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Scanning tunneling microscopy of a wheat seed storage protein reveals details of an unusual supersecondary structure

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ABSTRACT **Scanning tunneling microscopy has been used to demonstrate that a spiral structure based on β-reverse turns is adopted by the repeat sequences present in a group of wheat gluten proteins. This structure is similar to the β-spiral formed by a synthetic polypentapeptide based on a repeat sequence present in elastin. Wheat gluten and elastin are both elastomeric and it is possible that the spiral structure contributes to this property.**

Scanning tunneling microscopy (STM) and the derivative scanning probe techniques can produce high-resolution images of structures at the atomic and molecular levels. Already used as a tool in surface science (1), many of the properties of STM have great potential for the study of biopolymers. The STM can operate in air and even in liquid to image uncoated and unstained biomolecules deposited on a conducting surface. This allows biopolymers in their native hydrated state to be imaged (2–5). STM images of DNA have confirmed the details of the helical structure established by x-ray diffraction, giving confidence in this form of microscopy (6–10). STM can, therefore, be used to image structures that, to our knowledge, have not been described by other techniques. In the present study STM has been used to study the structure of a high molecular weight (HMW) subunit protein from wheat gluten for which an unusual structure has been predicted from the amino acid sequence and on the basis of other physicochemical studies.

The HMW subunits of wheat gluten appear to be largely responsible for the elastic behavior of dough. Analyses of genomic clones encoding several subunits have shown that they have similar structures (11, 12). Each protein consists of a central repetitive domain, varying in length from about 640 to 830 residues, flanked by shorter nonrepetitive N (81–104 residues)- and C (42 residues)-terminal domains. The HMW subunits are classified into two groups on the basis of their molecular weights, x-types (molecular weights in the range 83,000–88,000) and y-types (molecular weights in the range 67,000–74,000). The central repetitive domains are based on three motifs. Hexapeptides (consensus Pro-Gly-Gln-Gly-Gln-Gln) and nonapeptides (consensus Gly-Tyr-Tyr-Pro-Thr-Ser-Pro/Leu-Gln-Gln) are present in both x- and y-type subunits and tripeptides (consensus Gly-Gln-Gln) in x-type subunits only. The central domains are predicted to form regularly repeated β-reverse turns (13). Further evidence for the presence of β-reverse turns has come from spectroscopic (circular dichroism and Fourier-transform infrared) studies of synthetic peptides corresponding to the repeat motifs present in both x- and y-type subunits (14). Hydrodynamic studies of a single purified x-type HMW subunit from durum wheat showed an extended rod-like conformation and it was proposed that the β-reverse turns form a loose spiral (15). Modeling of several proteins containing β-reverse turns has indicated the possibility of spiral structures (16), and detailed studies of a synthetic polypentapeptide based on the repeat motif of elastin have provided direct evidence for β-spiral formation in a model system (17). In the present paper we use STM to demonstrate that a similar structure is formed by a homogeneous preparation of a wheat HMW subunit deposited from solution onto a graphite surface.

MATERIALS AND METHODS

HMW Subunit Preparation. A single HMW subunit [subunit 20, according to the nomenclature of Payne and Lawrence (18)] was prepared from durum wheat (*Triticum durum*) cv. Bidi 17 as described (15). The disulfide bonds were reduced and pyridethylated, to prevent disulfide bond reformation and aggregation. The HMW subunit was then purified to homogeneity by reverse-phase HPLC using a water/acetonitrile/trifluoroacetic acid solvent system (19).

Scanning Tunneling Microscopy. The HMW subunit was dissolved in trifluoroethanol at a concentration of 200 μg/ml. A 10-μl drop was deposited on a highly oriented pyrolytic graphite (HOPG; Union Carbide, Cleveland, OH) substrate. The protein has a hydrophobic nature and might be expected to adsorb well to the HOPG substrate. Solution behavior indicated a rod-like conformation (15) suggesting that an ordered adsorbed structure might be achieved by the liquid-crystal phase, as has been observed in STM studies of other molecules (20, 21). The solvent was allowed to evaporate slowly to permit the formation of ordered regions. The resulting thin layers were studied in an STM (WA Technology, Cambridge, U.K.). The microscope was operated in air in the constant-current mode, with typical values of bias and tunnel current of 800 mV and 70 pA. An electrochemically etched tungsten probe was used. Image magnification was calibrated against STM images of the graphite lattice with its known lattice constant.

Image Processing. Diffraction patterns were obtained by computer Fourier transformation of STM images. Noise reduction was achieved by partial subtraction of the nonperiodic component in the Fourier transforms followed by inverse transformation of all the diffraction spots (22).

RESULTS AND DISCUSSION

STM images were obtained of the HMW subunit preparation deposited onto the HOPG substrate. When the protein was deposited by slow evaporation of a solution in trifluoroethanol, the individual protein subunits formed a sheet in which the molecules were aligned. Fig. 1*A* is an unprocessed image

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Abbreviations: STM, scanning tunneling microscopy; HMW subunit, high molecular weight subunit; HOPG, highly oriented pyrolytic graphite.

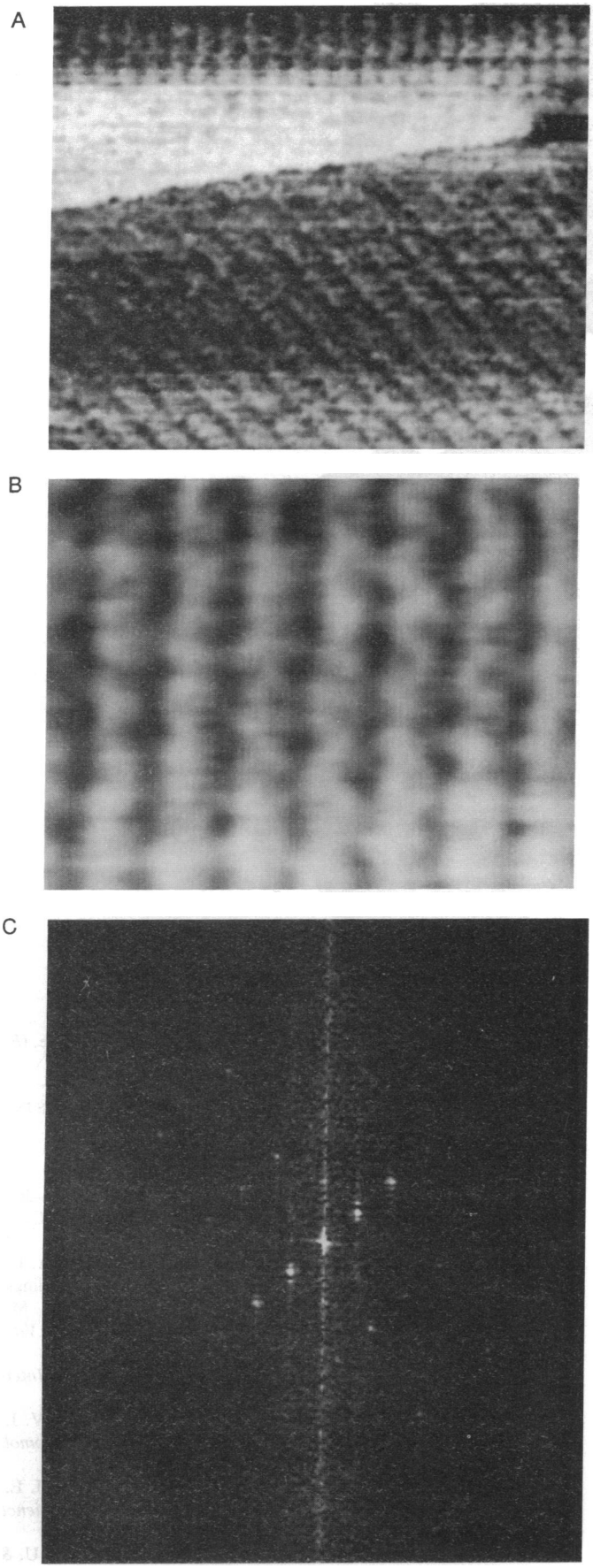

FIG. 1. (*A*) Unprocessed STM image (60 nm × 41 nm) of HMW subunit protein molecules, showing two ordered domains of rod-like molecules. (*B*) Unprocessed STM image at higher magnification, showing periodicity along rod-like structures. (*C*) Two-dimensional Fourier transform of lower domain in *A*.

of such a preparation and shows a region where aligned rods in different orientations meet, possibly at a step in the graphite surface. It is unlikely that these adsorbed domains are more than one layer thick, because of the size of the gap between the tip and graphite surface under the tunneling conditions employed. An image of the HOPG substrate at this magnification showed no detail (image not shown). The image observed is, therefore, unlikely to be an artifact of the substrate surface. In some of the structures observed it was possible, at higher magnification of an unprocessed image, to observe a periodicity along the rod (Fig. 1*B*). Such images were reproducible, being obtained on several occasions with different protein solutions. They were also stable, having been observed for more than 1 hr. The N- and C-terminal domains of the HMW subunits are thought to be predominantly α-helical when the protein is dissolved in trifluoroethanol (15). They could not be identified in the STM images but may have been present in the poorly resolved regions. The reason for this is not known but could result from the absence of a regular structure in the hydrated solid state.

Fourier transformation of the lower domain in Fig. 1*A* resulted in a diffraction pattern (Fig. 1*C*) consistent with a degree of order greater than that of a liquid-crystal structure. This indicated that the rods, and the structures along their length, lie on a two-dimensional lattice. The diffraction pattern suggests two-dimensional order with a periodicity along the molecule of 1.49 nm (1.58 nm in the upper domain of Fig. 1*A*) and a lateral spacing of the molecules of 3.10 nm. The apparent space between the rods may result from the interaction between protein molecules mediated by solvent molecules, which are not visible in the STM. The effect could also be one of epitaxy, the HMW subunits aligning themselves along the lattice of the graphite surface. Deformation of proteins due to interaction with the substrate is a potential problem in all STM or conventional electron microscopy studies. In the present case we do not consider that this would cause major alteration in the structure. This is because the HMW subunit structure is known to be stable in a range of solvents and temperatures and resistant to denaturation (15). The adsorption forces, therefore, would not be expected to cause a distortion of its conformation greater than involved in its putative functional role as an elastomer. The possibility that the structure is itself induced by interaction with the graphite is unlikely. Comparison of DNA dimensions derived from STM and x-ray crystallography shows that they are very similar (23), indicating that the molecule–graphite interaction had not significantly affected the structure.

Fourier filtering of the images was used to enhance the periodic diffraction signals along the molecules, resolving clear diagonal striations (Fig. 2). From these processed images the apparent diameter of the molecule is 1.95 nm. This value is consistent with results from hydrodynamic measurements (15), which suggest a rod-like structure for the HMW subunit with dimensions of 55 × 1.8 nm in 0.05 M acetic acid or 50% (vol/vol) aqueous 1-propanol and 65 × 1.55 nm in trifluoroethanol. The images indicate a loose spiral structure but do not reveal fine details, in part due to the effect of the filtering. The diameter of the spiral is similar to that formed by the synthetic polypentapeptide of elastin (diameter, 1.8 nm) (23), but the pitch is greater, 1.49 nm for the HMW subunit and 0.95 nm for the polypentapeptide. This difference would be anticipated, as there is no sequence homology between the polypentapeptide of elastin (Val-Pro-Gly-Val-Gly) and the repeat motifs of the HMW subunit (Pro-Gly-Gln-Gly-Gln-Gln, Gly-Tyr-Tyr-Pro-Thr-Ser-Pro/Leu-Gln-Gln, Gly-Gln-Gln).

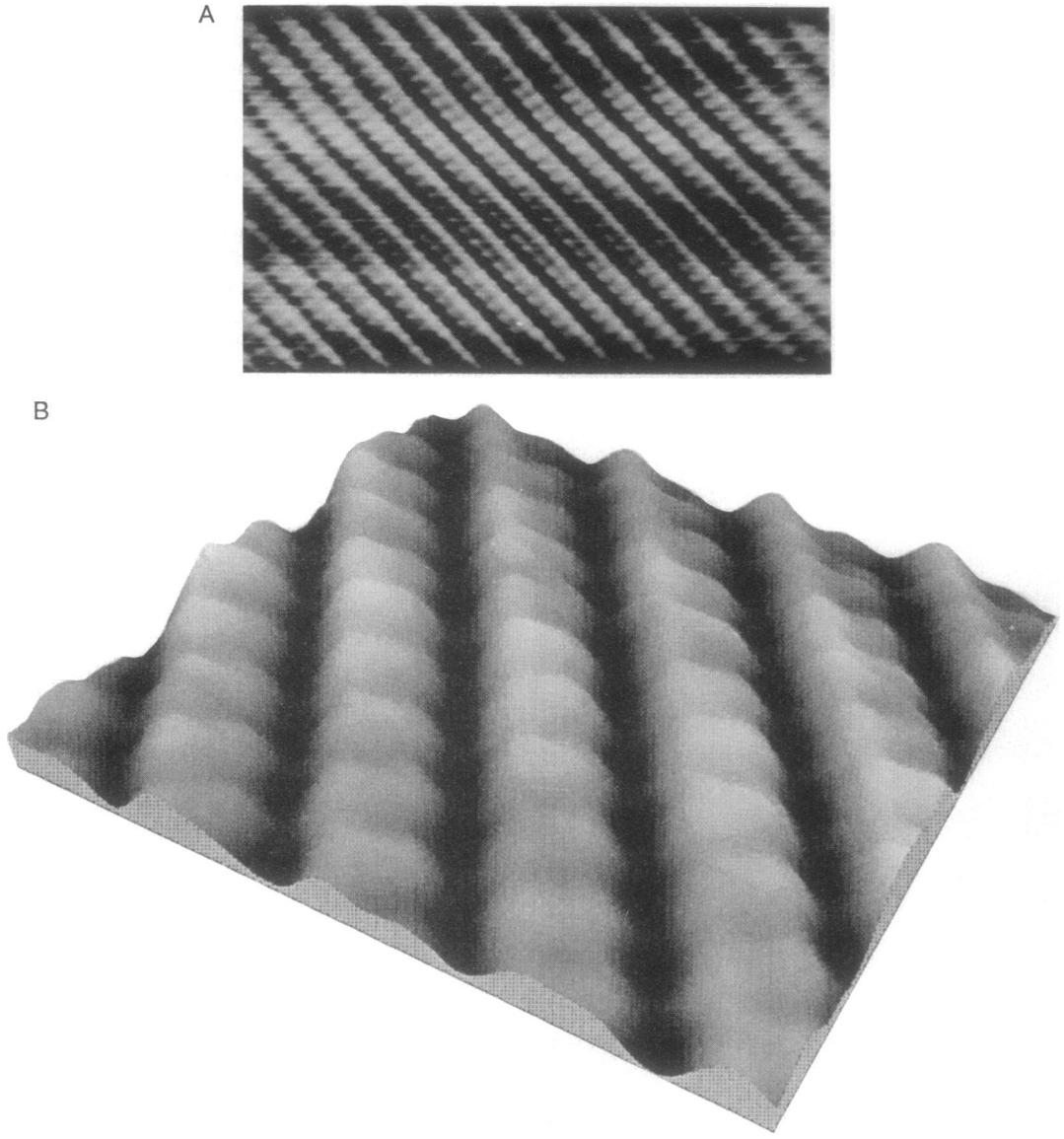

FIG. 2. (*A*) Fourier-filtered STM image (54.5 nm × 37.4 nm). Inverse transform of Fig. 1*C* after partial subtraction of background noise. (*B*) Three-dimensional representation of part of *A* (16 nm × 11 nm).

The STM images of the HMW subunit protein shown in Figs. 1 and 2 provide information on the dimensions and periodicities of the molecule. The mechanisms by which the images are produced are not clearly understood (20, 24, 25), but for proteins they probably result from a combination of the shape (topography) of the molecule on the graphite surface and a function of the conductance of electrons.

Measurements of the thickness of the layer of HMW subunits give an average value of approximately 1.2 nm, which is less than expected if the diameter of the spiral is 1.95 nm. This could result from the protein being an imperfect conductor. Values of thickness obtained in several recent STM studies of biological molecules have been in the range of one-third to one-half of the expected thickness. Our value for the HMW subunit fits into this pattern.

Thus with earlier spectroscopic and hydrodynamic studies, the STM images in Figs. 1 and 2 demonstrate that the repeat motifs present in the HMW subunit form a spiral supersecondary structure, based on regularly repeated β-reverse turns. It remains to be determined whether this structure has any functional role in the elastomeric properties of wheat gluten. The cross-linked polypentapeptide of the elastin repeat motif exhibits elastomeric behavior (26), but the relevance of this to the elastomeric properties of elastin is not established.

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