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**REVIEW**

# Palm Tree Peroxidases

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**Abstract**—Over the years novel plant peroxidases have been isolated from palm trees leaves. Some molecular and catalytic properties of palm peroxidases have been studied. The substrate specificity of palm peroxidases is distinct from the specificity of other plant peroxidases. Palm peroxidases show extremely high stability under acidic and alkaline conditions and high thermal stability. Moreover, these enzymes are more stable with respect to hydrogen peroxide treatment than other peroxidases. Due to their extremely high stability, palm peroxidases have been used successfully in the development of new bioanalytical tests, the construction of improved biosensors, and in polymer synthesis.

*Key words*: peroxidase, palm tree, purification, substrate specificity, stability, application

Peroxidase (EC 1.1.11.7) is one of the key enzymes controlling plant growth, differentiation, and development. The enzyme participates in the construction, rigidification, and eventual lignification of cell walls, biosynthesis of ethylene from 1-aminocyclopropane-1-carboxylic acid and $H_2O_2$, regulation of auxin level, protection of tissue from damage and infection by pathogenic microorganisms as well as in the oxidation of indoleacetic acid [1-3]. *In vitro* this enzyme is widely employed in microanalysis [4-6] and in the construction of enzyme electrodes [7, 8]. Peroxidases have been used also for the synthesis and biotransformation of organic molecules [6, 9, 10].

Although peroxidases are widely distributed in the plant kingdom, at the present time the major source of commercially available peroxidase is horseradish roots (*Armoracia rusticana*). However, availability of peroxidases with higher stability and different substrate specificity would improve immunoenzymatic analytical kits and promote the development of new analytical methods and industrial processes [4, 6]. Presently extensive investigations of peroxidases isolated from different origins are being carried out [8, 11-14]. Taking into consideration the huge biodiversity of tropical flora, peroxidases from tropical plants have a good potential for practical use in biotechnology.

*Abbreviations*: RPTP) royal palm tree peroxidase; AOPTP) African oil palm tree peroxidase; HRP) horseradish peroxidase; ABTS) 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid).

## SCREENING AND PURIFICATION

During screening of tropical plants [15], it was found that the leaves of some palm trees exhibit high peroxidase activity. The highest activity was detected in leaves of royal palm (*Roystonea regia*), date palm (*Phoenix dactylifera*), African oil palm (*Elaeis guineensis*), ruffle palm (*Aiphanes cariotifolia*), and windmill palm (*Trachycarpus fortunei*). Moreover, the peroxidase activity does not depend upon the age of palm trees and remains essentially constant over the whole year. Therefore, palm peroxidase is a constitutive enzyme.

Peroxidases have been purified to homogeneity from the leaves of royal and African oil palm trees [15, 16]. Extraction of the peroxidases under neutral conditions (10 mM phosphate buffer, pH 7.0) is the most efficient. The addition of NaCl, Triton X-100, and EDTA into the extraction buffer does not change the yield at the extraction step, suggesting that the peroxidase in palm leaves is not appreciably bound to cell walls but are present in a soluble form.

Like many extracts of plant tissues, the palm leaf extracts contain large amounts of pigments. These compounds (polyphenols) are chemically active and can modify irreversibly chromatographic supports during enzyme isolation. To prevent the modification, a simple and quick procedure for removal of pigments has been developed. This method is based on the separation of peroxidase and pigments in an aqueous two-phase system containing poly(ethylene glycol) and ammonium sulfate

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[16]. The maximal removal of pigments and highest recovery of the peroxidase activity are obtained in the presence of 20% ammonium sulfate and 14% poly(ethylene glycol).

Further purification of palm peroxidases was carried out by chromatography on phenyl-Sepharose, Sephacryl S-200, and DEAE-Toyopearl columns. The specific activities of purified royal palm tree peroxidase (RPTP) and African oil palm tree peroxidase (AOPTP) toward guaiacol were 4300 and 4900 units/mg, respectively.

## MOLECULAR PROPERTIES

Purified RPTP and AOPTP migrate in SDS-electrophoresis as proteins with molecular weights of 51 and 57 kD, respectively. These values are higher than those reported for peroxidases from other sources, namely, for horseradish (HRP) (44 kD) [17], tobacco (37 kD) [14], sweet potato (37 kD) [18], peanut (37-40 kD) [19], and soybean peroxidases (37 kD) [20]. Only isoperoxidases isolated from radish and pepper fruit show similar molecular weights [21, 22]. A relatively high molecular weight of palm peroxidases is possibly due to extensive glycosylation. The carbohydrate sequences as well as the amino acid sequences for palm peroxidases are unknown at present.

Isoelectrofocusing experiments demonstrate that RPTP and AOPTP are anionic enzymes with the p*I* values of 3.5 and 3.8, respectively [15, 16]. Previously, other anionic peroxidases have been detected in sweet potato, tobacco, horseradish, and tomato [18, 23-25].

The spectrum of palm peroxidases has a Soret maximum at 403 nm, which is typical for plant peroxidases [26]. The spectrum shows also a CT band at 633 nm. The occurrence of these bands in the combination with a Q band at 492 nm suggests the presence of a high-spin heme. Here it should be noted that no detailed spectroscopic studies of palm peroxidases have been carried out.

## SUBSTRATE SPECIFICITY

The substrate specificity of palm peroxidases has been examined using well-known peroxidase substrates (Table 1) [15, 27]. Since the optimal conditions for the catalysis by different peroxidases are not identical [28-30], the favorable conditions for the oxidation reactions with each substrate in the presence of palm peroxidases were determined. For all the substrates, the pH optima of the peroxidases occur between 5.0 and 5.5, except for ABTS for which it is 3.0-3.5, 1.5-2 units lower than the corresponding value for HRP [28]. It should be emphasized that at pH 5.0 RPTP showed only 7% of its maximum catalytic activity.

To evaluate the catalytic capacity of the peroxidases, the values of the second-order rate constant ($k_{app}$) for the reaction between peroxidase compound II (Cpd II) and hydrogen donor substrates ($AH_2$) (Eq. (3)) have been estimated with Eq. (4):

$$E + H_2O_2 \rightarrow \text{Cpd I} + H_2O; \quad (1)$$

$$\text{Cpd I} + AH_2 \rightarrow \text{Cpd II} + AH^{\cdot}; \quad (2)$$

$$\text{Cpd II} + AH_2 \rightarrow E + AH^{\cdot} + H_2O; \quad (3)$$

$$\text{rate} = k_{app}\,[\text{EII}]\,[AH_2], \quad (4)$$

where E is the ferric enzyme (resting state), Cpd I and Cpd II are compound I and compound II, the oxidized intermediates of peroxidase; $AH_2$ and $AH^{\cdot}$ are an electron donor substrate and the primary radical product of its one-electron oxidation, respectively.

Initial concentrations of the peroxidase were used instead of compound II concentration in calculations because the reaction of compound II with electron donor substrate ($AH_2$) is the rate-limiting step of peroxidase catalysis [1]. In the case of peroxidases, the use of $k_{app}$ is more informative compared to the parameters "specific

**Table 1.** Substrate specificity of plant peroxidases [27]

| Substrate | $k_{app}$, $\mu M^{-1} \cdot sec^{-1}$ | | | | | | |
|---|---|---|---|---|---|---|---|
| | African oil palm tree | royal palm tree | soybean | horseradish | tobacco | peanut | alfalfa |
| ABTS | 17 | 52 | 0.36 | 4.0 | 1.1 | 0.37 | 1.0 |
| Ferulic acid | 18 | 63 | — | — | — | — | — |
| *o*-Dianisidine | 3.9 | 0.97 | 0.39 | 4.3 | 2.0 | 2.0 | 2.4 |
| *o*-Phenylenediamine | 0.85 | 2.9 | 0.04 | 0.49 | 0.032 | 0.22 | 0.042 |
| Guaiacol | 0.21 | 1.2 | 0.64 | 1.6 | 0.51 | 2.4 | 15 |

activity", "apparent catalytic constant ($k_{cat}$)", and "apparent Michaelis constant ($K_m$)", which depend on the $AH_2$ concentration used. By contrast, the $k_{app}$ value is dependent solely upon the catalytic capacity of peroxidases.

Comparison of the $k_{app}$ values measured under optimal conditions reveals that the efficiency of catalysis of palm peroxidases depends on the chemical nature of the substrate (Table 1). The best substrates are ferulic acid and ABTS. The values of $k_{app}$ for palm peroxidases toward ABTS are significantly higher than those reported for other plant peroxidases (Table 1). For the other substrates, such as *o*-dianisidine and *o*-phenylenediamine (aromatic amines) and guaiacol (phenol), the $k_{app}$ values are 20-60-fold lower than that for ferulic acid and ABTS. Comparison of $k_{app}$ values for plant peroxidases from different sources measured against *o*-dianisidine, *o*-phenylenediamine, and guaiacol shows that all enzymes except alfalfa peroxidase oxidize these substrates with similar efficiency. It should be also emphasized that vanillin, (±)catechin, ascorbic acid, veratryl alcohol, KI, and ferrocyanide, which are oxidized by other peroxidases, are not oxidized by palm peroxidases. Thus, the substrate specificity of the novel peroxidases is not identical to that of peroxidases from horseradish roots, soybeans, peanuts, tobacco leaves, and alfalfa [23].

## STABILITY

At ambient temperature, palm peroxidases are highly stable enzymes over a broad pH range (Fig. 1). These enzymes differ advantageously from HRP, which looses its secondary and tertiary structures at pH 4.5 and below [31] and is inactivated quickly under acidic conditions [32-34]. Increasing temperature up to 70°C has no effect on the stability of palm peroxidases under neutral conditions; however, combination of high temperature with acidic or alkaline conditions decreases their stability dramatically.

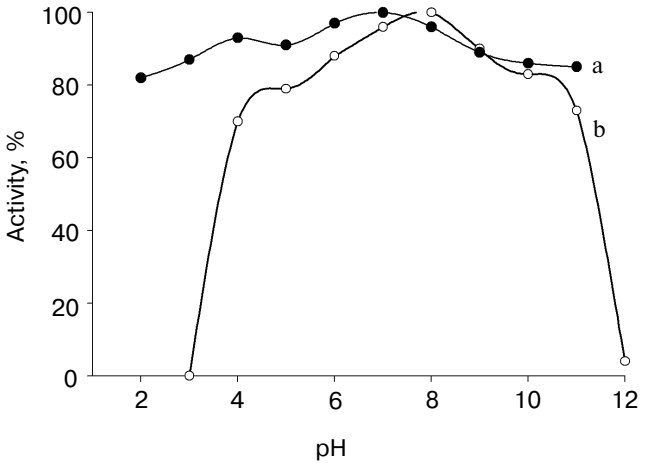


**Fig. 1.** Effect of pH on the stability of the African oil palm tree peroxidase at 25 (a) and 70°C (b).

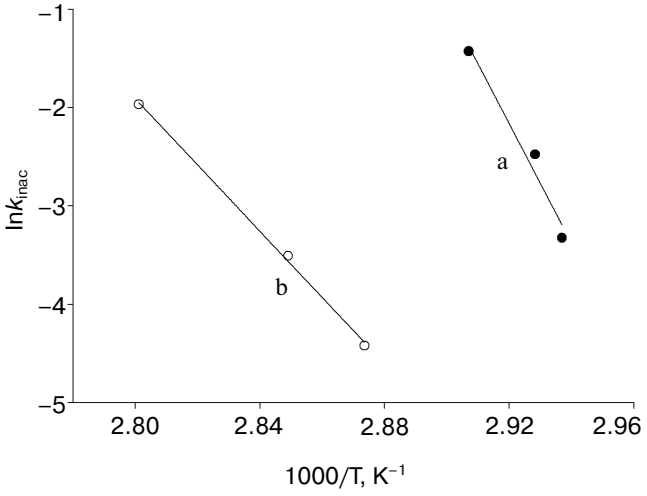


**Fig. 2.** Thermal inactivation of the African oil palm tree peroxidase at pH 10.5 (a) and 3.0 (b).

The stability of AOPTP has been analyzed in detail [35, 36]. Since the maximum stability of AOPTP is observed under neutral conditions, its thermal stability was studied at pH 7.5. Under these conditions, the enzyme completely preserves its catalytic activity after 1 h incubation at temperatures up to 70°C. Under the same conditions, HRP loses its activity within 5 min. Only soybean peroxidase shows comparable thermal stability [37].

Under alkaline conditions, AOPTP is also a highly stable enzyme. At 65°C and pH 10.5, no loss of the enzyme activity is detected for at least 80 min. At higher temperatures, alkaline inactivation obeys first-order kinetics. At pH 10.5, the inactivation energy for AOPTP was calculated to be 67 kcal/mol (Fig. 2). Since AOPTP shows high stability under strongly alkaline conditions, it may be used successfully as a potent dye-bleaching agent in detergents [38].

Many plant peroxidases are labile at acidic pH. As mentioned above, HRP is unstable under acidic conditions [32-34]. By contrast, under acidic conditions and at ambient temperature, AOPTP loses its activity very slowly; hence, AOPTP inactivation was studied at higher temperatures (Fig. 2). At pH 3.0, the AOPTP inactivation obeys first-order kinetics like during alkaline denaturation. From the values of the inactivation constants measured at pH 3.0, the inactivation energy for AOPTP was calculated to be 104 kcal/mol [35, 36]. This value is significantly higher compared to that reported for HRP under the same conditions (38 kcal/mol) [34].

Comparative study of the effects of three common organic solvents on the activity of AOPTP and HRP [16] showed that at low concentration (10-20% v/v) of diox-

**Table 2.** The activity of African oil palm and horseradish peroxidases in different organic solvents [16]

| Solvent concentration, % | Enzyme activity, % | | | | | |
|---|---|---|---|---|---|---|
| | dioxane | | dimethylsulfoxide | | ethanol | |
| | AOPTP | HRP | AOPTP | HRP | AOPTP | HRP |
| 0 | 100 | 100 | 100 | 100 | 100 | 100 |
| 10 | — | — | 51 | 53 | 81 | 80 |
| 20 | 26 | 27 | 27 | 9.7 | 49 | 46 |
| 30 | 13 | 4.9 | 11 | 6.3 | 20 | 14 |
| 40 | 2.1 | 0.4 | 5.2 | 3.4 | 4.2 | 3.6 |
| 50 | 1.0 | 0 | 3.2 | 0.9 | 1.4 | 0.4 |
| 60 | 0.17 | 0 | 0.5 | 0 | 0.4 | 0.07 |

ane and ethanol, both enzymes had similar activity (Table 2). However, at increasing concentrations of the solvents the palm peroxidase shows higher activity compared to horseradish enzyme. In the case of dimethylsulfoxide, the difference is already pronounced at 20% solvent concentration. This fact may be important in the development of enzymatic approaches in organic synthesis of chemicals.

Hydrogen peroxide inactivates plant peroxidases [39-41]. Inactivation of AOPTP by hydrogen peroxide was studied under neutral and alkaline conditions [35]. These conditions mimic those used in practical applications of peroxidases [38, 42]. At pH 10.5 and 40°C, AOPTP is inactivated slowly in the presence of 0.2 mM $H_2O_2$ with pseudo-first order inactivation constant ($k_{inac}$) of 0.003 $min^{-1}$. At increasing $H_2O_2$ concentration, AOPTP stability is decreased, and at 20 mM $H_2O_2$, the $k_{inac}$ value is 0.19 $min^{-1}$.

Under neutral conditions, AOPTP is more stable toward hydrogen peroxide treatment than at alkaline pH values [35]. At pH 7.5 and 40°C, palm peroxidase is fully active in the presence of 0.2 mM $H_2O_2$. At increasing $H_2O_2$, concentration the stability of AOPTP is decreased, although even at 20 mM $H_2O_2$ the inactivation rate constant ($k_{inac}$) is only 0.03 $min^{-1}$. At decreasing temperature from 40 to 29°C, the $k_{inac}$ value is decreased sharply to 0.002 $min^{-1}$. Therefore, under neutral conditions AOPTP may be used even at high hydrogen peroxide concentrations.

## APPLICATION

**A. Chemiluminescence assay.** Miles and Hales [43] described how enzymes could be used instead of isotopes in an immunometric assay. HRP is used as a label in immunochemical reagents because it is commercially available in a highly purified state, inexpensive, stable, and highly active.

There are three general methods for the determination of the activity of the peroxidase label: colorimetric, fluorimetric, and luminescent. The last method, using luminol as the substrate, is the most sensitive. However, HRP-catalyzed oxidation of luminol operates a low chemiluminescence signal. This problem was solved by developing an enhanced chemiluminescence assay in which the luminol oxidation is performed in the presence of enhancers. A typical kinetic curve of HRP-catalyzed luminol oxidation by hydrogen peroxide in the presence of *p*-iodophenol (the best enhancer) is presented in Fig. 3 (curve (a)). In this case, the chemiluminescence intensity increases, reaches a maximum, and then decreases. The unstable chemiluminescence signal is a source of errors in the immunoassay.

Palm peroxidases can be used to overcome this problem [44]. Luminol oxidation in the presence of palm per-

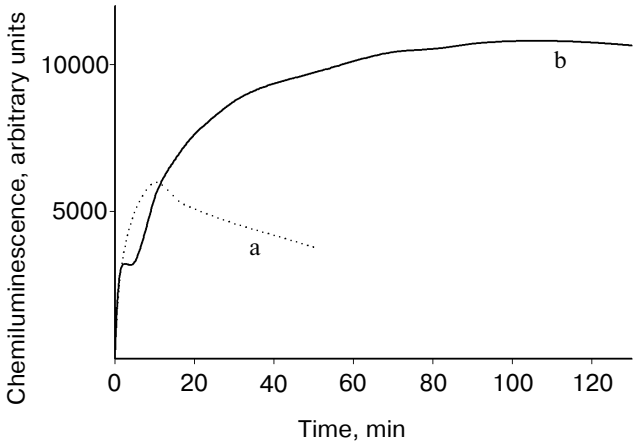


**Fig. 3.** Kinetic curves of chemiluminescence intensity during luminol oxidation by hydrogen peroxide catalyzed by horseradish (a) and African oil palm tree (b) peroxidases.

oxidases results in a high and long-term luminescence signal (Fig. 3, curve (b)). Moreover, these peroxidases can efficiently oxidize luminol in the absence of enhancers. Thus, taking into consideration their unusual stability, palm peroxidases are promising enzymes for immunoassays with chemiluminescence detection.

**B. Enzyme electrodes.** In addition to immunoassays, peroxidases can be used in the construction of electrode biosensors [45-47]. Peroxidases are known to catalyze the oxidation of various electron donor substrates by a ping-pong mechanism (Eqs. (1)-(3)). If peroxidase molecules are immobilized, the electrode can substitute for the electron donor substrate ($AH_2$) in the peroxidase reaction cycle. In this case, peroxidase is oxidized by hydrogen peroxide according to reaction (Eq. (1)) and is subsequently reduced by the electrons provided by the electrode (Eq. (5)):

$$\text{Cpd I} + 2e^- + 2H^+ \rightarrow \text{E} + H_2O. \qquad (5)$$

A number of biosensors for detection of hydrogen peroxide and organic hydroperoxides have been constructed using HRP [48, 49]. Unfortunately, the working $H_2O_2$ concentration range for HRP-containing electrodes is not wide (10-200 μM) (Fig. 4, curve (b)) due to the inactivation of the enzyme at higher concentrations of $H_2O_2$. As mentioned above, palm peroxidases are more stable with respect to $H_2O_2$ treatment than HRP. This allowed us to develop a $H_2O_2$-sensitive biosensor with a working range up to 700 μM $H_2O_2$ (Fig. 4, curve (a)) [50]. Moreover, this biosensor has lower detection limit (1 μM). It should be also noted that RPTP-based electrodes are able to measure hydrogen peroxide concentration in samples under acidic conditions (pH 4.5), unlike biosensors with HRP. Thus, the use of palm peroxidases allows constructing $H_2O_2$-sensitive electrodes with improved characteristics.

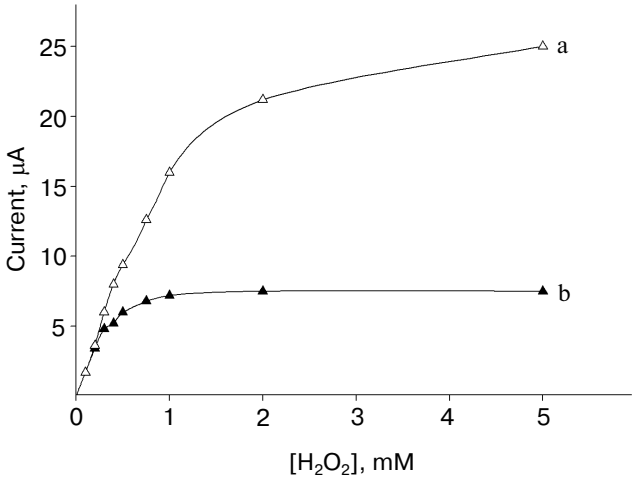


**Fig. 4.** Effect of hydrogen peroxide concentration on the current produced by the African oil palm tree (a) and horseradish (b) peroxidases immobilized on graphite electrodes.

**C. Polymer synthesis.** There is presently tremendous interest in the production of conducting polymers. Polyaniline is one of the most extensively investigated conducting polymers because of its high environmental stability and promising electronic properties. It is used in organic lightweight batteries, light-emitting diodes, optical displays, anticorrosive protection, bioanalysis, etc. [51-53]. The synthesis of polyaniline was reported first in 1840 by Fritzsche; hence, polyaniline is the oldest electroactive synthetic polymer. Presently, polyaniline is commonly synthesized by oxidizing monomer aniline with ammonium persulfate as the initiator of polymerization. Unfortunately, this chemical method has a severe drawback, namely, the reaction is not environmentally friendly because it is carried out at very low pH values (usually in 1 M $H_2SO_4$) in the presence of a strong oxidant.

The enzymatic polymerization of aniline is an attractive alternative to the chemical synthesis. Previously, HRP has been used in the synthesis of polyelectrolyte complexes of polyaniline and soluble polymers having sulfonic and phosphoric groups [54, 55]. It should be noted that unlike polyaniline itself, the polyelectrolyte complexes of polyaniline do not precipitate during their synthesis but form a stable suspension of small particles and, hence, the processability of the complexes is higher than that of polyaniline itself. The advantage of the enzymatic approach compared to the chemical one consists of the fact that the production of doping polyaniline is performed under mild conditions.

Unfortunately, HRP shows low activity toward aniline and low stability at pH 4.5 and below, i.e., in the pH range where the polyelectrolyte complex between polyaniline and negatively charged polymeric templates can be formed. Since palm peroxidases show higher stability under acidic conditions, a "green" synthesis of RPTP-catalyzed complex of polyaniline and sulfonated polystyrene has been developed [56]. Varying pH of the reaction medium, it was shown that maximum rate of the polymerization reaction is observed at pH 3.5. It should be noted that at this pH value, polyaniline ($pK_a$ of 4.6) interacts with sulfonated polystyrene ($pK_a$ of 0.7), yielding an ionic complex. Therefore, favorable pH values for the enzymatic polymerization of aniline and the formation of the polyelectrolyte complex are the same. Characterization of the complex of polyaniline and sulfonated polystyrene by UV-Vis spectroscopy, EPR, and cyclic voltammetry showed that the polyelectrolyte complex was electroactive [57]. Therefore, the use of RPTP allows synthesizing conducting polyaniline under environmentally friendly conditions.

Thus, novel plant peroxidases have been purified from the leaves of African oil palm tree *Elaeis guineensis*

and royal palm tree *Roystonea regia*. The study of pH-, thermal, and oxidative stability of palm peroxidases showed that these enzymes are the most stable peroxidases reported so far. Although the reasons of stabilization of palm peroxidases are not clear yet, their extremely high stability in a combination with wide substrate specificity opens good promise for the practical use of these enzymes.

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## REFERENCES


1. Dunford, H. B. **(**1999) *Heme Peroxidases*, Willey-VCH, New York.
2. Farrell, R. L., Murtagh, K. E., Tien, M., Mozuch, M. D., and Kirk, T. K. (1989) *Enzyme Microb. Technol.*, **11**, 322-328.
3. Wakamatsu, K., and Takahama, U. (1993) *Physiol. Plants*, **88**, 167-171.
4. Krell, H.-W. (1991) in *Biochemical, Molecular and Physiological Aspects of Plant Peroxidases* (Lobarzewski, J., Greppin, H., Penel, C., and Gaspar, Th., eds.) University M. Curie-Sklodowska and University Geneva, Lublin-Geneva, pp. 469-478.
5. Tijssen, P. (1985) *Practice and Theory of Enzyme Immunoassay*, Elsevier, Amsterdam.
6. Egorov, A. M., Gavrilova, E. M., and Sakharov, I. Yu. (2000) in *Integrated Plant Systems* (Greppin, H., Penel, C., Broughton, W. J., and Strasser, R., eds.) University Geneva, pp. 369-385.
7. Lindgren, A., Ruzgas, T., Gorton, L., Csöregi, E., Bautista Ardila, G., Sakharov, I. Yu., and Gazaryan, I. G. (2000) *Biosensors Bioelectronics*, **15**, 491-497.
8. Gaspar, S., Popescu, I. C., Gazaryan, I. G., Bautista, G., Sakharov, I. Yu., Mattiasson, B., and Csöregi, E. (2000) *Electrochem. Acta*, **46**, 255-264.
9. Colonna, S., Gaggero, N., Richelmi, C., and Pasta, P. (1999) *Trends Biotechnol.*, **17**, 163-168.
10. Adam, W., Lazarus, M., Saha-Moller, C. R., Weichold, O., Hoch, U., and Schreier, P. (1999) *Adv. Biochem. Eng.*, **63**, 74-108.
11. Duarte-Vázquez, M. A., Whitaker, J. R., Rojo-Domínguez, A., García-Almendárez, B. E., and Regalado, C. (2003) *J. Agric. Food Chem.*, **51**, 5096-5102.
12. López-Molina, D., Heering, H. A., Smulevich, G., Tudela, J., Thorneley, R. N. F., García-Cánovas, F., and Rodríguez-López, J. N. (2003) *J. Inorg. Biochem.*, **94**, 243-254.
13. Gray, J. S. S., and Montgomery, R. (2003) *J. Agric. Food Chem.*, **51**, 1592-1601.
14. Gazaryan, I. G., and Lagrimini, L. M. (1996) *Phytochemistry*, **41**, 1029-1034.
15. Sakharov, I. Yu., Vesga Blanco, M. K., Galaev, I. Yu., Sakharova, I. V., and Pletjushkina, O. Yu. (2001) *Plant Sci.*, **161**, 853-860.
16. Sakharov, I. Yu., Castillo Leon, J., Areza, J. C., and Galaev, I. Yu. (2000) *Bioseparation*, **9**, 125-132.
17. Welinder, K. G. (1979) *Eur. J. Biochem.*, **96**, 483-502.
18. Castillo Leon, J., Alpeeva, I. S., Chubar, T. A., Galaev, I. Yu., Csöregi, E., and Sakharov, I. Yu. (2002) *Plant Sci.*, **163**, 1011-1019.
19. Van Huystee, R. B., Sun, Y., and Lige, B. (2003) *Crit. Rev. Biotechnol.*, **22**, 335-354.
20. Gilliken, J. W., and Graham, J. S. (1991) *Plant Physiol.*, **96**, 214-220.
21. Lee, M. Y., Choi, Y., Park, J. H., Jang, S. G., and Kim, S. S. (1991) in *Biochemical, Molecular and Physiological Aspects of Plant Peroxidases* (Lobarsewski, J., Greppin, H., Penel, C., and Gaspar, Th., eds.) University M. Curie-Sklodowska and University Geneva, Lublin-Geneva, pp. 159-168.
22. Pomar, F., Bernal, A., Diaz, J., and Merino, F. (1999) *Phytochemistry*, **46**, 1313-1317.
23. Gazaryan, I. G., Reshetnikova, I. A., Doseeva, V. V., and Bekker, E. G. (1995) *Biochemistry (Moscow)*, **60**, 767-772.
24. Marangoni, A. G., Brown, E. D., Stanley, D. W., and Yada, R. Y. (1989) *J. Food Sci.*, **54**, 1269-1271.
25. Kwak, S.-S., Kim, S.-K., Lee, M.-S., Jung, K.-H., Park, I.-H., and Liu, J.-R. (1995) *Phytochemistry*, **39**, 981-984.
26. Smulevich, G. (1996) in *Plant Peroxidases. Biochemistry and Physiology* (Obinger, C., Burner, U., Ebermaun, R., Penel, C., and Greppin, H., eds.) University Agriculture of Vienna and University Geneva, Geneva-Vienna, pp. 13-19.
27. Sakharov, I. Yu., Vesga Blanco, M. K., and Sakharova, I. V. (2002) *Biochemistry (Moscow)*, **67**, 1043-1047.
28. Childs, R. E., and Bardsley, W. G. (1975) *Biochem J.*, **145**, 93-103.
29. Abelskov, A. K., Smith, A. T., Rasmussen, Ch. B., Dunford, H. B., and Welinder, K. G. (1995) *Biochemistry*, **34**, 4022-4029.
30. Rasmussen, Ch. B., Dunford, H. B., and Welinder, K. G. (1997) *Biochemistry*, **36**, 9453-9463.
31. Chattopadhyay, K., and Mazumdar, S. (2000) *Biochemistry*, **39**, 263-270.
32. Liu, W., Cholli, A. L., Nagarajan, R., Kumar, J., Tripathy, S., Bruno, F. F., and Samuelson, L. (1999) *J. Am. Chem. Soc.*, **121**, 71-78.
33. Berezin, I. V., Ugarova, N. N., Kerschengoltz, B. M., and Brovko, L. Yu. (1975) *Biokhimiya*, **40**, 297-300.
34. Pina, D. G., Shnyrova, A. V., Gavilanes, F., Rodríguez, A., Leal, F., Roig, M. G., Sakharov, I. Yu., Zhadan, G. G., Villar, E., and Shnyrov, V. L. (2001) *Eur. J. Biochem.*, **268**, 120-126.
35. Sakharov, I. Yu., and Sakharova, I. V. (2002) *Biochim. Biophys. Acta*, **1598**, 108-114.
36. Rodríguez, A., Pina, D. G., Yélamos, B., Zhadan, G. G., Castillo León, J. J., Villar, E., Gavilanes, F., Roig, M. G., Sakharov, I. Yu., and Shnyrov, V. L. (2002) *Eur. J. Biochem.*, **269**, 2584-2590.
37. McEldoon, J. P., and Dordick, J. S. (1996) *Biotechnol. Progr.*, **12**, 555-558.
38. Cherry, J. R., Lamsa, M. H., Scheider, P., Vind, J., Svendsen, A., Jones, A., and Pedersen, A. H. (1999) *Nature Biotechnol.*, **17**, 379-384.
39. Baynton, K. J., Bewtra, J. K., Biswas, N., and Taylor, K. E. (1994) *Biochim. Biophys. Acta*, **1206**, 272-278.
40. Chung, N., and Aust, S. D. (1995) *Arch. Biochem. Biophys.*, **316**, 851-855.

41. Arnao, M. B., Acosta, M., del Rio, J. A., Varon, R., and García- Cánovas, F. (1990) *Biochim. Biophys. Acta*, **1038**, 85-89.
42. Wright, H., and Nicell, J. A. (1999) *Bioresource Technol.*, **70**, 69-79.
43. Miles, L. E., and Hales, C. N. (1968) *Nature*, **219**, 186-189.
44. Sakharov, I. Yu. (2001) *Biochemistry (Moscow)*, **66**, 515-519.
45. Gorton, L. (1995) *Electroanalysis*, **7**, 23-45.
46. Chaubey, A., and Malhotra, B. D. (2002) *Biosensors Bioelectronics*, **17**, 441-456.
47. Wang, B., Li, B., Cheng, G., and Dong, S. (2001) *Electroanalysis*, **13**, 555-558.
48. Ruzgas, T., Csöregi, E., Eménus, J., Gorton, L., and Marko-Varga, G. (1986) *Anal. Chim. Acta*, **330**, 123-138.
49. Ghindilis, A. L., Atanasov, P., and Wilkins, E. (1997) *Electroanalysis*, **9**, 661-674.
50. Alpeeva, I., Nistor, M., Castillo, J., Csöregi, E., and Sakharov, I. (2003) *Proc. 2 Int. Conf. Biotechnology*, Moscow, Vol. 2, pp. 194-195.
51. MacDiamid, A. G. (2002) *Synth. Metals*, **125**, 11-22.
52. Heeger, A. J. (2002) *Synth. Metals*, **125**, 23-42.
53. Raitman, O. A., Katz, E., Buckmann, A. F., and Willner, I. (2002) *J. Amer. Chem. Soc.*, **124**, 6487-6496.
54. Lui, W., Cholli, A. L., Nagarajan, R., Kumar, J., Tripathy, J., Bruno, F. F., and Samuelson, L. (1999) *J. Amer. Chem. Soc.*, **121**, 11345-11355.
55. Nagarajan, R., Tripathy, J., Kumar, J., Bruno, F. F., and Samuelson, L. (2000) *Macromolecules*, **33**, 9542-9547.
56. Sakharov, I. Yu., Vorobiev, A. Ch., and Castillo Leon, J. J. (2003) *Enzyme Microb. Technol.*, **33**, 661-667.
57. Sakharov, I. Yu., Ouporov, I. V., Vorobiev, A. Kh., Roig, M. G., and Pletjushkina, O. Yu. (2003) *Synth. Metals*, **142**, 127-135.