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Colorimetric detection of human chorionic gonadotropin using catalytic gold nanoparticles and a peptide aptamer†

Chia-Chen Chang,[a] Chie-Pein Chen,[b] Chung-Han Lee,[a] Chen-Yu Chen*[bcd] and Chii-Wann Lin*[aef]

We combined catalytic gold nanoparticles (AuNPs) with an hCG-specific peptide aptamer to create a simple, sensitive, label-free colorimetric assay for hCG. The applications of this colorimetric biosensor may be expanded by changing the peptide aptamer.

Human chorionic gonadotropin (hCG), a hormone produced by the placenta, is considered the major indicator of embryo implantation in pregnancy. Moreover, it is an important clinical parameter for the early diagnosis of ectopic pregnancy and in prenatal screening for Down's syndrome.[1] In addition to pregnancy, hCG is associated with male health problems such as testicular cancer.[2] Accordingly, the development of sensitive hCG immunoassays is desirable for both the investigation of pregnancy-related changes and the early diagnosis of cancer. In general, lateral flow immunochromatographic tests are used for hCG detection, which employ a gold-conjugated monoclonal antibody. Despite their user-friendly format and rapid, low-cost analysis, they only provide qualitative information, which is insufficient to elucidate biodevelopmental processes.[3] In this regard, a host of immunoassays using antibody recognition and based on electrochemistry, surface plasmon resonance, fluorescence, and mass spectrometry have been developed to detect hCG in recent years.[4] Although these approaches represent promising platforms for highly sensitive detection, they still suffer from drawbacks, including the necessity for strict washing processes, professional operation, and data interpretation, and thus are impractical for on-site detection. Moreover, the use of antibodies as the recognition element is controversial because of batch-to-batch variation in quality and the complexity of their production.[5] Therefore, alternative antibody-free detection strategies with the advantages of convenience, low cost, and ease of operation are in demand.

Of the alternatives to antibody-based sensing techniques, aptamer-based methods have become popular over the past decade because of their specificity and affinity for their targets and stability in various media, including natural environments and living organisms.[6] Although the application of peptide aptamers as biosensors is seldom reported, they have numerous advantages, including easy generation and low cost, over antibodies such as nucleic acid aptamers.[7] Moreover, peptide aptamers offer the diverse structural and functional features of proteins for molecular interactions. The development of a peptide aptamer against hCG offers an alternative approach to hCG detection. Using the phage display technique, Yang's group successfully selected an hCG-binding peptide aptamer and utilized this in a liquid crystal (LC)-based assay.[8] This approach is superior to immunometric methods from a practical viewpoint because it is label-free and does not require expensive antibodies. Nevertheless, a noteworthy limitation of this method is its relatively poor sensitivity (LOD = 1000 mIU mL^{-1}).

Recently, gold nanoparticles (AuNPs) have demonstrated great utility in chemical and biomedical detection because of their unique optical and electrical properties. In particular, they have been developed and investigated for their surface plasmon resonance, fluorescence, and Raman properties in detecting biological targets.[9] To date, however, few biodetection assays exploit the catalytic activity of AuNPs.[10] Although gold is chemically inert, AuNPs were recently revealed to be extremely useful artificial enzymes.[11] These findings showed that a catalytic AuNP-based strategy has enormous potential in bioanalysis and can be exploited to develop biosensors for a wide variety of protein targets. Enlightened by the above facts, we present a simple, sensitive method for the quantitative analysis of hCG based on

[a] *Institute of Biomedical Engineering, National Taiwan University, Taipei 106, Taiwan. E-mail: cwlinx@ntu.edu.tw*
[b] *Department of Obstetrics and Gynecology, Mackay Memorial Hospital, Taipei 104, Taiwan. E-mail: f122481@mmh.org.tw; Fax: +886-2-23620586; Tel: +886-2-33665271*
[c] *Mackay Junior College of Medicine, Nursing, and Management, Taipei 112, Taiwan*
[d] *Mackay Medical College, Taipei 252, Taiwan*
[e] *Institute of Biomedical Electronic and Bioinformatics, National Taiwan University, Taipei 106, Taiwan*
[f] *Center for Emerging Material and Advanced Devices, National Taiwan University, Taipei 106, Taiwan*

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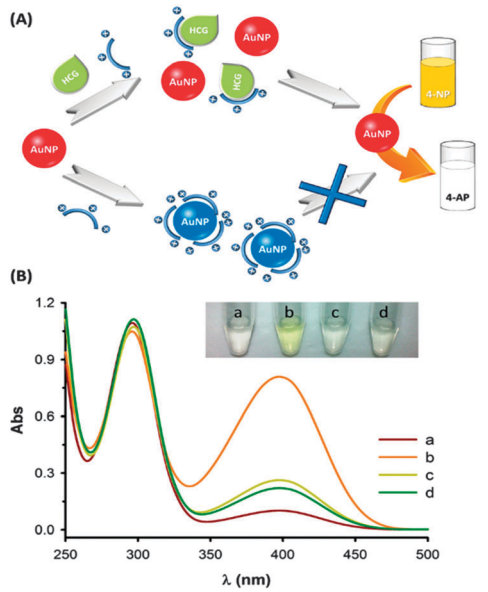

Fig. 1 (A) Schematic illustration of the proposed colorimetric detection of human chorionic gonadotropin (hCG) based on peptide-regulated gold nanoparticles (AuNPs) catalysis. (B) Ultraviolet-visible spectrophotometry absorption spectra of (a) AuNPs, (b) AuNP + peptide aptamer, (c) AuNP + peptide aptamer + hCG, and (d) AuNP + hCG. Inset: the corresponding photographs.

peptide-regulated AuNP catalysis. Using our strategy, detection of the target protein was possible using the naked eye or with the aid of ultraviolet-visible spectrophotometry (UV-vis). Furthermore, our strategy did not require any modification of the aptamer peptide, making it cost-effective.

Fig. 1A outlines the concept of the catalysis-based colorimetric assay. The total charge of the hCG-binding oligopeptide (PPLRINRHILTR) is positive at pH 7.4 because arginine (R) is positively charged.[8] In the absence of hCG, AuNPs were incubated with a peptide aptamer under conditions favoring the formation of the AuNP–aptamer complex through electrostatic interaction. The bound peptide decreases the active surfaces of the nanoparticles, resulting in a decrease in the catalytic abilities of AuNPs. Once hCG molecules are present in the solution, the peptide aptamer specifically recognizes and binds to hCG, rendering its attachment to citrate-capped AuNPs impossible and restoring their catalytic activity. Subsequently, AuNPs catalytically convert a yellow-colored chemical compound (4-NP) to a colorless product [4-aminophenol (4-AP)], resulting in a gradually intensifying change in solution color evident on sensitive colorimetric analysis.

To confirm the feasibility of our concept, the spectral responses of the catalytic-based colorimetric assay were observed under different conditions (Fig. 1B). The spectral value of free AuNPs in solution at 400 nm was 0.09 absorbance units (a.u.) (Fig. 1Ba), explained by the reduction of yellow 4-NP to colorless 4-AP by catalysis. After adding the peptide aptamer, this value (Fig. 1Bb) increased to 0.8 a.u. because of the deactivation of catalysis on the AuNP surfaces, a result indicating that the catalytic AuNP surfaces were effectively masked by the peptide. When hCG molecules were present in the solution (Fig. 1Bc), the peptide–AuNP complex could not be formed and more AuNPs were free, permitting 4-NP to access the AuNP surface and undergo conversion to 4-AP. As expected, the addition of hCG alone caused a change in solution color (Fig. 1Bd), resulting from the easy access of 4-NP to AuNPs. These results confirm that the positively charged peptide aptamer is the main cause of AuNP-dependent catalysis because of the different affinities of the peptide–AuNP and peptide–hCG complexes. Similarly, aggregation-based methods cause a remarkable shift in the absorption peak in the presence of only a peptide probe (Fig. S1, ESI†). These results confirm the electrostatic interaction between AuNPs and the peptide, consistent with the results of the catalysis-based approach. The catalytic properties of AuNPs were further investigated by monitoring UV-vis spectral changes at different hCG concentrations. As shown in Fig. S2 (ESI†), ΔA increased with an increase in hCG concentration and the response reached saturation in about 90 min with 1000 mIU mL^{-1} hCG. Therefore, ΔA at 90 min was used for hCG quantification.

To determine the sensitivity of the assay, we used a range of hCG concentrations between 5 and 2000 mIU mL^{-1}, as shown in Fig. 2A. With increasing hCG concentration, the color of the solution changed from yellow to pale yellow to colorless, suggestive of the hCG concentration-dependent 4-NP catalysis. Moreover, examination of the absorption spectra of the solutions (Fig. 2B) showed that the intensity at 400 nm (A_{400}) decreased with increasing hCG concentration, also suggestive of the gradually increasing 4-NP catalysis. The concentration curve is not a linear response, as is generally obtained for immunoassays. Thus, using a logarithmic scale it was possible to fit the experimental points from 15 mIU mL^{-1} to 1000 mIU mL^{-1} into a calibration curve between the change in absorbance (ΔA) and the hCG level with a linear

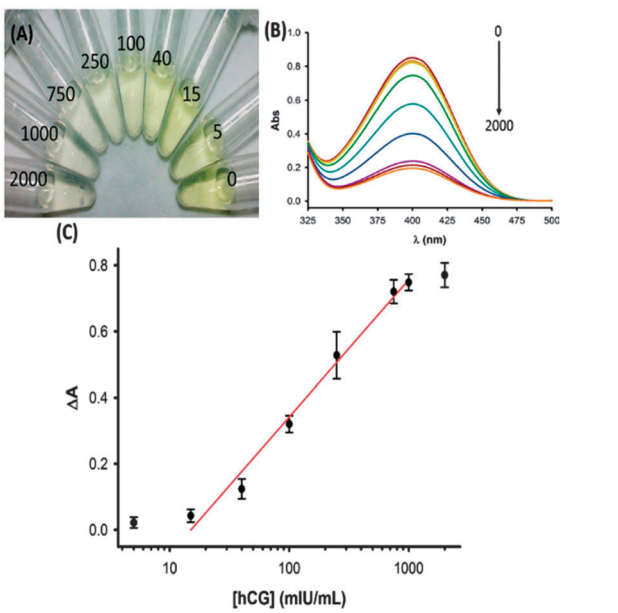

Fig. 2 (A) Photographs of solution in the presence of a 125 nM peptide aptamer probe, and the subsequent addition of 0, 5, 15, 40, 100, 250, 750, 1000, and 2000 mIU mL^{-1} human chorionic gonadotropin (hCG). (B) Ultraviolet absorption spectra of solutions in the presence of different hCG concentrations. (C) Plots of change in absorbance (ΔA) *versus* hCG concentration. $\Delta A = (A_0 - A)/A_0$, where A and A_0 are the absorption intensities at 400 nm with and without hCG addition, respectively.

relationship (R^2 = 0.98). Interestingly, we were able to identify the solution color change from yellow to colorless using the naked eye, even at hCG concentrations as low as 40 mIU mL^{-1}. LOD of this catalysis-based colorimetric strategy can be reduced to 15 mIU mL^{-1} (1.5 ng mL^{-1}) using UV-vis spectrophotometry, which is 20-fold lower than that of the aggregation-based colorimetric assay (Fig. S3, ESI†). Considering the sample volume (400 μL) to the concentration of hCG, the amount of hCG detected with this proposed colorimetric sensor was estimated to be down to the picogram level (600 pg). Although we performed no further optimization, LOD of this detection assay was more than 66-fold lower than a previously described peptide-based LC sensor[8] and comparable to the antibody-based sensors for label detection (Table S1, ESI†).[4a,b,12]

To investigate the specificity of our catalytic AuNP-based colorimetric assay, the effects of potentially interfering chemical substances, such as ascorbic acid, glucose, uric acid, urea, acetaminophen, and acetylsalicylic acid,[13] and other biomarkers or proteins, for example, IgG, IgE, interleukin-12 (IL-12) and interferon-gamma (INF-γ), were monitored and compared with those of hCG. The comparison was made using the low-concentration hCG and high-concentration interfering components with the change in the spectral response. The Δ*A* and corresponding photographic images of AuNP solutions containing 1000 mIU mL^{-1} (equal to 100 ng mL^{-1}) hCG, 50-fold excess of interfering molecules, and 5-fold excess of proteins are shown in Fig. 3. Only hCG caused a significant solution color change through activation of the catalytic AuNP system; the excess of interfering components had only slight effects on the catalytic ability of AuNPs and solution color under the same testing conditions. These results clearly indicate the high specificity of the catalytic AuNP-based colorimetric immunoassay resulting from the strong affinity between hCG and the peptide aptamer.

A further aspect to consider relates to the potential applications of the assay in complex fluids, such as human urine or serum. Thus, spiked solutions were prepared using appropriate dilutions of urine and serum by the addition of a standard sample of hCG within the concentration range of 0–750 mIU mL^{-1}. As can be seen in Fig. S4 (ESI†), Δ*A* increased with the increment of the dilution fold of biological samples. When the biological samples were both diluted by more than 100-fold, Δ*A* matched the result in the buffer solution much better. The reason for such an interference is not quite clear and needs additional analysis, but the presence of some matrix components in biological solutions may be one of the possibilities.[14] Either using the filtration steps or diluting samples could minimize the interference. The latter method would be simple for the practical applications in our study. Comparison between the results obtained with hCG in biological fluids and buffer systems showed that the assay also works in diluted human serum and urine samples (Fig. S5, ESI†). Also, the prepared catalytic AuNP-based sensor was used for the analysis of 4 clinical serum specimens with different hCG concentrations. The results were compared with those obtained by using the enzyme-linked immunosorbent assay (ELISA) provided by the hospital. The concordance correlation coefficient (CCC) is employed to determine precision and accuracy values.[15] The correlation plot showed

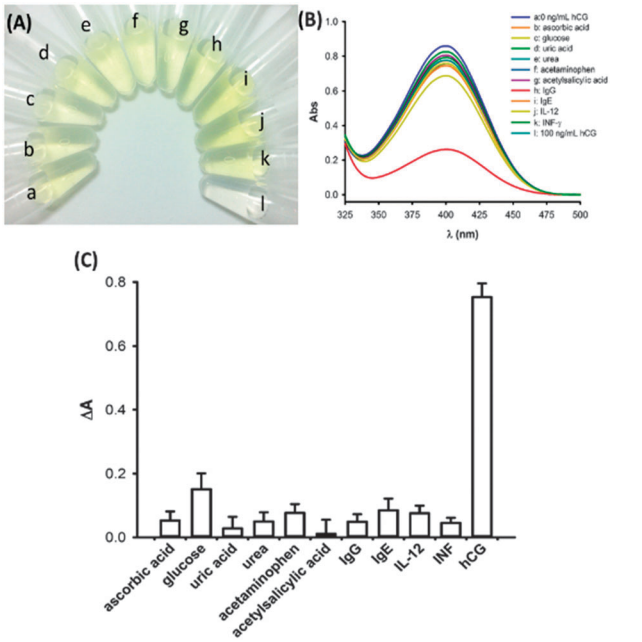

Fig. 3 Selectivity evaluation of this colorimetric approach toward the detection of human chorionic gonadotropin. (A) Photographs and (B) ultraviolet absorption spectra of catalytic gold nanoparticle solution against 5000 ng mL^{-1} of (b) ascorbic acid, (c) glucose, (d) uric acid, (e) urea, (f) acetaminophen, (g) acetylsalicylic acid, 500 ng mL^{-1} of (h) IgG, (i) IgE, (j) IL-12 (k) INF-γ, and response to (a) 0 ng mL^{-1} and (l) 100 ng mL^{-1} (1000 mIU mL^{-1}) hCG. (C) Plots of change in absorbance *versus* concentrations of 5000 ng mL^{-1} chemical interferences, 500 ng mL^{-1} biological substances, and 100 ng mL^{-1} hCG. In the case of hCG, Δ*A* in excess of 5-fold was observed compared with other interferences.

a linear correlation between the AuNP assay and ELISA with a Pearson correlation coefficient of 0.976 (Fig. S6, ESI†). A value of CCC is determined to be 0.968 by using MedCalc. Thus, the resulting accuracy is calculated to be 0.992. The above results indicate that our bioassay can be successfully applied to hCG analysis in practice. However, further efforts are required to minimize the matrix components present in undiluted biological matrices.

The level of hCG in healthy women is as low as 5 mIU mL^{-1}, whereas the concentrations of 25–50 mIU mL^{-1} are an indicator of early pregnancy.[16] An increase of 1000 mIU mL^{-1} within 48 h is effective in screening for abnormal pregnancy in serial hCG measurements.[17] Furthermore, a high hCG level (greater than 1000 mIU mL^{-1}) indicated a significant risk factor for germ-cell tumors.[18] Thus, even without further optimization, given the LOD of 15 mIU mL^{-1} with a linear range up to 1000 mIU mL^{-1} for the present assay, it holds great potential for development into the diagnosis and management of hCG-secreting diseases. In the future, several factors such as the size and concentration of catalytic AuNPs, and the ratio of borohydride to 4-aminophenol might be exploited to improve the detection limits, speed of detection, and quantification in hCG-related diseases.

In summary, we developed a label-free colorimetric assay for hCG detection using a peptide-regulated catalytic AuNP-based mechanism. The presence of hCG activates catalytic AuNPs, resulting in 4-AP production. Use of an hCG-binding peptide aptamer facilitates simple detection with considerable sensitivity

and selectivity. Further development will be required to optimize the concentration of AuNPs, which critically affects the catalytic reaction by providing the catalytic active sites, to achieve the best biosensing capability. In contrast to previous reports, this assay does not require special chemical modification of the probe or any complex equipment, making this approach particularly suitable for low-cost on-site testing. Furthermore, given the advances in peptide technologies, this simple design can be made widely applicable for the analysis of peptide–protein interactions and the selective detection of a wide range of analytes by changing the charged peptide sequence.

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