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|---|---|
| TYPE: | Article CC:CCG |
| JOURNAL TITLE: | Archives of biochemistry and biophysics |
| USER JOURNAL TITLE: | Archives of Biochemistry and Biophysics |
| ARTICLE TITLE: | Paul F. Fitzpatrick: A life of editorial duties and elucidating the mechanism of enzyme action |
| ARTICLE AUTHOR: | SOBRADO, |
| VOLUME: | 742C |
| ISSUE: | |
| MONTH: | |
| YEAR: | 2023 |
| PAGES: | 109635- |
| ISSN: | 0003-9861 |
| OCLC #: | |

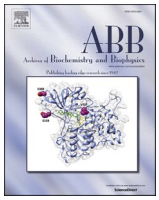
Processed by RapidX: 12/11/2023 8:41:22 AM

Archives of Biochemistry and Biophysics 742 (2023) 109635

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

journal homepage: www.elsevier.com/locate/yabbi



Review article

# Paul F. Fitzpatrick: A life of editorial duties and elucidating the mechanism of enzyme action

## 1. Training and professional path

Paul Fitzpatrick grew up in Detroit, MI, and attended the University of Detroit High School, where he became fascinated with chemistry and mathematics. After completing a Biology degree at Harvard College, Paul entered the Ph.D. program in the Department of Biological Chemistry at the University of Michigan, joining the laboratory of Vincent Massey. During his Ph.D., Paul learned how to conduct rigorous science and learned about the power of rapid-reaction and steady-state kinetics to provide mechanistic insights. It was also in Ann Arbor that Paul married Colette Daubner. Paul then moved to Penn State University for postdoctoral training in the laboratory of Dr. Joseph Villafranca, where he gained proficiency in organic synthesis and kinetic isotope effects.

In 1986, Paul and his family moved to College Station, Texas (TX), where he worked as an assistant professor in the Department of Biochemistry and Biophysics at Texas A&M University (TAMU). There, he rose to the rank of Professor before moving to the University of Texas Health Science Center-San Antonio (UTHSCSA) in 2009.

After 33 years of a very successful career, Paul retired from academia in 2019. Many former trainees, colleagues, and friends gathered in San Antonio, TX, to celebrate this important occasion with him (Fig. 1). In 2022, Paul stepped down as the Editor-in-Chief of *Archives of Biochemistry and Biophysics (ABB)*, a position he had held in for 23 years.

## 2. Our personal views of Paul as a friend, colleague, and mentor

José Luis Neira (JLN) met Paul F. Fitzpatrick and his wife for the first time in Madrid (Spain), when Colette was on sabbatical at a local college. JLN took Paul and Colette sightseeing in Segovia, and they quickly became friends. Paul's open-minded, serious character and Collette's charming friendliness beguiled JLN during their visit to the Roman Aqueduct and Segovia's fortress (the Alcazar).

Over the years, JLN learned a tremendous amount from Paul about how to handle manuscripts, how to separate the "chaff from the wheat" when reviewing a manuscript, how not to burden expert reviewers with too many manuscripts, and how to assess the efforts that all of us, as authors, do when trying to answer the questions raised by reviewers. In our editorial meetings at ABB, Paul was very strict about (i) the journal quality parameters (i.e., the value of reviewers' reports, the amount of time to get those reports back, etc.); and (ii) who to get onto the editorial board. He was also full of good ideas on how to promote the journal at conferences and congresses. In the first years of training in how to be an editor, JLN felt he was very lucky to have Paul, Helmut Sies (one of the former editors-in-chief of ABB), and Anthony Newman (the Elsevier publisher) on his side, correcting and guiding him. JLN enjoyed this because he was "seeing what went on behind the curtain" and was hanging around three very nice and interesting men. Paul was of a similar mind to JLN regarding the scientific aspects of biochemistry (i.e., kinetic rates of the binding of certain protein systems, the dimerization state of a domain of tyrosine hydroxylase) and would back JLN up. JLN learned that Paul's apparent bluntness was one of the most interesting features of his character. Paul was also an extremely dedicated teacher and scientist and could succinctly explain difficult concepts to scientists and non-scientists alike, in an amazingly clear and precise way.

Paul mentored dozens of postdocs, graduate students, and undergraduate students at TAMU and UTHSCSA. Pablo Sobrado (PS) was one of Paul's graduate students at TAMU. The high quality and rigorous publications from Paul's laboratory attracted top-notch postdoctoral scientists and students who embraced the use of multiple biochemical techniques to rigorously probe the mechanism of enzyme action. Paul had a "hands off" mentoring style, which allowed trainees to develop at their own pace; however, students were always motivated by Paul's profound questions about their research during group meetings, which inspired his students to read the scientific literature and innovate. Experiments in the early days included the isolation of enzymes from pig hearts (e.g., D-amino acid oxidase) and bovine adrenal medulla (e.g., tyrosine hydroxylase) but the laboratory quickly moved to recombinant gene expression, which facilitated structural elucidation and site-directed mutagenesis studies. The repertoire of biophysical and biochemical techniques used to probe enzyme action in Paul's laboratory included steady-state and rapid-reaction kinetics, pH studies, kinetic isotope effects, product inhibition studies, viscosity effects, structural elucidation by X-ray diffraction, and NMR, EPR, and Mossbauer studies, among others. It was expected that publications from Paul's laboratory would include many of these techniques and several equations for data analysis. The impact of Paul's mentorship can be measured by the careers of former laboratory members who implemented Paul's innovative and rigorous approaches to their independent research programs and who are now leaders in academia and industry.

## 3. The research focus of Paul's life in science

Paul dedicated his scientific life to elucidating the catalytic mechanisms of several enzymes, among them those containing a flavin prosthetic group (flavoenzymes) and the enzyme members of the non-heme iron and tetrahydrobiopterin-dependent aromatic amino acid hydroxylase (AAAH) family. Below, we will highlight some of the key discoveries from Paul's laboratory on these topics.

https://doi.org/10.1016/j.abb.2023.109635
Received 8 May 2023; Accepted 9 May 2023
Available online 18 May 2023
0003-9861/

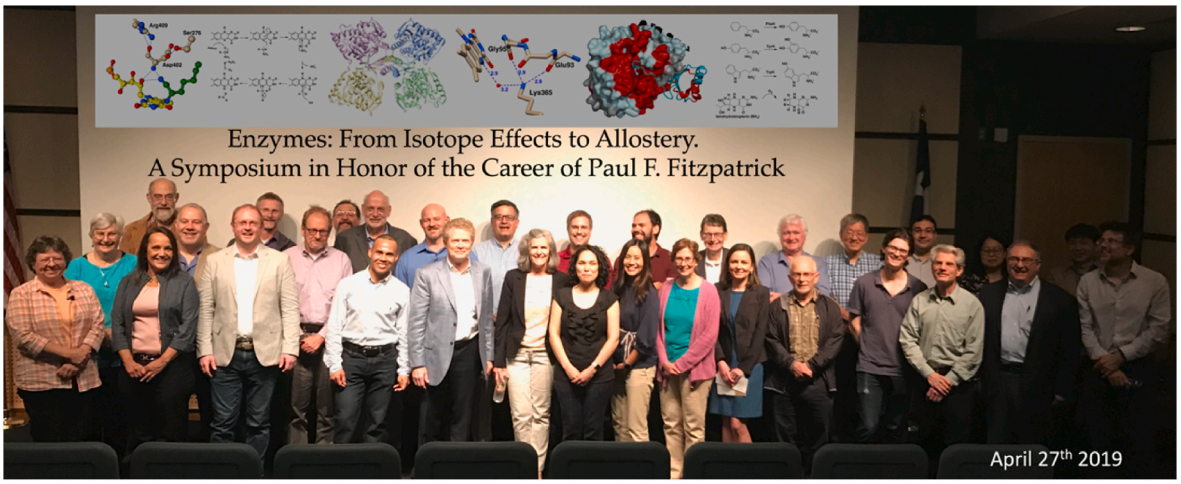



**Fig. 1.** Students, colleagues, family, and friends at the symposium in honor of Paul Fitzpatrick's scientific career.

### 3.1. Flavoenzymes

Paul's laboratory at TAMU focused on the kinetic and chemical mechanism of several flavin-dependent oxidases. The prototypic enzyme D-amino acid oxidase (DAAO) was the subject of steady-state and rapid reaction kinetic analyses using pH and kinetic isotope effects to probe the mechanism of C–H bond cleavage, which was a main focus of investigation by many other groups [1]. Two main mechanisms were proposed: direct hydride transfer or carbanion intermediate [2]. It was until Paul's implementation of heavy atom kinetic isotopes and pH effects, that it was demonstrated that in the reaction of DAAO with D-alanine, the amino group became deprotonated upon binding, and a hydride was then transferred in the rate-limiting transition state [3]. This work provided the first evidence of a hydride transfer in the family of flavin oxidases (Fig. 2A). The mechanism of DAAO was also supported by structures of the enzyme in complex with substrate that were subsequently published by other groups [4]. Paul's laboratory also expanded studies into hydroxy acid oxidases, of which the chemical mechanism was also controversial. It was proposed that the reaction occurred *via* the formation of a carbanion intermediate or by a concerted direct hydride transfer [5]. Using flavocytochrome b2 (Flb2) as a model system, the chemical mechanism was probed with primary and solvent kinetic isotope effects, viscosity, and pH studies, on both wild-type and mutant enzymes. The experiments demonstrated that the reaction catalyzed by Flb2 was more complicated than previously thought. Surprisingly, the kinetic solvent isotope and the viscosity effects showed an increase in the $k_{cat}/K_m$ value (e.g., inverse effects) and this was interpreted as originating from an equilibrium step coupled to deprotonation of the hydroxyl group, which formed an alkoxide intermediate. This reaction was followed by direct hydride transfer from the alpha carbon of lactate to the flavin N5-atom. Thus, the mechanism of Flb2 was determined not to occur *via* the formation of a carbanion or a concerted hydride (Fig. 2B) [6–8]. Similar mechanistic approaches were implemented on related flavin oxidases, where an analog mechanism has been proposed [9,10].

The studies on DAAO and Flb2 in Paul's laboratory provide an elegant example of how to use isotope effects to elucidate enzyme mechanisms. The proposed mechanisms for DAAO and Flb2 have stood the test of time and helped our understanding of the mechanisms of related enzymes. Similar biochemical and structural approaches have been applied to other flavin-dependent oxidases, such as tryptophan 2-monooxygenase/oxidase, triethylamine dehydrogenase, L-hydroxy nicotine oxidase, polyamine oxidase, and spermine oxidase. These studies established a hydride transfer mechanism and the role of active

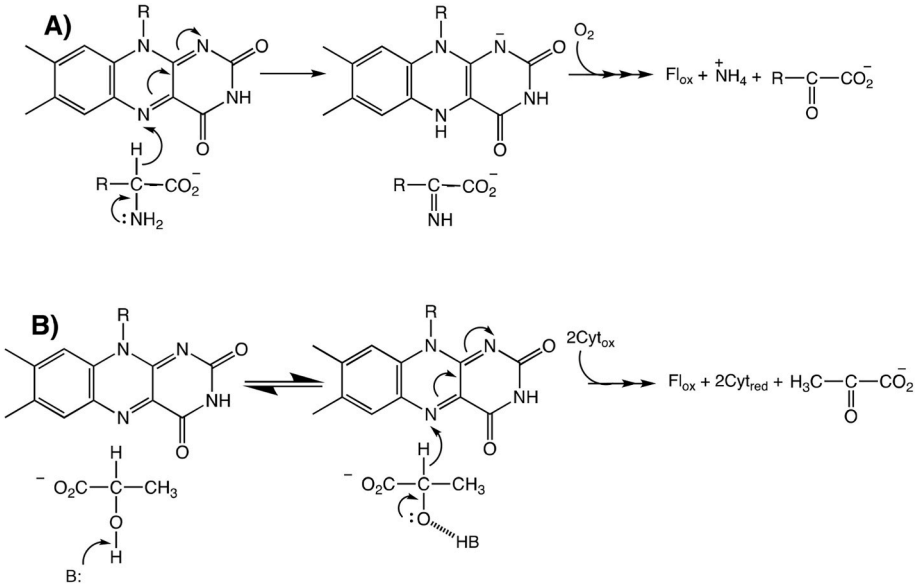



**Fig. 2.** A) Hydride transfer mechanism of amine oxidases. B) Hydride transfer mechanism of hydroxyl acid dehydrogenases/oxidases.

site residues for these enzymes [11–14].

The Fitzpatrick laboratory also worked on an enzyme that was reported to oxidize nitroalkanes. This enzyme is present in the fungi *Fusarium oxysporum* and was first isolated from this fungi in an inactivated form. After testing numerous conditions and performing absorbance and mass spectral analysis, it was determined that the protein was isolated as a nitrobutyl-FAD [15,16] and it was later demonstrated that the chemical mechanism of nitroalkane oxidase involves a transient FAD-N5 covalent adduct [17–19]. This was the first characterized flavin-dependent covalent mechanism with native substrates (Fig. 3) [20–22].

Paul's work in flavin-dependent enzymes firmly established the chemical mechanism of amine oxidases, hydroxy acid oxidases, and nitroalkane oxidases, providing elegant examples of how to use kinetic isotope effects, pH profiles, and viscosity effects, in combination with mutant enzymes as probes of enzyme mechanism.

### 3.2. AAAHs

In eukaryotic organisms, there are three AAAHs corresponding to each aromatic residue. Tyrosine hydroxylase (TH) catalyzes the hydroxylation of L-tyrosine to L-3,4-dihydroxyphenilalanine (L-DOPA), the first and rate-limiting step in catecholamine (CA), dopamine (DS), and nor-adrenaline and adrenaline syntheses. In the brain, CAs are key neuromodulators and neurotransmitters. The second member of the family is phenylalanine hydroxylase (PheH), which catalyzes the hydroxylation of excess phenylalanine to tyrosine during catabolism; loss of PheH results in the disease phenylketonuria. The third member of the family is Tryptophan hydroxylase (TPH), which hydroxylates L-tryptophan to 5-hydroxy-tryptophan and is the rate-limiting step in the biosynthesis of the neurotransmitter serotonin. From a structural point of view, mammalian AAAHs are mainly present as homo-tetramers, with a three-domain subunit structure. First, an N terminus regulatory domain (RD) formed by an N-terminal, mainly disordered, tail of varying length is followed by a well-folded ACT (aspartate kinase-chorismate mutase TyrA) domain, which is usually involved in feedback inhibition and allosteric processes in other proteins. Second, the central catalytic domain (CD) contains the active site iron and binding-sites for $BH_4$ and substrate. Finally, a C-terminal oligomerization domain (a coiled-coil helix) is responsible for protein oligomerization (OD). The CDs of the three proteins are similar: a single non-heme iron atom is bound in place by two histidines and a glutamate. Paul's team has contributed to an in-depth understanding of the catalytic processes of the three AAAHs; this mechanism in the three proteins involves the formation of a Fe(IV)O intermediate [23].

When Max Perutz was working to explain the allosteric mechanism of hemoglobin in the presence of $O_2$, he used a top-down approach: he solved the structure of myoglobin (along with Kendrew) and then the structure of hemoglobin. With those structures in hand, he then tried to explain how the conformational features of the protein changed in the presence of $O_2$ [24]. When Paul started working with AAAHs there were no structures of the intact (i.e., containing the three domains) proteins. Therefore, his team used a bottom-up approach. From the findings from low-resolution techniques, they were able to obtain key information about the mechanism of AAAHs. Many of their suggestions for the mechanism of AAAHs, firmly rooted in a plethora of different orthogonal techniques, have been confirmed by the recently published structures of: (i) full-length TH (cryo-EM) [25], (ii) full-length PheH (X-ray) [26], and (iii) 47-N-terminally truncated TPH (cryo-EM) [27]. Before his retirement, Paul's team solved the solution (NMR) structure of the isolated RD of TH, showing that it consisted of a 70-residue-long flexible N-terminal tail, followed by an ACT domain [28] (Fig. 4). That finding showed that many of the general catalysis models they had proposed over the years were correct. Since then, the efforts of Paul's team were mainly focused on TH and PheH; only in the last few years did they start focusing on elucidating the oligomerization state of the isolated RD of TPH [29]. Therefore, we will focus on the most important and valuable contributions of the first two AAAHs.

In the human RD of TH there are three serine phosphorylation sites: Ser19, Ser31, and Ser40. Activation of TH by phosphorylation is well-established [30,31] and it was found that those different sites regulate TH through binding to 14-3-3 proteins (Ser19); cellular localization to synaptic vesicles and Golgi (Ser31); and the activity and catalysis mechanisms of TH (Ser40). TH inhibition by CA is competitive and CA uptake from the TH active site occurs by incubation with $BH_4$ or phosphorylation at Ser40. The rate constants of dissociation of CA are increased by three-orders of magnitude compared to unphosphorylated TH [29]. Phosphorylation at Ser40 is performed by a cAMP-dependent protein kinase (PKA), among other kinases, and the phosphatase that catalyzes dephosphorylation is protein phosphatase 2A. The rate of phosphorylation at Ser40 is increased by phosphorylation at Ser19 [29]. Until the advent of the structure of the full-length TH, use of an array of biophysical probes allowed Paul's team to propose structural models of the function of RD and its 70-residue-long N-terminus. Binding of dopamine at the active site of the enzyme leads to rigidity of the N-terminal region of the RD of TH (as tested by proteolysis and

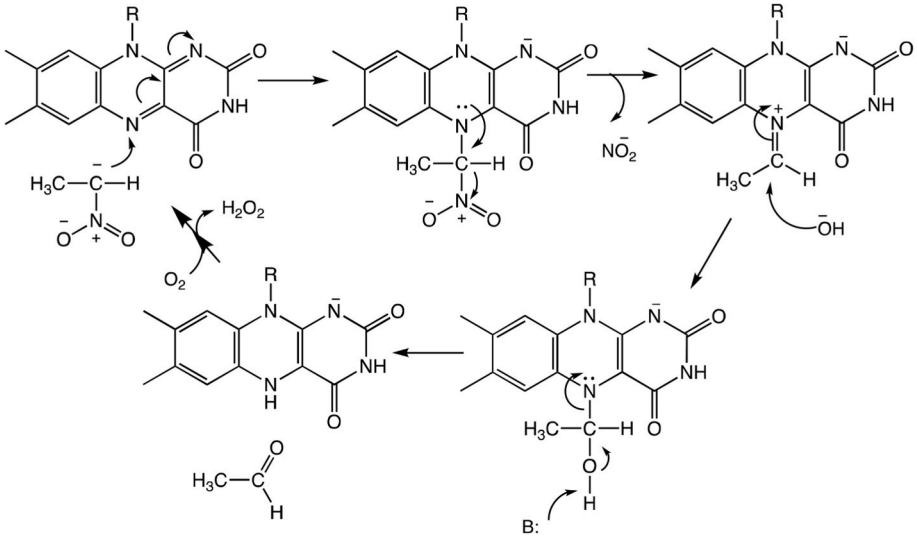



**Fig. 3.** Chemical mechanism of nitroalkane oxidase.

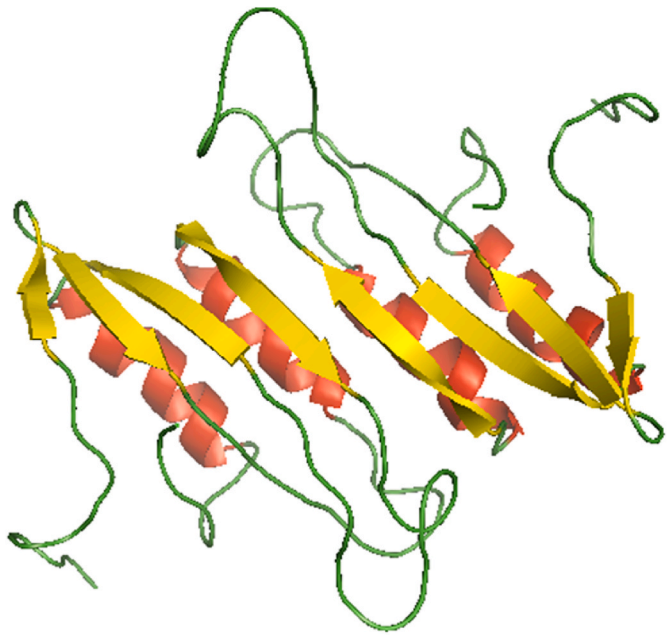


**Fig. 4.** The NMR structure of the dimeric RD of TH [28]. The elements of secondary structure are in different colors: β-strands in yellow; α-helices in red; and disordered regions in green (PDB number: 2MDA). The figure was produced with Pymol.

hydrogen/deuterium exchange mass spectrometry) [29]. Conversely, when Ser40 is phosphorylated, the N-terminal region of RD becomes more flexible, as indicated by fluorescence anisotropy measurements, and suggested by hydrogen-exchange mass spectrometry [29]. These hydrogen-exchange experiments also indicated that a region at the brink of the active site of the enzyme, in the CD, changes its solvent accessibility by the position of the RD, suggesting proximity between a polypeptide patch and the N-terminal region of RD when dopamine is present in the active site of TH. All these findings led Paul's team to suggest that there are two forms of the enzyme: an open form and a closed one. In the closed form, the 70-residue-long N-terminal region of the RD gets close to the active site. This form is stabilized by CA binding, which locks the inhibitor into the active site and shifts the conformational equilibrium of the region comprising residues 41 to 58 (in the RD) towards a well-formed, rigid α-helix. Phosphorylation breaks down the interaction between the active site and the fully-formed α-helical region of the N terminus of RD, leaving the active site open and allowing dissociation of CA (the open form). In the unphosphorylated species (resting form), the N-terminal region of RD is flexible enough to enable a fully accessible active site in which there are still some interactions; these interactions are hampered in TH phosphorylated at Ser40. The recent cryo-EM structures of the closed (phosphorylated with CA at the active site), open (phosphorylated without CA at the active site), and resting (unphosphorylated without CA at the active site) forms [25] have fully confirmed all features of the general model provided by Paul's team at the beginning of the 2010s. Thus, we can say that the work of Paul's team in TH has firmly established the basis for the knowledge of the structure and regulation of TH through feedback inhibition by dopamine and its reversal by PKA-mediated Ser40 phosphorylation, establishing the N-terminal region of the protein as a crucial player in TH modulation.

Although the work on PheH has not been as abundant as that on TH (probably because there were more PheH structures available than TH structures at the time), Paul's team has also tried to address the mechanism of PheH, finding key answers to the allosteric mechanism of the enzyme. PheH is allosterically regulated by Phe [32,33]. Since the RD of PheH contains an ACT motif, it was suggested that the allosteric site was located on the RD. Paul and his laboratory were the first to experimentally test for the presence of a Phe binding site in the RD of PheH [34]; furthermore, they showed that the binding of Phe to RD shifts the conformational equilibrium of the RD of PheH to its dimeric species, which also happens with other ACT domains. Thus, the activated forms of PheH (i.e., with Phe) are dimeric species through the RD interface [35], where the two Phe residues bind (i.e., two residues per dimer). Phosphorylation at Ser16, at the N-terminal region of the RD, reduces the amount of Phe needed to activate PheH by shifting the equilibrium between the activated (dimeric) and inactivated species (without altering the affinity for Phe of the inactivated form) [36], as shown by the fluorescence changes of a Trp close to the allosteric binding site [29]. This general scheme was confirmed when the X-ray structure of dimeric RD with two Phe residues bound was obtained [37], and more recently, with the structure of full-length PheH [26].

With this brief editorial, we wish to thank Paul for his long-standing dedication to *Archives of Biochemistry and Biophysics*, his commitment to the quality of the journal and his team, and the life-long friendship that the interactions with the editorial board have resulted in.

## Data availability

No data was used for the research described in the article.

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Handling Editor: J.P. Jin