J. Serb. Chem. Soc. *69*(2)93–106(2004)
JSCS – 3133

UDC 57.088:681.723
Original scientific paper

Scanning probe microscopy characterisation of immobilised enzyme molecules on a biosensor surface: visualisation of individual molecules

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(Received 2 June 2003)

Abstract: Scanning probe microscopy techniques were used to study immobilised enzyme molecules of glucose oxidase (GOD) on a biosensor surface. The study was carried out in order to optimise atomic force microscopy (AFM) imaging and reveal the molecular resolution of individual GOD molecules. Chemically modified AFM tips and the light tapping mode were found to be the optimal conditions for imaging soft biomolecules such as GOD. The information obtained from the AFM images included spatial distribution and organization of the enzyme molecules on the surface, surface coverage and shape, size and orientation of individual molecules. Two typical shapes of GOD molecules were found, spherical and butterfly, which are in accordance with the shapes obtained from scanning tunnelling microscopy (STM) images. Using a model of the orientation of the GOD molecules on the surface, these shapes are assigned to the enzyme standing and lying on the surface. After AFM tip deconvolution, the size of the spherical shaped GOD molecules was found to be 12 ± 2.1 nm in diameter, whereas the butterfly shapes were 16.5 ± 3.3 nm ×10.2 ± 2.5 nm. Corresponding STM images showed smaller lateral dimensions of 10 ± 1 nm × 6 ± 1 nm and 6.5 ± 1 nm × 5 ± 1 nm. The disagreement between these two techniques is attributed to the deformation of the GOD molecules caused by the tapping process.

Keywords: atomic force microscopy, scanning tunnelling microscopy, enzyme biosensors, enzyme immobilisation, glucose oxidase, self-assembled monolayers.

INTRODUCTION

Surface immobilisation of biomolecules has drawn great attention recently because it has found applications in a variety of areas including biosensors, bioelectronics and biotechnology.[1] Particularly the covalent binding of the targeting biomolecule onto an electrode modified with self-assembled monolayers (SAMs) has received considerable attention for biosensor fabrication.[2,3] This approach provides fabrication of enzyme electrodes

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with a high degree of reproducibility, molecular level control over the spatial distribution of the immobilized enzymes and the immobilisation of the enzyme close to the electrode allowing direct electron transfer to be achieved.[4]

In order to improve the systematic reliability of biosensors, the study of biosensing down to the scale of individual molecules is particularly desirable, when conducted in conjunction with conventional surface analysis and response characterization. Among several other parameters, the immobilisation of the enzyme to the electrode surface is one of crucial parameters in defining biosensor performance. Understanding the immobilisation process at a molecular level and the influence of various parameters on the resulting enzyme organization, surface distribution and enzymatic activity are of common concern.[5,6] Information about individual biomolecules, their orientation on surfaces and their functionality, as well as the homogeneity of the surface coverage could be the key for solving many challenging questions in the biosensor field.[7]

Scanning probe microscopes constitute a whole new class of instruments capable of generating 3D surface profiles of molecular structures with nanometer resolution and so provide a new approach for the study of biosurfaces and/or interfaces.[8] The number of investigations of biomolecules by atomic force micrscopy (AFM) and scanning tunnelling microscopy (STM) has increased rapidly in the last ten years. Studies have focused on all common biomolecules, including DNA, nucleic acids, enzymes, proteins and antibodies.[9,10] Many different approaches have been applied such as imaging of topography (3-D visualization), force curve measurement (*e.g.*, ligand-receptor) and compositional mapping (friction and force mapping).[9] In the biosensor field these techniques present a potentially exciting strategy for both exploring the biorecognition surface and studying the recognition events. Among biosensors, the most commonly used enzyme is glucose oxidase (GOD) and, surprisingly, there are only a few reports related to AFM and STM studies. The earliest report using AFM for characterization of an enzyme immobilisation matrix for biosensor fabrication was reported by Gooding *et al.* where the matrix of the enzyme and alkyl acrylate polymers were investigated.[11] The first molecular resolution AFM image of glucose oxidase was claimed by Quinto *et al.*[12] Study of the correlation between the topography and the activity of the immobilized enzyme presented by Zheng *et al.* showed that AFM could be a useful tool for the characterization of a biosensor surface.[13] However, a GOD biosensor prepared by immobilisation onto SAMs on gold has not been investigated by SPM and visualization of individual GOD molecules on a biosensor surface has not yet been reported.

Previously, we have studied many aspects of enzyme biosensors based on SAMs where glucose oxidase (GOD) was used as the biorecognition element.[4–6,14–19] The primary focus was to determine which parameters are important for biosensor response with regard to fabricating reproducible devices. AFM was used to study the immobilisation process of GOD on a SAM to probe the influence of enzyme concentration, deposition time and topography of the gold surface.[20] STM was used to study individual GOD molecules on a gold surface.[21] In this work, the characterisation of immobilized GOD molecules on biosensor surfaces using AFM with focus on the visualisation of individual enzyme molecules is presented. Parameters including imaging mode, tip modification, tip

geometry, applied force of the tip and image deconvolution were investigated in order to provide images giving more detail of the organization and spatial distribution, size and shape and orientation of enzyme molecules and of the biosensor surface.

EXPERIMENTAL

Materials

Gold foil (99.95 %) 25 mm × 25 mm was supplied from Peter W. Beck, Adelaide, Australia. 3-Mercaptopropanoic acid (MPA), (3-mercaptopropyl)trimethoxysilane (MPS) were obtained from Aldrich. Glucose oxidase (GOD) from *Aspergillus niger* (type VII-S), *N*-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) were obtained from Sigma. Potassium chloride, potassium dihydrogen orthophosphate, dipotassium hydrogen orthophosphate and ethanol were supplied by Ajax Chem. Pty. Ltd. (Sydney, Australia). All chemicals were of the highest quality available and used without further purification. All aqueous solutions were prepared with Milli-Q grade reagent water.

Sample preparations/GOD enzyme immobilisation

Thin gold film substrates were prepared by thermal vacuum evaporation onto freshly cleaved muscovite mica and used as precursors for the preparation of flat gold films.[18,22] Freshly prepared flat gold substrates were immersed in 20 mM MPA in an ethanol-water solution (75:25) for 12–24 hours, followed by activation in 0.002 M EDC and 0.005 M NHS for 1 h and incubation in a GOD solution (490 mg dm^{-3} in pH 5.5 buffer) for 90 min as described in our previous work.[5,16] The prepared GOD electrodes, which consisted of GOD covalently bonded onto MPA modified gold, were used for AFM characterisation.

Surface characterisation

AFM characterisation was performed using a Dimension 3000 (Digital Instruments, Santa Barbara, USA). The contact mode was used for the initial experiments and noncontact (tapping) mode was applied for the rest of this study, both employed under ambient conditions. For the tapping mode AFM measurements commercial Si cantilevers/tips (Olympus) were used. Unmodified AFM tips were used as received. Prior to chemical modification, the tips were cleaned in water plasma. The MPS modification of the tip involved a 16 h exposure to an MPS solution (1 % v/v in ethanol) followed by rinsing with ethanol and drying. In the imaging mode, topographic (height) and phase images were obtained simultaneously. Background images of bare flat gold and gold modified with MPA were taken prior to imaging of GOD modified surfaces. During an AFM experiment the optimal instrument condition (set point, amplitude, scan size, scan speed and feedback control) was adjusted to allow the best resolution of images. The different forces described as "light" and "hard" tapping, were adjusted by the magnitude of the free air amplitude (A_0) and ratio (r_{sp}) of the engaged (A) or set point amplitude to A_0 ($r_{sp} = A/A_0$). The size and shape of individual GOD molecules were analysed from images using DI off-line software using cross section analysis. Two methods for AFM tip characterisation were used, including silicon grating (TGT-01 produced by MikroMasch-Silicon-MDT ltd.) and by gold nanoparticles. Having calibrated the tip, deconvolution processes were used to obtain the corrected size of GOD molecules as described elsewhere.[23,24]

STM imaging was performed under ambient conditions using a Nanoscope II (Digital Instruments, Santa Barbara, USA). All images were obtained in the constant current mode using mechanically cut Pt/Ir tips. The bias voltage and tunnelling current set point were adjusted to allow optimal images (typically 0.2 to 0.5 V and 0.1 – 3 nA).

RESULTS AND DISCUSSION

AFM imaging of immobilized enzyme molecules

Initial attempts to image the enzyme immobilized on a gold electrode using AFM were performed employing the contact mode in air. It was observed that the AFM tip

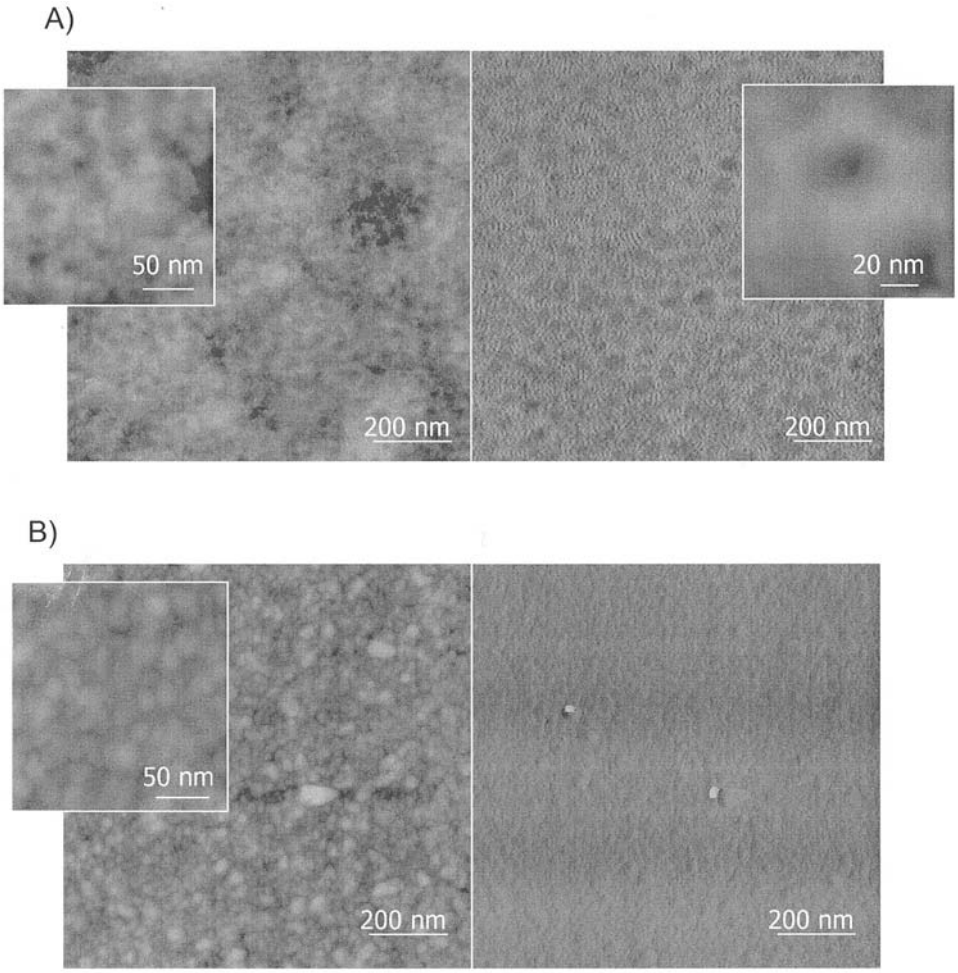

Fig. 1. AFM images of immobilized GOD on SAM (MPA) modified gold using the tapping mode, A) Typical topographic and phase images obtained with an unmodified AFM tip. Image size 1 μm × 1 μm, *z*-scale is 10 nm for the height and 30 º for the phase image. Enlarged images in the top right and left corners show characteristic ring structures. B) Typical topographic and phase images obtained with a modified MPS/tip. Image size 1 μm × 1 μm, *z*-scale is 10 nm for the height and 30 º for the phase image. The enlarged height image is in the top left corner.

dragged the enzyme molecules around, meaning it was impossible to observe molecular features (data not shown). Force curves showed large hysteresis in the retracting portion which is believed to have been due to adhesion from capillary forces. These results show that the contact mode in air is not the optimal method for imaging soft biomaterials such as GOD molecules. The non-contact (tapping) mode was considered as the next option which could give better results for these biomolecules and hence was further used.

Figure 1A shows typical AFM images of immobilised GOD molecules on MPA modified gold obtained using the tapping mode in air. Both topographic (left) and phase images (right) show clearly features on the surface which, when compared with the background images (bare gold and MPA modified gold), can be attributed to the presence of GOD molecules. The AFM images show that the structure of the GOD monolayer is dominated by an array of characteristic ring structures of clusters. They are mainly interlinked across the surface but occasionally isolated clusters are observed as shown in the magnified image (right box). A hexagonal structure of six GOD molecules in each cluster was the most frequent but clusters with five GOD molecules were also observed. The size of individual GOD molecules was difficult to determine precisely because of either poor image resolution or the fact that the GOD molecules appear highly interlinked. Poor resolution of the topographical images indicates that significant interaction and perhaps distortion of the enzyme molecules occurred between the tip and the surface. Capillary forces between the tip and sample caused by the water layer or the hydrophilicity of the soft GOD molecule associated with the chemical nature of unmodified AFM tip (used as received or stored over time) could be the explanation for the observed poor resolution, difficulty in adjustment of the parameters and irreproducibility in imaging. These results demonstrate that the tapping AFM mode should be used to image GOD molecules immobilised on biosensor surfaces but further optimisation is still required to make the characterisation more efficient.

Improved AFM imaging using a chemically modified tip and "light" tapping

The first strategy explored was the use of chemically modified AFM tips. The minimisation of the tip-sample interfacial free energy by chemical modification of the tip has recently been demonstrated for imaging soft materials and chemical composition mapping of samples.[25,26] In this work, a similar approach was applied to improve the imaging of enzyme molecules. A variety of tip modifications, such as cleaning the tip in a plasma with water or ethanol and surface functionalisation with dodecylamine, 3-aminopropyltrimethoxysilane, 3-mercaptopropanoic acid, 3-mercaptopropyltrimethoxysilane, and cyanoacrylates were explored.[27] After modification, the tips were characterised to check the tip geometry before and after the imaging experiment. It was found that all the modified tips, except the polymer modification, showed no changes in tip geometry. In comparison with unmodified tips, imaging of the enzyme surface with modified tips showed improved resolution. In particular, the MPS/tip performed extremely well allowing easy adjustment of the parameters for optimal imaging and providing images with the highest resolution. A typical AFM image of immobilised GOD surface obtained using a modified tip (MPS/tip) is shown in Fig. 1B. The topographic images (left) show a number of small spherical or ellipsoidal features randomly dispersed on the surface while the corresponding phase image (right) shows poor contrast resolution. In comparison with the array organization of the GOD molecules obtained with an unmodified tip (Fig. 1A), this image shows more "ball-like" topography with much clearer resolution of single molecules. The force curves obtained

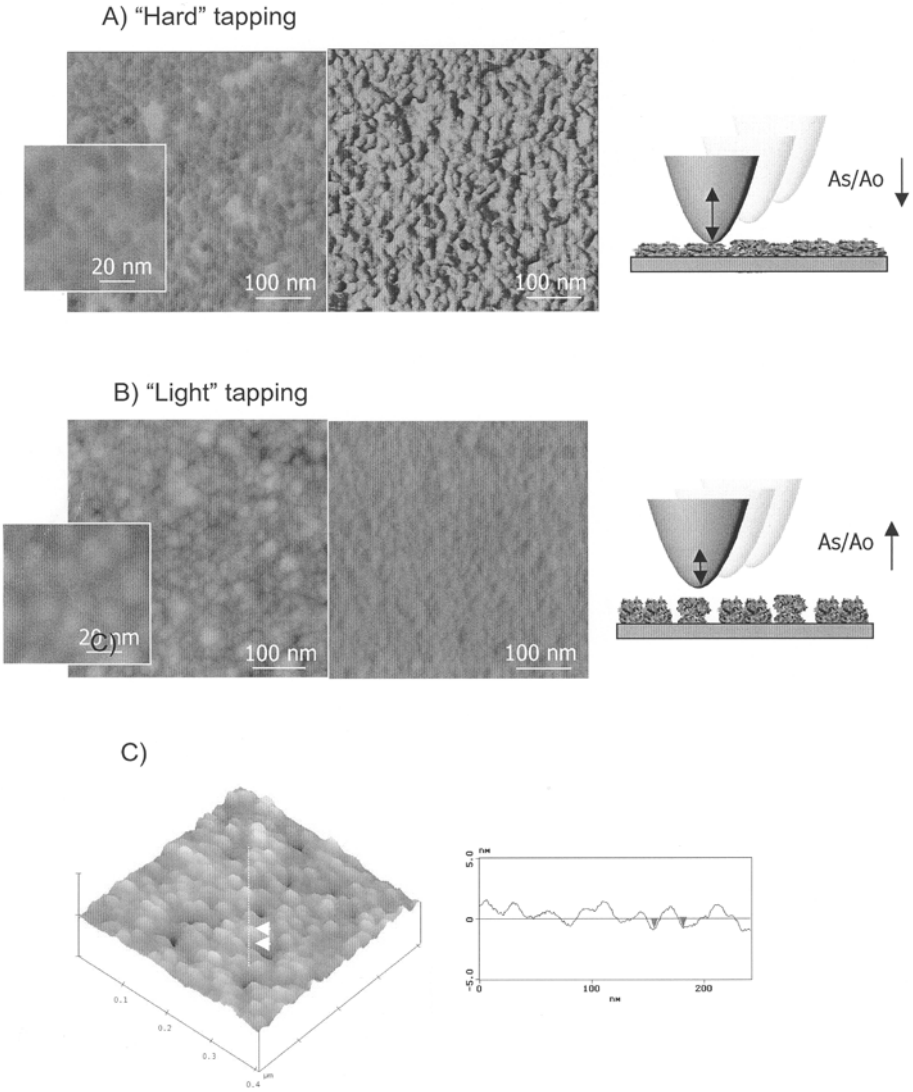

Fig. 2. Comparative AFM images of immobilised GOD molecules obtained with modified AFM tip using A) higher force (hard tapping) and B) lower force (light tapping). Height images are on the left, phase images are on the right. The size of the images are 500 nm × 500 nm. Higher resolution height images (size 100 nm × 100 nm) are shown in the inset (left). Scheme of the tip/sample interaction and the deformation of the molecules are shown on the right, for hard tapping (top right) and light tapping (bottom right). C) A typical 3-D topographic AFM image of a GOD electrode surface (size 400 nm × 400 nm) obtained by light tapping with the corresponding cross section profile.

using the chemically modified tips also show lower interaction between the tip and the surface after the approaching and retraction of the tip (data not shown). The explanation for the better resolution of soft enzyme molecules using this method can be attrib-

uted to the increase in the hydrophobicity of the tip which results in a decreased tip-sample interaction and a lowering of the impact of the condensed water layer on the surface. Similar observations were made by others when imaging monolayers with different chemical functionalities.[25]

Two main advantages in using chemically modified AFM tips were observed. Firstly, as described previously, improved AFM images of GOD molecules are obtained enabling the shape and size of individual molecules to be resolved. Secondly, the tip modification enabled the application of a larger range of forces between the tip and biological surface than was the case when unmodified tips are used. This could result in the minimisation of the tip-sample interfacial free energy. Further investigations were undertaken to determine what advantages this effect could have on AFM images. Experiments were performed with tracking of the tip on the GOD surface with adjustment from the minimum force ("light" tapping) to the maximum force ("hard" tapping), which allowed high resolution imaging in both topographic or phase images. This approach was successfully applied to achieve high resolution imaging of nanostructures of polymers, SAMs and humidity sensitive biomaterials.[26] AFM images obtained using a chemically modified tip (MPS/tip) with different amplitude set-ups and schematic presentation of the AFM tip/GOD molecules interaction are shown in Fig. 2. The AFM images obtained by "hard" tapping (Fig. 2A) and "light" tapping (Fig. 2B) show a difference in the GOD surface within terms of both the height and phase images. The topographic images obtained using soft tapping show resolved individual GOD molecules (of spherical shape) while imaging of the same GOD surface by hard tapping shows arrays topography with unclear single molecules and distorted features. The hard tapping topographic images look similar to the images obtained using an unmodified AFM tip at the beginning of this AFM study (Fig. 1A). Comparative phase images show no contrast when light tapping was used which indicates the tip tapping on the top of the GOD molecules was used with sufficiently low force to give no change in the phase between the GOD and uncovered MPA/Au surface. On the other hand, the phase images obtained by hard tapping show very high contrast of the GOD surface which is attributed to the difference of GOD molecules and uncovered gold. The decrease of the surface roughness from Ra = 1.4 nm (light taping) to Ra = 0.9 nm (hard tapping) also proves that hard tapping flattens the surface. To explain the observed differences in the AFM images, a schematic AFM/tip GOD molecule interaction is presented in Figs. 2 A-B (right). When the higher force is applied, the tip oscillates more into the GOD molecules and molecular deformations (probably inelastic) occur, resulting in dramatic changes in both the topographic and phase images (Fig. 2A). When a low force between the tip and the GOD molecules is applied, the tip oscillates on the top of surface and the shape of the GOD molecules can be imaged with little or no deformation (Fig. 2B). Larger deformations of GOD molecules are particularly clear on height images where visualisation of the individual GOD molecules is very difficult. The conclusion from these results is that gentle "tapping" is the preferable method for providing high-resolution images of soft biomolecules such as enzymes.

AFM visualisation of individual GOD molecules

Typical 3-dimensional topographic and high resolution AFM image of immobilised GOD obtained using a modified AFM tip (MPS/tip) and optimised light tapping is shown in Fig. 2C with the corresponding cross section. From this image various information about the GOD biosensor surface and the individual GOD molecules could be obtained, including the spatial distribution, coverage, size, shape and orientation on the surface. The GOD molecules are well dispersed across the surface and attempt was made to recognise a pattern in the organization of the GOD molecules in the monolayer. Random organization is mainly observed but one typical pattern of grouped GOD molecules is commonly observed, as it is shown in Fig. 2B (inset). A similar pattern (hexagonal or pentagonal ring structure) was observed as the dominant one in the phase images of the initial AFM experiment (Fig. 1A). A clear explanation for the formation of these structures is unknown, but it may indicate the existence of some repulsive force between the molecules, which leaves an unoccupied space in the middle. A similar structure has been observed for other proteins, such as membrane proteins.[10]

The surface coverage by GOD molecules on the surface was determined using images to be an average of 1.0×10^{12} molecules cm^{-2} or 1.6 pmol cm^{-2}. These results are consistent with our previous study using both fluorometric and Quartz Crystal Microbalance (QCM) characterisation of these surfaces.[27] The lower than theoretical (2.5 pmol cm^{-2}) density of GOD molecules on the surface can be attributed to repulsive forces between the GOD molecules which prevent close packing. An AFM study showed that with higher amounts of GOD in the solution (> 600 μg dm^{-3}) and longer deposition time (> 120 min), agglomeration or multilayer formation occurs. Images of the GOD biosensor surface confirmed that the fabrication protocol based on SAMs allows good reproducibility in the preparation of this biorecognition surface with similar spatial distributions of enzymes and organization of the monolayer or submonolayer of GOD without agglomeration or multilayer formation. These results are in good agreement with the observed reproducibility of GOD enzyme electrodes fabricated in the same way.[4,5,19]

In order to further understand the impact of the surface, the orientation of individual GOD molecules was investigated. Two typical shapes were found, one spherical and the other ellipsoidal, as shown in Fig. 3 A. The spherical features are similar on most samples. However, the ellipsoidal features range from a highly distorted spherical shape to two closely joined spheres. In our previous STM study of individual GOD molecules, very similar shapes of the GOD molecules were observed.[21] Figure 3B shows the STM image of the two typical shapes of a GOD molecule: spherical and butterfly shape. In order to interpret the structure of the individual GOD molecules, a model of native GOD molecule and its possible orientation on the surface was considered. Glucose oxidase from *Aspergillus niger* is a dimeric protein with a secondary structure (28 % helix, 18 % sheet).[28] The overall dimensions of the dimer are 7.0 nm × 5.5 nm × 8.0 nm according to X-ray crystallography data.[29] Two orientations of adsorbed native GOD molecule are possible: parallel to the major axis

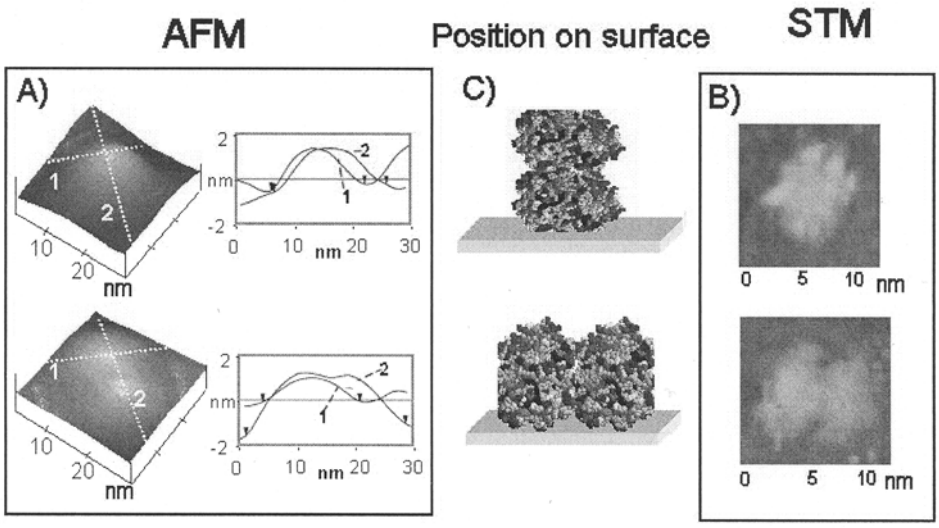

Fig. 3. Individual GOD molecules on a biosensor surface. A) AFM images of individual GOD molecules with observed spherical shape (top) and double spherical or butterfly shape (bottom) with the corresponding cross section graphs, B) STM images of individual GOD molecules indicating the two typical shapes: spherical (top) and butterfly (bottom), C) Schematic illustration of the possible orientations of a GOD molecule on the surface, standing position (top) and lying position (bottom).

(lying position) and along the perpendicular direction (standing position), as shown in Fig. 3C. The model shows that this shape could be used as the primary criteria to distinguish the appearance of the standing and lying positions of the enzyme on the surface, where the spherical shape presents the standing and the butterfly shape the lying position. Both AFM and STM images of GOD molecules (Figure 3A-B) with spherical and butterfly shape are in good agreement with the corresponding model of the GOD positions on the surface.

Cross sectional analysis of many examples of each form allows the shape and the lateral dimensions of the observed features of the enzyme to be matched to the expected geometries. The average size (diameter) of the standing isolated GOD molecule was determined by AFM as 24.0 ± 3.5 nm and height 2.4 ± 0.2 nm. Not surprisingly, the observed results give an overestimation of the GOD molecular size and an underestimation of the height in comparison to the native size (crystallographic data) and the STM observation.[29] To obtain more accurate information of the GOD molecules using the AFM images, the effect of the tip force on the sample was taken into account to allow corrections of the size of the features.

AFM image deconvolution toward real structure recovery of GOD molecules

The two major problems that have been confronted when imaging with AFM are distortion of the image and overestimation of the lateral size due to the varying geometry and characteristics of the scanning tip.[30–32] The AFM imaging process is a result of the interaction between the tip and the sample and the image obtained is a convolution of their geom-

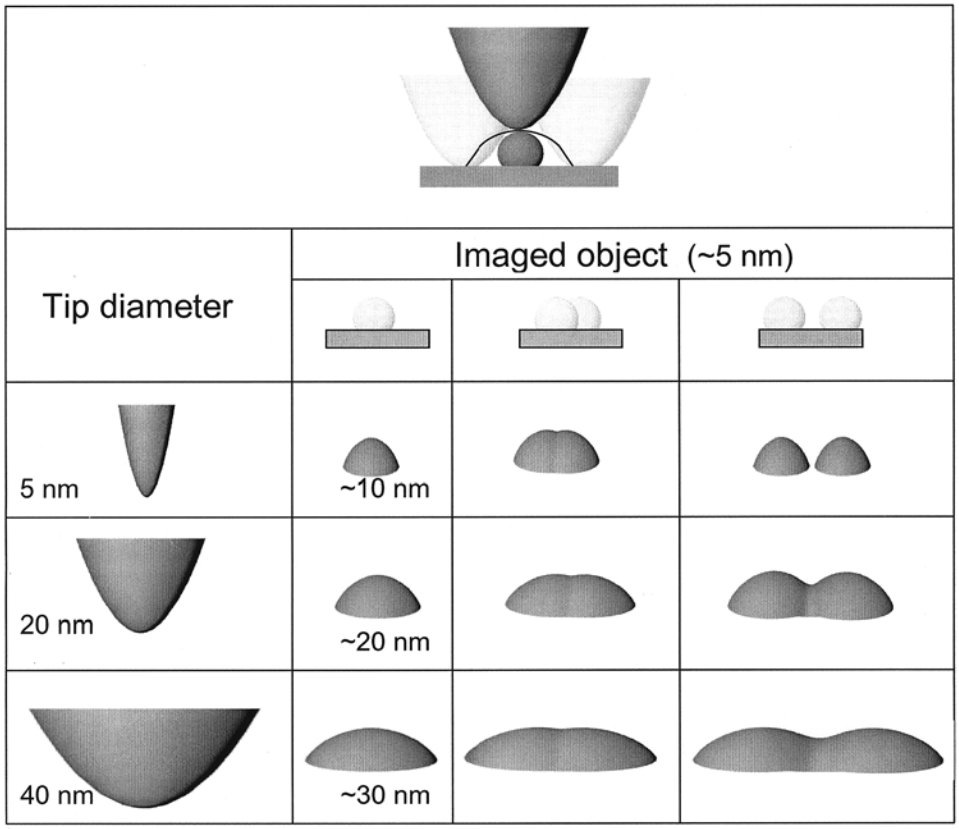

Fig. 4. Presentation of tip/sample convolution and the impact of the tip diameter on the imaging of small objects. Relation between the observed lateral dimension of an object and the diameter of the AFM tip. Theoretical images of 5 nm object (isolated, two linked and two separated) are drawn in dependence of tip size (5 nm, 10 nm and 20 nm).

etries. Moreover, when the surface feature is sharper than the tip, the shape of the tip will dominate the image (*e.g.*, the feature will effectively image the tip). The model of the tip/sample convolution effect is schematically shown in Fig. 4 where the sample is a spherical object with a size of 5 nm which is presented as isolated, as two linked and as two separated. The deconvoluted AFM images as a result of the tip/sample AFM images for all these objects were constructed by computer software using three different tips with average radius of 5 nm, 20 nm and 40 nm. It was assumed there was no deformation of the molecule. In the first case, when the tip is sharper than the sample, only minor distortion is observed in the image and the lateral dimension of the image is similar to the dimensions of the molecule. Increasing the tip radius increases the distortion in the simulated image and the observed lateral dimensions exceed the real size of the molecule significantly and there is no possibility of resolving individual object when they are separated by a distance less than their diameter. The model shows no impact of the tip shape on the height of an object.

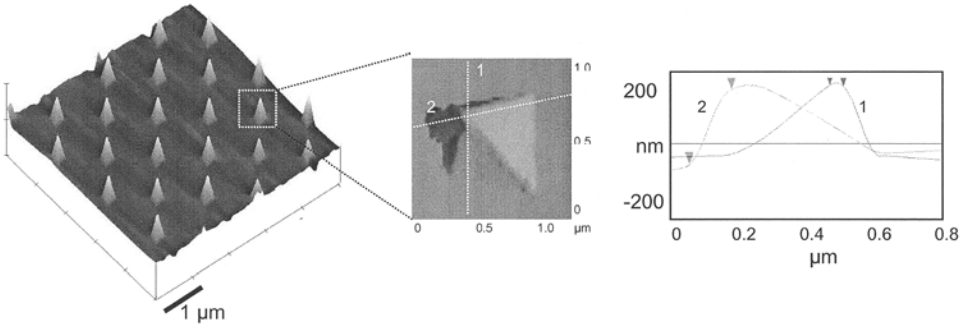

Fig. 5. Calibration procedure for AFM tips using a silicon calibration grating. Typical inverted AFM image on the left (size 5 µm × 5 µm), and 3-D image of a single AFM tip obtained by the tapping mode with the corresponding cross section (right).

The characterisation of the tip was the first step towards the true surface reconstruction and determination of the accurate size of a GOD molecule. In this work, two methods were used including calibration gratings and gold nanoparticles. Each of these methods was tested on a series of images of immobilised GOD and used for the reconstruction of the distorted images to demonstrate the appropriateness of these methods for the evaluation of AFM modified tips and to determine the overestimation of the size of a GOD molecule. Typical example of AFM tip calibration using a silicon grating test structure is shown in Fig. 5. Scanning an AFM tip over these structures provides an inverted image of the AFM tip itself, yielding a mirror transformation of the real tip shape, as is shown in the topographic image and corresponding cross section (Fig. 5). An unwanted change in the radius of curvature of the AFM tip could be easily observed as a consequence of inappropriate modification processes or tip contamination. This method is fast and provides reliable visual information of the AFM tip and is very useful for frequent control of the tip during AFM experiments, particularly in cases of expected tip contamination from the surface. The disadvantages are that the hard spikes on the gratings could remove or damage the modification layer on the tip during the imaging process and this method is not recommended in cases when the chemical nature of the tip/sample is particularly important. Tip characteristics, such as radius and angle, are determined by appropriate software (*e.g.*), Deconvlo 1.1 or DI) and further used for the deconvolution process to obtain the corrected size of the imaged structure (data not shown).

A more appropriate method for AFM tip characterisation, particularly in the case when biomolecules are studied, is based on gold nanoparticles. Since the globular GOD molecules appear very similar in shape and size to nanoparticles, this similarity is attractive and allows a more accurate calculation of the tip convolution effect. Among several reported methods, the procedure based on the work of Hu and Arndorf[23,24] was used in this study. The cross section (data not shown) represents a convolution graph between gold nanoparticles (20–30 nm) and the AFM tip. This convolution graph was used as the basis

for the calculation of tip characteristics (radius of tip curvature) and further used for the correction of the tip convolution effect on images of GOD molecules. The corrected size of the GOD molecule was calculated for a large number of molecules from several AFM images obtained using a MPS modified tip. The average size of isolated GOD molecules after deconvolution was determined to be 12.4 ± 2.1 nm in diameter for the spherical shape and $16.5 \pm 3.3 \times 10.2 \pm 2.5$ nm for ellipsoidal shape of GOD molecules. In comparison with the native size of GOD molecules (7 nm × 5.5 nm × 8 nm), this overestimation is explained as a consequence of mechanical deformation of GOD molecules during the imaging process. Further investigation is proposed to obtain more qualitative and quantitative information about the impact of this.

CONCLUSIONS

The results presented here show that AFM using the tapping mode is an effective tool for the characterisation of immobilized enzymes on biosensor surface. AFM provides valuable information, including spatial distribution of the GOD molecules on the surface, surface coverage, size and shape of individual molecules, their orientation, and intermolecular organization. Chemically modified AFM tips provide an improvement in the imaging of GOD relative to unmodified tips. They allow improved molecular resolution of the enzyme molecules, particularly in the topographic mode, providing the possibility of determining the shape and size of individual GOD molecules more accurately and give flexibility in adjusting the force between the tip and sample from light to hard tapping.

In comparison with STM, AFM leads to an overestimation of the dimension of individual GOD molecules. To avoid this broading effect caused by tip/sample convolution, the calibration of tip is an essential part of an AFM experiment. Evaluation of several methods for tip characterisation show that gold nanoparticles provide a good quality calibration sample having similarity in size and shape with the investigated GOD molecules. Deformation caused by tip/sample interaction is another effect which must be considered when using tapping mode AFM.

Acknowledgments: We would like to acknowledge AINSE for funding a visit to the Australian Nuclear Science Technology Organisation (ANSTO) to allow the acquisition of AFM imaging.

ИЗВОД

КАРАКТЕРИЗАЦИЈА МОЛЕКУЛА ЕНЗИМА ИМОБИЛИЗОВАНИХ НА ПОВРШИНИ БИОСЕНЗОРА ПРИМЕНОМ ТЕХНИКЕ СКЕНУЈУЋЕ МИКРОСКОПИЈЕ: ВИЗУАЛИЗАЦИЈА ИНДИВИДУАЛНИХ МОЛЕКУЛА

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У овом раду приказана је примена технике скенујуће микроскопије за карактеризацију молекула ензима (глукоза-оксидазе – GOD) имобилизоване на површини биосензора. Циљ ове студије је проналажење оптималних услова за примену скенујуће атомске микроскопије (AFM) за визуализацију индивидуалних GOD молекула. Показано је да хемијски модификован AFM шиљак и „лагани" типкајући начин рада пружају низ предности за добијање слике високе резолуције „нежних" ензимских биомолекула као што је GOD. Добијен је одређен број корисних информација као што су покривеност и организација GOD биомолекула на површини електроде, њихов облик (форма), димензија и оријентација индивидуалних молекула. Регистрована су два типична облика GOD молекула: сферни и лептир који су у корелацији са сличним облицима добијеним применом скенујуће тунелујуће микроскопије (STM). Према 3-d моделу GOD молекула који показује две могуће оријентације молекула на површини добијени резултати су послужили као доказ о присуству ензима у „стајаћем" (сферни) и „лежећем" (лептир) положају. Димензија GOD молекула одређена је после корекције као резултат шиљак/узорак конволуцијског ефекта. Величина пречника сферног облика GOD молекула одређена је као 12 ± 2,1 nm, док су димензије облика лептира утврђене као 16,5 ± 3,3 nm × 10,2 ± 2,1 nm. Резултати добијени применом STM технике показали су значајно мању димензију GOD молекула (10 ± 1 nm × 6 ± 1 nm и 6,5 ± 1 nm × 5 ± 1 nm) који су ближи њиховој природној величини. Објашњење за ову уочену димензијску разлику је физичка деформација GOD молекула изазвана AFM шиљком у току процеса сканирања.

(Примљено 2. јуна 2003)

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