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Short communication

Scanning tunnelling microscopy in biology

Are the current carriers tunnelling electrons or hydrated protons?

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Since its initial development and use in physical systems by Binnig and Rohrer [1,2], there has been a burgeoning interest in the application of scanning tunnelling microscopy (STM) to biology and biotechnology (e.g. refs. 3–6). The principle of the technique, by now well known, is that a probe, whose tip is virtually of atomic sharpness, is scanned across a (conductive) surface and the current between the tip and the surface measured. The relationship between the current and the (horizontal and) vertical position of the tip is then used to derive an image of the surface and any superincumbent molecules, either directly or (in "constant current" mode) via feedback.

I would like to point out that two assumptions continue to be made about the images derived from biomolecules such as proteins and oligonucleotides using this technique: (i) that they are actually based on a tunnelling current, and (ii) that electrons are the current carriers. Since it is only an electrical current that is measured and used in the feedback loop which positions the probe, neither of these assumptions should pass untested.

There is an extensive literature [7–10] on the conductivity of hydrated biomacromolecules in macroscopic samples. From this, one may discern, inter alia, (i) that the conductivity of proteins is strongly dependent on their hydration, and (ii) that proteins retain water of hydration even at extremely low water activities. Thus, even under the conditions of high vacuum sometimes (but not invariably) used in STM, it is probable that the dominant carriers taking the current between the upper surface of the biomolecule and the surface on which it is positioned are not in fact electrons but (hydrated) protons.

A piece of evidence that is sometimes taken to be consistent with electron tunnelling as the source of the image in the STM of biomolecules is that the distance dependence of the current observed is consistent with the theory [11] for semiconducting systems. However, Haggerty et al. [5], for instance, studied lysozyme and chymotrypsinogen A, whose known smallest dimensions (from X-ray analysis) are respectively 30 and 40 Å, distances far larger than an electron is known to be able to tunnel with any significant probability.

The extent to which, or even whether, (hydrated) protons are (contributors to) the current in STM could be tested in many ways. Perhaps the simplest way is by changing the electrode metal used as the probe, from materials such as Wo or Pt, to Pd under a H_2 atmosphere, the latter but not the former being capable of generating protons. This should be expected to affect the apparent image in predictable ways.

A second way is to vary the electronic conductivity of the protein. Heller and colleagues (e.g. ref. 12) have described means by which the genuinely electronic conductivity of a protein may be increased substantially via chemical modification, for use in amperometric biosensing devices, presumably (in that enzymatic activity is retained) without gross structural changes. If the STM current around/through the protein is mainly electronic, such modified proteins should have an apparently much smaller height; by contrast, if the current is largely protonic, their size should not appear substantially to differ from their unmodified parent proteins.

Similarly, one may change the protonic conductivity of a protein in a manner that does not cause significant structural changes, either by varying its extent of hydration or the pH. If the STM current around/through the biomolecules is mainly protonic, their apparent size should vary greatly as a function of such treatments; by contrast, if the current is largely electronic, their size should not appear to change significantly. Equally, substitution of D_2O for H_2O will also have a predictable effect on protonic conductivity [13] but a negligible one on electronic tunnelling.

Lastly, the conductivity of proteins is well known to be frequency-dependent [7–10] (and, indeed, many of the probes used in STM have interfaces that are virtually blocking under dc conditions). Thus analysis of the frequency dependence of the electrical currents measured (and of the possible generation of frequencies other than those applied [14,15]) might serve as a powerful mechanism for assisting the elucidation of the current carriers involved.

If the dominant current carriers are indeed protons, this might substantially affect the interpretation of the images of biomacromolecules derived using STM.

Another piece of evidence that one might use to distinguish electronic tunnelling from proton hopping is to study the i/V relationships of the STM. Indeed, Welland et al. [16] showed that whilst the i/V curve when an STM probe was held over graphite was symmetric about 0 V and approximately parabolic, that obtained when the probe was over the protein vicilin was qualitatively different, being both concave and highly asymmetric.

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