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# Chemiluminescence detection of proteins from single cells

(avidin/biotin/immunoblot/photoreceptor)

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ABSTRACT **The analysis of proteins from single cells requires techniques of supreme sensitivity. Although radiochemical procedures are capable of detecting small amounts of electrophoretically separated proteins, their sensitivity falls short of that required for routine detection of minor components of single cells. Utilizing the avidin–biotin interaction and the alkaline phosphatase substrate 3-(4-methoxyspiro{1,2-dioxetane-3,2′-tricyclo-[3.3.1.1$^{3,7}$]decan}-4-yl)phenyl phosphate (AMPPD), we have developed an alternative, chemiluminescence-based method for protein detection whose sensitivity exceeds that of other methods. Applying this method to a purified protein, we could detect as little as 63 fg (0.9 amol) of biotinylated bovine serum albumin. The sensitivity of the method was demonstrated by the detection of proteins from individual photoreceptor outer segments, including proteins constituting ≈1% of the total. Chemiluminescence detection also proved extremely sensitive for immunoblotting: a comparison of five methods for detection of antibody–antigen interactions showed that the AMPPD technique was more sensitive than detection with a colorimetric alkaline phosphatase substrate, $^{125}$I-labeled protein A, $^{125}$I-labeled anti-mouse IgG, or colloidal gold-conjugated anti-mouse IgG.**

When analyzing cellular constituents, biochemists perennially strive to detect smaller amounts of protein. Since the introduction of SDS/PAGE, this motivation has resulted in a progressive reduction in the threshold for protein detection from ≈100 ng with Coomassie blue staining (1) to ≈100 pg with silver stains (2). The most sensitive technique in general use today involves the autoradiographic detection of proteins after covalent labeling with radiochemicals. Despite the sensitivity afforded by radioisotopes, concern about their associated hazards and disposal problems has stimulated the continuing development of sensitive detection methods. Several of these techniques have proven successful: silver staining of miniature gels (3), electroblot-based colorimetric methods using horseradish peroxidase (4, 5) and alkaline phosphatase (6, 7), and colloidal gold labeling (8, 9) with silver-mediated enhancement (8). None of these techniques, however, is capable of detecting the subpicogram amounts of individual proteins in a single small cell.

Chemiluminescence methods can potentially detect extremely small amounts of protein: in solution, the light output from the activity of fewer than 1000 molecules of alkaline phosphatase may be measured (10). Chemiluminescence methods based on horseradish peroxidase (11) and alkaline phosphatase (12–14) have been developed for the detection of very small amounts of DNA. Adaptation of these assays to protein detection has been impeded, however, by the high background that characteristically accompanies the signal.

In this report, we describe chemiluminescence methods optimized for usefulness in the analysis of proteins. We find that if the background is minimized by a judicious choice of the membrane-blocking solution, one may use biotinylation, electroblotting, and chemiluminescence to detect remarkably small amounts of protein. We can, in fact, analyze the proteins from individual rod outer segments. The same methods also serve well in the detection of proteins by antibodies on immunoblots. The advantages of the chemiluminescence detection method favor it over all the other techniques we examined.

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## EXPERIMENTAL PROCEDURES

**Sources.** The immunogold conjugate anti-mouse IgG kit (AuroProbe BLplus), $^{125}$I-labeled protein A, and $^{125}$I-labeled anti-mouse IgG were obtained from Amersham. Avidin-agarose, bovine casein, and bovine hemoglobin were obtained from Sigma. *N*-Hydroxysuccinimidobiotin (NHS-biotin) and *N*-hydroxysulfosuccinimidobiotin (sulfo-NHS-biotin) were obtained from Pierce. Gradient gels of 3–17% acrylamide were obtained from Jule (New Haven, CT). Other materials were obtained from the suppliers indicated below or in ref. 15.

**Biotinylation.** Bovine serum albumin (BSA) was biotinylated at room temperature with NHS-biotin in 25 mM Hepes (pH 8.0). The reaction was stopped by the addition of lysine to 100 mM. The extent of biotinylation was monitored by trichloroacetic acid precipitation, Pronase digestion, and competition of the biotin-labeled peptides with the dye 4′-hydroxyazobenzene-2-benzoic acid for avidin binding sites (16). For the experiment depicted in Fig. 1, BSA was diluted in SDS/PAGE sample buffer containing 100 mg of unlabeled lysozyme per liter.

Sealed rod outer segments from bullfrogs (*Rana catesbeiana*) were purified on a Percoll gradient (17) and resuspended in 110 mM NaCl/2 mM KCl/2 mM $MgCl_2$/3 mM D-glucose/25 mM Hepes, pH 8.0. Outer segments were permeabilized by electroporation (18) or with 20–40 mg of saponin per liter. After 30 min labeling with 2.5 mM sulfo-NHS-biotin, outer segments were diluted into buffer solution layered on a pad of 68% Percoll in a 35-mm plastic dish. While being observed under a dissecting microscope with dark-field illumination, outer segments could then be isolated by gently sucking them into a glass micropipette. Samples were treated with SDS/PAGE sample buffer at room temperature for at least 30 min.

The shift in apparent molecular mass of biotinylated BSA upon SDS/PAGE was proportional to the number of biotin moieties conjugated and approximated 400 Da per mol of biotin (data not shown). Based on this calibration factor, proteins typically incorporated 1–10 mol of biotin per mol of enzyme when exposed to 2.5 mM NHS-biotin or sulfo-NHS-biotin for 15–30 min.

Abbreviations: AMPPD, 3-(4-methoxyspiro{1,2-dioxetane-3,2′-tricyclo-[3.3.1.1$^{3,7}$]decan}-4-yl)phenyl phosphate; BCIP, 5-bromo-4-chloro-3-indolyl phosphate; BSA, bovine serum albumin; NBT, *p*-nitro blue tetrazolium chloride; NHS-biotin, *N*-hydroxysuccinimidobiotin; sulfo-NHS-biotin, *N*-hydroxysulfosuccinimidobiotin; PVP-40, polyvinylpyrrolidone 40.

**SDS/PAGE and Blotting.** Proteins were separated by SDS/PAGE on minigels and were transferred to nylon blotting membranes. For total protein detection, proteins were transferred at 4°C to charged nylon (ZetaProbe; Bio-Rad) in 10 mM 3-(cyclohexylamino)-1-propanesulfonic acid (pH 10.8). A field of 625 V/m was applied for 16 hr with plate electrodes (Bio-Rad). For immunoblotting, proteins were transferred to charged nylon in 10 mM 3-(cyclohexylamino)-1-propanesulfonic acid as described above or to uncharged nylon (Tropix, Bedford, MA) in 15.6 mM Tris base/120 mM glycine/20% (vol/vol) methanol. The optimal time of transfer was determined for each protein that was examined by immunoblotting. After transfer, membranes were incubated in transfer solution or 68 mM NaCl/75 mM sodium phosphate, pH 7.4 (PBS) for at least 12 hr.

**Chemiluminescence Detection of Total Protein.** After protein transfer, remaining protein-binding sites on the membrane were saturated for 2–4 hr with a blocking solution of 6% casein/1% polyvinylpyrrolidone 40 (PVP-40)/3 mM $NaN_3$/10 mM EDTA/PBS, pH 6.8. To reduce alkaline phosphatase activity contaminating the casein and to aid dissolution, this solution was heated to 65°C for 1 hr and then cooled to room temperature before the addition of $NaN_3$. The solution was stored at 4°C prior to use. To reduce the concentration of contaminating biotin and biotinylated proteins, an aliquot of the blocking solution was agitated for 16 hr with 10 ml of avidin-agarose per liter, which was capable of binding ≈1 μmol of biotin per liter of blocking solution. The treated solution was filtered through a sintered-glass funnel and used immediately. The membrane was incubated for 2 hr with 1:30,000 streptavidin/alkaline phosphatase (Tago) in the blocking solution. Higher background ensued if the streptavidin/alkaline phosphatase was diluted in a solution of lower casein concentration. The membrane was next washed with five 5-min changes of 0.3% Tween 20/PBS and five 5-min changes of 1 mM $MgCl_2$/50 mM sodium carbonate-bicarbonate, pH 9.6. The membrane was then incubated for 5 min in the latter solution containing 400 μM 3-(4-methoxyspiro{1,2-dioxetane-3,2′-tricyclo-[3.3.1.1$^{3,7}$]decan}-4-yl)phenyl phosphate (AMPPD; Tropix), blotted lightly with filter paper to remove surface moisture, and wrapped in plastic wrap. After a 20-min preincubation at room temperature, the membrane was exposed for 5–1200 s to x-ray film (XAR or XRP; Kodak).

AMPPD solutions could be reused several times if the concentration of the substrate was monitored. To determine AMPPD concentration, we injected samples onto a reverse-phase HPLC column (ODS Hypersil, 100 × 2.1 mm; Hewlett–Packard) equilibrated with 0.15% trifluoroacetic acid in water. When the column was developed with a 12-min gradient of 60% acetonitrile/40% water/0.12% trifluoroacetic acid, AMPPD was eluted after 10.3 min. We found that satisfactory results could be obtained until the substrate concentration declined below half its original value.

**Chemiluminescence Detection of Antigen–Antibody Interactions.** After protein transfer, the blotting membrane was blocked for 2–4 hr with the blocking solution used for total protein detection (untreated with avidin-agarose) or with 4% casein/2% hemoglobin/1% PVP-40/3 mM $NaN_3$/PBS, pH 6.8. The membrane was next incubated for 1–2 hr with primary antibody that had been diluted in blocking solution, then washed with four 5-min changes of 0.3% Tween/PBS. To detect antibody–antigen interactions, the membrane was incubated for 1 hr with an alkaline phosphatase-conjugated secondary antibody (Southern Biotechnology Associates, Birmingham, AL) diluted 1:1000 in the blocking solution. The membrane was successively washed with 0.3% Tween/PBS and $MgCl_2$/carbonate, incubated with AMPPD, wrapped in plastic wrap, and exposed to film as described above.

**Other Methods.** The concentration of BSA was determined by measuring the optical density at 280 nm ($\varepsilon = 4.4 \times 10^6$ $M^{-1} \cdot m^{-1}$; ref. 19). Blots developed with alternative detection methods were developed as described in the legend of Fig. 3. Comparison of the sensitivity and dynamic range of the immune detection methods was performed with a laser densitometer (Ultroscan XL; Pharmacia LKB).

## RESULTS

**Use of Chemiluminescence for Detection of Total Protein.** To analyze very small amounts of protein, such as the constituents of a single cell, we first labeled the proteins with biotin esters of *N*-hydroxysuccinimides. These readily available compounds form stable, covalent linkages with α- and ε-amino groups under mild reaction conditions (20). Biotinylated proteins were then separated by SDS/PAGE and transferred onto charged nylon membranes. The electroblots were probed with streptavidin/alkaline phosphatase, which bound tightly to the biotin moieties of the derivatized proteins. Finally, we used the alkaline phosphatase substrate AMPPD, which slowly decomposes after dephosphorylation to yield photons that can be detected with standard x-ray film (21, 22).

The choices of transfer membrane and blocking solution proved critical for attaining the maximal signal/noise ratio in the detection of biotinylated proteins. Nylon membranes are advantageous in that they enhance light production in the assay procedure (14). Uncharged nylon membranes are of limited use for total protein detection, for proteins of widely disparate molecular size cannot be quantitatively transferred to and retained by such membranes (23). We therefore chose for our experiments positively charged nylon membranes (24), which allowed the transfer to and retention by the membrane of nearly 100% of proteins of molecular mass <100 kDa and >75% of proteins as large as the 400-kDa heavy chain of laminin (15, 23). The high protein-binding capacity of these membranes, which substantially increased the background, mandated an unusually effective blocking agent. As in other chemiluminescence detection methods (13, 14), casein proved to be the most effective single blocking agent. We initially used as a blocking solution 4% casein and 2% hemoglobin, a combination that improved the signal/noise ratio substantially over 6% casein or 6% hemoglobin alone. We later found, however, that the best signal/noise ratio attainable was limited by contamination of the casein with biotinylated proteins and perhaps free biotin (data not shown). To improve the signal/noise ratio, we therefore removed these contaminants from the blocking solution with avidin-agarose.

Application of the method described here to purified, biotinylated BSA demonstrated the sensitivity of the technique (Fig. 1). We were able to detect as little as 63 fg ($63 \times 10^{-15}$ g) of BSA; this amount of BSA corresponds to 0.9 amol ($0.9 \times 10^{-18}$ mol), or ≈600,000 molecules. Fig. 1 also shows that treatment of the blocking and streptavidin-dilution solutions with avidin-agarose improved detection by a factor of ≈8; 500 fg of biotinylated BSA was the least that could be detected if the casein blocking solution had not been treated. Although the output of light remained substantial for at least 20 hr (14), the membranes of Fig. 1 were exposed to film for only several minutes; the background limited the detection of still smaller amounts of protein.

**A Case Study: Detection of Proteins from Individual Rod Outer Segments.** To demonstrate more graphically the sensitivity of this detection method, we examined biotinylated proteins from single outer segments of rod photoreceptors. Because they contain well-known amounts of easily identified proteins, frog outer segments provide an excellent test of the sensitivity demonstrated with purified proteins. One such

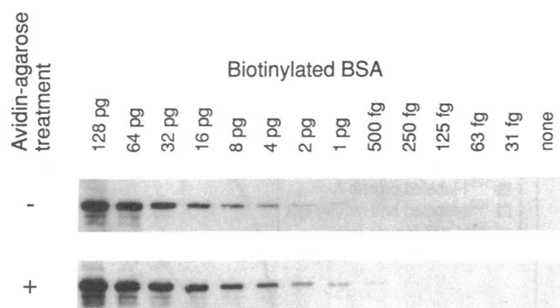


FIG. 1. Threshold for protein detection by chemiluminescence. BSA, conjugated at a level of 10 mol of biotin per mol of protein, was diluted to the specified amounts and electrophoresed on a 12% acrylamide gel. Blot 1, the blocking and streptavidin/alkaline phosphatase dilution solution contained 6% casein; the minimum detectable band contained 500 fg of BSA. The film was exposed for 15 min. Blot 2, the blocking and streptavidin/alkaline phosphatase solution buffers contained 6% casein that had been treated overnight with 10 ml of avidin-agarose per liter; the highest sensitivity was accordingly attained. Although not visible in the photographic reproduction, the band that contained 63 fg of BSA could be seen by eye. The film was exposed for 4 min. Treatment with avidin-agarose reduced the background due to biotinylated proteins in the blocking solution. In addition, by removing biotin from the solution used to dilute the streptavidin/alkaline phosphatase conjugate, the treatment increased the effective concentration of the conjugate applied to the nylon membrane. The exposure time was thus shorter for the blot that used avidin-agarose-treated solutions. The minor bands were not the result of heterogeneity in the number of biotins conjugated but rather were derived from contaminants that could be demonstrated after SDS/PAGE and silver-staining of the unbiotinylated BSA preparation.

outer segment contains $3 \times 10^9$ molecules of rhodopsin, $3 \times 10^8$ molecules of transducin, $8 \times 10^7$ molecules of arrestin, and $3 \times 10^7$ molecules of the cGMP phosphodiesterase (17, 25).

By permeabilizing outer segments and biotinylating their constituents with sulfo-NHS-biotin, we labeled both intracellular and extracellular proteins. We then isolated single outer segments and electrophoresed their proteins on a gradient gel (Fig. 2*A*). Rhodopsin, the $\alpha$ subunit of transducin, and arrestin were readily identified. Even less abundant proteins, such as the $\alpha$ and $\beta$ subunits of the cGMP phosphodiesterase, could easily be seen in the gel lanes from individual outer segments. Low molecular weight protein bands that may correspond to the $\gamma$ subunits of transducin and phosphodiesterase were also routinely observed. The chemiluminescence signal from each of these bands decidedly exceeded the background. Coomassie blue staining of gels containing proteins from biotinylated and unbiotinylated outer segments (Fig. 2*B*) demonstrated the similarity in protein patterns and the minor shifts in molecular mass of proteins induced by biotinylation.

**Use of Chemiluminescence Detection in Immunoblotting.** The success of total protein detection by chemiluminescence encouraged us to perform immunoblotting with this system. To observe antibody–antigen interactions, we probed nylon blotting membranes with an alkaline phosphatase-conjugated secondary antibody; AMPPD hydrolysis was then detected as before. In association with enhancers of chemiluminescence (10) and carbonate-free buffers, the use of poly(vinylidene difluoride) membranes also afforded excellent sensitivity with immunoblots.

We used the chemiluminescence detection method successfully with a variety of monoclonal and polyclonal antibodies. To compare the sensitivity of the chemiluminescence method with that of other high-sensitivity techniques for the detection of antibody–antigen interactions, we examined the

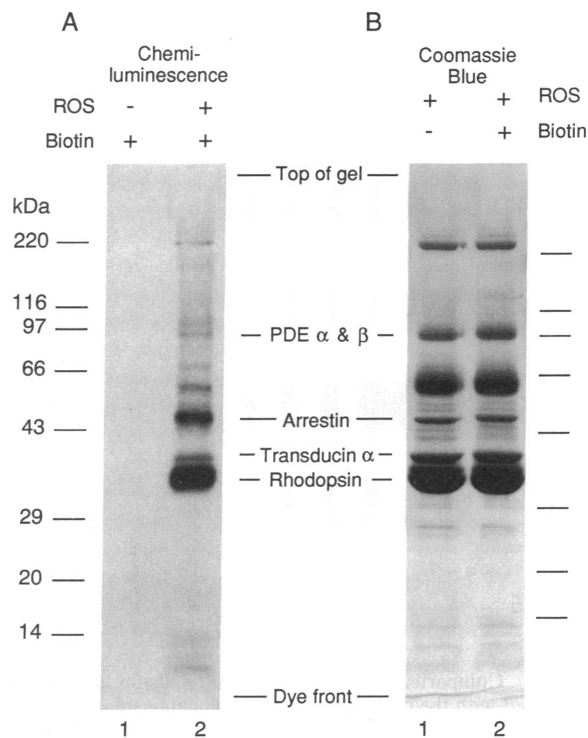


FIG. 2. Demonstration of high-sensitivity protein detection in a single cell. Samples were subjected to SDS/PAGE on 3–17% acrylamide gradient gels and stained with Coomassie blue or transferred to a charged nylon membrane. (*A*) Chemiluminescence detection of proteins from a single rod outer segment. Lane 1, a control sample of buffer solution from the outer segment-containing preparation; lane 2, a single outer segment from a bullfrog retina. (*B*) Coomassie blue staining of proteins from several million outer segments (≈0.1 retina). Outer segment proteins before (lane 1) or after (lane 2) biotinylation. Some rhodopsin dimers formed in the Coomassie blue-stained sample, in which the concentration of rhodopsin was several millionfold greater than in the sample used in lane 2 of *A*. Outer segment proteins were identified by their behavior upon moderate and low ionic strength extraction in the presence or absence of GTP (26) and by their relative molecular masses. Molecular mass values displayed on the left of *A* also apply to *B*.

immune detection on slot blots of the microtubule motor protein kinesin (27, 28). After applying specific amounts of purified kinesin to strips of uncharged nylon, we probed the membranes with a monoclonal antibody directed against the heavy chain of kinesin (H2; ref. 29). The AMPPD method was used as described. Four other detection procedures were used for a comparison: anti-mouse IgG-conjugated alkaline phosphatase hydrolysis of the colorimetric substrate pair 5-bromo-4-chloro-3-indolyl phosphate (BCIP) and *p*-nitro blue tetrazolium chloride (NBT) (6), colloidal gold-conjugated anti-mouse IgG (9), $^{125}$I-labeled protein A (30), and $^{125}$I-labeled anti-mouse IgG (4).

Examination of the developed immunoblots revealed that the chemiluminescence detection method was the most sensitive. The smallest amount of kinesin on the membrane, 10 pg, was easily detected in a 5-min exposure (Fig. 3*A*, blot 1). As little as 1 pg of kinesin (3 amol, or ≈2 million kinesin tetramers) could be detected on blots with smaller amounts of kinesin (data not shown). The minimum amount of kinesin detected with BCIP and NBT was 10 pg; the other detection methods were even less sensitive. The detection limits for both $^{125}$I-labeled protein A and $^{125}$I-labeled anti-mouse IgG were as low as 300 pg, but exposures of 2–5 days were required. Detection with colloidal gold-conjugated anti-mouse IgG was even less sensitive.

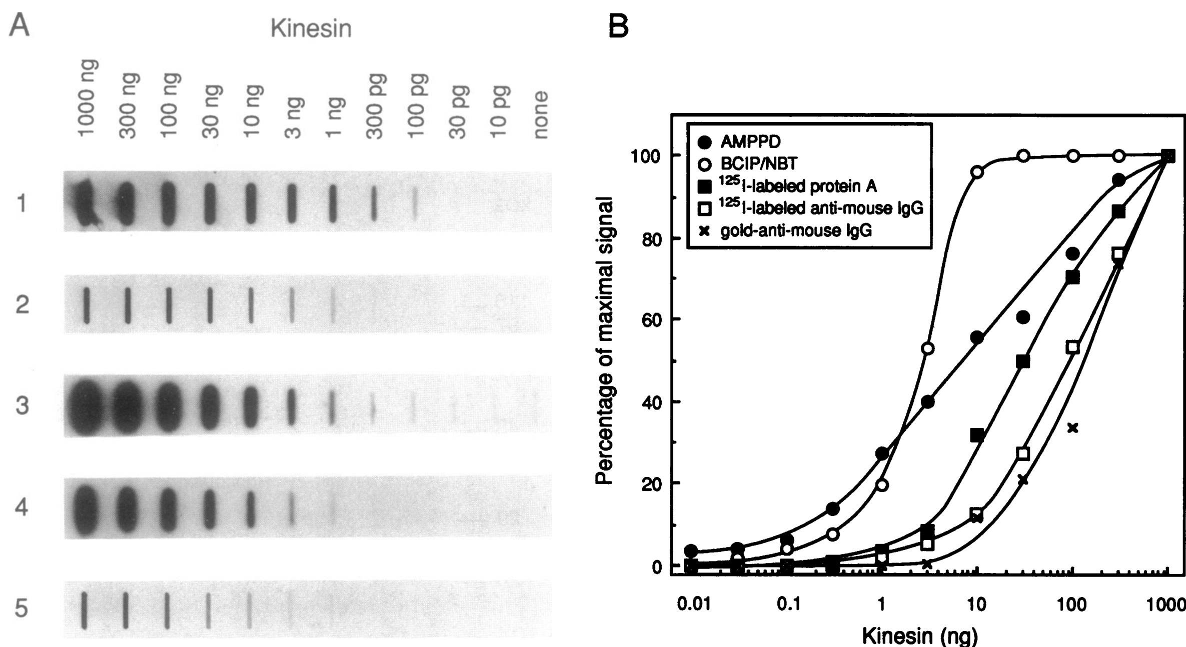


FIG. 3. Comparison of five sensitive methods for antigen–antibody detection. (*A*) Detection of kinesin on slot blots. Purified bovine kinesin was diluted with the casein/hemoglobin blocking solution diluted 1:1000 with PBS; the indicated amounts were applied to an uncharged nylon membrane with a slot-blot device. The membranes were blocked for 3 hr, incubated with 1:5000 anti-kinesin ascites fluid for 1 hr, washed, and detected with secondary reagents as indicated. Blot 1 (chemiluminescence), detection was as described in *Experimental Procedures*. The film was exposed for 5 min. Blot 2 (BCIP and NBT), the membrane was blocked with 6% casein/1% PVP-40/3 mM $NaN_3$/PBS; antibody–antigen complexes were detected with alkaline phosphatase-conjugated anti-mouse IgG. Blots were developed with a mixture of 350 $\mu$M BCIP and 350 $\mu$M NBT until background staining became apparent in ≈2 hr. Blot 3 ($^{125}$I-labeled protein A), antibody–antigen complexes were detected with an unlabeled anti-mouse IgG followed by a 2-hr incubation with 740 kBq of $^{125}$I-labeled protein A per liter as described (31). Detection of amounts below 300 pg was limited by the reaction of protein A with immunoglobulins contaminating the casein solution used to dilute kinesin. The film was exposed with an intensifying screen for 2 days at −70°C. Blot 4 ($^{125}$I-labeled anti-mouse IgG), antibody–antigen complexes were detected with 370 kBq of $^{125}$I-labeled anti-mouse IgG per liter. The film was exposed with an intensifying screen for 5 days at −70°C. Blot 5 (silver-enhanced colloidal gold-conjugated anti-mouse IgG), antibody–antigen complexes were detected by following the manufacturer's instructions. (*B*) Densitometric analysis of blots 1–5. The integrated area of each signal was normalized to the maximal response and plotted against the amount of kinesin applied. The blank values from slots in which no kinesin was applied, which were significant only with $^{125}$I-labeled protein A, were not subtracted from the data. The curves were drawn by eye.

To quantify the advantages of the chemiluminescence detection system, we subjected the slot blots of Fig. 3 to laser densitometry. The integrated area of each kinesin detection signal, relative to the maximal response, was plotted against the amount of kinesin (Fig. 3*B*). This graph illustrates the extreme sensitivity, linearity on a logarithmic scale, and wide dynamic range of the AMPPD assay. Although the colorimetric detection method was only 10-fold less sensitive, the dynamic range of this method was considerably narrower. The broad dynamic range of the chemiluminescence method makes it particularly useful for quantitative immunoblotting when samples containing widely varying amounts of an antigen are to be analyzed.

## DISCUSSION

The chemiluminescence technique for total protein detection is remarkably sensitive. Under optimal conditions, we can consistently detect <100 fg of a common protein such as BSA. The assay can, in other words, detect less than an attomole of protein, or <600,000 molecules. This threshold implies that a protein of modest abundance—0.1% of a cell's total—can be detected from a single cell of modest size—10 $\mu$m in diameter.

Although radioiodine-based methods for total protein detection (32–34) or immunoblotting (4, 30) can detect small amounts of protein, their use can require multimillicurie manipulation and protracted exposures of autoradiographs. These techniques also suffer from diffuse protein bands due to the spread of the $\gamma$-radiation. In addition to its sensitivity, the virtues of the chemiluminescence detection method are its short film exposures, sharply defined protein bands, and lack of associated hazards. While AMPPD is not inexpensive, substantial savings are accrued by elimination both of the cost of radiochemicals and of disposal expenses. In addition, if care is taken to avoid contamination, AMPPD solutions can be reused several times.

To achieve the detection sensitivity described here, we covalently label proteins with biotin prior to electrophoresis. This procedure affords investigators two strategies for selectively examining cytoplasmic or extracellularly exposed proteins (15, 35–37). First, by using membrane-permeant or -impermeant biotinylation reagents, one may label respectively all proteins or only those on the cellular surface. Alternatively, one may use an impermeant biotinylation reagent, such as sulfo-NHS-biotin, and achieve specificity by labeling in the presence or absence of a membrane-permeabilizing reagent. We have successfully applied both approaches in the detection of scarce proteins from the sensory hair bundles of the frog's internal ear (15). Proteins might also be labeled after electrophoresis and transfer to membranes, which would eliminate the shift in molecular mass experienced by biotinylated proteins and allow examination of protein mixtures by two-dimensional electrophoresis. Posttransfer labeling methods may be plagued, however, by labeling of contaminants such as human skin keratin (15, 38).

Because the number of amino groups derivatizable by *N*-hydroxysuccinimide esters varies from protein to protein, the intensities of chemiluminescent bands may not precisely

reflect the relative abundances of the corresponding proteins. This problem, however, is not confined to the chemiluminescence detection technique. The frequently used Bolton–Hunter reagent (34) also relies on the reaction of an *N*-hydroxysuccinimide and thus skews labeling in the same manner. Techniques for iodination of tyrosyl residues (32, 33) are even less indicative of the abundance of each protein, for exposed tyrosyl residues are relatively rare. The capriciousness of protein staining with silver is also well documented (39). Although comparison of the chemiluminescence bands of Fig. 2*A* with the Coomassie blue-stained bands of Fig. 2*B* reveals some discrepancies in the labeling pattern, the chemiluminescence method nevertheless provides a reasonable picture of the protein complement of the outer segment.

The limiting factor in chemiluminescence detection is the background; although bands containing <100 fg of protein can be detected with only 4 min of exposure, background fogging of the film precludes detection of still smaller amounts of material. We believe that this background is due largely to the hydrolytic activity of the alkaline phosphatase conjugate nonspecifically adsorbed on the membrane. Because the light output remains substantial for at least 20 hr, further reduction of the background by the use of an alternative blocking agent or an improved membrane could allow an increase in exposure time—and hence of sensitivity—of several hundredfold.

In the detection of antibody–antigen interactions, the chemiluminescence technique results in a substantial increase in sensitivity compared with commonly used methods. Because the chemiluminescence method for protein detection on immunoblots does not rely on the avidin–biotin interaction, nonspecific background is reduced. When limited amounts of antigen are available, charged nylon membranes offer the advantage that blots can be stripped with 4 M $MgCl_2$ or 1% SDS and then reprobed with other antibodies (unpublished data). The chemiluminescence technique can easily replace any other detection method now used in immunoblotting: the sensitivity is greater and the hazards associated with radioactivity are eliminated.

In addition to permitting analysis of unprecedentedly small protein samples, the sensitivity provided by chemiluminescence protein detection allows innovative experimental approaches. For example, examination of the proteins from particular cells or organelles could follow whole-cell recording of membrane currents using an electrode filled with a biotinylation reagent. The expression of cloned proteins in single *Xenopus* oocytes could be confirmed by immunoprecipitation of biotinylated antigen or by immunoblotting. A single cell can now serve routinely as the starting material for protein analysis.

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