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

G. Belvedere, D. Blezza and L. Cantoni

Istituto di Ricerche Farmacologiche 'Mario Negri', Via Eritrea, 62, I-20157 Milano (Italy), 27 July 1981

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

tables. Ferredoxin reductase and ferredoxin concentrations were calculated on the basis of the following molecular extinction coefficients: 8240 at 465 nm for ferredoxin[12] and 10,740 at 456 nm for the flavoprotein[13]. The reaction mixtures were preincubated for 5 min at 37 °C and the reaction was started by addition of 20 µl of styrene (40 mM) dissolved in acetonitrile. After 10 min of incubation at 37 °C the reaction was terminated by adding 0.4 ml of H_2SO_4 (0.6 N). After overnight incubation at room temperature to hydrolyze chemically the styrene oxide formed enzymatically, 0.4 ml of NaOH (0.6 N) were added and the styrene glycol was extracted twice with 3 ml of ethyl acetate. The solvent was evapored under N_2 and the dry residue was dissolved in 1 ml of toluene and reacted with trifluoroacetic anhydride. Formation of the glycol was assayed by a sensitive gas chromatographic procedure using an electron capture detector[14].

Results and discussion. In the assay for the formation of styrene oxide catalyzed by ferredoxin reductase a method for the study of styrene monooxygenase in microsomal preparations was used[15-17]. The styrene oxide formed enzymatically is chemically quantitatively hydrated by acidification to the glycol, a more suitable compound for gas chromatographic analysis. For this reason the amounts of styrene oxide are reported as nmoles of styrene glycol. Table 1 shows that ferredoxin reductase oxidizes styrene to styrene oxide in the presence of NADPH. The formation of styrene oxide is confirmed by the absence of glycol in samples containing glutathione, which is known to react with epoxides[18], and by a much smaller amount of glycol in samples extracted after addition of NaOH in place of H_2SO_4 (data not shown).

Addition of ferredoxin to the incubation system partially inhibited styrene oxide formation (table 2). Since it has been reported that ferredoxin reductase forms a 1:1 molar complex with the nonheme iron protein ferredoxin, depressing NADPH oxidation[7,19], it is likely that NADPH oxidation is involved in styrene oxide formation.

It has been also observed that ferredoxin reductase, in the absence of ferredoxin, can oxidize NADPH with production of H_2O_2 and $O_2^{-\cdot}$[7,20]. In our conditions the addition of either superoxide dismutase or catalase suppressed styrene oxidation (table 1), suggesting that both $O_2^{-\cdot}$ and H_2O_2 were involved in this reaction. However the xanthine oxidase/xanthine system that in our conditions produced $O_2^{-\cdot}$ in a larger amount than ferredoxin reductase (data not reported), was considerably less active (table 2). For this reason it is likely that $O_2^{-\cdot}$ is not directly responsible for styrene oxidation; in fact a direct role of $O_2^{-\cdot}$ has been demonstrated in only a small number of reactions[21].

H_2O_2 is known to support substrate hydroxylation in the presence of cytochrome P-450 and horseradish peroxidase[3], by a peroxidative mechanism. H_2O_2 also supported styrene oxide formation when added to ferredoxin reductase in the absence of NADPH (table 1). However, a typical peroxidase such as horseradish peroxidase, added in equimolar concentration to that of the reductase, showed only very low activity (table 2). These findings seem to indicate that styrene oxidation to styrene oxide catalyzed by ferredoxin reductase can be supported by the NADPH mediated formation of $O_2^{-\cdot}$ and H_2O_2 or by direct addition of H_2O_2; however, whether these 2 reactions proceed by similar mechanisms remains to be established.

Table 1. Styrene oxidation to styrene oxide catalyzed by ferredoxin reductase

Additions[a]	Styrene glycol[b] nmole/nmole protein
Ferredoxin reductase + NADPH	15.7 ± 0.7
−NADPH	n.d.[c]
+ Glutathione (10 mM)	n.d.
+ Catalase (1520 units)	0.7 ± 0.1
+ Superoxide dismutase (1520 units)	1.9 ± 0.2
−NADPH + H_2O_2 (0.1 mM)	28.1 ± 2.8
Ferredoxin reductase (boiled) + NADPH	n.d.

[a] Incubation mixtures consisted of 0.2 M Na^+-phosphate buffer pH 7.4 ferredoxin reductase (0.49 µM) and NADPH (5.3 µM). Samples were incubated with styrene (0.8 mM) at 37 °C for 10 min. Styrene glycol formed in control samples consisting of buffer and NADPH or H_2O_2 was substracted. [b] The styrene oxide formed enzymatically was chemically converted to styrene glycol (see Materials and methods). [c] Not detectable.

Table 2. Styrene oxidation to styrene oxide catalyzed by different enzymatic systems

Systems[a]	Styrene glycol nmole/nmole protein
Ferredoxin reductase (0.49 µM) + NADPH (5.3 µM)	15.7 ± 0.6
Ferredoxin reductase + NADPH + ferredoxin (4.1 µM)	4.9 ± 0.5
Xanthine (4 mM) + xanthine oxidase (0.49 µM)	0.01 ± 0.001
Horseradish peroxidase (0.49 µM) + H_2O_2 (0.1 mM)	0.02 ± 0.002

[a] Samples were incubated in 0.2 M Na^+-phosphate buffer pH 7.4 with styrene (0.8 mM) at 37 °C for 10 min. Styrene glycol formed in control samples consisting of buffer and NADPH or H_2O_2 was subtracted.

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