Gold nanoparticle-based competitive colorimetric assay for detection of protein–protein interactions†

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Received (in Cambridge, UK) 23rd May 2005, Accepted 29th June 2005
First published as an Advance Article on the web 28th July 2005
DOI: 10.1039/b507237a

A gold nanoparticle-based competitive colorimetric assay uses the ensemble of Concanavalin (ConA) and manno-oligoside-encapsulated gold nanoparticles (Man-GNPs) to identify the binding partners for ConA and the binding constants are determined based on the wavelength changes.

Protein–protein interactions play key roles in structural and functional organization of living cells. A variety of techniques have been developed for the investigation of these interactions including protein microarrays, two-hybrid analysis, immunoassays, mass spectrometric analysis by affinity capture, fluorescence spectroscopy, and calorimetry. However, development of facile and sensitive identification of protein–protein interactions, especially in high-throughput, is still one of the most challenging as well as important tasks in proteomics research.

Gold nanoparticles (GNPs) smaller than 60 nm have extremely high extinction coefficients at ~520 nm. Moreover, different agglomeration states of GNPs can result in distinctive color changes. These extraordinary optical features make GNPs an ideal color reporting group for signaling molecular recognition events and render the nanomolar concentration detection possible. While elegant biosensor designs utilizing these concepts, such as DNA-functionalized GNPs for colorimetric detection of DNA and Pb(II) as well as carbohydrate-modified GNPs for selectively sensing lectins, have been demonstrated, it is desirable to expand this rather facile/sensitive detection methodology to new applications. In this communication we would like to describe an adequate technique based on the cognate substrate-modified gold nanoparticles, namely gold nanoparticle-based competitive colorimetric assay, for sensitive and expedient detection of protein–protein interactions in solution. Direct visualization without the need for protein labeling or the aid of instruments is possible.

A detection scheme for protein–protein interactions is illustrated in Fig. 1. A protein, denoted by X, binds the ligands protruding from the GNP surface and promotes agglomeration of the particles via multivalent ligand–protein interactions giving rise to a blue colored solution (Fig. 1b). However, the addition of a putative protein, denoted by Y, capable of interacting with protein X, could influence the binding between X and GNPs. It is therefore possible to undo the agglomeration of GNPs and cause the dissipation of GNPs. As a result, the color of the solution can change from blue to the original burgundy color (Fig. 1c).

Lectins are carbohydrate-binding proteins and most of them are multivalent and capable of agglutinating cells. Interaction of cellular proteoglycans, glycoproteins and glycolipids with carbohydrate-binding proteins is involved in a number of important biological processes. Accordingly, we have exploited concanavalin (ConA) and manno-modified-GNP (Man-GNP) to demonstrate proof-of-concept of the methodology. Thyroglobulin, a glycoprotein known to bind ConA strongly, was selected to verify the mode of sensing. Six lectins along with three other proteins were tested for their capability of interacting with ConA.

The Man-GNP used in the present work comprised a thiol appended mannose ligand attached to 32-nm-diameter gold nanoparticles. The Man-GNPs in 0.01% aqueous sodium citrate solution showed an absorption band with \( \lambda_{max} \) at 528 nm and displayed the largest red-shifts and the most pronounced change of absorption intensity in the presence of ConA, whereas there was no appreciable absorption change upon the addition of other proteins (see ESI†), indicating Man-GNPs specific binding to ConA. The spectral changes are attributed to a severe aggregation of GNPs mediated by ConA.

We next evaluated the possibility of using the preformed Man-GNPs/ConA complex for a competitive colorimetric assay. Among 10 proteins considered herein, four proteins i.e. thyroglobulin, BS-I, SBA, and MAL are found to have very drastic effects on the absorption spectrum of Man-GNPs/ConA compared to the remaining proteins tested. For these proteins, the wavelength was blue-shifted and the absorption intensity increased. The color changed from blue to burgundy indicating these four proteins were able to compete with Man-GNPs binding to ConA and disrupt

† Electronic supplementary information (ESI) available: Synthetic procedures for manno-oligoside, UV-vis spectra of Man-GNPs in the presence of individual tested protein, MALDI-TOF-MS spectra, Job’s plot, Hill plot for stoichiometry determination and the curve fitting for the binding constant. See http://dx.doi.org/10.1039/b507237a
agglomeration of the particles. In fact, the color change can be easily detected by the naked eye within 2 min and the detection limit can be as low as 5 nM. When the sequential addition order of Man-GNPs was changed so that ConA first mixed with these four proteins followed by the addition of Man-GNPs, the color of Man-GNPs remained burgundy. The result indicates that the presence of a binding partner prevents ConA from cross-linking Man-GNPs (Fig. 2).

In accordance with our expectation, thyroglobulin disrupts the agglomeration of the particles giving rise to a burgundy color. We demonstrate that BS-I, SBA, and MAL are capable of interacting with ConA for the first time. To provide further support for this result, we examined the interactions by SDS-PAGE. The GNPs can penetrate a gel under the correct conditions and the proteins that entered the gel were separated as shown in Fig. 3. Two bands are evidenced in lanes 3, 4, and 5, respectively, and the color of the corresponding solutions is burgundy. In contrast, only one band corresponding to ConA is observed in lanes 6, 7, and 8 and the color of the solutions is blue. The results demonstrated that the tested proteins-BS-I, SBA, and MAL interacted with ConA, which is consistent with spectroscopic studies.

To further validate some aspects of the data, matrix-assisted laser desorption ionization-time of flight-MS was employed to analyze the components of biomolecules adsorbed on the Man-GNPs. The mass traces (see ESI†) clearly provided evidence for the formation of a ConA and BS-I complex. This result along with the gel analysis allows us to confirm that the protein–protein interactions can indeed be evaluated by direct color visualization of the surface plasmon change.

The new technology that we have developed here provides not only qualitative but also quantitative evaluation of protein–protein interactions. Fig. 4a shows representative UV-vis spectra of ConA (98 nM) with various concentrations of BS-I in the presence of a constant concentration of Man-GNP. A clear color progression from blue to purple to burgundy with increasing concentrations of BS-I can be observed since disruption of the cross-linked particles is induced by ConA/BS-I interaction influencing the interactions between ConA and Man-GNP. The extent of particle dissipation, in terms of wavelength changes at maximum absorption, is proportional to the BS-I concentration added and leveled off once the concentration of BS-I reaches 49 nM; half of the concentration of ConA used in the study. It indicates the stoichiometry for the binding of BS-I to ConA as 1:2 (Fig. 4b). Job’s plot also confirms the stoichiometry and the Hill plot further shows that the binding is cooperative with Hill’s coefficient close to 1.8 (see ESI†). Based on the wavelength changes, the binding constant of ConA/BS-I is calculated to be 1.5 x 10^15 by a nonlinear regression curve fitting program (see ESI†). The rather large binding constant is not surprising if the large surface area of proteins possible for interactions and the 1:2 stoichiometry for the binding of BS-I to ConA are taken into account.
perceived by the naked eye. There is also no need to use analytical instruments or labeled lectins. By using this expedient methodology, a broad range of proteins can be rapidly evaluated for their abilities to interact with the protein of interest in real time. The method is easily generalized for studying other protein–protein interactions simply by capping GNPs with corresponding cognate substrates, rendering high-throughput screening of protein–protein interactions possible. It should also be straightforward to use this methodology to screen molecular libraries containing potential drugs to disrupt cell–cell adhesion mediated by lectins. Further work in this direction and the application of this colorimetric assay in proteomic analysis are underway.

The authors gratefully acknowledge the National Science Council and Ministry of Education, Taiwan for financial support and Prof. Sunney Chan for suggestions.

Notes and references


3. For 13- and 50-nm diameter gold nanoparticles are 2.7 × 10⁻⁸ and 1.5 × 10⁻⁶ M⁻¹ cm⁻¹, respectively. For details see R. Jin, G. Wu, Z. Li, C. A. Mirkin and G. C. Schatz, J. Am. Chem. Soc., 2003, 125, 1643–1654.


5. FAB-MS: calcd for C₂₃H₄₅NO₉S( M + 2); FAB-HRMS: calcd for C₂₃H₄₅NO₉SNa⁺ ( M + Na⁺).

6. Six lectins are bandiera simplicifolia lectin I (BS-I), soybean agglutinin (SBA); maackia amurensis (MAL), erythrina cristagalli lectin (ECL), wheat germ agglutinin (WGA), bandeiraea simplicifolia lectin II (BS-II). Three other proteins are RNome, trypsin inhibitor, bovine serum albumin (BSA).

7. Spectroscopic data for the mannopyranoside thiol derivative used in the preparation of Man-GNP: ⁴H-NMR (400 MHz, CD₃OD, 25 °C, TMS): δ = 8.02 (bs, 1 H, NH), 4.81 (d, J = 1.5 Hz, 1 H), 3.88–3.80 (m, 3 H), 3.74–3.52 (m, 13 H), 3.39–3.32 (m, 2 H), 2.68 (t, J = 7.3 Hz, 2 H), 2.49 (t, J = 7.3 Hz, 1 H, SH), 2.20 (t, J = 7.5 Hz, 2 H), 1.70–1.56 (m, 4 H), 1.42–1.26 (m, 12 H); ¹³C-NMR (CD₃OD, 100 MHz): δ = 175.5, 101.4, 74.4, 72.4, 72.0, 71.5, 71.3, 71.2, 70.6, 68.5, 67.6, 62.9, 40.5, 39.9, 37.2, 35.4, 30.8, 30.7, 30.6, 30.4, 29.7, 29.7, 27.3; IR (KBr) 3416, 3323, 2925, 2859, 1649, 1553, 1467, 1135, 1062 cm⁻¹; FAB-MS: m/z 512.3 (M⁺ + 1), 534.2 (M + Na⁺); FAB-HRMS: calcd for C₆₃H₇₂Na₂O₁₂S (M⁺ + 1) 512.2894, found 512.2885.

8. Different sizes of Man-GNPs were prepared. It turns out that 32-nm Man-GNPs give the best optical signals in our studies. Transmission electron microscopic images of synthesized 32-nm Man-GNP alone and in the presence of ConA are included in the ESI.


10. Six lectins are bandiera simplicifolia lectin I (BS-I), soybean agglutinin (SBA); maackia amurensis (MAL), erythrina cristagalli lectin (ECL), wheat germ agglutinin (WGA), bandeiraea simplicifolia lectin II (BS-II). Three other proteins are RNome, trypsin inhibitor, bovine serum albumin (BSA).

11. Spectroscopic data for the mannopyranoside thiol derivative used in the preparation of Man-GNP: ⁴H-NMR (400 MHz, CD₃OD, 25 °C, TMS); δ = 8.02 (bs, 1 H, NH), 4.81 (d, J = 1.5 Hz, 1 H), 3.88–3.80 (m, 3 H), 3.74–3.52 (m, 13 H), 3.39–3.32 (m, 2 H), 2.68 (t, J = 7.3 Hz, 2 H), 2.49 (t, J = 7.3 Hz, 1 H, SH), 2.20 (t, J = 7.5 Hz, 2 H), 1.70–1.56 (m, 4 H), 1.42–1.26 (m, 12 H); ¹³C-NMR (CD₃OD, 100 MHz); δ = 175.5, 101.4, 74.4, 72.4, 72.0, 71.5, 71.3, 71.2, 70.6, 68.5, 67.6, 62.9, 40.5, 39.9, 37.2, 35.4, 30.8, 30.7, 30.6, 30.4, 29.7, 29.7, 27.3; IR (KBr) 3416, 3323, 2925, 2859, 1649, 1553, 1467, 1135, 1062 cm⁻¹; FAB-MS: m/z 512.3 (M⁺ + 1), 534.2 (M + Na⁺); FAB-HRMS: calcd for C₆₃H₇₂Na₂O₁₂S (M⁺ + 1) 512.2894, found 512.2885.

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