chain apparently reverses the direction of its propagation at the bottom part of the image and loops back onto itself through 180°, as previously predicted by Edsall. Fig. 3b shows the schematic sketch of a possible interpretation for the helix propagation by tracing the bright features in Fig. 3a. It is, however not yet possible for us to determine either the detailed structures at those bending positions or the ends of the chain from our present information.

The final point, or probably a major concern we want to address is that the artifacts which are mimic to the

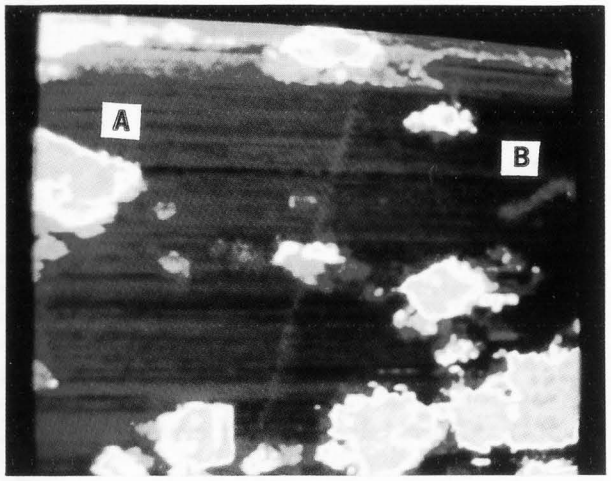

Figure 1.

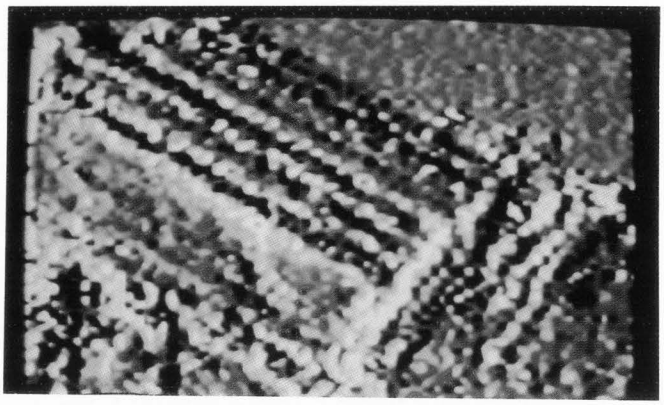

Figure 2.

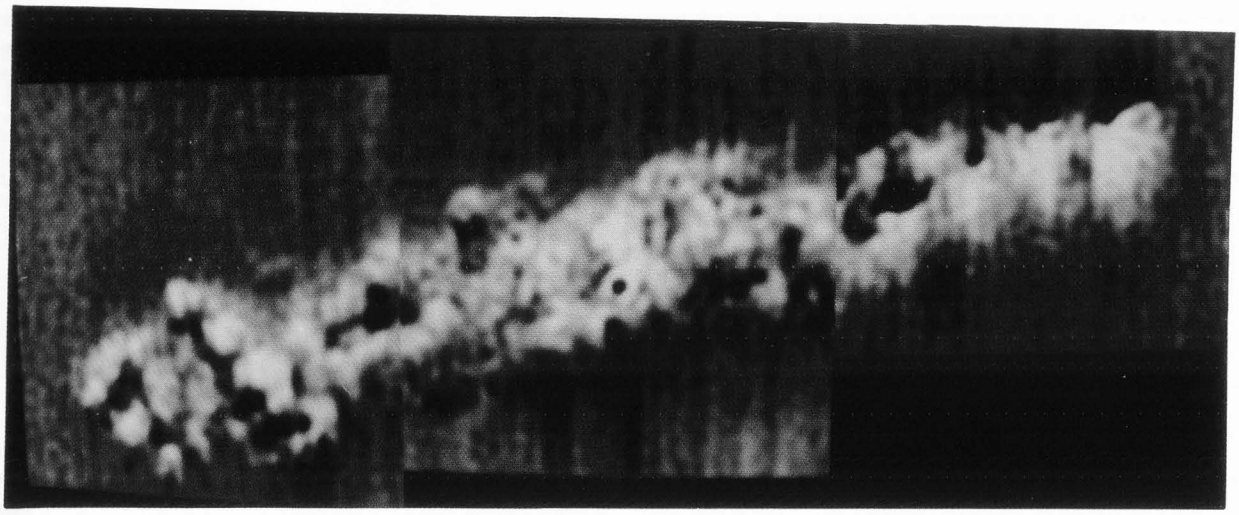

Figure 3a.

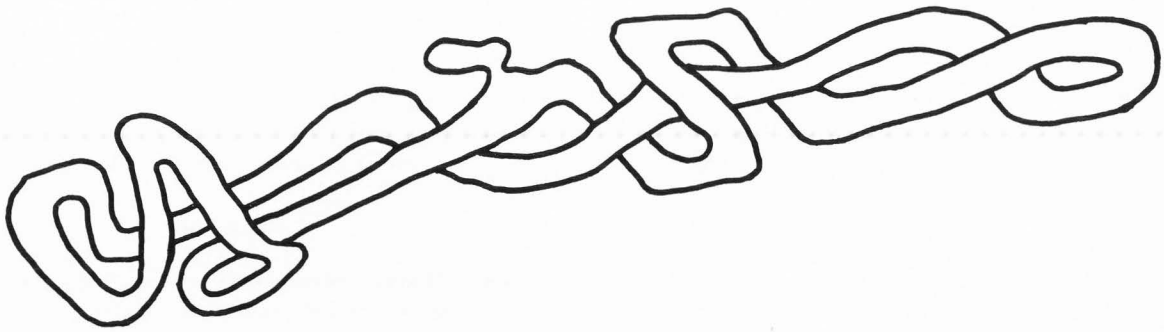

Figure 3b.

deposited biomolecule structures can be introduced by using HOPG as a substrate. It is, therefore a great challenge to develop new techniques which enable differentiating biomolecules from substrate artifacts. The major problem here is the steplines of graphite where various quasi one-dimensional structures can falsely be interpretated as DNA chains. This is obviously not the problem in our case since the images we presented are not associated with surface steps. Graphite flakes of various sizes can also exist as reported by Clemmer and Beebe[4]. However, these flakes were typically found in disrupted surface areas. From images of large areas scanned, we did not see any surface defects. The underlying surface of Fig. 3a is flat up to several thousand angstroms and regular graphite structure can be easily seen at high resolution. Similar results have been obtained on different samples which are prepared by the same method. Features are extremly stable and reproducible. Aggregations were found at both flat and stepped surface areas. We have also examined bare graphite surfaces without poly-L-proline and never saw those aggegates, although we occasionally observed features of long straight bright lines, as reported by other groups. All these observations indicate that features we presented above are related to the deposited poly-L-proline molecules instead of the artifacts of HOPG subtrates.

Conclusions

We have presented high-resolution STM images of poly-L-proline which reveal the existence of two fundamentally different conformational structures of poly-L-proline. One structure (type B) exhibits the repeating unit cell of poly-L-proline I helical chains with *cis*-peptide bonds. The other more aggregated and stacked structure (type A) correlates well with the structure of parallel-linked poly-L-proline II chains. Discussion of further details concerning the nature of the bonds between individual chains would require a careful determination of the height of the individual aggregates which, at present, cannot be evaluated quantitatively. The intriguing observations, presented here, point towards future possibilities and challenges to unravel the architecture of more complex proteins thus allowing a deeper comprehension of their special biological function.

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References

1. Blout ER, Bovey FA, Goodman M, Lotan N. (1974). Peptides, Polypeptides and Proteins, Wiley J. & Sons, New York, pp. 26-189.

2. Caswell DS, Spiro TG. (1987). Proline signals in ultraviolet resonance Raman spectra of proteins: *cis-trans* isomerism in poly proline and ribonuclease A. J. Am. Soc. 109: 2796-2800.

3. Chao YYH, Bersohn R. (1987). C- and H-NMR studies of *cis-trans* conformers of oligoprolines. Biopolymers 17: 2761-2767.

4. Clemmer CR, Beebe Jr TP. (1991). Graphite: A mimic for DNA and other molecules in scanning tunneling microscope studies. Science 251: 640-642.

5. Cowan PM, McGavin S. (1955). Structure of poly-L-proline. Nature 176: 501-503.

6. Edsall JT. (1954). Configuration of certain protein molecules. An inquiry concerning the present status of our knowledge. J. Polym. Sci. 12: 253-280.

7. Edsall JT, Wyman J. (1958) Biophysical Chemistry, Vol. 1, Academic Press, New York, pp. 14-135.

8. Gould S, Marti O, Drake B, Hellemans L, Bracker CE, Hansma PK, Keder NL, Eddy MM, Stuky GD. (1988). Molecular resolution images of amino acid crystals with the atomic force microscope. Nature 332: 332-334.

9. Isemura T, Okabayashi H, Sakakibara S. (1968). Steric structure of L-proline oligopeptides. I. Infrared absorption spectra of the oligopeptides and poly-L-proline. Biopolymers 6: 307-321.

10. Jericho MH, Blackford BL, Dahn DC, Frame C, Maclean DJ. (1990). Scanning tunneling microscopy imaging of uncoated biological material. Vac. Sci. Technol. A8: 663-666.

11. Lindsay SM, Thundat T, Nagahara L, Knipping U, Rill RL. (1989). Images of the DNA double helix in water. Science 244: 1017-1116.

12. Madison V, Schellman J. (1970). Location of proline derivatives in conformational space. I. Conformational calculations; optical activity and NMR experiments. Biopolymers 9: 511-567.

13. Pauling L. (1940). A theory of the structure and process of formation of antibodies. J. Am. Chem. Soc. 62: 2643-2657.

14. Pauling L, Corey RB. (1951). The structure of proteins: Two hydrogen-bonded helical configurations of the polypeptide chain. Proc. Natl. Acad. Sci. U. S. 37: 205-211. Atomic coordinates and structure factors for two helical configurations of polypeptide chains. Natl. Acad. Sci. U. S. 37: 235-285. Configurations of polypeptide chains with favored orientations around single bonds: Two new pleated sheets. Natl. Acad. Sci. U. S. 37: 729-740.

15. Salmeron M, Beebe T, Odriozola J, Wilson T, Ogletree DF, Siekhaus W. (1990). Imaging of biomolecules with the scanning tunneling microscope: Problems and prospects. Sci. Technol. A. 8: 635-641.

16. Traub DW, Shmueli U. (1963). Structure of poly-L-proline I. Nature 198: 1165-1166.

Discussion with Reviewers

B.L. Blackford: These are interesting, intriguing images of Poly-L-Proline helixes, if they can be taken at face-value. The problem is that HOPG was used as the substrate and it is now becoming increasingly clear that bare HOPG can produce many images which look

deceptively like the desired bio-structures. See the dramatic recent results of Clemmer and Beebe, Science 251, 640 (1991). Please comment.

Author: We agree that the graphite artifacts can be falsely interpreted as the deposited molecules when used as a substrate in biological application of STM. Like other research groups, we have examined a large number of uncoated graphite surfaces and found that most of these artifacts were associated with the surface steps, as well as those long straight line features which were probably the carbon fibers. However, features we presented in the text were only observed after the poly-L-proline samples were deposited. Our belief in these STM images as real poly-L-proline features relied on the following several observations, although not direct proofs: the right dimensions as we measured from Fig. 3a, similar results, similar shape and average size of aggregates which we could obtained on different samples and using different probing tips.

Reviewer #4: How reproducible is the image shown in Fig. 3a? Is it only seen when poly -L-proline is applied to the graphite? What was the basis for the drawing in Fig. 3b. Is that the only interpretation that could be made of the image?

Author: The image shown in Fig. 3a is very reproducible and is so stable that we were able to acquire this mosaic image by scanning over dfferent sections of the helix at high magnification. Uncoated graphite surfaces were also imaged and we never found features similar to what we presented in the text. We agree that there do exist other possible intepretations of Fig 3a. By comparing with other STM images of the same feature acquired at both low and high magnfications, we believe the conformation shown in Fig. 3b is the likely one. Nevertheless, much better molecular resolution images are definitely needed in order to clarify the detailed structures at those bending positions and this is also the goal of our future work.