Mounting proteins on metal nanoparticles: Statistical analysis of AFM images

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ABSTRACT

A reliable procedure for controlled mounting of proteins on gold nanoparticles has been developed. The procedure is exemplified using Glucose Oxidase (GOX) as a model protein. Complex characterization of protein-nanoparticle conjugates was performed using absorbance spectrometry, dynamic light scattering (DLS), activity assays combined with atomic force microscopy (AFM).

Keywords: colloidal nanoparticles, protein, AFM, image analysis

INTRODUCTION

It has long been recognised that colloidal gold and silver nanoparticles can serve as versatile substrate for immobilisation of biomolecules. Presence of gold colloids gives the conjugates number of interesting properties not existing in proteins. We can roughly divide these new properties into "active" and "passive". On one hand, nanoparticles can act as a solid support and anchor for an active component therefore immobilizing it a given location. Properly sized nanoparticles (50-100 nm) can be used to deliver and retain attached drug at a predefined location in the organism preventing its fast removal and exertion on the one hand and accumulation in liver, spleen and kidney on the other hand [1]. The same property makes nanoparticle-enzyme conjugates a promising choice in biocatalysis where it could reduce washing out of enzyme. On the other hand gold and silver nanoparticles can play an active role in sensing applications. As those particles support localised plasmon excitations they can interact with each other and incoming light and greatly enhance the electric field on their surface. This has recently lead to the whole range of new applications including colorimetric assays based on localized surface plasmons [2,3], amplification of surface plasmon resonance assays [4], surface enhanced fluorescence and Raman [4]. The effects of the geometry of the conjugates, the degree of aggregation as well as the influence of nanoparticle conjugate formation on biocatalytic activity are of paramount importance for functioning of the nanoparticle-protein conjugates and therefore attract large attention.
In the present paper we report an effective and reliable procedure for controlled formation of gold nanoparticle – protein conjugates and discuss initial measurements on conjugate structure and activity.

MATERIALS AND METHODS

Materials. Glucose Oxidase, 16-Mercaptohexadecanoic acid (MHA), N-Hydroxysuccinimide (NHS), 1-ethyl-3-(3-dymethylaminopropyl)carbodiimide hydrochloride (EDC), Gold (III) chloride trihydrate ($AuCl_4H$), trisodium citrate, D-sorbitol, D(+) Glucose, aceton, horseradish peroxidase 50000 units (POD), were purchased from Sigma-Aldrich. Polyoxyethylene (20) sorbitan monolaurate (TWEEN 20) was purchased from Fluka. Colloidal gold 30 nm solution was purchased from British Biocell. All materials were used without further purification.

Nanoparticle preparation.

Gold nano particles were prepared by dissolving 1mM Au(III) Cl in deionized water. 20ml of the Au solution was brought to boiling and 2ml of a 1% trisodium citrate solution was added. The solution was kept at boiling temperature until the color of the solution changed to a dark red-violet solution. The dark red-violet solution was taken off the heater plate and let to cool to room temperature. During the cooling the colour changed to a ruby red. This procedure yields gold anno particles of a narrow size distribution of around 20nm radius.

Gold particle conjugation. Tween 20 was added to the stock solution of gold nanoparticles and left incubating on a shaker for 2 hours for physisorption of Tween 20 to the gold particles. Separately 20mM solution of MHA was activated with a mixture of 20ul of 10mM EDC and 20ul of 10mM NHS and left standing for 2 hours. After 2 hours the solution of activated MHA was diluted by stock solution of MHA to create a mixture of activated and non-activated molecules. Upon addition to gold nanoparticles/Tween 20 solution it displaces physisorbed Tween 20 and forms a mixed self-assembled monolayer. The solution is centrifugated, supernatant discarded and the pellet is resuspended in PBS buffer. GOX is introduced in the solution and left reacting for 2 hours. The mixture is centrifugated 3 times, after each cycle supernatant is discarded and the pellet resuspended in PBS.

Substrate preparation. Silicon wafer was cut into small pieces, sonicated in acetone for 60 min, blow dried and exposed to 3% APTES solution in absolute ethanol for 60 minutes. Substrates were soaked in absolute ethanol for another 60 min, blow dried and baked on a hot plate at

100°C. Gold nanoparticles or nanoparticle conjugates solutions were deposited on the prepared substrates and left incubate for 1-1.5 hours. Afterwards the substrates were gently rinsed with DI water and blow dried.

Atomic Force Microscopy. AFM images were collected using a Digital Instuments (Veeco) Nanoscope IIIa microscope operating in tapping mode. High resolution NSG10S silicon tips (NT-MDT) with resonance frequencies around 250kHz and a specified tip curvature 10nm or better were used. All images shown are unfiltered, background and linear slope are subtracted.

RESULTS AND DISCUSSION

We have performed glucose oxidase immobilization on both self-made and commercially available 30nm gold colloids using the same technique described above. The suspension of nanoparticle-glucose oxidase conjugates showed characteristic reddish colour and did not show any degradation within the period of experiment (several days). Absorbance spectra (Fig.1) of the conjugates reveals two peaks at 260 nm and 540 nm indicating presence of the protein and localized plasmon absorption of the gold particles. The later is red-shifted relatively to the gold particles solution (520 nm) indicating a change in local particle environment due do proteins attached on the surface. DLS measurements indicate broader size distribution with a larger average hydrodynamic radius of the conjugated gold particles in comparison with stock gold particles solution (50nm against 20 nm before conjugations).

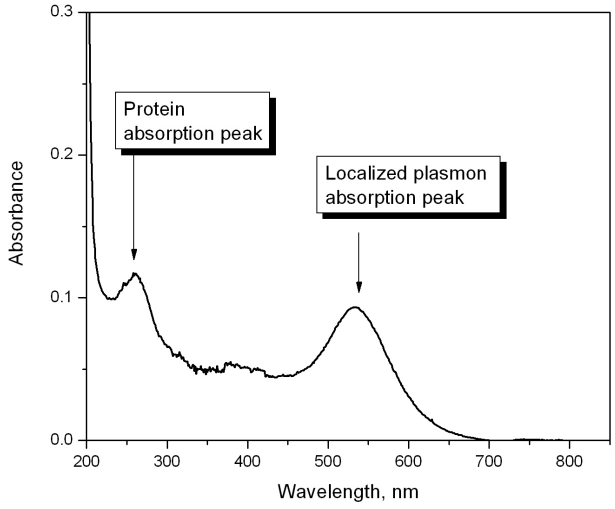

Fig.1 Absorbance spectrum of gold nanoparticle – GOX conjugates.

Corresponding AFM images show that this is related both to increase of particles size due to attachment of the enzyme molecules (with approximate diameter 8 nm) and in some cases a partial aggregation as well. Figures 2B and.

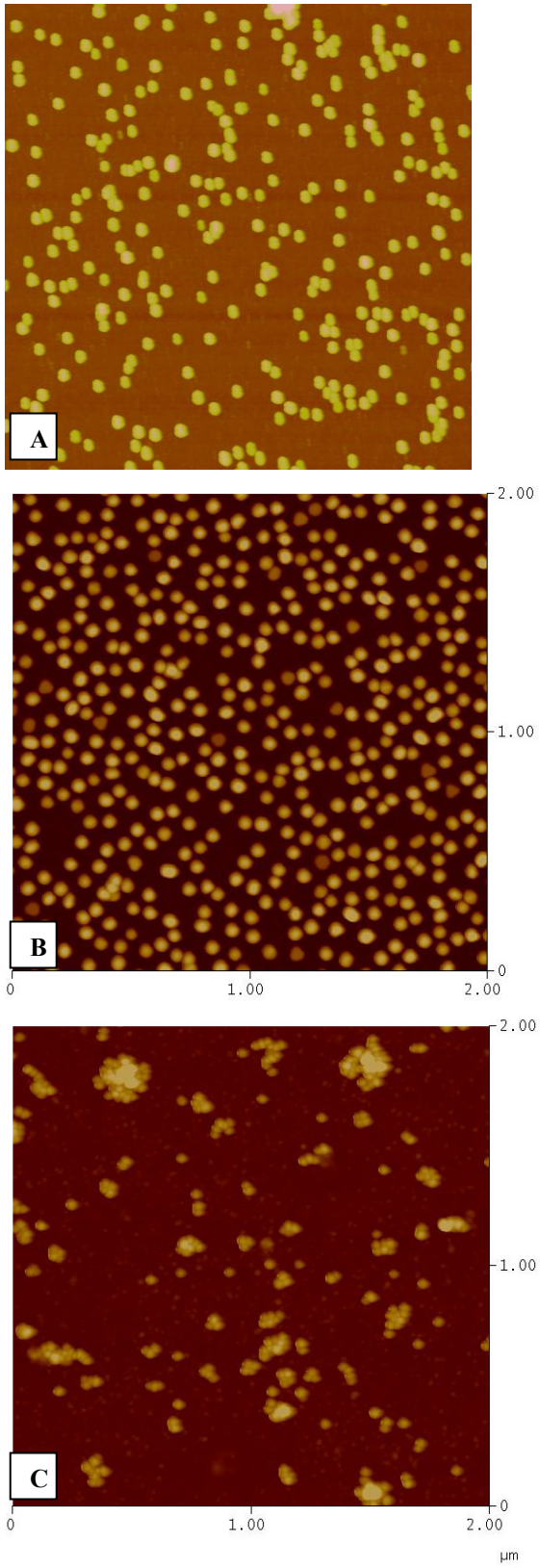

Fig.2 A) AFM image of a gold colloids deposited on APTES-modified silicon substrate. B-C) AFM images of gold-glucose oxidase conjugates showing different degree of aggregation.

2C show images of two conjugate solutions with a different degree of aggregation. Although AFM cannot image protein molecules on the curved surface of nanoparticles, statistical properties of the particle distribution reflect presence of conjugated proteins. We have performed statistical analysis of AFM images calculating distribution of height, size and distance to nearest neighbors. It reveals good correlation between particle distribution on the surface and volume data obtained by DLS.

In addition we have performed activity assays based on coupled reaction with horseradish peroxidase and colored substrate. All assays on particle-conjugate solutions demonstrated similar degree of activity corresponding to approximately 0.5ng/ml of pure GOX. We speculate that the actual concentration of the GOX is most probably higher but the activity of enzyme is degraded due to steric hindrance.

CONCLUSIONS

We have developed a procedure for controlled mounting of proteins on gold nanoparticles. Mixed self-assembled monolayer of activated and non-activated MHA improves stability of the conjugate solution and allows us to control amount of protein attached to nanoparticles. Statistical properties of 2-dimensional nanoparticles distribution obtained from AFM images corresponds well to the size distribution obtained from DLS and provides additional insight into particle morphology. In combination with bulk measurement technique AFM images provide meaningful information on conjugates present in the solution, including size distribution, degree of aggregation etc. thus combining imaging of individual conjugates with statistical information.

REFERENCES

[1] John D. Hood, Mark Bednarski, Ricardo Frausto, Samira Guccione, Ralph A. Reisfeld, Rong Xiang, and David A. Cheresh, “Tumor Regression by Targeted Gene Delivery to the Neovasculature”, Science 296, 2404-2407 (2002).

[2] N.Nath and A.Chilkoti, “A colorimetric gold nanoparticle sensor…”, Anal. Chem. 74, 504-509 (2002)

[3] K.Aslan, J.R.Lakowicz, C.D. Geddes, “Nanogold-plasmon-resonance-based glucose sensing”, Anal. Biochem. 330, 145-155 (2004)

[4] A. Lyon, M.D. Musick, and M.J. Natan, "Colloidal Au-Enhanced Surface Plasmon Resonance Immunosensing," Anal.Chem. 70, 5177-5183, (1998).

[5] K. Aslan, J. Malicka, E. Matveeva, J. RRRr. Lakowicz, C.D Geddes, ”Metal-Enhanced fluorescence: an emerging tool in biotechnology”, Current Opinion in Biotechnology 16, 55-61 (2005)