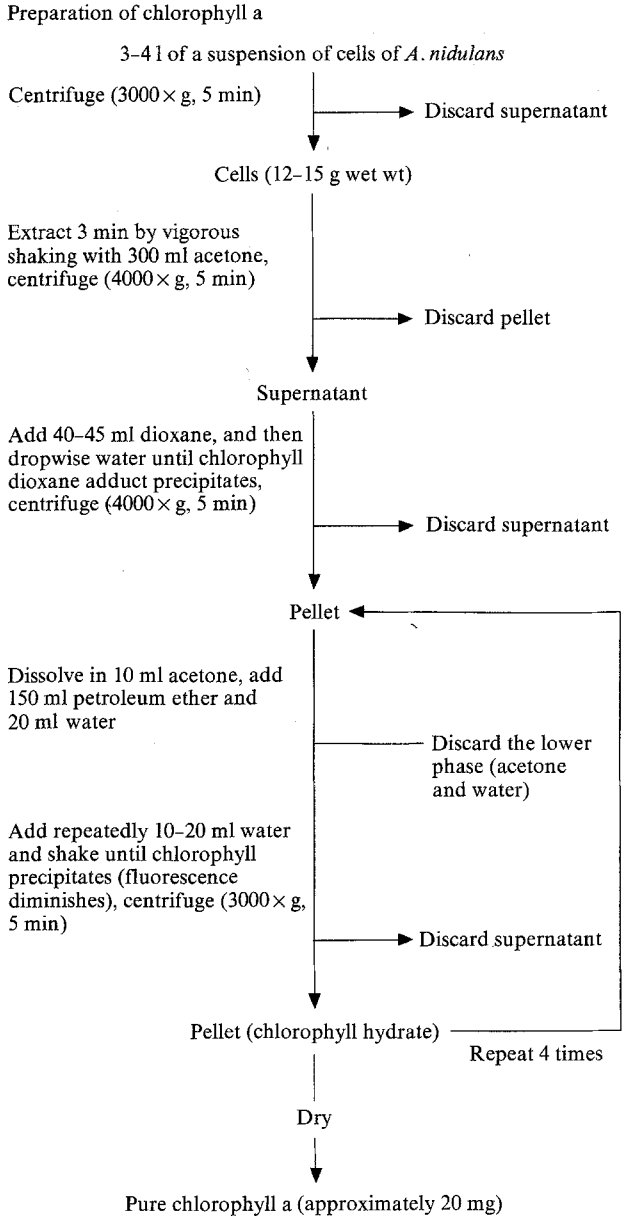

is the only chlorophyll in blue-green algae, and that it can be precipitated as chlorophyll dioxane adduct[9] and as chlorophyll hydrate[3]. The chlorophyll hydrate, also termed polycrystalline chlorophyll a, is characterized by its broad absorption peak around 740 nm[3]. A flow sheet of the preparation of chlorophyll a is given in the figure. All work was done under a dim light and with cooled solvents (0–5 °C). The final precipitation of chlorophyll hydrate was carried out from 2-methylbutane, which has a low boiling-point (28 °C) and is therefore easy to remove during drying. The purity of chlorophyll a was checked by elemental analysis, determination of the absorption coefficient, thin layer chromatography and spectrography in the UV, visible and IR regions. All data were in excellent accordance with those known for the purest chlorophyll a, isolated from spinach[2,3,10].

Analysis by HPLC of a routine sample shows 1 peak of chlorophyll a and small amounts (< 1%) of impurities (xanthophyll?, pheophytin a). The fact that chlorophyll a from spinach and *A. nidulans* have the same retention time in HPLC gives - in addition to the other analytical data - further evidence for the identity of the 2 chlorophylls. Mixtures of chlorophyll a with derivatives (methyl chlorophyllide a, pheophytin a, chlorophyll a') are resolved excellently by HPLC. Especially the completeness of the separation of chlorophyll a' (a stereoisomeric chlorophyll a) from the parent chlorophyll a demonstrates the superiority of HPLC to conventional sugar chromatography. The table shows the retention times of the pigments.

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Comparison of chemiluminescence and absorptiometry in enzyme immunoassays for protein quantification[1]

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Summary. The use of chemiluminescence in competitive binding assays for human serum albumin, human alpha-fetoprotein and human immunoglobulin G and in double antibody sandwich enzyme immunoassays for cytomegalovirus and herpes simplex virus increased the sensitivity of the detection of antigen or antibody 16- to 95-fold above that obtained by conventional absorptiometric methods.

Absorptiometry and luminometry have been applied clinically in enzyme-linked immunosorbent assay (ELISA) for the quantification of a variety of medically important substances[3–5]. ELISA which uses an enzyme as the immunoglobulin marker instead of a γ-emitting isotope, was developed as a non-isotopic alternative to radioimmunoassay (RIA)[6]. The assay uses an enzyme-antibody conjugate which becomes bound to a solid-phase support through a series of antigen-antibody reactions and is subsequently detected by the addition of an appropriate substrate. A

visible reaction product can be measured spectrophotometrically[7], while a photo-emissive substrate is measured in a luminometer or a liquid scintillation counter[8].

Although both methods of enzyme detection claim good sensitivity, often approaching that of RIA, no direct comparison between the 2 detection systems has yet been made. This study directly compares absorbance (ABS) and chemiluminescence (CL) measurement in ELISA for the quantification of human serum albumin (HSA), alphafetoprotein (AFP), immunoglobulin G (IgG), cytomegalovirus (CMV) and herpes simplex virus (HSV) and shows that the application of CL increases the sensitivity of the detection of antigen (or antibody) above that obtained absorptiometrically.

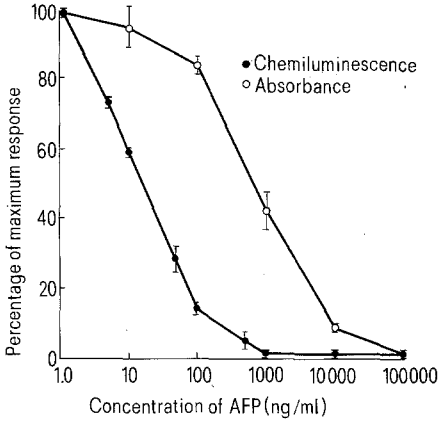
Solid-phase competitive binding assays were developed for quantification of HSA, AFP and IgG and standardized by absorptiometry as described[9]. Human AFP was partially purified from human cord serum and quantified by radial immunodiffusion against a previously established standard[10]. Double antibody sandwich ELISA was developed for quantification of CMV[11] and HSV[12]. CMV (strain AD169) was grown in human fibroblastic cells and titrated by 50% tissue culture infectious dose endpoint (TCID50). HSV type 1 (MacIntyre strain) was grown in primary rabbit kidney cells and infectivity was assayed by plaque production[13].

The assays were performed using antigen or antibody adsorbed to 6.4 mm diameter polystyrene spheres with specular finish obtained from Precision Ball Co., Chicago, IL. All reagents were pretested to determine the limiting dilution i.e., the lowest concentration of reactant that yielded the highest response in the assay. Unless stated otherwise, incubation was performed for 30 min at 37 °C using 400 µl of solution prepared with 1% bovine serum albumin in phosphate buffered saline (PBS) as the diluent and was followed by 3 washings with 0.05% Tween 20 in PBS (TP). The spheres were prepared for human protein assay (albumin, alpha-fetoprotein or immunoglobulin G) by incubation in a solution of antigen (10 ng/ml in 0.1 M sodium carbonate buffer pH 9.6) for 18 h at 4 °C or for viral antigen assay in a solution of rabbit or goat antiviral antiserum (diluted 1:20 for HSV and 1:50 for CMV in the carbonate buffer). The coated spheres were washed 6 times with TP.

Protein assay was performed by incubating 200 µl of a test solution of antigen with an equal volume of a solution of a 1st antibody (of which each 200 µl contained the amount of antibody that was capable of reacting with approximately 60% of the antigen adsorbed to a sphere). The mixture was then incubated with an antigen coated sphere which was subsequently washed. For the IgG assay, the antibody was labelled with peroxidase and the washed spheres were assayed immediately. For the albumin or AFP assays, the spheres were reincubated, in turn, with an excess of a peroxidase-labeled 2nd antibody which was directed against the 1st antibody. The reincubated spheres were washed and assayed. Viral antigen assay was performed by incubating antiviral antibody coated spheres with the test material, washing, reincubating the spheres in human antiviral antiserum (diluted 1:160 for HSV and 1:200 for CMV), washing again, adding peroxidase-labelled anti-human IgG antibody (diluted 1:3000) and repeating the incubation and washing steps before assay.

Absorptiometry was performed by incubating each sphere with 300 µl of a 4.6 mM o-phenylenediamine and 1.8 mM hydrogen peroxide solution in 0.1 M sodium citrate buffer, pH5, for 30 min at 22 °C, stopping the reaction with 150 µl of 2 N sulfuric acid, then recording the optical density of the mixture at 493 nm[14].

Chemiluminescence was assayed by adding, to a vial which contained a sphere in 1 ml distilled water, 300 µl of 1 mM



The bars indicate ± SD for 3 separate assays, each assay having consisted of 2 determinations of absorbance (open circles) or 3 determinations of chemiluminescence (closed circles). The dose response curve for AFP determined by a competitive enzyme linked immunosorbent assay using absorptiometry and chemiluminescence.

Comparison of chemiluminescent and absorptiometric detection systems in enzyme immunoassays for protein quantification

Substance measured	Type of assay[b]	Chemiluminescence Range of assay or minimal amount detected	Mean variance (%) Intra-test	Inter-test	Absorbance Range of assay or minimal amount detected	Mean variance (%) Intra-test	Inter-test	Increase in sensitivity[f]
Human serum albumin	CBA	0.4–7.2 µg/ml	6.5	3.5	1.9–180 µg/ml	5.8	4.3	16
Human alpha-fetoprotein	CBA	1.9–190 ηg/ml	5.1	2.6	0.03–8.5 µg/ml	4.3	3.8	42
Human immunoglobulin G	CBA	0.04–2.5 µg/ml	5.3	6.0	1.0–600 µg/ml	3.0	1.8	95
Cytomegalovirus	DAS	$\geq 10^{0.3}TCID_{50}$/sample[c]	3.6	4.4	$\geq 10^{2.0}TCID_{50}$/sample	4.5	4.8	50
Herpes simplex virus[a] type 1	DAS	≥ 40 PFU/sample[d]	4.0	ND	≥ 25000 PFU/sample	0.9	ND[e]	64

[a] Adapted from Pronovost et al.[12]. [b] CBA, competitive binding assay; DAS, double antibody sandwich. [c] $TCID_{50}$, tissue culture infectious dose (50%). [d] PFU, plaque forming unit. [e] ND, not determined. [f] The multiplicand by which the sensitivity of the chemiluminescent assay exceeded that of absorptiometry at the 50% inhibitory dose in competitive binding assays for albumin, alpha-fetoprotein and immunoglobulin G and in the minimal amount of viral antigen detected per sample (300 µl) by double antibody sandwich. A dilution of viral antigen which gave a response ≥ 10% above the background control (uninfected cells) was considered positive.

EDTA in 1 M glycine buffer, pH 10.5, and 30 µl of 0.1 mM isoluminol in 1 M glycine buffer, pH 9, mixing, then adding 1 ml of 0.3 mM hydrogen peroxide in distilled water, and measuring the emitted light[8,12] by means of a liquid scintillation counter with the coincidence circuitry disconnected[15]. The table provides a direct comparison between CL and ABS for antigen quantification in ELISA. Detection by CL was 16–95 times more sensitive than ABS in assays for HSA, AFP, IgG, CMV and HSV-1. Comparable increases in sensitivity were obtained in competitive binding and double antibody assays though the assays differed in detail. The variances exhibited by CL and ABS within runs and between runs were comparable. The data shows that CL provides a more sensitive means for the detection of enzyme label in enzyme immunoassays. The use of luminogenic substrates with higher quantum efficiencies and peak light intensities may further increase sensitivity[8].

The results of absorptiometric measurements of HSA, AFP, IgG, HSV-1 and CMV were comparable to those reported by others[12,16–18]. The results of HSA measurement by CL were comparable to those reported by Whitehead et al.[4]. By contrast, IgG was measured with greater sensitivity (approximately 500-fold) using the present method than that of Hersch et al.[9].

The use of CL does not appear to have been reported previously for AFP. The figure illustrates the inverse relationship in the assay between the amount of tested antigen and the intensity of the enzymatic reaction. The inverse relationship occurred because the more antibody was bound by a high dose of antigen in the test solution then the less antibody remained for reaction with the spheres which, in turn, subsequently bound less indicator, enzyme-linked 2nd antibody.

The sensitivity of the present method approaches that of RIA[16]. The fact that CL is more sensitive than ABS for the detection of viral antigen may prove useful for rapid viral diagnosis[19].

Measurement of light emission in enzyme immunoassays employing peroxidase appears to be a sensitive, rapid and safe means for quantification of antigen or antibody at very low concentration e.g. in urine, cerebrospinal fluid or in vitro, and may provide a convenient alternative to radioimmunoassay.

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Ferredoxin reductase catalyzes styrene oxidation to styrene oxide

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Summary. The flavoprotein ferredoxin reductase catalyzed the oxidation of styrene to styrene oxide in the presence of NADPH. This reaction was inhibited by the addition of catalase and superoxide dismutase. The addition of the nonheme iron protein ferredoxin partially inhibited styrene oxidation. H_2O_2 was also able to catalyze this reaction when added to the enzyme in the absence of NADPH.

Enzymes and hemoproteins, other than cytochrome P-450, have been shown to metabolize xenobiotics in the presence of NADPH, H_2O_2 and organic peroxides. Benzo(a)pyrene is oxidized to quinones by a peroxide produced in the course of prostaglandin synthesis[2]; N-demethylation of aminopyrine occurred in the presence of horseradish peroxidase and H_2O_2[3], and aniline hydroxylation was observed in the presence of hemoglobin and NADPH[4].

Ferredoxin reductase (reduced NADP ferredoxin oxidoreductase EC 1.6.99.4) is an FAD containing enzyme isolated from plant chloroplasts[5]. This enzyme is known to transfer electrons from the iron sulfur protein ferredoxin to NADP[6] or to catalyze the reverse reaction transferring electrons from NADPH to ferredoxin and other acceptors[7–9].

In the course of an investigation on the activation of styrene to the potentially toxic and mutagenic styrene oxide[10,11] by enzymes other than microsomal monooxygenases, the activity of ferredoxin reductase was studied. In this paper we report that this flavoprotein is able to oxidize styrene to styrene oxide in the presence of NADPH or H_2O_2.

Materials and methods. Ferredoxin reductase, ferredoxin, superoxide dismutase and NADPH were obtained from Sigma (St.Louis, Miss., USA); xanthine oxidase and horseradish peroxidase were purchased from Boehringer (Mannheim, FRG); xanthine and H_2O_2 were obtained from Merck (Darmstadt, FRG).

The incubation system consisted of 1 ml of 0.2 M Na^+-phosphate buffer pH 7.4 and the concentrations of the different chemicals and enzymes were as indicated in the