

Immunochemical characterization of NADPH–cytochrome *P*-450 reductase from Jerusalem artichoke and other higher plants

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Polyclonal antibodies were prepared against NADPH–cytochrome *P*-450 reductase purified from Jerusalem artichoke. These antibodies inhibited efficiently the NADPH–cytochrome *c* reductase activity of the purified enzyme, as well as of Jerusalem artichoke microsomes. Likewise, microsomal NADPH-dependent cytochrome *P*-450 mono-oxygenases (cinnamate and laurate hydroxylases) were efficiently inhibited. The antibodies were only slightly inhibitory toward microsomal NADH–cytochrome *c* reductase activity, but lowered NADH-dependent cytochrome *P*-450 mono-oxygenase activities. The Jerusalem artichoke NADPH–cytochrome *P*-450 reductase is characterized by its high M_r (82000) as compared with the enzyme from animals (76000–78000). Western blot analysis revealed cross-reactivity of the Jerusalem artichoke reductase antibodies with microsomes from plants belonging to different families (monocotyledons and dicotyledons). All of the proteins recognized by the antibodies had an M_r of approx. 82000. No cross-reaction was observed with microsomes from rat liver or *Locusta migratoria* midgut. The cross-reactivity generally paralleled well the inhibition of reductase activity: the enzyme from most higher plants tested was inhibited by the antibodies; whereas *Gingko biloba*, *Euglena gracilis*, yeast, rat liver and insect midgut activities were insensitive to the antibodies. These results point to structural differences, particularly at the active site, between the reductases from higher plants and the enzymes from phylogenetically distant plants and from animals.

INTRODUCTION

In plants, cytochrome *P*-450-dependent mono-oxygenases have been described in several species (Higashi, 1985). They are involved in important metabolic pathways leading to lignins (Potts *et al.*, 1974; Benveniste & Durst, 1974; Grand, 1984), phytoalexins (Fujita *et al.*, 1982; Kochs & Grisebach, 1986), pigments (Hagmann *et al.*, 1983), hormones (Hasson & West, 1976), steroids (Petersen & Seitz, 1985; Rahier & Taton, 1986), alkaloids (Madyastha *et al.*, 1976), cutins (Soliday & Kolattukudy, 1977) and hydroxy-fatty acids (Salaün *et al.*, 1978; Benveniste *et al.*, 1982*b*). Plant cytochrome *P*-450s are also implicated in the metabolism of exogenous compounds (Frear *et al.*, 1969; Young & Beevers, 1976; Fonné-Pfister *et al.*, 1988) and in the detoxification of herbicides (Cabanne *et al.*, 1987). Most of these oxygenases occur in a microsomal fraction and all are dependent upon molecular oxygen and reducing equivalents provided preferentially by NADPH, and to a lesser extent by NADH.

Previous work using selective competitive inhibitors of the microsomal NADPH–cytochrome *c* reductase pointed out the central role of this flavoprotein in the electron transfer from both NADPH and NADH to cytochromes *P*-450 catalysing the hydroxylation of cinnamic acid (Benveniste *et al.*, 1977) and lauric acid (Salaün *et al.*, 1978) in Jerusalem artichoke microsomes. On the basis of immunochemical studies with polyclonal

antibodies raised against purified Jerusalem artichoke NADPH–cytochrome *P*-450 reductase, this report confirms the importance of this flavoenzyme in the reduction of cytochrome *P*-450 by NADPH. The electron flow from NADH to the cytochrome *P*-450 remains unclear. In addition, this paper presents data on the cross-reactivity of these immunoglobulins with NADPH–cytochrome *c* reductase from a large variety of plants, yeast and animals. The lack of recognition of the reductases from primitive plant species and from animals by the antibodies to the Jerusalem artichoke enzyme indicates significant structural differences between the enzymes.

MATERIALS AND METHODS

Plant materials

Jerusalem artichoke (*Helianthus tuberosus* L., var. Blanc commun.) tubers were sliced and incubated for 24 h in well-aerated distilled water, or for 42 h in a 20 mM-aminopyrine solution, at room temperature in the dark. Potato (*Solanum tuberosum* L., var. Jose) tubers were sliced and aged for 17 h in distilled water oxygenated by a stream of hydrated filtered air. Tulip (*Tulipa gesneriana* L., var. Orange emperor) bulbs were cut into 1 mm thick slices and aged for 48 h in distilled water. Avocado (*Persea americana* L.) mesocarp was extracted without any induction treatment. *Vicia faba* seedlings were

Abbreviations used: PMSF, phenylmethanesulphonyl fluoride; DCPIP, dichlorophenolindophenol; PBS, phosphate-buffered saline (10 mM- $\text{Na}_2\text{PO}_4/0.9\%$ NaCl, pH 7.4).

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germinated on filter paper for 4 days in the dark and were then immersed for 48 h in a well-aerated clofibrate emulsion (2 mM). Leek (*Allium porrum* L.) seedlings were grown on filter paper for one week in the dark. The day before extraction they were illuminated by two red- (660 nm) light periods of 5 min each, separated by a 6 h interval. Sunflower (*Helianthus annuus* L.) seedlings were grown on filter paper for 7 days in the dark and then illuminated for 24 h by far red (730 nm) light. Maize (*Zea mays* L., var. LG 11) was germinated in moist vermiculite for 48 h, then the axes were cut from the caryops and used as a source of microsomes. Cell suspension cultures constituted the source of material for bramble (*Rubus fruticosus* L.) and for *Ginkgo biloba*. For *Euglena gracilis* Z., the bleached form was used. Yeast (*Saccharomyces cerevisiae*) was grown overnight under vigorous shaking in a medium containing 10% (w/v) glucose.

Preparation of microsomes

All plant materials were homogenized at 4 °C in 100 mM-sodium phosphate buffer, pH 7.4, containing 25 mM-2-mercaptoethanol, 1 mM-EDTA and 250 mM-sucrose. Phenylmethanesulphonyl fluoride (PMSF) (1 mM) was added to the extraction medium before grinding, to avoid solubilization of the hydrophilic form of the NADPH-cytochrome *c* reductase by endogenous proteases. Tubers and seedlings were homogenized with a Moulinex mixer. Cells from suspension cultures were first disrupted by grinding the liquid N₂-chilled material in a mortar and were then homogenized with a Moulinex mixer. *Euglena* cells were disrupted by sonication. Yeast cells were ground with glass beads by stirring on a Vortex. Microsomes were then prepared by the procedure described previously (Benveniste *et al.*, 1986).

Solubilization of the microsomes

Microsomes from different plants were solubilized by 1.5% Emulgen 911 (v/v) for 30 min at 4 °C, in the presence of 1 mM-PMSF. After centrifugation at 100 000 *g* for 1 h, NADPH-cytochrome *c* reductase activity was recovered exclusively in the supernatant.

Purification of the Jerusalem artichoke NADPH-cytochrome *c* reductase

The lipophilic form of the reductase was purified as described previously (Benveniste *et al.*, 1986) by anion-exchange chromatography and by affinity chromatography.

Preparation of polyclonal antibodies

The polyclonal antibody prepared previously (Benveniste *et al.*, 1986) was used in the present study. Antigen (0.5 ng) could be detected with a 10⁻³ dilution of the serum, in dots revealed by the peroxidase method described below.

For enzyme inhibition studies, these antibodies were further purified by rivanol precipitation of serum proteins (Hardie & Van Regenmortel, 1977). The IgGs were then precipitated by 50% saturated ammonium sulphate solution. Immunoglobulins from non-immune rabbit sera were prepared in the same way.

Western blot analysis

The solubilized microsomal proteins were separated

by SDS/polyacrylamide-gel electrophoresis and electrotransferred on to nitrocellulose (Schleicher and Schüll, D-3354 Dassel, Germany, 45 µm pore size) under 0.1 A overnight at 4 °C (Towbin *et al.*, 1979). The remaining protein-binding sites on the nitrocellulose were then blocked by 3% bovine serum albumin in solution in phosphate-buffered saline (PBS) (10 mM-sodium phosphate buffer, pH 7.4/0.9% NaCl). The immunoglobulins (825 µg of IgG/30 ml of PBS buffer containing 0.1% bovine serum albumin) reacted with the antigens for 4 h at room temperature. After careful washing of the antibody, the nitrocellulose was incubated overnight with anti-rabbit antibody coupled with peroxidase. The secondary antibody was then washed off and the antigen-antibody complexes were revealed by addition of α-chloronaphthol and hydroperoxide.

Enzyme assays

NADPH- or NADH-dependent reduction of cytochrome *c* was monitored at 26 °C by the increase in absorbance at 550 nm, in the presence of 100 mM-sodium phosphate buffer, pH 7.4, and 0.05 mM cytochrome *c*. When microsomal activities were measured, 1 mM-KCN was added to inhibit the cytochrome *c* oxidase activity of possible mitochondrial contaminations. When the activity of the purified enzyme was determined, KCN was omitted, but 2.5 µM-FMN, a labile prosthetic group of the reductase, was added (Benveniste *et al.*, 1986). The volume of the reaction mixture was 1 ml. A molar absorption coefficient of 21 mM⁻¹·cm⁻¹ for horse heart cytochrome *c* was used.

NADPH- or NADH-dependent reduction of K₃Fe(CN)₆ (1 mM) was measured at 26 °C, in the presence of 100 mM-sodium phosphate buffer, pH 7.4, by the decrease of absorbance at 420 nm. The molar absorption coefficient of K₃Fe(CN)₆ at 420 nm is 1.02 mM⁻¹·cm⁻¹.

NADPH- or NADH-dependent reduction of dichlorophenolindophenol (DCPIP) (100 µM) was determined at 26 °C, in 100 mM-sodium phosphate buffer, pH 7.4, by the increase of absorbance at 600 nm, using a molar absorption coefficient of 21 mM⁻¹·cm⁻¹.

Cinnamic acid hydroxylase, lauric acid in-chain hydroxylase and chlortoluron demethylase activities were measured radiochemically as described by Benveniste *et al.* (1977), Salaün *et al.* (1978) and Fonné (1985) respectively.

Enzymes were preincubated with antibodies for 15 min at room temperature before addition to the reaction mixtures.

RESULTS AND DISCUSSION

In a previous report (Benveniste *et al.*, 1986), the purification and characterization of the NADPH-cytochrome *P*-450 (cytochrome *c*) reductase from Jerusalem artichoke has been described. This purified enzyme was used to immunize a rabbit, and polyclonal immunoglobulins were isolated from the antisera. These antibodies were used to investigate further the electron transport route involved in the NADPH- and NADH-dependent cytochrome *P*-450 mono-oxygenases.

Fig. 1 shows the effect of increasing concentrations of anti-reductase immunoglobulins on the activity of cinnamic acid (Fig. 1*a*) and lauric acid (Fig. 1*b*) hydroxylases sustained by NADPH or by NADH in Jerusalem artichoke microsomes. For both activities, NADPH was

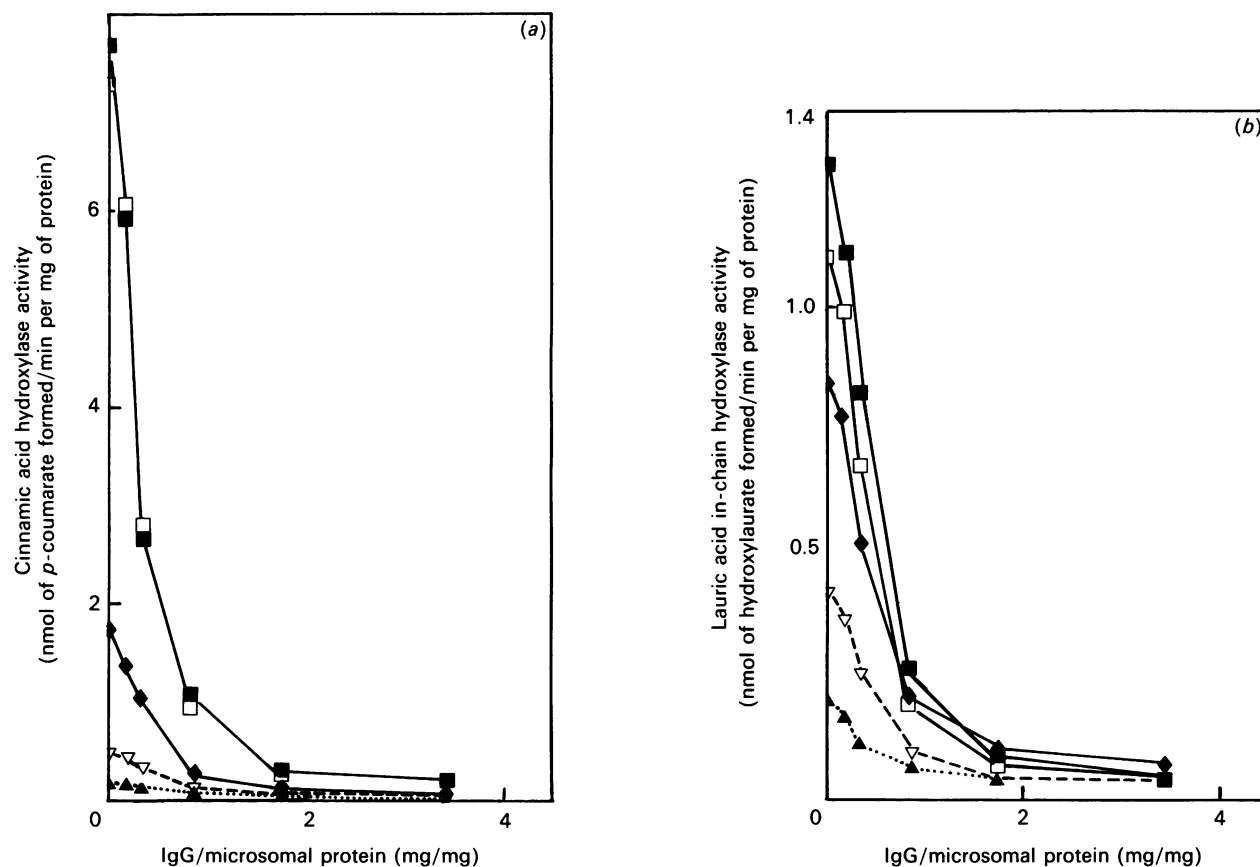


Fig. 1. Inhibition of cinnamic acid hydroxylase (a) and lauric acid in-chain hydroxylase (b) activities from Jerusalem artichoke microsomes by antibodies directed against the purified NADPH-cytochrome *P*-450 reductase from Jerusalem artichoke

Microsomes were preincubated with increasing concentrations of anti-reductase IgG for 15 min at room temperature. (a) Cinnamic acid hydroxylase was measured by incubating microsomal protein (21 μ g with NADPH, 53 μ g with NADH) at 25 $^{\circ}$ C for 20 min, in presence of different concentrations of NADPH (\square , 0.5 mM; \blacksquare , 2 mM) or NADH (\blacktriangle , 0.5 mM; ∇ , 2 mM; \blacklozenge , 10 mM). (b) Lauric acid hydroxylase activity was measured with 106 μ g of microsomal protein at 27 $^{\circ}$ C for 20 min. The NADPH concentration was 0.5 mM (\square) or 2 mM (\blacksquare), and that of NADH was 0.5 mM (\blacktriangle), 2 mM (∇) or 10 mM (\blacklozenge).

by far the most efficient reductant. However, NADH, at relatively high concentrations (0.5, 2 and 10 mM), allowed mono-oxygenase activity, and was a better electron donor for the lauric acid in-chain hydroxylase than for the cinnamic acid hydroxylase. An immunoglobulin dose-dependent inhibition was observed for both hydroxylases in the presence of NADPH or NADH. These activities were nearly totally inhibited by 2 mg of IgG/mg of microsomal proteins. Non-immune immunoglobulins were without effect. We observed, however, that the non-immune serum, in contrast with purified non-immune IgG, produced 50% inhibition of the lauric acid hydroxylase. This inhibition probably results from the binding of lauric acid to serum albumin (Pedersen *et al.*, 1986), leading to a diversion of substrate for the lauric acid hydroxylase.

The activity of chlortoluron demethylase, another cytochrome *P*-450-dependent mono-oxygenase from Jerusalem artichoke (Fonné, 1985) was also drastically lowered by the anti-reductase antibodies, the electrons being furnished by NADPH or by NADH.

Since the antibodies were raised against purified NADPH-cytochrome *c* (cytochrome *P*-450) reductase, their inhibitory effect on different cytochrome *P*-450 mono-oxygenase activities confirms the central role of

this flavoprotein in the transfer of reducing equivalents from NADPH and NADH to cytochrome *P*-450. These studies corroborate previous results obtained with specific inhibitors of the microsomal flavoproteins (Benveniste *et al.*, 1982a). AADP⁺ (3-aminonicotinamide adenine dinucleotide phosphate) competitively inhibited the microsomal NADPH-cytochrome *c* reductase with a K_i of 16 μ M, the K_i for the microsomal NADH-cytochrome *c* reductase reaching 46 mM. In contrast, AAD⁺ (3-aminonicotinamide adenine dinucleotide) inhibited very selectively the microsomal NADH-cytochrome *c* reductase with a K_i of 100 μ M, while its K_i for the NADPH-cytochrome *c* reductase was 45 mM. Only AADP⁺ inhibited NADPH- and NADH-dependent cinnamic acid hydroxylation, whereas AAD⁺ did not affect them significantly.

In an attempt to distinguish NADPH-cytochrome *P*-450 reductase from NADH-cytochrome *b*₅ reductase and to test the selectivity of the polyclonal antibodies towards these microsomal flavoproteins, we utilized several artificial electron acceptors in the presence of NADPH or NADH. Fig. 2 shows the immunotitration of the reduction of cytochrome *c* (Fig. 2a), DCPIP (Fig. 2b) and ferricyanide (Fig. 2c) over a wide range of IgG concentrations.

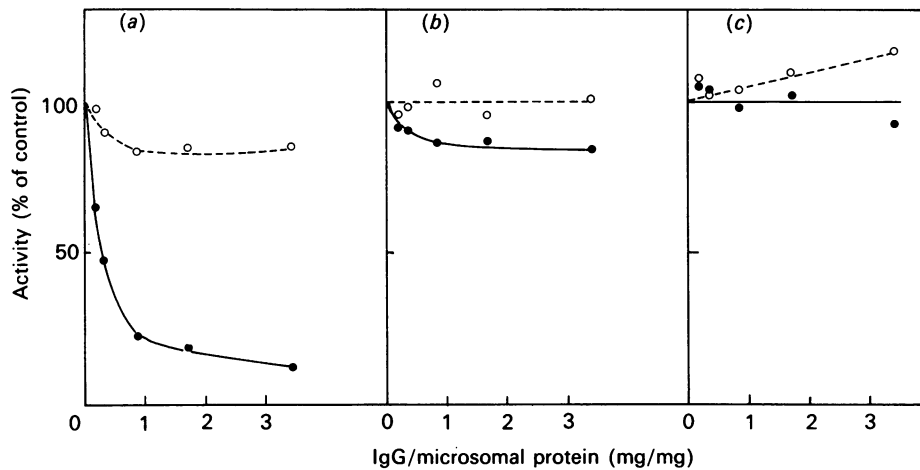


Fig. 2. Effect of Jerusalem artichoke anti-reductase IgG on the microsomal NADPH- and NADH-dependent reductions of artificial electron acceptors: cytochrome *c* (a), DCPIP (b) and potassium ferricyanide (c)

Jerusalem artichoke microsomes were preincubated with increasing amounts of anti-reductase IgG at room temperature for 15 min. NADPH- (●) and NADH- (○) dependent activities were measured spectrophotometrically at 26 °C in the presence of 18 μg of microsomal protein. The concentration of NADPH or NADH was 2 mM in the tests. The reference activities, measured in the absence of IgG, were as follows (nmol/min per mg of protein): NADPH-cytochrome *c* reductase, 266; NADH-cytochrome *c* reductase, 705; NADPH-DCPIP reductase, 421; NADH-DCPIP reductase, 571; NADPH-ferricyanide reductase, 5900; NADH-ferricyanide reductase, 6600.

When NADPH was the source of reducing equivalents, cytochrome *c* reduction was increasingly inhibited by an increasing amount of antibody. A good correlation was observed between the inhibition of the reduction of cytochrome *c* (Fig. 2a) and cytochrome *P*-450 (Figs. 1a and 1b). Approx. 10% of the microsomal NADPH-cytochrome *c* reductase activity seemed insensitive to the antibodies. This 'resistant' activity could be due to a microsomal diaphorase, interacting with NADPH and cytochrome *c*, which would have no structural relationship to NADPH-cytochrome *P*-450 reductase.

DCPIP- and ferricyanide-reductase activities (Figs. 2b and 2c) were only slightly or not diminished by the IgG. Similar observations were reported by Masters (1978) with pig liver microsomes and by Feyereisen & Vincent (1984) with housefly microsomes. The interaction of the NADPH-dependent reductase with cytochrome *P*-450, its physiological electron acceptor, or with cytochrome *c*, an artificial proteinic electron acceptor, would require a precise conformation of the proteins to permit the electron flow. Indeed, interactions between cytochrome *c* and NADPH-cytochrome *P*-450 reductase involve complementary charge-pairing between a positively charged surface of cytochrome *c* and negatively charged surfaces of the reductase (Dailey & Strittmatter, 1980). This protein-protein association could be altered by the antibodies. The interaction of small acceptor molecules with the reductase would not require such a strict conformation of the enzyme and would not be affected by the IgG.

When the electrons were furnished by NADH, the microsomal NADH-ferricyanide reductase, which essentially measures the activity of the NADH-cytochrome *b*₅ reductase, was not inhibited but was in fact slightly stimulated by the antibody. Under the same conditions, DCPIP reductase was unaffected and cytochrome *c* reduction only weakly inhibited (Fig. 2). These results seem to exclude an interaction of the IgG with the NADH-cytochrome *b*₅ reductase.

It is noteworthy that the parallel inhibition by the IgG of the microsomal NADPH-cytochrome *c* reductase and cytochrome *P*-450-mediated mono-oxygenase activities was not observed when the reductase activity was lowered by NADP⁺, a competitive inhibitor. Fig. 3 shows that cinnamic acid and lauric acid hydroxylases are substantially less inhibited than the reductase. One plausible explanation is that the polyclonal antibody mainly decreases the re-oxidation of the flavoprotein, i.e. its capacity to interact with, and transfer electrons to, cytochromes *c* and *P*-450. On the other hand, NADP⁺ inhibits the reduction of the enzyme, thus favouring the smaller and hydrosoluble cytochrome *c* over cytochrome *P*-450.

The effect of the antibody was tested on the NADPH- and the NADH-cytochrome *c* reductase activities of the purified enzyme. Fig. 4 shows a gradual inhibition of NADPH-cytochrome *c* reductase by increasing concentrations of antibody: the activity was almost totally abolished, whereas non-immune IgG had no effect or stimulated slightly the activity.

Surprisingly, the (low) NADH-cytochrome *c* reductase activity of the purified enzyme was not affected by the IgG. In fact, we have measured a reduction of cytochrome *c* by NADH without enzyme. This reduction was non-saturable as a function of NADH concentration. According to these results, NADH would not constitute a substrate for the NADPH-cytochrome *P*-450 reductase. These observations raise the problem of the electron transport route for mono-oxygenation when NADH is the reductant. The existence of an intermediate step between NADH and cytochrome *P*-450 reductase could explain these data.

The antibodies were also used to examine the immunological relationships with equivalent enzymes from various origins. Western blot analysis was performed on microsomal proteins prepared from a large variety of plants, comprising a monocotyledon and dicotyledons, a prespermaphyte, a unicellular alga and a

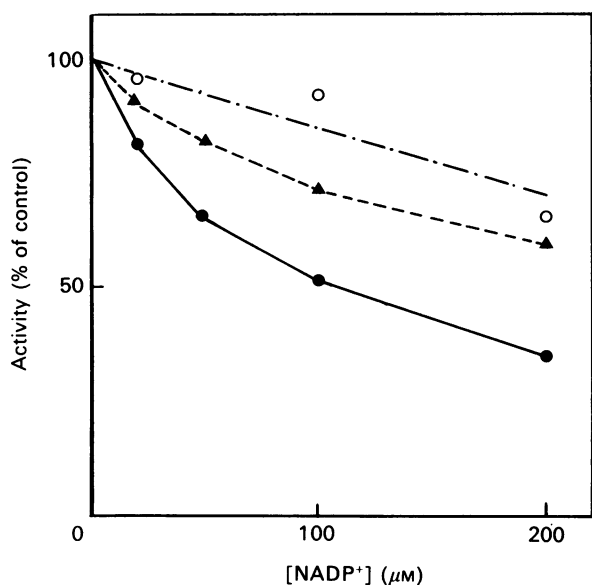


Fig. 3. Inhibition of microsomal NADPH-cytochrome *c* reductase, cinnamic acid hydroxylase and lauric acid hydroxylase as a function of NADP⁺ concentration

100% lauric acid hydroxylase activity (○) was 17 pmol of hydroxylaurate formed/min per mg of protein for a laurate concentration 10 times greater than the K_m . 100% cinnamic acid hydroxylase activity (▲) was 2.55 nmol of *p*-coumarate formed/min per mg of protein for a cinnamate concentration 60 times greater than the K_m . 100% NADPH-cytochrome *c* reductase activity (●) was 114 nmol of cytochrome *c* reduced/min per mg of protein. In all incubations NADPH was present at a concentration of 100 μM.

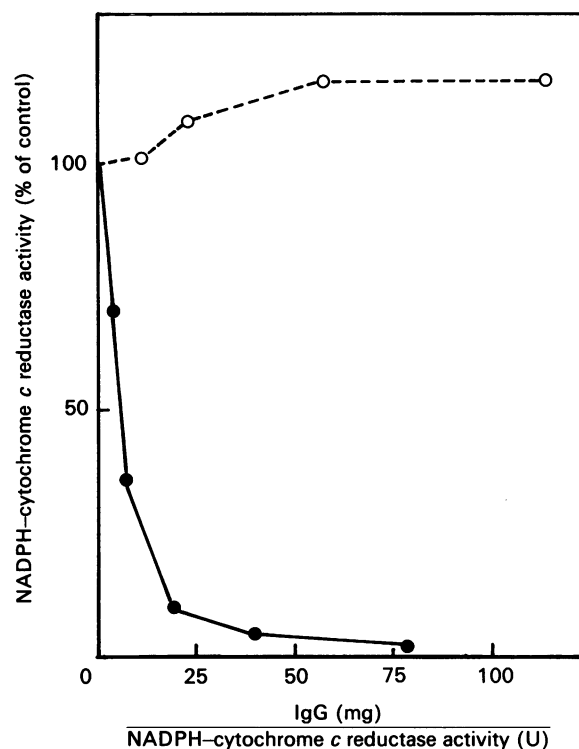


Fig. 4. Immunotitration curves of the NADPH-dependent cytochrome *c* reduction activity of purified Jerusalem artichoke NADPH-cytochrome *P*-450 reductase

After preincubation for 15 min at room temperature with increasing concentrations of anti-reductase IgG (●) or with non-immune IgG (○), 8.3 mU of the purified reductase were added to the test and NADPH-cytochrome *c* reductase activity was measured spectrophotometrically at 26 °C.

yeast. Among animals, rat liver, pig liver and grasshopper midgut were utilized.

Mammalian liver and *Locusta migratoria* midgut enzymes did not react with the Jerusalem artichoke anti-reductase IgG. Since mammalian and insect microsomes contained high NADPH-cytochrome *c* reductase activity, the absence of immunoreaction would reflect important structural differences between higher plant and animal reductases. A lack of interaction was also observed with *Euglena* and *Ginkgo biloba* microsomes. This may be due to the very low amount of the reductase in these materials and also to large structural differences.

In contrast, a strong immunological reaction occurred (Fig. 5) with microsomes from Jerusalem artichoke, maize, potato, avocado, bramble, tulip and leek: in each case, a protein with an M_r of 82000 (near that of the purified tuber reductase) was the major protein revealed. A weak response was obtained with *Vicia faba* and sunflower microsomes (Fig. 5). This is probably due to the small amount of enzyme present and not to marked structural differences since, for example, the antibodies inhibited very efficiently the cytochrome *P*-450-dependent hydroxylations of decanoic, dodecanoic and tetradecanoic acids in *Vicia faba* microsomes (Simon, 1987), in spite of the weak response in Western blot analysis. *Saccharomyces cerevisiae* (not shown) mostly presented a positive response for a protein with a very high M_r . However, sometimes a weak band was observed at the level of the Jerusalem artichoke reductase. This positive band could represent the yeast NADPH-

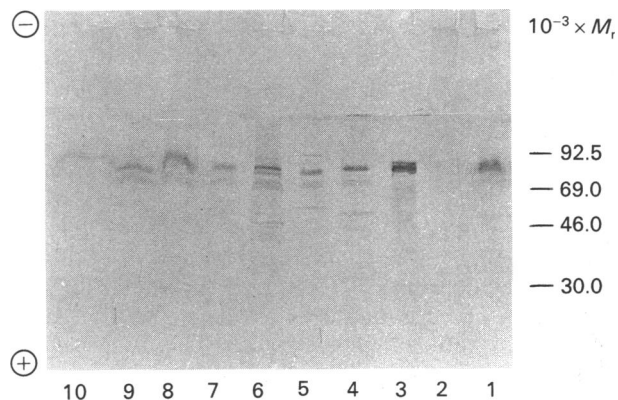


Fig. 5. Western blot analysis on microsomal protein from different higher plants

The microsomal proteins, solubilized by 2 mg of Emulgen 911 per mg of protein, were loaded on a 12.5% polyacrylamide gel in the presence of SDS. Lane 1, Jerusalem artichoke (82 μg of protein); lane 2, sunflower (225 μg); lane 3, purified Jerusalem artichoke reductase (3 μg); lane 4, maize (65 μg); lane 5, potato (33 μg); lane 6, avocado (38 μg); lane 7, bramble (59 μg); lane 8, tulip (80 μg); lane 9, leek (53 μg); lane 10, *Vicia faba* (140 μg). The minor bands of lower M_r probably resulted from protein degradation in some microsomal preparations.

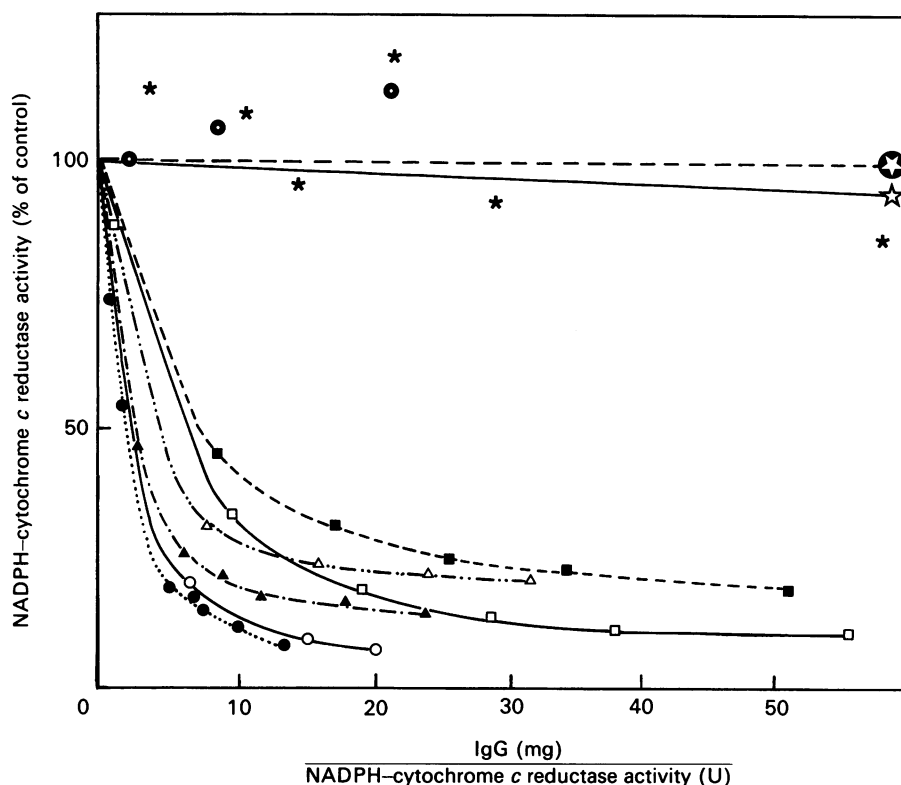


Fig. 6. Dose-dependent inhibition of microsomal NADPH-cytochrome *c* reductase of different species by anti-(Jerusalem artichoke reductase) IgG

The microsomes and IgG were preincubated at room temperature for 15 min. Activity is expressed as a percentage of control incubations without IgG. Microsomes were from: ●, rat liver; ★, *Saccharomyces cerevisiae*; ⊕, *Euglena gracilis*; ●, Jerusalem artichoke; △, sunflower; ■, bramble; □, *Vicia faba*; ▲, avocado; ○, maize; ☆, *Gingko*.

cytochrome *P*-450 reductase, since Aoyama *et al.* (1978) measured an M_r of 83000 for the purified enzyme.

In all cases where an immunological interaction was detected, the M_r determined by Western blotting was approx. 82000 or slightly higher. This observation confirms that the native form of the plant reductase has a higher M_r than the mammalian enzyme (76000–78000) (Yasukochi & Masters, 1976).

The degree of similarity was also investigated at the level of the active site of the reductases, by measuring the inhibitory effect of the artichoke anti-reductase IgG on the activities of NADPH-cytochrome *c* reductases from different sources. Fig. 6 shows the inhibition of different NADPH-cytochrome *c* reductases by increasing concentrations of immunoglobulins, as a function of the IgG/activity ratio of each material. The insensitivity of the reductases from rat liver, *Euglena* and *Gingko* to the tuber anti-reductase IgG appeared again clearly (in spite of the high IgG concentration used). These results confirm those obtained by Western blot analysis and indicate the presence of different antigenic sites, even at the active site of the enzymes.

Yeast reductase may possibly constitute an exception, since its activity was not inhibited by the antibodies, but a protein with similar M_r was sometimes recognized by the tuber IgG in Western blots. A low degree of conservation at the level of the active site seems however surprising. Microdomains concerned with catalytic activity are likely to be preserved during evolution. In animals, three regions involved in the binding of FMN

and FAD were found to be well conserved among rabbit, rat and pig enzymes (Porter & Kasper, 1985; Katagiri *et al.*, 1986). Unfortunately, such structural data are not yet available for plant reductases.

A preliminary study of the amino acid composition of the proteolytic form of the Jerusalem artichoke reductase has shown the following differences: compared with rat and rabbit reductases, methionine is present in a very low amount, serine is under-represented; threonine, proline, phenylalanine and lysine are more abundant. Structural differences between animal and plant reductases have previously been reported (Benveniste *et al.*, 1982a), since antibodies against pig liver reductase were unable to inhibit the NADPH-cytochrome *c* reductase and cytochrome *P*-450-dependent hydroxylases from plants. The present results corroborate these previous observations.

In contrast to animals and primitive plants, the microsomal NADPH-cytochrome *c* reductase from different higher plants cross-reacted with the Jerusalem artichoke anti-reductase IgG (Fig. 5). The most efficient inhibitions were obtained with Jerusalem artichoke and maize microsomes, a dicotyledon and a monocotyledon. The reductase from avocado fruit was also largely inhibited. The enzyme from sunflower, which belongs to the same genus as Jerusalem artichoke, showed a weaker sensitivity to the antibody. *Vicia faba* reductase was poorly represented in the microsomes (see above), and seemed less susceptible to the antibodies. Clearly, the extent of cross-reaction did not depend on the phylo-

genetic distance between the higher plants from which the enzymes were isolated. Nevertheless, these antibodies represent an interesting tool with which to study the regulation and expression of the NADPH-cytochrome *P*-450 reductase, not only in Jerusalem artichoke but in many heterologous higher plant systems.

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