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REPORT

IV SYMPOSIUM "PLANT PEROXIDASES: BIOCHEMISTRY AND PHYSIOLOGY" (VIENNA, AUSTRIA, JULY 6–10, 1996)

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The IV Symposium on plant peroxidase (PO) was excellently organized by R. Ebermann, C. Obinger and coll. from the Institute of Chemistry in the Agricultural University, Vienna. It took its sources from an 11-year-old tradition, namely the I Symposium organized by H. Greppin, C. Penel and Th. Gaspar (Geneva, Switzerland, 1985) followed by the Symposia in Lublin, Poland (1990) and Elsinore, Denmark (1993); in all these events the support of the Geneva University is to be underlined.

Some 150 communications (oral presentations and posters) were presented by about 170 researchers from 34 countries; two representatives from Bulgaria (N. Bakardjieva and A. Edreva) were invited. It was fascinating that most of the contributions resulted from the joint efforts of international research groups. The Proceedings of the Symposium (C. Obinger, U. Burner, R. Ebermann, C. Penel and H. Grepin, eds., University of Agriculture, Vienna, and University of Geneva, 1996) involve a selection of 70 contributions.

The main topics were: structure; enzymology; ascorbate peroxidase; genetics; plant development; stress and disease; applications.

The opening session accentuated on enzyme structure as the most fundamental aspect of PO research. Previously (in 1993) plant POs were defined as a superfamily divided into three classes which show less than 20% similarity: the class I appears to be of prokaryotic origin; class II comprises extracellular fungal PO, and class III – the plant secretory PO (Welinder and coll., Denmark). As stated by Veitch, Smith and coll. (England), "the recent publication of the first three-dimensional structure of a plant peroxidase heralds something of a turning point in the study of these enzymes". Since the last PO meeting a large number of new peroxidase crystal structures have been solved including peanut PO (Schuller and coll., USA, Canada, England), horseradish peroxidase C (HRPC) (Gajhede and coll., Denmark, USA, England), and barley PO (Henriksen and coll., Denmark). It was established that the peanut PO is a trimetal glyco protein containing 1 mole Fe, 2 moles Ca and 1 mole Mn per 1 mole protein, and 3 glycan chains, Ca being essential for the enzyme activity; its loss causes conformational changes at and around the active site and the heme. Mn was not expected to occur in higher plant PO (Van Huystee and coll., Canada).

The solution of the crystal structure of cytochrome C PO (class I) from the bacterium *Pseudomonas aeruginosa* reveals the existence of a new class, di-heme PO. The single

polypeptide chain contains 2 covalent c hemes: one of them is a low-polential (–330 mV) centre where H_2O_2 is reduced (the peroxidatic site); the other is a high-potential (+320 mV) centre which feeds electrons to the peroxidatic site from soluble electron - shuttle proteins like cytochrom *c* and azurin. Thus, the enzyme works without the need for creation, storing and stabilization of free radicals during the catalytic cycle; this property may lead to a longer-lived protein which is likely to be less affected by mutations than radical PO (Fülöp, England).

The discovery of another member (named bas 1) of a new group of plant PO, thioredoxin-dependent peroxide reductase, homologue of animal and bacterial proteins, was reported. Its primary and secondary structures were determined, pointing that bas 1 is distinct from the previously discovered member of this family, the phospholipid hydroperoxide glutathione peroxidase (PHGPx). Both bas 1 and PHGPx are the only plant alkyl hydroperoxide scavenging enzymes known so far. They are induced by light; PHGPx is also induced by ozone and salt stress whereas bas 1 is not, this suggesting a specific role of the latter in oxidative stress (Baier and Dietz, Germany).

Studies on amino acid and cDNA sequences, as well as on isoenzyme diversity of PO in *Arabidopsis* were carried out contributing to a better understanding of higher plant PO gene family; evolutionary tree of plant PO was proposed, and evolutionary aspects of plant PO were examined (Welinder and coll., Denmark; Simon and coll., Switzerland, Bakardjieva and coll., Bulgaria). Evidence for dimeric PO structure was presented (Krzakowa, Poland).

New insights into the structure–activity relationships in PO were reported. They flowed from the use of a modern molecular experimental approach, the site-directed mutagenesis, combined with the currently applied physical methods (proton nuclear magnetic resonance – ^{1}H NMR, resonance Raman spectroscopy, electronic absorption spectroscopy). This approach is based on directed substitution of single amino acid residue in the polypeptide chain achieved by PCR technique; as a result mutant protein molecules are constructed.

It was demonstrated that the structure of the heme pocket in the polypeptide chain of PO is of crucial importance for both heme and substrate binding, i.e. for the catalytic reaction.This may be accounted for by the specific co-ordinative and spin states of heme influenced by the proximal and distal amino acid environment. A model of the heme pocket in HRPC was proposed where the key phenylanine residue in the pocket, responsible for the aromatic substrate binding, is identified (Veitch and coll., England, Orlova and coll., Russia). Comparative studies on heme pocket of members of class I, II and III PO superfamily were carried out, and essential variations were revealed (Veitch and coll., England, Denmark). PO of class III are characterized by a strong distortion of heme from planarity, whereas in class I and II PO the heme is planar, this resulting from specific heme–heme pocket interactions (Smulevich, Italy). By site-directed mutagenesis, the conversion of myoglobin to peroxidase was effectuated (Ozaki et coll., Japan).

The enzymological studies of PO concerned mainly the oscillatory mechanism of peroxidase–oxidase reaction, the mechanism of oxidation of IAA, halides and thiols, lignin biosynthesis and inactivation of PO by H_2O_2. It was demonstrated that the PO catalyzed oxidation of IAA involves direct binding of Fe^{3+} – PO to O_2 as an initial key step; hence PO acts as an IAA oxygenase (Gazaryan and coll., Russia, USA, England). The PO catalyzed oxidation of iodine was reported to be the best example of an “enzyme clock”, where the reaction starts

after a well defined time (Kummer and coll., Germany). Thiols were shown to be oxidized by PO in the absence of H_2O_2 via a free radical mechanism (Obinger and coll., Austria). Specific isoperoxidases responsible for lignin biosynthesis in poplar and beech were identified using molecular approaches; in poplar coregulation of lignification genes (coding for cinnamoyl alcohol dehydrogenase and PO) was established (Christensen and coll., Denmark, Obinger and coll., Austria). In the absence of a substrate for PO, H_2O_2 can replace it; in this case inactivation of PO was recorded (Acosta and coll., Spain).

Ascorbate PO is known to occur in green algae and higher plants. The fact that the enzyme was found in one of the most primitive unicell red algae is important for understanding its molecular evolution (Sano and coll., Japan). Thylacoid localization and dual function of ascorbate PO in scavenging active oxygen species and dissipation of excess photon energy were reported (Asada and coll., Japan).

In several communications new data were presented that the relation of PO to growth and development is based on its involvement in auxin catabolism and cell wall structure and metabolism. By using transgenic tobacco plants, over- and underexpressing PO, Lagrimini (USA) proved that: PO activity and plant growth are inversely related; IAA is a specific effector of the expression of anionic PO gene; and PO can degrade IAA. Gaspar and coll. (Belgium, Switzerland, Luxembourg, France) demonstrated the role of PO in initiating rooting and flowering, PO being indissociable factor of auxin and polyamine metabolism; the importance of the putrescine catabolism through GABA (γ-aminobutyric acid) pathway was underlined. The structural and functional importance of PO in cell walls was recognized; the specific binding of PO to cell wall pectins (via Ca^{2+}) was established (Penel and coll., Switzerland). The use of iso-PO as markers of rooting in *Quercus suber* (Roldao and coll., Portugal) and embryogenesis in *Phoenix dactylifera* cultures (Baaziz and coll., Morocco) was reported.

The involvement of PO in abiotic and pathogenic stress phenomena was reported in numerous communications. A wide array of stress stimuli, namely high and low temperature, heavy metal ions, ozone, SO_2, polychlorinated biphenyls, as well as pathogens were shown to induce an overall increase of PO activity. However, the differential specific response of the individual isoperoxidase components was underlined, this being examined as a tool for fine metabolic regulation and adjustment of plants to the adverse environment (Bakardjieva and coll., Bulgaria; Macek and coll., Czech Republic; Erdei and coll., Hungary). It was also shown that PO is a component of the molecular bases underlying plant-obligatory pathogen relationships (Edreva and coll., Bulgaria, France).

The widening of the range of PO applications in biotransformation of organic compounds and bioremediation of polluted environment was substantiated (Macek and coll., Czech Republic).

Most probably the new experimental approaches would open new horizons in PO research. At the next PO meeting which will be held in Ohio, USA, 1999, fascinating results are to be expected.

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