Scanning Tunnelling Microscopy of Poly(γ-benzyl L-glutamate)

The technique of scanning tunnelling microscopy[1,2] (STM) offers great potential for the study of biological macromolecules. Although the exact mechanism for electron tunnelling through biopolymers is still unclear, STM offers the advantages of relatively straightforward sample preparation, imaging under "native" conditions in liquid or gaseous environments, and the possibility of achieving atomic resolution.[3] A range of biological systems have been studied including lipid bilayers,[4] bacteriophage,[5] proteins,[6-8] DNA,[9-14] and DNA complexes.[15] Studies of proteins are still limited[6-8] and have failed so far to produce images of the secondary structural features of these biopolymers.

This paper reports studies on the synthetic polypeptide poly(γ-benzyl L-glutamate) (PBLG). Preliminary STM studies of PBLG have been published elsewhere.[16] Such biopolymers can be used as models for the secondary structures adopted by naturally occurring peptide sequences in proteins. Extensive studies[17-19] have been made on the secondary structures adopted by PBLG in different solvents. At room temperature dilute solutions of PBLG in dimethyl formamide (DMF) contain molecularly dispersed α-helices. At polymer volume fractions above 0.15 these helices associate to form lyotropic liquid-crystalline structures.[20,21] Fibrillar aggregates can be obtained by precipitating PBLG from DMF or certain other solvents,[22-24] while slow casting from solution produces ordered crystalline structures.[25]

PBLG of molecular weight 70 000 (Sigma Chemicals, U.K.) was dissolved in DMF at a concentration of 1 mg mL^{-1}. A 5-μL drop of this solution was then deposited onto a freshly cleared surface of highly oriented pyrolytic graphite (HOPG) of dimensions 5 × 5 × 1 mm (Union Carbide, Geneva, Switzerland). The HOPG surface was covered and the sample allowed to dry for 18–24 h before examination by STM. The objective of the preparation technique was to slowly evaporate the DMF, thus inducing liquid-crystalline ordering and favoring the formation of an ordered structure on the HOPG surface. The advantages of this approach are that large ordered structures are easy to locate on the substrate, the molecules are unlikely to be moved during scanning, and the ordered array facilitates image enhancement methods.

The STM was a commercial instrument (WA Technology, Cambridge, U.K.). It was operated in air at ambient temperature and pressure. The interatomic spacings of a graphite lattice were used to calibrate the apparatus. Tips were produced by electrochemically etching tungsten in a 2 M KOH solution. Typical STM operating conditions were a tunnel current of 0.1 nA and a bias voltage of 500 mV. The STM was operated in the constant-current mode, and acquisition times for a 256 × 256 pixel raster ranged from 46 to 404 s. Images of regular repeating structures were enhanced by computer filtration using a fast Fourier transform method to enhance periodic features and reduce noise.

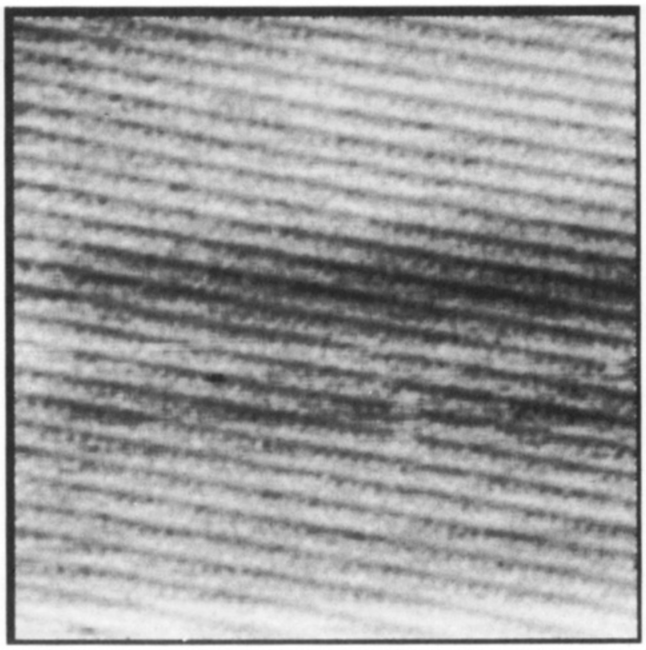

Figure 1. Low-magnification image of PBLG on HOPG. The scan area is 800 × 800 Å. The tunnel current and bias voltage were 0.1 nA and 500 mV, respectively. This image has been low-pass filtered.

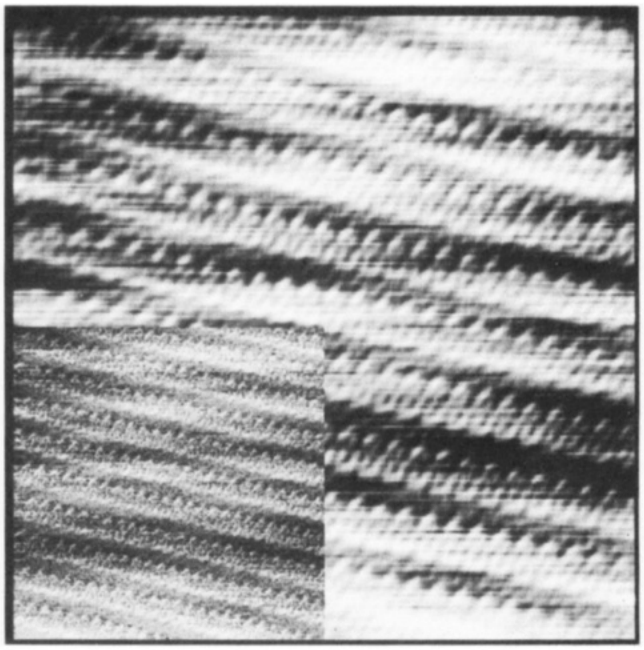

Figure 2. Main figure is a 400 × 400 Å scan, which was processed using a Fourier transform method. The inset shows the raw data to the same scale as the main figure. It is apparent that this image processing has merely removed noise, leaving periodic features intact. Tunnelling conditions were 0.1 nA and 500 mV.

Figure 1 shows a typical large area scan of PBLG on HOPG. This type of regular, long-range ordered array of rodlike PBLG molecules was observed to occur over areas of at least 4000 Å^2. Fourier filtering of the images emphasizes the helical character of the PBLG molecules (Figure 2). The nonperiodic component of the diffraction pattern was removed, and the periodic features were back-transformed to produce the Fourier-filtered image. The inset in Figure 2 shows the unprocessed data. Figure 2 shows a bright helical structure of pitch 1.61 nm, width 2.9 nm, and a row spacing of 4.12 nm. Figure 3 shows a Fourier-filtered image of another area obtained at a higher magnification. The helical parameters are similar with a pitch of 1.65 nm and a width of 2.3 nm. However, the row spacing of 3.3 nm is considerably smaller. Figure 4 is the Fourier transform used to process Figure 2. It can be seen that the pattern is more akin to a two-dimensional crystal lattice than a liquid-crystalline structure.

PBLG characteristically adopts an 18_5 α-helical structure. The axial rise per residue is 0.15 nm, the axial rise per helical turn is 0.54 nm, and the helix repeats every 18

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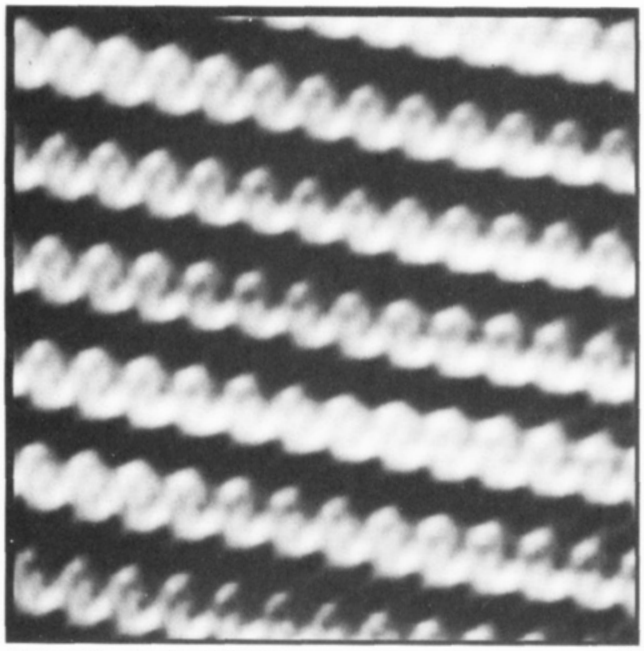

Figure 3. Fourier-filtered image of scan area 200 × 200 Å. Tunnelling conditions were 0.1 nA and 500 mV. The helical nature of the image is clearly visible.

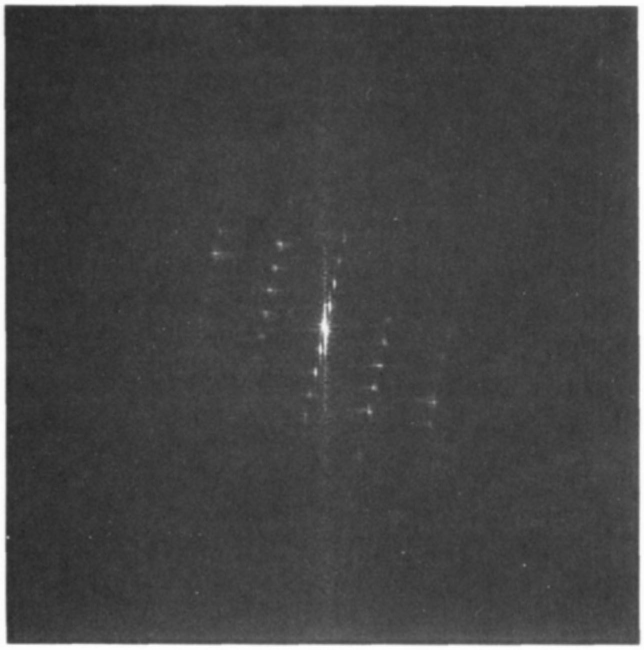

Figure 4. Fourier transform used to process Figure 2.

residues forming 5 turns of the helix with a repeat distance or pitch of 2.7 nm. PBLG films cast from DMF exhibit a number of crystalline forms,[25,26] and thus it is perhaps not too surprising that different row spacings are observed in Figures 2 and 3. If the bright helical structure represents the PBLG molecules, then it is necessary to explain the observed width and pitch of the helix.

Coiled coils of the three- or six-strand type[27,28] or an aggregated supercoil of some form[24] could explain the width of the bright region, but the pitch of these structures would be too large. When the PBLG molecules pack together as an ordered structure, the orientation and disposition of the side chains, and more particularly the benzyl groups, are of interest. X-ray crystallography of DMF-wetted PBLG[29,30] has suggested a cooperative stacking of the benzene rings between nearest neighbors in a quasi-helical arrangement with a periodicity of 5.5 nm. A similar stacking arrangement has also been observed in solid films[25] and may be produced at the expense of the backbone conformation. The benzene rings are particularly interesting because such aromatic rings appear to be clearly imaged by STM.[31] It is possible that the present images represent a projection, of a helical array of benzyl substituents on the circumference of the normal helical backbone structure of PBLG, onto the graphite surface and viewed through the "invisible" backbone peptide helix. Complete extension of the side chains would be necessary to account for the width of the bright helical regions. Despite the variability of the row spacings, the helical parameters in Figures 2 and 3 remain fairly constant. This suggests that intra- rather than interhelical interactions of the side chains, or interactions of the side chains with the graphite lattice, are important in determining helix stability. It is suggested that such intrahelical interactions of the side chains, or interactions of the side chains with the graphite surface, cause a slight distortion of the peptide backbone. The 18_5 helix shows a near repeat at 10.8 residues in 3 turns at a spacing of 1.62 nm. Such a structure is very similar to an 11_3 helix showing a repeat after 11 residues in 3 turns of 1.65 nm. This would account for the observed pitch of the experimental helix. Certainly Watanabe and Uematsu[25] have observed a metastable 7_2 helical structure for PBLG in films cast from solutions of PBLG in chloroform. In this case stabilization of the structure was attributed to an interhelical cooperative stacking of benzene rings at the ends of the side chains.

In order to explain the structure of the experimentally observed helix, it is necessary to assume that the intrahelical interactions of the side chains, or their interactions with the graphite surface, cause an orientation that, in projection through the peptide backbone onto the graphite surface, repeats with the helical pitch of 1.65 nm, rather than the pitch of 2.7 nm characteristic of the 18_5 helix. The proposed models, and the role played by the benzyl substituents, can be tested experimentally by studies on other substituted polyglutamate helices.

References and Notes

(1) Binnig, G.; Rohrer, H.; Gerber, Ch.; Wiebel, E. *Phys. Rev. Lett.* **1982**, *49*, 57.
(2) Quate, C. F. *Phys. Today* **1986**, *38*, 26.
(3) Hansma, P. K.; Elings, V. B.; Marti, O.; Bracker, C. E. *Science* **1988**, *242*, 209.
(4) Smith, D. P. E.; Bryant, A.; Quate, C. F.; Rabe, J. P.; Gerber, Ch.; Swalen, J. B. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 969.
(5) Baro, J. H.; Miranda, R.; Alaman, J.; Garcia, N.; Binnig, G.; Rohrer, H.; Gerber, Ch.; Carrascosa, J. L. *Nature* **1985**, *315*, 253.
(6) Dahn, D. C.; Watanabe, M. O.; Blackford, B. L.; Jericho, M. H.; Beveridge, T. J. *J. Vac. Sci. Technol., A* **1988**, *6*, 548.
(7) Welland, M.; Miles, M. J.; Lambert, N.; Morris, V. J.; Coombs, J. H.; Pethica, J. B. *Int. J. Biol. Macromol.* **1989**, *11*, 29.
(8) Feng, L.; Andrade, J. D.; Hu, C. Z. *Scanning Microsc.* **1989**, *3*, 399.
(9) Keller, D. J.; Bustamante, C.; Keller, R. W. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 5356.
(10) Arscott, P. G.; Lee, G.; Bloomfield, V. A.; Evans, D. F. *Nature* **1989**, *339*, 484.
(11) Lee, G.; Arscott, P. G.; Bloomfield, V. A.; Evans, D. F. *Science* **1989**, *244*, 475.
(12) Beebe, T. P.; Wilson, T. E.; Ogletree, D. F.; Katz, J. E.; Balhern, R.; Salmeron, M. B.; Siekhaus, W. J. *Science* **1988**, *243*, 370.
(13) Lindsay, S. M.; Thundat, T.; Nagahara, L.; Knipping, U.; Rill, R. L. *Science* **1989**, *244*, 1063.
(14) Dunlap, D. D.; Bustamante, C. *Nature* **1989**, *342*, 204.
(15) Amrein, M.; Stasiak, A.; Gross, H.; Stoll, E.; Travaglini, G. *Science* **1988**, *240*, 514.
(16) McMaster, T. J.; Carr, H. J.; Miles, M. J.; Cairns, P.; Morris, V. J. *J. Vac. Sci. Technol., A* **1990**, *8*, 648.
(17) Doty, P.; Bradbury, J. H.; Holtzer, A. M. *J. Am. Chem. Soc.* **1956**, *78*, 947.

(18) Kihara, H. *Polym. J.* **1977**, *9*, 443.
(19) Miller, W. G.; Russo, P. S.; Chakrabarti, S. *J. Appl. Polym. Sci., Appl. Polym. Symp.* **1985**, *41*, 49.
(20) Wee, E. L.; Miller, W. G. *J. Phys. Chem.* **1971**, *75*, 1446.
(21) Russo, P.; Miller, W. G. *Macromolecules* **1988**, *16*, 1690.
(22) Ishikawa, S.; Kurita, T. *Biopolymers* **1964**, *2*, 381.
(23) Tachibana, T.; Kambara, H. *Kolloidn. Zh.* **1967**, *219*, 40.
(24) Rybnikar, F.; Geil, P. H. *Biopolymers* **1972**, *11*, 271.
(25) Watanabe, J.; Uematsu, I. *Polymer* **1984**, *25*, 1711.
(26) Watanabe, J.; Imai, K.; Uematsu, I. *Macromolecules* **1986**, *19*, 1489.
(27) Crick, F. H. C. *Acta Crystallogr.* **1953**, *6*, 689.
(28) Pauling, L.; Corey, R. B. *Nature* **1953**, *171*, 59.
(29) Parry, D. A. D.; Elliott, A. *J. Mol. Biol.* **1967**, *25*, 1.
(30) Squire, I. M.; Elliott, A. *Mol. Cryst. Liq. Cryst.* **1969**, *7*, 457.
(31) Foster, J. S.; Frommer, J. E. *Nature* **1988**, *333*, 542.

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