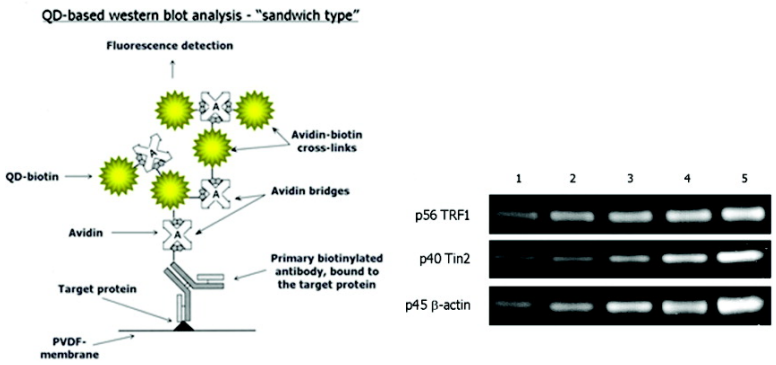
Communication

Quantum Dot-Based Western Blot Technology for Ultrasensitive Detection of Tracer Proteins

Rumiana Bakalova, Zhivko Zhelev, Hideki Ohba, and Yoshinobu Baba

J. Am. Chem. Soc., **2005**, 127 (26), 9328-9329• DOI: 10.1021/ja0510055 • Publication Date (Web): 04 June 2005



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 7 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
COMMUNICATIONS

Published on Web 06/04/2005

Quantum Dot-Based Western Blot Technology for Ultrasensitive Detection of Tracer Proteins

Rumiana Bakalova,*,† Zhivko Zhelev,† Hideki Ohba,*,† and Yoshinobu Baba‡

On-site Sensing and Diagnosis Research Laboratory, AIST-Kyushu, 807-1 Shuku-machi, Tosu 841-0052, Japan, and Department of Applied Chemistry, Graduate School of Engineering, Nagoya University, Nagoya, Japan

Received February 17, 2005; E-mail: r.bakalova-zheleva@aist.go.jp; h.ooba@aist.go.jp

The application notes of Quantum Dot Corporation (USA), published in the January 2005 issue of Nature Methods,[1] undoubtedly demonstrate the potential of quantum dot (QD)-based immunoblotting technology in multiplex detection of proteins or protein states from a single western blot. The described procedure takes the same time as standard western blot analysis with a simple colorimetric detection. The QD-labeled blots can be stored in a buffer at 4−8 °C with minimal loss of signal for imaging at a later date. Moreover, the narrow emission of QD-labeled protein blots enables simplified image acquisition and quantification that give a possibility to overcome the semiquantitative nature of western blot analysis.

The present study describes two different QD-based western blotting protocols and demonstrates one more privilege of this technology—the possibility for ultrasensitive detection of tracer proteins directly in cell lysate (obtained from control untreated cells), avoiding the preliminary procedures of their immunoprecipitation and concentration. With standard western blotting technology, this is extremely difficult and in many cases impossible. The described QD-based protocols are less time-consuming than the classical one, with a high quality of the blot images.

CdSe QDs were synthesized in our lab and conjugated with antibodies according to the methods described in our previous works.[2,3] QDs with emission maximum at 535 nm were used in this study. Telomere associated proteins—telomeric repeat binding factor (TRF1, a 56 kDa protein) and TRF1-interacting nuclear protein 2 (Tin2, ∼40 kDa protein)—were selected as proteins of interest. TRF1 and Tin2 are known to be poorly expressed in Philadelphia-positive (Ph+) cells derived from patients with chronic myelogenous leukemia (e.g., K-562 cells), and several modifications of the standard western blot procedure have to be done to identify both proteins in these cells.[4−6]

The conventional western blot analysis of tracer proteins consists basically of the following preliminary procedures[2−4] (details for TRF1 and Tin2 and principle scheme are described in the Supporting Information): the cells were lysed in appropriate buffer, and the lysates were centrifuged at 14 000*g*; the supernatant was precleared with IgG and protein G-Sepharose; the nonspecific antibody complexes and protein aggregates were removed by centrifugation, and the supernatant was used for immunoprecipitation of tracer proteins by specific monoclonal antibodies; the antigen−antibody complexes were collected on protein G beads, washed several times with buffer, and suspended in Laemmli buffer; the samples were either boiled or nonboiled (to prevent the IgG from co-migration with protein of interest); they were subjected to fractionation on SDS−PAGE and processed for immunoblotting on PVDF membranes using primary and Horseradish Peroxidase (HRP)-conjugated secondary antibodies; the blotted antigens were detected by the chemiluminescence of the HRP-catalyzed reaction of appropriate substrate (ECL Advance Western Blotting Detection Kit from Amersham Biosciences is used more often); the chemiluminescent blots were additionally exposed to X-ray or Polaroid films.

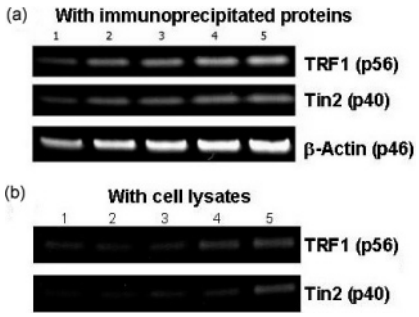


Figure 1. Standard western blot analysis of tracer proteins in K-562 cells. Typical concentration-dependent blots of TRF1, Tin2, and β-actin obtained with and without preliminary immunoprecipitation of the proteins and chemiluminescent detection (numbers 1−5 correspond to the following protein concentrations, applied to each gel patch for electrophoresis: 1−10 μg; 2−20 μg; 3−30 μg; 4−40 μg; 5−50 μg).

Results from conventional immunoblot analysis of TRF1 and Tin2 in K-562 cells are shown in Figure 1. The blots correspond to different protein concentrations. β-Actin (45 kDa protein, highly expressed in K-562 cells) was used as an internal standard. The chemiluminescence of TRF1 and Tin2 blots was weak despite of the preliminary procedures for immunoprecipitation and concentration of both proteins (Figure 1a). In contrast, the chemiluminescence of β-actin blots was so strong that it was difficult to distinguish the differences in protein concentration, especially in high concentration interval. Without preliminary immunoprecipitation of TRF1 and Tin2, it was practically impossible to detect both proteins in precleared K-562 cell lysates (Figure 1b).

QD-based western blot analysis of TRF1 and Tin2 excluded the preliminary steps of their immunoprecipitation and concentration, which (together with the simplified fluorescence detection of the blotted proteins) shortened the time of analysis. Briefly, cell lysates were centrifuged at 14 000*g*, and the supernatant was precleared with IgG and protein G-Sepharose; nonspecific antibody complexes and protein aggregates were removed by centrifugation; the supernatant was subjected to protein fractionation on SDS−PAGE and subsequent immunoblotting on PVDF membranes, using primary QD-labeled antibody (anti-TRF1, anti-Tin2, or anti-β-actin); the blotted QD-labeled antigens were detected directly by one of the following fluorescence gel imaging system: MultiImager (Alpha-InnoTech) or Fluor-S MultiImager (BioRad), at excitation with broad bandwidth UV light and emission at 530 nm. The described methodology was named "QD-based western blot analysis—mono-type" (details are described in the Supporting Information).

† AIST-Kyushu.
‡ Nagoya University and HTRC, AIST, Takamatsu, Japan.

10.1021/ja0510055 CCC: $30.25

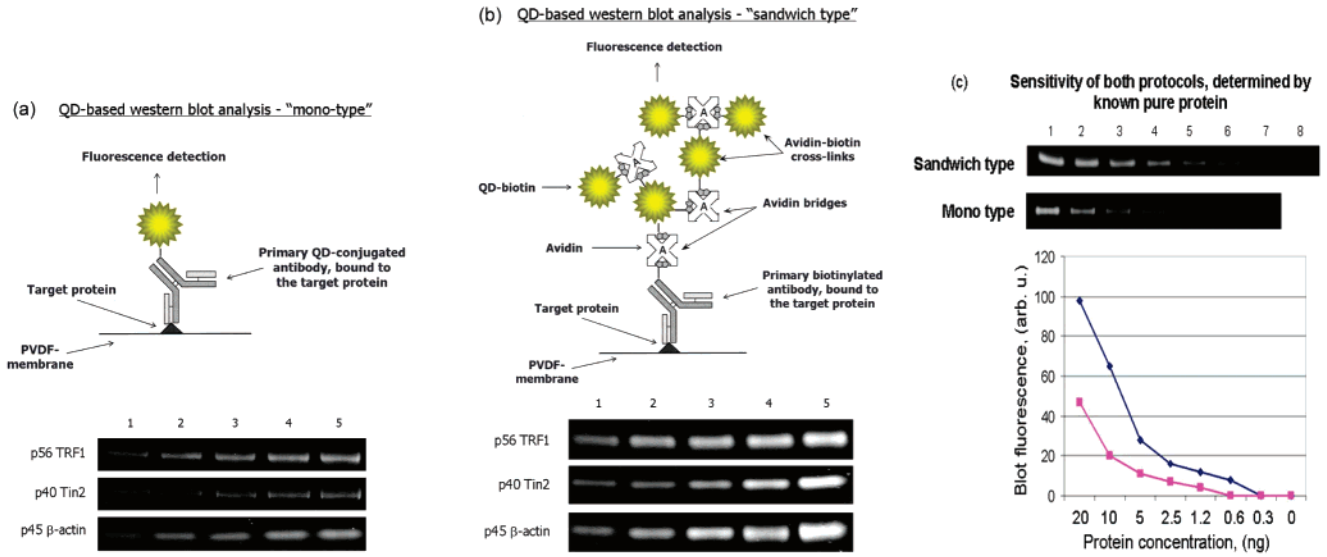


Figure 2. Quantum dot-based western blot analysis of tracer proteins (TRF1, Tin2) and β-actin in leukemia K-562 cells. (a) Mono-type; (b) sandwich-type; (c) sensitivity of both protocols, determined by pure soybean agglutinin. In (a) and (b), numbers 1−5 correspond to the following protein concentrations in cell lysates, applied to each gel patch for electrophoresis: 1−10 μg; 2−20 μg; 3−30 μg; 4−40 μg; 5−50 μg. In (c), the diagram represents mean data from four independent experiments (blue curve, sandwich-type; red curve, mono-type).

The experimental results in Figure 2a represent typical blots of TRF1 and Tin2, analyzed in cell lysates by QD-based "mono-type" protocol. Despite that the fluorescence of QD-labeled TRF1 and Tin2 blots was weak, it was possible to detect both proteins without preliminary immunoprecipitation and concentration procedures.

To improve the fluorescence images of QD-labeled protein blots, we applied a western blotting technology, named "sandwich-type" (Figure 2b). Precleared cell lysates were subjected to protein fractionation on SDS−PAGE and subsequent immunoblotting on PVDF membrane, using primary biotinylated antibody; the membrane was subjected to serial incubations in avidin and QD-labeled biotin; the blotted QD-labeled antigens were detected directly by a fluorescence gel imaging system (details are described in the Supporting Information). TRF1 and Tin2, detected in K-562 cell lysates by sandwich-type western blot technology, possessed a bright fluorescence as a result of biotin−avidin cross-links. In the absence of avidin, it was impossible to detect any blots on the membrane. The images in Figure 2a,b were generated using identical exposure time (15 min) and excitation/emission settings.

The fluorescence intensity of blots was enough to obtain good concentration-dependent curves either for TRF1/Tin2 or β-actin. The fluorescent signal was stable during continuous scanning in the gel imager. No changes in the fluorescence intensity were registered between 5 and 30 min scanning, a time enough for membrane imaging and data acquisition (Figure 2S in Supporting Information). The higher sensitivity of the QD-based methodology in comparison with that of classical analysis can be explained, at least partially, with the inherent sensitivity of fluorescence compared to chemiluminescence.

It was possible to markedly improve the brightness of blots with serial incubation of PVDF membrane in QD-labeled avidin and QD-labeled biotin. However, concentration-dependent saturation of the QD signal was detected at high protein concentrations of K-562 cell lysates (applied to each gel patch), and this procedure can be recommended for work with samples containing less than 20 μg of protein (data are not shown). Since QD−avidin and QD−biotin are commercially available, it is easy to improve the blot images using sandwich-type western blot technology in home-lab conditions. It is also possible to apply sandwich-type immunoassay using the microplate fluorescence reader that has already been described by Goldman et al.[7]

The proposed QD-based western blot procedures allow the possibility for quantification of the blot fluorescence, as well as the slightly shortened time of analysis of tracer proteins in comparison with the standard methodology (from ~31 to ~25 h). Since the quantum yield of water-soluble quantum dots offered by Quantum Dot Corporation (USA) is higher than that fabricated in our lab, there is a serious potential to increase the quality of the images even in the case of mono-type western blotting technology, avoiding sandwich-type avidin/QD−biotin washing steps. The sensitivity of our protocols was up to 1 and 5 ng of pure protein per gel patch for sandwich- and mono-type, respectively (Figure 2c). Ornberg et al.[1] have reported a sensitivity of their QD-based western blot analysis up to 20 pg. Both procedures in our study were developed for detection of TRF1 and Tin2 in K-562 cells, but they can be also used for detection of other tracer proteins directly in different cell lysates.

Supporting Information Available: Details of the experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

Note Added after ASAP Publication. In the version published on the Internet June 4, 2005, the protein concentrations in the figure captions should be in micrograms. On page 2, the sensitivity for the protocol is for pure protein. The final version, published June 7, 2005, and the print version are correct.

References

(1) Ornberg, R. L.; Harper; T. F.; Liu, H. *Nat. Methods* **2005**, *2*, 79−81.
(2) Bakalova, R.; Ohba, H.; Zhelev, Z.; Nagase, T.; Jose, R.; Ishikawa, M.; Baba, Y. *Nano Lett.* **2004**, *4*, 1567−1573.
(3) Zhelev, Z.; Ohba, H.; Bakalova, R.; Jose, R.; Fukuoka, S.; Nagase, T.; Ishikawa, M.; Baba, Y. *Chem. Commun.* **2005**, *15*, 1980−1082.
(4) Ohyashiki, J. H.; Hayashi, S.; Yahata, N.; Iwama, H.; Ando, K.; Tauchi, T.; Ohyashiki, K. *Int. J. Oncol.* **2001**, *18*, 593−598.
(5) Cook, B. D.; Dynek, J. N.; Chang, W.; Shostak, G.; Smith, S. *Mol. Cell. Biol.* **2002**, *22*, 332−342.
(6) Bakalova, R.; Ohba, H.; Zhelev, Z.; Kubo, T.; Fujii, M.; Ishikawa, M.; Shinohara, Y.; Baba, Y. *FEBS Lett.* **2004**, *564*, 73−84.
(7) Goldman, E. R.; Clapp, A. R.; Anderson, G. P.; Uyeda, H. T.; Mauro, J. M.; Medintz, I. L.; Mattoussi, H. *Anal. Chem.* **2004**, *76*, 684−688.

JA0510055