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Seeing is believing: Imaging amphiphiliccyclodextrin derived liposomes by atomic force microscopy

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COMMUNICATION

Seeing is believing: Imaging amphiphilic-cyclodextrin derived liposomes by atomic force microscopy

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The cyclodextrins are a class of cyclic oligosaccharides widely studied for their ability to include and transport a variety of organic molecules.[1] For their use in bio-pharmaceutical applications their relatively low load capacity, typically $<10\%$ active molecule by weight and the hemolytic problems associated with β-Cyclodextrin (β-CD), the most readily available molecule, have limited their use. The hemolytic problems are associated with the capacity of β-CD to include membrane components[2] and hence break down the cell membrane. We have, for a number of years, been concerned with the obtention of amphiphilic cyclodextrins with the dual aim of rendering these molecules more compatible with biological membranes and constructing liposomal delivery systems based on these novel amphiphiles.[3] Having achieved the synthesis of these systems,[4] the subsequent production of liposomes‡ proved simple,[5] and dynamic light scattering studies showed the presence of highly monodisperse assemblies with an apparent diameter of 150 nm.

The best adapted technique for imaging these assemblies is Atomic Force Microscopy (AFM),[6] an *in situ* method that does not require staining or replication. However, liposomes are very soft materials and using AFM in the standard contact mode requires very special attention to be pain to minimising the force applied by the cantilever. In fact even by using a system with a very low spring constant, liposomes are extremely difficult to image in the contact mode, resulting in deformed surfaces.

In this communication we report the AFM images of liposomes produced using the new 'Tapping' mode and compare these images with those produced using the contact mode.

The samples were prepared by placing a drop of an aqueous dispersion of the liposomes derived from the β-CD-2,3-dihexanoyl compound[4] onto a freshly cleaved mica surface and allowing the water to evaporate. The sample was mounted on a Digital Nanoscope III AFM and scanned in the two modes.

The contact mode of AFM provides excellent lateral resolution by sensing short range surface forces which arise when the sharp tip used comes into contact with the surface of a sample. Figures 1a and 1b show respectively the images obtained at xy scan dimensions of 4 μ and 500 nm. The analysis is carried out in a constant deflection mode in which the deflection o the cantilever is held constant using a feedback control and the displacement of the tip is measured. The cantilever used is of Si_3N_4 and has a spring constant

‡In this case liposome refers to a spherical structure formed amphiphilic molecules in water without implying the internal structure.

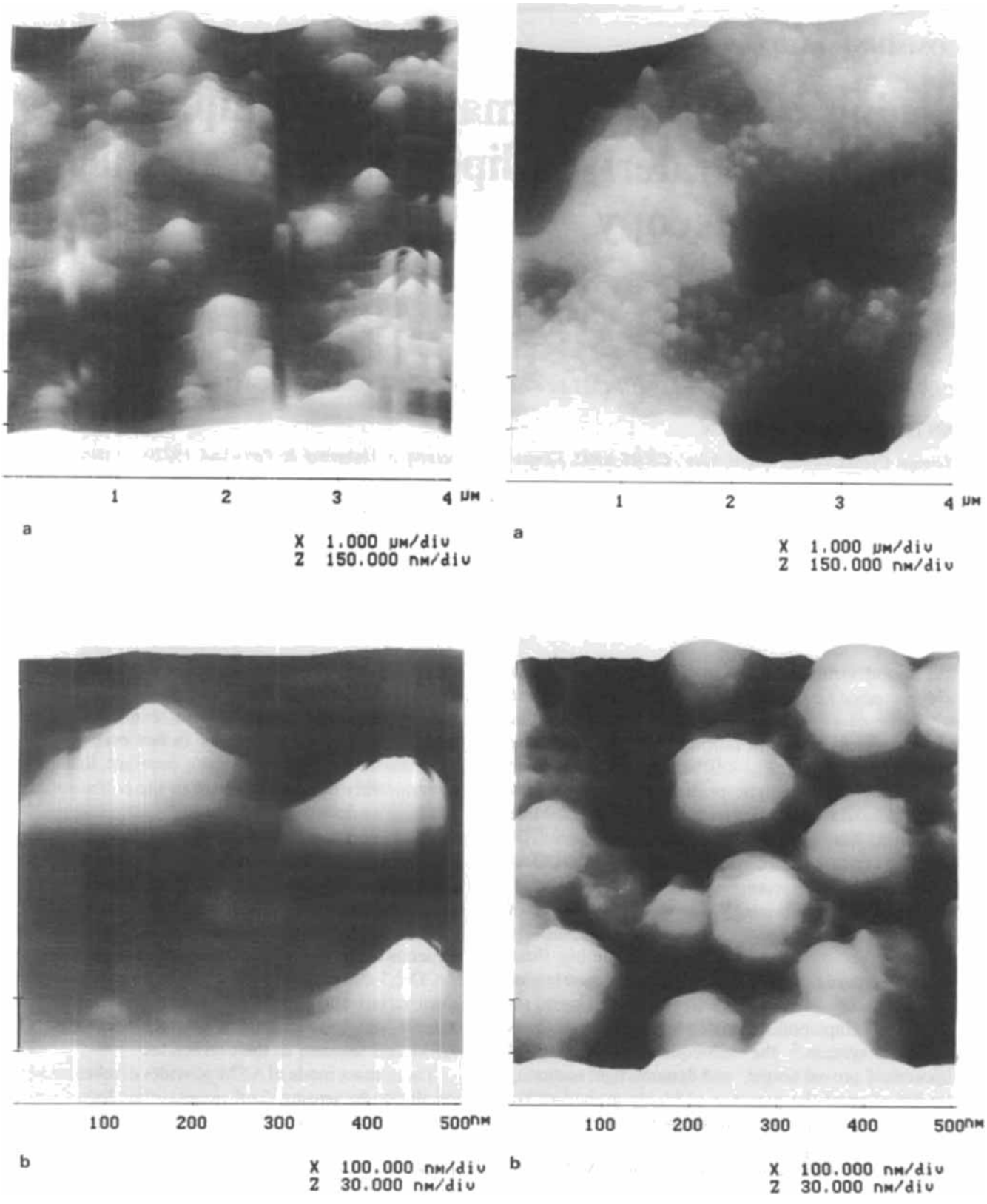

Figure 1 a) 4 m xy scan of a dried suspension of the CD-based liposomes in the Contact mode. b) 500 nm expansion of Fig 1a. (See color plate I at the back of journal.)

Figure 2 a) 4 m xy scan of a dried suspension of the CD-based liposomes in the Tapping mode, a vertical piling of the objects is clearly evidenced. b) 500 nm expansion of the lower left region of Fig 2a. (See color plate II at the back of journal.)

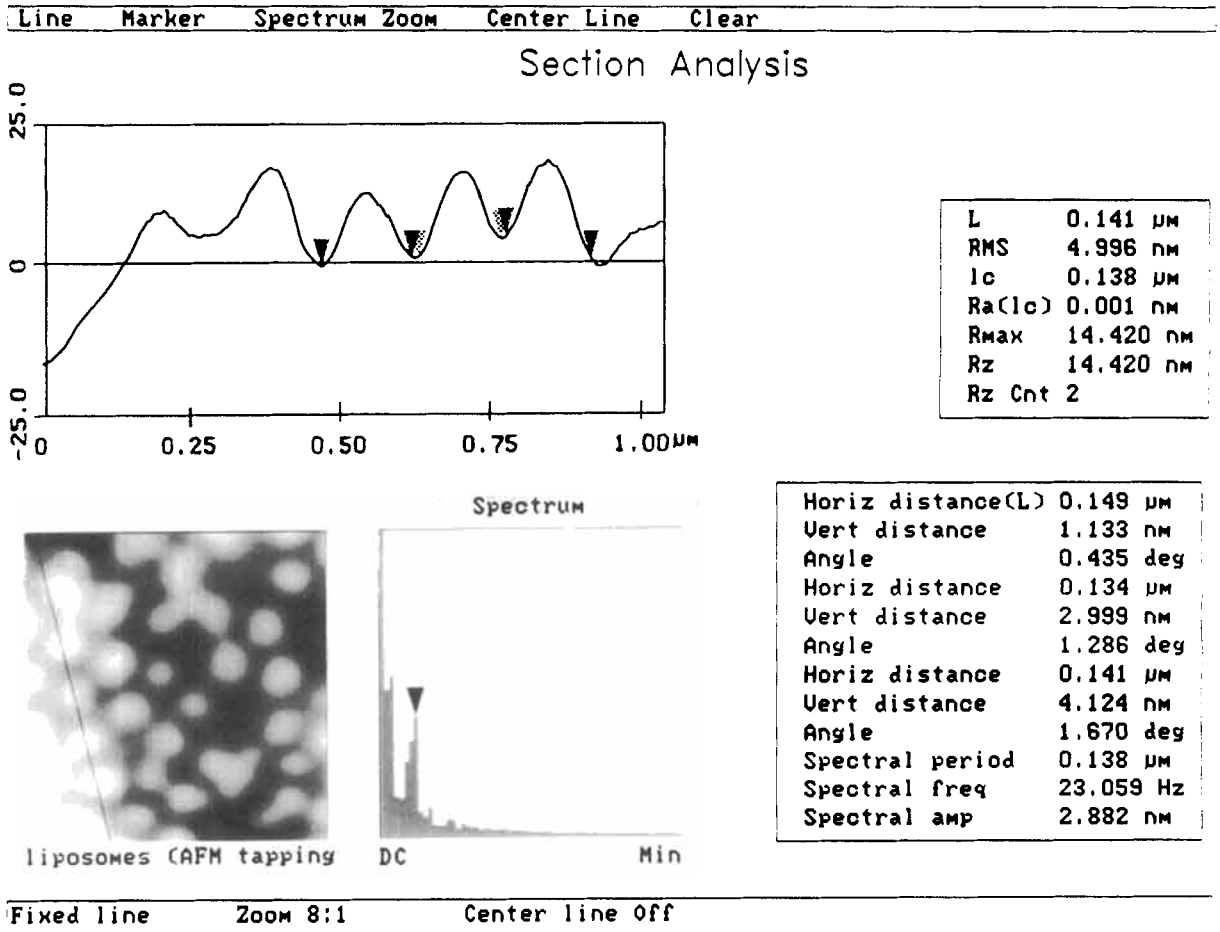

Figure 3 Cross sectional analysis of imaged objects. (See color plate III at the back of journal.)

of 0.12 Nm^{-1}, and the tip diameter is approximately 40 nm.

Even with such a low force applied to the surface, deformations of the liposomes are clearly evident. These are elongated and flattened by the tip during the scan. Differences of about 40 nm are observed in the scan direction (190 nm length) and in the orthogonal direction (150 nm width). Scratched scan lines are particularly visible on the large scale image. They are due to the high lateral forces applied to this soft sample which is also covered by a strong hydration shell.

The so-called 'Tapping' mode combines contact and non-contact modes.[7] The cantilever is oscillated at its resonant frequency and the amplitude of the oscillation is chosen to be sufficiently high so that the probe moves rapidly in and out of the contamination layer covering the sample and under these conditions the probe enters periodically into the repulsive (contact) mode. In these conditions, the resultant force applied to the surface is similar to that applied in the contact mode when the sample is observed in a liquid medium, that is to say two orders of magnitude less than the contact mode in air. The technique is particularly adapted to very soft samples and to scans in the range 200 nm to a few microns. The resolution obtained is approximately 10 nm.

In Fig 2a is shown the image obtained at a xy scan dimension of 4 μm in the 'Tapping' mode using an Si catilever with a tip diameter of about 10 nm. The liposomes are clearly visible as close packed spherical objects. The high concentration has resulted in the formation of a three-dimensional deposit in which the liposomes are piled up in a manner resembling the stacking of balls. In Fig 2b is shown an expansion of the lower left region of the image; the hexagonal packing expected for spherical objects of closely similar size is evident. This correlates well with the high degree of monodispersity observed in light scattering experiments.

The advantages in the 'Tapping' mode are very low compressive forces and the quasi absence of lateral forces responsible for the fluidity of images of very soft objects in the standard contact mode, and these are

clearly evident in Fig 3, (xy scan of 500 nm). Here the liposomes are imaged as distinct individual objects, allowing a size analysis to be undertaken as shown in the cross-section given in the lower right part of the figure. The three objects measured show diameters of 149 nm, 134 nm and 141 nm, giving a mean value of 138 nm, in close agreement with the solution value and with a dispersity within the limits measured by light scattering. The previous report of AFM on liposomes revealed only collapsed structures reflecting the lower stability of phospholipid liposomes as compared to those based on amphiphilic cyclodextrins.[8]

In conclusion we have shown that the Tapping mode of AFM is excellent for the imaging of very soft objects such as assemblies of amphiphilic molecules. The spherical geometry and high degree of monodispersity of the cyclodextrin derived liposomes has been confirmed.

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