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A nanogram-level colloidal gold single reagent quantitative protein assay

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ABSTRACT

We have developed a nanogram-level quantitative protein assay based on the binding of colloidal gold to proteins adhered to nitrocellulose paper. The protein–gold complex produces a purple color proportional to the amount of protein present, and the intensity of the stain is quantified by densitometry. Typical assays require minimal starting material (10–20 μl) containing 1 to 5 μg protein. A small volume (2 μl) of protein solution is applied to nitrocellulose paper in a grid array and dried. The nitrocellulose is incubated in colloidal gold suspension with gentle agitation (2–16 h), rinsed with water, and scanned. Densitometric analysis of the scanned images allows quantitation of the unknown sample protein concentration by comparison with protein standards placed on the same nitrocellulose grid. The assay requires significantly less sample than do conventional protein assays. In this report, the Golddots assay is calibrated against weighed protein samples and compared with the Pierce Micro BCA Protein Assay Kit. In addition, the Golddots assay is evaluated with several known proteins with different physical properties.

Analysis of protein concentration continues to be an important activity in most biomedical research laboratories. Procedures based on the ultraviolet absorbance of proteins, or their reactions with specific reagents, are abundant in the literature [1–4]. In general, these procedures require relatively large amounts of sample (μg to mg) and are biased based on the particular protein property exploited in the assay. Unbiased protein assays based on amino acid analysis are considered to be a standard but typically require large amounts of protein [5]. More recently, a fluorescent procedure has been developed with somewhat higher sensitivity than that of earlier procedures [6]. In our laboratory, we found that the established assays consumed significant quantities of valuable sample preparations. As we were using colloidal gold to stain proteins on nitrocellulose Western blots, we investigated the possibility that the same reaction could be used to estimate protein concentration quantitatively [7]. We developed a protein assay in which a small volume (2 μl) of protein solution is applied to nitrocellulose paper (NCP)[1] in a grid array and dried. The nitrocellulose is incubated in colloidal gold suspension with gentle agitation (4–16 h), rinsed with water, and digitized. Densitometric analysis of the scanned images allows quantitation of the unknown sample protein concentration by comparison with protein standards placed on the same nitrocellulose grid. In the current study, we show that the Golddots assay has a working range from 1.5 to 1500 ng protein/dot with a mean coefficient of variation of 5 to 7%. Above 1500 ng/2 μl spot, the assay begins to saturate. In addition, when tested against a panel of four different proteins, individual proteins exhibited differential staining intensity per nanogram protein analyzed [8]. Therefore, the Golddots assay is a sensitive and precise estimator of protein concentration, which is critical for proteomics and standard protein chemistry studies.

Materials and methods

Colloidal gold suspension was obtained from Bio-Rad Laboratories (Hercules, CA, USA). The NCP was Protran BA 45 (0.45 μm pore size, Schleicher & Schuell, Keene, NH, USA), and the sodium dodecyl sulfate (SDS) was ultrapure 10% stock solution from Gibco (Grand Island, NY, USA). Bovine serum albumin (BSA), bovine erythrocyte carbonic anhydrase, egg white lysozyme, and soybean trypsin inhibitor (SBTI) all were products of Sigma–Aldrich (St. Louis, MO, USA). The Micro BCA (bicinchoninic acid) Protein Assay Kit was obtained from Pierce (Rockford, IL, USA). The IntenSE BL Silver Enhancement Kit was obtained from GE Healthcare.

Golddots assay

For analysis, protein samples (20 μl) were adjusted to 0.1% SDS and heated at 80 °C for 10 min. Serial dilutions were made with 0.1% SDS such that 2-μl aliquots contained 1.5 to 1500 ng protein. In addition to unknown samples, a standard curve was constructed using BSA. All samples were spotted in duplicate on the same sheet of nitrocellulose. A template for guiding even rows and columns

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[1] *Abbreviations used:* NCP, nitrocellulose paper; SDS, sodium dodecyl sulfate; BSA, bovine serum albumin; SBTI, soybean trypsin inhibitor; BCA, bicinchoninic acid.

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with approximately 1-cm spacing was obtained by using the flat plastic, detachable, 1000-μl pipette tip holder tray support from the SpaceSaver rack series (Rainin Instrument). To deposit the samples, the nitrocellulose sheet is placed on absorbent tissue paper (e.g., Kimwipe), the template is positioned over the nitrocellulose, and the template is secured to the benchtop with pieces of tape. Aliquots (2 μl) were pipetted onto the nitrocellulose, as guided by the template, into a rectangular grid. This ordered array at 1-cm intervals provides ample distance between dots for accurate background measurement and the use of a grid reading capability of densitometric software for rapid quantitation of the entire array for up to 96 dots per sheet (Fig. 1).

Once all standards and samples were deposited on the nitrocellulose, the array was allowed to thoroughly dry at room temperature and was then placed into the colloidal gold suspension and incubated at room temperature with gentle rocking. The staining reached an endpoint after approximately 2 h, but it can be left overnight because there is no additional staining of protein dots or background after the endpoint of full color development has been achieved (Fig. 2).

On reaching the endpoint, the nitrocellulose was rinsed with water and digitized. Digitization can be carried out wet or dry.

Densitometry

The stained nitrocellulose sheet can be scanned by a standard desktop scanner or by a laser densitometer. Some of the data presented here were obtained using a Molecular Dynamics Personal Densitometer, and the stained spots were quantitated using Molecular Dynamics ImageQuant software (available from GE Healthcare). We also collected data using an Epson Perfection 1660 Photo Scanner, and we analyzed the images using UN-SCAN-IT gel software (Silk Scientific, Orem, UT, USA). Although the more expensive laser densitometer has some advantages for digitizing dots at the higher end of stain density, it is too powerful for some of the lighter staining dots. Indeed, the use of the Epson scanner revealed a more sensitive range for the assay, extending it down to 1.5 to 100 ng. The measured spot intensities were transferred to Quattro Pro (Corel) or Excel (Microsoft) for statistical analysis. Graphs were prepared using SigmaPlot (Systat Software).

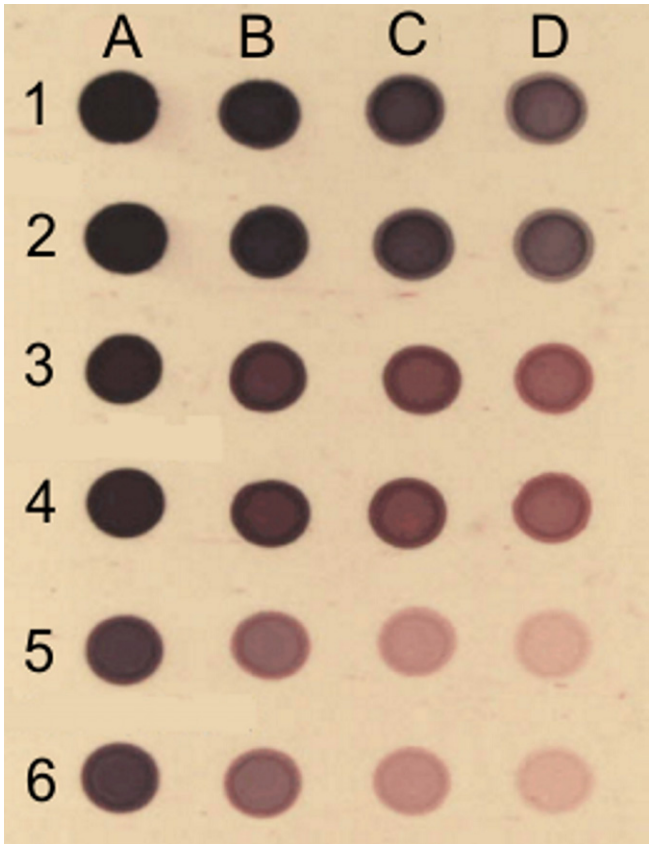

Fig. 1. Golddots assay. Duplicate samples (2 μl) of three proteins were spotted on NCP, dried at room temperature, and stained with colloidal gold suspension (6 h). The nitrocellulose was then dried and digitized. Each row in the grid is a dilution series composed of 1000 ng (A), 500 ng (B), 250 ng (C), or 125 ng (D). Rows 1 and 2 are BSA, rows 3 and 4 are carbonic anhydrase, and rows 5 and 6 are nonfat dry milk (Blotto).

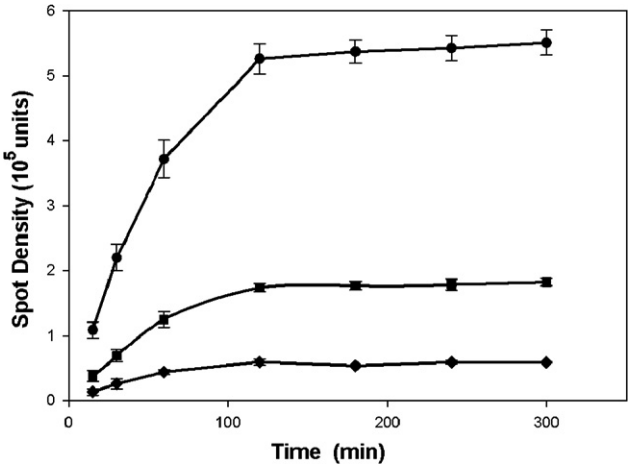

Fig. 2. Time course of Golddots assay. Triplicate samples of three concentrations of BSA were spotted on seven identical pieces of NCP and incubated with colloidal gold as described in materials and methods. At the indicated times, one piece of paper was rexmoved, dried, and analyzed by densitometry. The samples contained 5.35 ng (♦), 22.5 ng (■), or 169 ng (•) of BSA per dot. Each point is the mean of three determinations, and the error bars indicate the standard deviation.

Results and discussion

The appearance of a typical Golddots assay is shown in Fig. 1. The figure demonstrates a regular grid of Golddots 1 cm apart representing a dilution series of BSA, bovine erythrocyte carbonic anhydrase, and casein (Blotto) in duplicate ranging from 125 to 1000 ng protein, as determined by dry weight. The data in the figure show the reproducibility of the assay and the differences in staining sensitivity among these proteins. To optimize the assay, we determined the spot density for three concentrations of BSA as a function of time (Fig. 2). As shown in the figure, the spot density increases with time up to approximately 2 h, after which it remains stable.

Quantitative dose–response Golddot analyses of BSA are presented in Fig. 3. Using the Epson scanner, two linear ranges of spot density were revealed: a low range (1.5–100 ng) and a higher range (100–400 ng). The upper range can be further expanded through the use of the Molecular Dynamics Personal Densitometer, where spots up to 1500 ng can be measured (not shown). One advantage of the Golddots method is that the same blot can be scanned by different instruments to maximize the data range. As is evident in Fig. 3, the two linear ranges have different slopes. In the figure, the regression lines are calculated as a two-segment linear piecewise fit using SigmaPlot. Alternatively, a nonlinear least squares fit to polynomial expression can be used to cover the whole range with a single expression (not shown). The differences in densitometric measurement reflect the two different methods used. The Molecular Dynamics Personal Densitometer measures transmitted light, whereas the Epson scanner measures reflected light. Thus,