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Early years of cytochrome P450 research in Berlin-Buch: its present state and origin of the biochemical and biophysical conferences

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The very beginning of hemoprotein research in Berlin/Berlin-Buch

The origin of hemoprotein research in Berlin goes back to the 1930s, when Wolfgang Heubner was head of the Institute of Pharmacology of the Kaiser-Wilhelm-University in Berlin. Shortly before World War II, Heubner was confronted with human intoxication by nitro compounds, resulting in the formation of methemoglobin observed in industrial workers producing explosives. His idea that pharmacological and toxicological effects can be reduced to physical and chemical processes led him to study these maladies.

These clinical observations were the origin of studies not only on the interactions of different nitro compounds with erythrocytes and hemoglobin but also on the physicochemical properties of hemoglobin to better understand how the mechanism of intoxication by different blood poisons proceeds to produce methemoglobin and in this way inhibits reversible oxygen binding. His assistants, for example, Fritz Jung, Robert Havemann, and Herbert Remmer, were entrusted with this task.

For those who are interested in historical data it may be of interest that Herbert Remmer [1], who discovered the induction together with Kato, Conney, and Ernster [2–4] and also Kalow [5] as Remmers doctorand, with his pioneering work on polymorphism of pseudocholinesterase, originated from the Heubner School in Berlin.

Shortly after World War II Fritz Jung, working at the Pharmacological Institute of the University in Würzburg, used his offer of an appointment to the university in Berlin—now Humboldt University—to continue studies on the mechanism of blood poisoning. This project was not only performed at the Pharmacological Institute of Humboldt University in the center of the city, which was largely destroyed during World War II, but also at the former Brain Research Institute of the Kaiser Wilhelm Society in Berlin-Buch in the near surroundings of the city. In this building—later one of the Institutes of the Academy of Sciences—a research group under the leadership of Werner Scheler was established by Fritz Jung to pursue the physicochemical analysis of hemoproteins. Also Robert Havemann, originating from the Heubner School, became director of the Institute of Physical Chemistry of the University and continued studies on hemoglobin. An important result was independently elaborated by Scheler [6] and Havemann [7] using magnetic and optical measurements of methemoglobin derivatives [8]. They found a correlation between the position of the Soret band in the absorption spectrum and the magnetic susceptibility of the derivatives. At this time the finding was of no functional importance because methemoglobin is an artificial product of hemoglobin. Nevertheless, this result should be emphasized because this correlation is used to interpret spectral changes at substrate binding to P450 shifting the low-spin ferric state to the high-spin state.

After an offer of appointment, Werner Scheler left Berlin-Buch for Greifswald (1959), and Klaus Ruckpaul took over the leadership of the hemoprotein group in Berlin-Buch. By means of various optical methods (ORD, CD, MORD) the interaction of globin with heme was analysed to understand the specific function of hemoproteins such as reversible oxygen binding (hemoglobin), splitting of hydrogen peroxide (catalase), and electron transfer (cytochrome *c*).

At this time Braunitzer und Konigsberg independently elucidated the amino acid sequence of hemoglobin, and Perutz and Kendrew succeeded in the elucidation of the three-dimensional structure of myoglobin and hemoglobin. By their results the question of

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the influence of the protein moiety on the oxygen binding function of hemoglobin obtained a molecular basis. Therefore the studies on methemoglobin were complemented by physiologically relevant studies on the binding behaviour of oxygen and alkylisocyanides (as models for oxygen) in dependence on allosteric effectors such as organic (ATP, ADP) and inorganic (for instance, inositolhexa (-penta) phosphates [9,10]. These investigations also included questions of the stability of the protein and the relationship between the amino acid composition and the binding behaviour of hemoglobin. For these studies—following the methodological tradition—in addition to optical UV–Vis and IR spectroscopic methods, derivative spectroscopy, circular dichroism, stopped-flow kinetics, ESR and NMR spectroscopy, and calorimetry were used to better understand structure/function relationships.

P450 research in Berlin-Buch

As pharmacologists we followed with great interest the progress of P450 research in the beginning of the 1970s, as P450 is one of the most important systems in the biotransformation of drugs and of hormones. The decision to use our physicochemical knowledge to determine the structural peculiarities that define the function of cytochrome P450, compared to other hemoproteins, and the structural differences that exist between the various P450 enzymes was based on the excellent physicochemical studies on P450cam of the Gunsalus group [11] and on Jud Coon's discovery that microsomal mammalian P450 can be solubilized and reconstituted in an enzymatic active form at the end of the 1960s [12–14].

Three main research topics characterized the scientific profile of the P450 research of the group in Berlin-Buch: (i) studies at the molecular level aimed at understanding structure/function relationships and regulation mechanisms of membrane-bound microsomal systems; (ii) phospholipid/protein interactions; and (iii) studies on alkane hydroxylating microorganisms, in particular yeasts, to transfer results of basic research to biotechnology (e.g., protein synthesis, synthesis of special fatty acids).

Regulation at the molecular level

With regard to the molecular level, our strategy was directed at answering three open questions:

(i) What makes a hemoprotein from an oxygen binding protein into an oxygen splitting enzyme?
(ii) What regulates the enzymatic activity?
(iii) What amino acid residues are involved in the formation of the active center and in the electron pathway?

(i) The specific function of hemoproteins is determined mainly by the axial heme iron ligands. In contrast to oxygen binding proteins such as myoglobin and hemoglobin, in which the fifth heme coordination position is occupied by a histidine residue, cytochrome P450 is distinguished by a cysteine residue in this position. This important peculiarity was experimentally evidenced by EPR [15] and Raman resonance [16] studies and finally by X-ray structure analysis [17,18]. The Berlin-Buch group extended these results by spectral studies in the near UV [19] and infrared [20] region, showing that the typical position of the Soret band of the CO complex is due to the thiolate ligand. Experimental and theoretical analysis [21,22] showed that a strong pi-back donation from the iron to dioxygen is a prerequisite for reductive dioxygen splitting. Quantum chemical calculations of dioxygen heme complexes with thiolate and imidazole nitrogen as fifth ligands revealed that in the reduced cytochrome P450 dioxygen model the electron supplied is predominantly localized (67%) in the axial pi system, compared to only 32% in the hemoglobin model [22,23], which favours reductive splitting of dioxygen by lowering of the dioxygen antibonding pi bond (for detailed reviews, see [23,24]).

(ii) Regulation of activity at the molecular level is mainly understood as a structural change in cytochrome P450. Such structural changes may be effected by low-molecular-weight components (substrate or cosubstrate) as well as by interacting components of the monooxygenase system (cytochrome P450 reductase, cytochrome *b*5, and phospholipids). Regulation proceeds predominantly through interaction of cytochrome P450 with substrates. The target for the substrate-controlled regulation is provided by an equilibrium of two conformers with different functional properties. Substrates shift the spin equilibrium toward the high-spin conformation. This reaction is connected with a redox potential shift to a more positive value, resulting in a greater electromotive force for a subsequent electron transfer from the reductase to the monooxygenase. A correlation between spin and redox state and the reduction rate as well could be evidenced and indicates the regulatory significance of the substrate dependent spin equilibrium. This regulatory function was shown not to be limited to the first electron transfer but extended through the whole reaction cycle by use of a homologous series of substrates. Based on thermodynamic data the spin-redox coupling could be rationalized in a corresponding model.

From temperature difference spectra of phenobarbital-induced rabbit liver microsomes the existence of a low-spin $(S = 1/2)$ high-spin $(S = 5/2)$ equilibrium which is shifted to the high-spin conformer with increasing temperature [25] analogously to that in the P450cam system [26] was derived and thermodynamically analyzed [27]. Substrate binding also induces a high-spin shift which provides the energy to force the

conformational changes necessary for starting the reaction cycle. Furthermore, it was shown that the spin state of the heme iron regulates the redox equilibrium [27,28] and this way also the reduction rate [29]. Hence, the Berlin-Buch group provided experimental evidence for a temperature- and substrate-controlled spin state equilibrium of microsomal cytochrome P450 as an essential regulatory principle. The reader is referred to comprehensive reviews in [23,24].

(iii) The active center of P450 is characterized by the ability to bind a multitude of different compounds. Studies of the substrate binding area by means of spin-labeled type I substrate analogues (e.g., *n*-propylisocyanide) showed a remarkable degree of motional freedom indicating a relatively high conformational flexibility of the substrate binding region of CYP 2B4 (P450 LM2) [30,31]. That may explain (at least in part) the ability of P450 LM2 to bind substrates differing in stereochemical structures.

Studies to identify such amino acids, which were involved in electron transfer pathways and in the formation of the active center chemical modification, were used as appropriate means. Besides labeling with fluorescein isothiocyanate (FITC) to define the localization of the N-terminus [32] (see below in "Phospholipid/protein interaction"), chemical modification of amino groups using 2-methoxy-5-nitropone provided evidence for Lys 139, 251, and 384 as being part of the reductase-binding site and for these sites being extramembraneously located [33]. These studies were extended to CYP1A2 (rabbit liver P450 LM4) providing support that residues Tyr 243 and Tyr 271 nitrated by tetranitromethane are functionally involved in the interaction with reductase [34] and later supplemented by spin-labeled substrate analogues and site-directed mutagenesis [35,36]. In 1995 R. Bernhardt took over the chair of biochemistry at the University of Saarbrücken and continued studies on the mitochondrial P450 system.

Phospholipid/protein interactions

Since the solubilization and reconstitution of a microsomal P450 system to an enzymatically active monooxygenase which required phospholipid(s) for optimal activity, it became clear that structural peculiarities of the membrane would exert some control upon P450 function. By the end of the 1970s several important experiments had been undertaken to elucidate certain features of the membrane topology and P-L interaction. A review summarizing the knowledge at that time as well as historical aspects has been published [37].

Based on these results, the strategy in that field was (i) development of a novel reconstitution system providing "microsomal-like" lipid bilayer vesicles for better simulation of the physiological membrane environment, (ii) EPR (spin label) studies of the motion and aggregational state of P450 in the membrane, (iii) freeze–fracture electron microscopy to study membrane topology, (iv) chemical modification to analyze the location of the amino-terminus and/or P450/reductase interaction, and (v) stopped-flow kinetics of P450 reduction to elucidate specific effects of lipids (membrane) on P450 function and the electron transfer between reductase and P450.

Schwarz of the Berlin-Buch group developed a reconstitution technique using for the first time octylglucoside as detergent and combined dialysis/adsorption to incorporate P450 into large unilamellar bilayer vesicles with appropriate properties for rotational diffusion and electron microscopy experiments, thus simulating the natural microsomal membrane with regard to lipid composition, L/P ratio, P450/reductase ratio, negative membrane charge, and size [38,39].

By means of EPR spin label studies (saturation transfer EPR), rotational diffusion of P450 was applied to analyze the motional and aggregational state of CYP2B4 (P450 LM2) in liposomal and microsomal membranes. The data suggest limited rotational diffusion of an oligomeric P450 aggregate for both kinds of membranes from which a model was derived according to which a hexameric P450 complex is anchored via its six single transmembrane NH_2-terminal helices rotating slowly about an axis perpendicular to the membrane surface [40]. Later, this hypothesis was supported by freeze–fracture electron microscopy, unambiguously revealing a transmembrane localization of microsomal CYP2B4 [41].

To experimentally evidence the orientation of P450 within the microsomal membrane, fluorescein isothiocyanate (FITC) was used to label the NH_2-terminal amino group of rabbit liver microsomal CYP2B4 (P450 LM2). The data indicate that the NH_2 terminus is located on the cytosolic side of the ER membrane, and would be consistent with the hairpin-model and two transmembrane segments [42].

The component interaction in reconstituted P450 systems is dependent on different lipids [43–45] and was studied by Blanck and colleagues using the anaerobic NADPH-dependent reduction of CYP1A2 and CYP2B4 (P450 LM4 and LM2) as functional tool. They found a stimulation of the reduction rate of P450 after introducing negatively charged phospholipids such as phosphatidylserine. In general, the reduction of P450 exhibits a two-exponential (biphasic) kinetics with the physiologically relevant rapid reaction phase proceeding via a functional 1:1 complex with reductase. In vesicles (not in DLPC-reconstituted micelles) reductase cycling is observed and is proposed to occur in P450/reductase clusters consistent with the model of oligomeric P450 associates. On the other hand, the slow phase is due to randomly distributed P450 in the membrane.

Since the beginning of the 1990s, P450 research on phospholipid P450 interactions has turned to

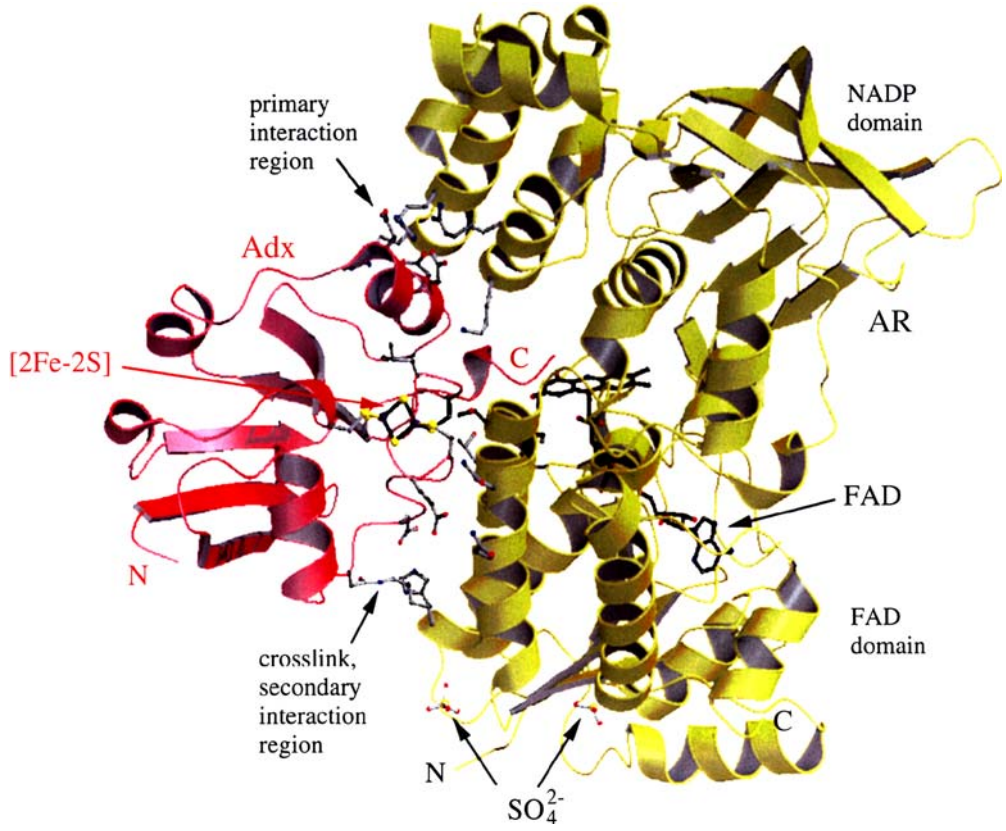

Fig. 1. Architecture of the adrenodoxin/adrenodoxin-reductase complex. Crystal structure of the Adx/AR-complex. Adx/AR contacts occur at the primary and secondary interaction regions and the region between the [2Fe-2S] cluster of Adx and the isoalloxazine ring of FAD of AR. The side chains of some residues involved in polar Adx/AR interactions are displayed [49].

mitochondrial P450, mainly CYP11A1 (P450scc), as a fruitful cooperation with the Stier group in Göttingen, colleagues from Minsk (Pyotr Kisselev, Sergey Usanov, Alexey Chernogolov), and Schmid (Stuttgart). Two results were of special importance. First, mitochondrial P450s do not exhibit a transmembrane membrane anchor like microsomal P450s, probably caused by the absence of any hydrophobic N-terminal segment of the mature form of CYP11A1 (as evidenced by freeze–fracture experiments of reconstituted CYP11A1). The data favor peripheral association with a localization of the membrane-binding segment(s) not deeper than the center of the bilayer [46]. Second, nonbilayer lipids such as cardiolipin—a component of the mitochondrial membrane—and branched phosphatidylcholines strongly stimulate the side-chain cleavage activity of the enzyme. The data indicate that membrane properties as the nonbilayer phase propensity rather than specific lipid interactions are important for the enzymatic activity as well as other CYP11A1 features such as cholesterol binding, membrane binding, and protein exchange between vesicles [47,48].

The data suggest that microsomal and mitochondrial P450 monooxygenases exhibit different topological features. Future work is needed to support or reject this hypothesis. Certainly, techniques such as NMR, X-ray crystallography, and AFM will contribute to obtaining detailed knowledge of membrane topology and interaction. Site-directed spin labeling using Cys scanning provides another promising spectroscopic tool to reveal the membrane structure and localization of the N-terminal hydrophobic segment of P450s.

Finally, to sum up the contributions of the Berlin-Buch group at the molecular level a recent result should be mentioned. To understand the structural basis of electron transfer as pivotal function of the monooxygenatic system resulting in reductive splitting of molecular oxygen, the three-dimensional structure of the adrenodoxin/adrenodoxin-reductase complex was elucidated by X-ray structure analysis [49] and is to be complemented by crystallization of the Adx/P450scc complex (Fig. 1).

Cytochrome P450 research on microorganisms

This part of the Berlin-Buch P450 research started as early as the middle of the 1970s. At that time, *n*-alkane-assimilating yeasts attracted much research attention primarily because of the interest of petroleum compa-

nies in implementing processes for the production of single-cell protein (SCP), dicarboxylic acids, and other valuable metabolites. The dream of converting oil to SCP dates back to the early 1960s and included the promise of opening a novel, rich source of foodstuff. In the 1970s, the oil crisis put an end to this dream in the Western countries. However, until the political revolutions in Eastern Europe, large-scale industrial SCP production remained an important goal in the former Soviet Union and in East Germany, where—for strategic and economic reasons—other protein sources used in animal feeding could be substituted. What has remained worldwide until the present are (i) the great expectations and efforts directed to the use of microbial biocatalysts for performing regio- and stereospecific oxygenation reactions which are difficult to obtain in classical chemistry and (ii) a steady interest in the biochemistry, genetics, and biotechnological potential of "non-conventional yeasts" and of their unique P450 systems.

Using a strain of *Candida maltosa*, a series of studies in Berlin-Buch showed the presence of a microsomal system catalyzing the NAD(P)H-dependent oxidation of *n*-alkanes and identified P450 and NADPH-P450 reductase as the essential components of this system. The importance of the P450 system also became evident after the assimilation of *n*-alkanes by cultured yeast cells was completely blocked by low CO concentrations which still allowed an unaffected growth on fatty alcohols, the primary products of P450-catalyzed alkane oxidation. The expression of this P450 system was shown to be inducible by *n*-alkanes, further enhanced during oxygen-limited growth, and repressed by glucose.

Taken together, these results of the Berlin-Buch group and those of several other laboratories (reviewed in [50,51]) clearly demonstrated that P450 systems catalyze the first and rate-limiting step of alkane assimilation in yeasts. Thus, after P450cam was shown by Dr. I.C. Gunsalus and co-workers to play a key role in bacterial camphor degradation, the alkane-hydroxylating yeast P450s provided a second example of the occurrence of specific P450 enzymes in such microorganisms that developed the capacity to utilize certain inert hydrocarbons as a sole source of carbon and energy.

Soon after the first P450 sequences were cloned from alkane-assimilating yeasts [52–57] it became obvious that there is not a single P450 but a whole new multigene family (termed CYP52). Studies performed in close collaboration between the groups in Berlin-Buch and at Tokyo University revealed that four of the eight CYP52 genes in *C. maltosa* are directly involved in alkane degradation and that each of the P450 isoforms is distinguished by a unique substrate specificity [58–63]. For example, CYP52A3 was clearly the most active alkane omega-hydroxylase, CYP52A4 showed a preference for lauric acid, CYP52A5 equally accepted both *n*-alkanes and fatty acids, and CYP52A9 was the most active omega-hydroxylase of palmitic and oleic acid. Other CYP52 members which alone were unable to initiate alkane degradation showed significant activities toward lauric acid but failed to hydroxylate *n*-alkanes.

Thus, the remarkable result in the evolution of the CYP52 family is the development of P450 forms that share an almost absolute regiospecificity in favor of omega-hydroxylation but differ in the preferred class (*n*-alkanes or fatty acids) and chain-length of their substrates.

The intracellular localization of alkane-hydroxylating P450s was largely unclear for a long period of time and the speculations ranged from (i) the plasma membrane as the site of *n*-alkane uptake, and (ii) the endoplasmic reticulum (ER) as the usual localization of microsomal P450s to (iii) the peroxisomes as the major site of fatty acid β-oxidation. Subcellular fractionation [64] and eventually immunoelectron microscopy [65] performed in Berlin-Buch provided clear evidence for a localization of alkane-induced P450 and NADPH-P450 reductase in the ER. As shown in subsequent studies, these yeast proteins are—like their mammalian counterparts—targeted to and retained in the ER via N-terminal signal-anchor sequences and their catalytic domains are exposed to the cytoplasm [66,67]. Substantiating this type of membrane topology, site-specific proteolysis just after the predicted membrane anchor yielded the cytosolic domain of CYP52A3 as a soluble and enzymatically fully active P450 protein [68].

A surprising result of the immunoelectron microscopical studies was that the ER membranes harboring the alkane-induced P450 proteins formed largely extended tubules that occurred in close vicinity to the plasma membrane and to the peroxisomes, thus specifying the former speculations on a P450 localization as favorable to an efficient subcellular organization of the alkane degradation pathway. Subsequent studies revealed that high-level expression of ER-resident P450 proteins and of the NADPH-P450 reductase is alone sufficient to trigger enhanced formation of ER membranes in the *Saccharomyces cerevisiae* and *C. maltosa* host cells (reviewed in [69]). The structure of the proliferated membranes (extended tubular networks, "karmellae" or other forms of cisternal stacked membranes) was found to depend on the membrane protein overproduced and to be largely determined by both the specific membrane anchor and the "folding state" [70,71] of the particular membrane protein. Further studies demonstrated that P450-induced ER proliferation is accompanied by an increase in both cellular phospholipids and certain ER-resident proteins, suggesting the operation of several signal transduction pathways regulating ER composition and extension in response to increased P450 biosynthesis [72–74].

Most of the projects described above were completed by the end of the 1990s and since then our group has shifted to the P450-dependent metabolism of arachidonic acid in mammals. Main objectives are to understand the role of P450-derived eicosanoids in the regulation of renal and vascular function, to identify the specific P450 isoforms involved, and to gain insight into the significance of alterations in P450-dependent arachidonic acid metabolism for the development of hypertension and inflammatory renal damage. This novel part of P450 studies in Berlin-Buch is performed in close collaboration with the Franz Volhard Clinic and the reader is referred to some of our recent publications [75–77] for more detailed information on this topic.

Conferences

The broad functional capability and the physiological importance of mammalian cytochrome P450 systems brought together physicians, pharmacologists, toxicologists, and biochemists. Moreover, from the very beginning medicinal and biotechnological aspects showed the possibilities of application, which was the reason for the interest of the pharmaceutical industry in these biotransforming systems. Based on this multidisciplinarity and the necessity to exchange ideas, it became obvious that a discussion forum should be started, which led to a series of conferences titled Microsomes and Drug Oxidations. The first took place in 1968 in Bethesda, followed by a second one 1972 in Stanford. The conferences are listed in Table 1.

The first conferences dealt mainly with pharmacological and biochemical aspects such as broad substrate specificity, identification and characterization of different isozymes, and induction phenomena. With increasing knowledge of biological and biochemical details of microsomal and mitochondrial systems, the number of participants increased in parallel.

The beginning studies on P450 in Berlin-Buch were complicated. At that time there existed only limited possibilities of cultivating *Pseudomonas putida* and there was a lack of experience in solubilization of microsomal P450.

First experiments on microsomal P450 at the beginning of the 1970s led us to a very fruitful cooperation with Dr. Maricic from Zagreb (Croatia, former Yugoslavia). A huge amount of biophysical and biochemical data in microbial, mammalian microsomal, and mitochondrial systems brought about the idea of a further discussion forum for exchange of results in addition to the MDO conferences. A second motivation was to bring together colleagues from eastern and western Europe and from the US and Japan—where the main activities in P450 research were concentrated—to overcome the barriers which had divided Europe since the end of World War II. This led us to organize the first International Conference on Biochemistry and Biophysics on Cytochrome P450 in Primosten (Yugoslavia) in 1976.

The first conference was organized by Dr. Maricic from Zagreb (Yugoslavia) and brought together a small group of about 40 leading scientists from several eastern and western European countries and several colleagues from the US. The conference was characterized by presentation of biophysical results such as quantum-chemical calculations of the importance of heme ligands and analysis of mechanisms of oxygen activation and substrate control using the advantage of soluble P450 systems and solubilized preparations. This provided the

Table 1
International symposia on microsomes and drug oxidations

Year	Venue of the conference	Organizers
1968	Bethesda, USA	James R. Gillette
1972	Stanford, USA	George J. Cosmides, Ronald W. Estabrook, James R. Gillette, Ronald G. Kuntzman, and Gabriel L. Plaa
1976	Berlin, Germany	Alfred Hildebrandt
1979	Ann Arbor, USA	Minor J. Coon
1981	Tokyo, Japan	Ryo Sato and Ryuichi Kato
1984	Brighton, England	Donald Davies
1987	Adelaide, Australia	Donald Birkett
1990	Stockholm, Sweden	Sten Orrenius and Jan-Ake Gustafsson
1992	Jerusalem, Israel	Jaime Kapitulnik
1994	Toronto, Canada	Gail D. Bellward
1996	Los Angeles, USA	Oliver Hankinson
1998	Montpellier, France	Patrick Maurel
2000	Stresa, Italy	Francesco De Matteis
2002	Sapporo, Japan	Tetsuya Kamataki
Forthcoming meetings		
2004	Mainz, Germany	Franz Oesch
2006	Budapest, Hungary	Laszlo Vereczkey

possibility of comparing membrane-bound with soluble systems and of studying the influence of the membrane on functional parameters of the enzyme. An interesting result was that the optical and magnetic properties of the membrane-bound P450 presented by the Berlin-Buch group and the soluble system from *Pseudomonas putida* presented by the Gunsalus group were qualitatively equal. Both forms are characterized by a temperature-dependent spin equilibrium which at higher temperature is shifted to the high-spin form. On this occasion we became acquainted with Dr. Gunsalus, who encouraged us to continue analyzing quantitatively the membrane-bound mammalian P450.

The second conference was organized in immediate pursuance of the FEBS Meeting 1978 in Dresden and took place in Eberswalde near Berlin. It followed the biophysical and biochemical main topics which were started so successfully in Primosten. The number of participants increased to about 60. But what was likewise important first cooperations with Coons lab in Ann Arbor resulted in joint papers. That was only the beginning and developed in the following years in cooperations with the Gunsalus and Estabrook laboratories and with labs in Sweden, France, and Great Britain. See Table 2.

Remarkable support for inviting outstanding experts from abroad came from IUB, where Jud Coon as the responsible member of an Interest Group took care of financial support. This help was an important means of ensuring the tradition of excellence of this series.

Already at the Stockholm conference in 1980 the number of participants increased to more than 300. Molecular biology was included as new topic which proved an excellent idea and essential topic for the following conferences. The next conferences in Kuopio, Budapest, and Vienna followed the principle of alternating between capitalistic and socialistic countries as locations for the conferences.

The feature of the respective conference was determined by the organizer. Consequently, the Kuopio conference in 1983 was focused on environmental implications of P450 and especially on monitoring fish pollution. Other topics following molecular biology introduced at the Stockholm conference were fundamental principles of cloning but also biochemical studies on characterization of different CYPs and pathways of oxygen cleavage.

The Budapest Conference in 1985 dealt with the consequences of induction and molecular details of the induction pathway through the AHH receptor. For the first time, pilot X-ray data on P450cam were reported.

The Vienna Conference in 1988 was the largest conference in this series. Its specific focus was dynamics of structure and function, models, and calculations. The peculiarity of the Vienna Conference was the invitation of experts who discussed problems of general importance such as electron transport through protein structures, phospholipid–protein interactions, or principles of crystallization of membrane bound proteins. Based on the elucidated three-dimensional structure of P450cam, models and calculations of docking of electron-donor components were presented. And finally site-directed mutagenesis, regulation of expression, and stable expression in yeast and mammalian cells were discussed.

Because of political reasons and uncertainties, the Moscow Conference in 1991 took place mainly with participants from former socialistic countries with only few exceptions. The Moscow group presented a P450 database and thus introduced bioinformatics into the P450 conferences completed by contributions of thorough sequence analyses. Further topics were the expression of human CYPs in yeast cells, regulation of

Table 2
Conferences on biochemistry and biophysics of cytochrome P450

Year	Venue of the conference	Organizers
1976	Primosten, Yugoslavia	Sinisi Maricic
1978	Eberswalde, GDR	Klaus Ruckpaul
1980	Saltsjöbaden, Sweden	Jan-Ake Gustafsson
1982	Kuopio, Finland	Osmo Haenninen
1985	Budapest, Hungary	Laszlo Vereczkey
1988	Vienna, Austria	Inge Schuster
1991	Moscow, Russia	Alexander Archakov
1993	Libon, Portugal	Maria Celeste Lechner
1995	Zurich, Switzerland	Wolf D. Woggon
1997	San Francisco, USA	John H. Dawson
1999	Sendai, Japan	Yoshiaki Fujii-Kuriyama and Yuzuru Ishimura
2001	La Grande Motte, France	Reinhard Lange
2003	Prague, Czech Republic	Pavel Anzenbacher
Forthcoming meetings		
2005	Dallas, USA	Julian A. Peterson and Sandra E. Graham
2007	Bled, Slovenia	Katja Breskvar and Damjana Rozman
2009	Saarbruecken, Germany	Rita Bernhardt

Table 3
Conferences on cytochrome P450 and biodiversity

Year	Venue of the conference	Organizers
1991	Berlin, Germany	Hans-Georg Mueller
1993	Tokyo, Japan	Takagi, M., Okhawa, H., Yoshida, Y. and Ishimura, Y.
1995	Woods Hole, USA	Loper, John C., O'Keefe, Daniel, P. and Coolbaugh, Ronald C.
1998	Strasbourg, France	Durst, Francis, Kelly, S.L., Werck- Reichhart, D.
2000	Copenhagen, Denmark	Halkier, Barbara Ann; Möller, Birger Lindberg; Peitersen, Karina Juul
2002	Los Angeles, USA	Fulco, Armand, J.; Cho, Arthur, K.; Fukuto, Jon; Poulos, Thomas; Goodman, Doris, V. N.

gene expression, and the presentation of molecular details of P450 BM3.

The topics of the Lisbon Conference in 1993 were molecular biology (regulation of gene expression) and immunotoxicology. NO-synthase was presented as a multicomponent hemoprotein with P450 like properties.

After the fall of the Berlin wall in 1989 it was no longer necessary to organize conferences alternatively in Eastern and Western countries, enabling scientists especially from the GDR to attend international meetings. Therefore in 1993 the Scientific Advisory Board decided to change the European Conferences to International Conferences with the consequence that the next conferences were organized in San Francisco and Sendai due to the outstanding contributions of American and Japanese groups.

Since the beginning of the 1990s and especially during the Lisbon Conference, it became obvious that contributions of studies on microbial and plant P450 had increased. Therefore colleagues dealing with nonmammalian P450s decided to organize a special series of conferences titled Cytochrome P450 on Biodiversity following the research field that I.C. Gunsalus had begun with the discovery of P450cam in 1965 and which so successfully developed (see Table 3).

It would break the frame of this article to characterize all conferences in so much detail. But it may be of interest to go back to 1988 when Ron Estabrook listed some unanswered questions: factors influencing protein folding; mechanisms of electron tunneling for reduction of CYP; influence of oxygen affinity; new chemistry from new CYPs; new crystal structures; polymorphisms and microheterogeneity of structure; P450 and behavioral biology; P450 impact on gene therapy. Some of these questions have been answered, some of them are still to be answered, and more questions have arisen. And the existence of many open questions is the best sign for a still developing field which has nothing lost of its fascination.

The discovery and comprehensive characterization of P450cam, the camphor hydroxylase of the bacterium *Pseudomonas putida*, by I.C. Gunsalus have provided fundamental data for a thorough understanding of the properties of this multicomponent enzyme system. Its solubility in aqueous solutions opened facilities for extensive biophysical studies to analyze the reaction mechanism in detail. The pioneering, multidisciplinary research of I.C. Gunsalus and his collaborators on the biochemical, biophysical, and genetic parameters of cytochrome P450cam has decisively determined and broadened our knowledge about cytochrome P450. That is the reason why the P450cam system still serves today as prototype also for membrane bound mammalian P450 systems.

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