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# Memories of a Senior Scientist

# Focusing on phosphomonohydrolases: a timely choice

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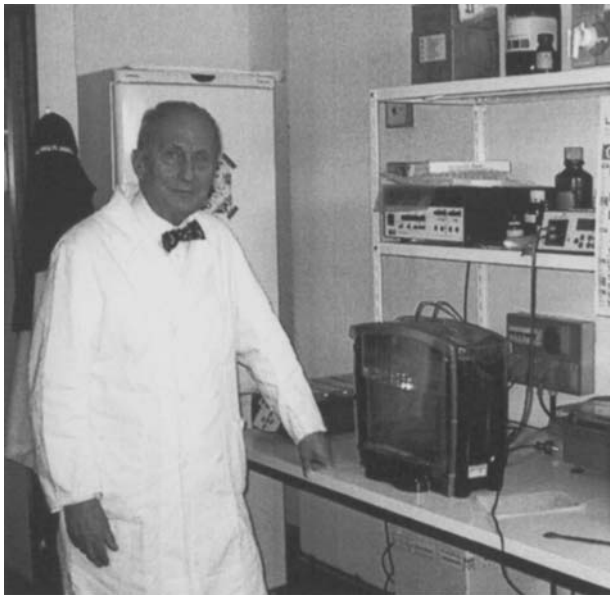

Giampietro Ramponi, (photograph taken in 2003). Biochemist and President of The Italian Society of Biochemistry and Molecular Biology. Throughout his career, he has examined the role of phosphomonohydrolases, in particular focusing on their structure/function relationship.

I was born in Pistoia, a small city in Tuscany, where I achieved maturity at the Classic Grammar School Niccolo' Forteguerri in 1949. They were hard years, years of war, and for some periods I was not able to go to school regularly, because of the bombardments. My family moved to Cutigliano, in the countryside of the Apennines of Tuscany-Emilia, where I now return to spend weekends and vacation periods.

My decision to do a Bachelor's degree in pharmacy was made for family and economic considerations, and because of my passion for chemistry and science. I chose the University of Florence and immediately became enthusiastic about organic chemistry, and the complex procedures of synthesis of the alkaloids and their pharmacological use. My passion was noticed by a professor of pharmaceutical chemistry, who invited me to attend the laboratory of organic synthesis as an internal student. I was involved in a plan of synthesis of some guanidinic molecules, which were used then as antidiabetic drugs. It was a happy period of my life where the past (the chemistry lab was housed in the former stables of the Grand Duke of Tuscany Leopoldo) and the present (alembics and spectrophotometers) cohabited among unpleasant odors and smoke, not always owing to chemical reactions, but to the carelessness of the students.

Experimental research changed my aspirations and, after obtaining my bachelors degree in pharmacy, I also graduated in Biological Sciences, but my definite decision to dedicate myself to research and in particular to biochemistry was taken after reading the book of Baldwin 'Dynamic Biochemistry'. After a short experience in general pathology, where I studied the mechanisms of action of the transaminases, the invitation of Vincenzo Baccari to take the chair of Biochemistry at the Faculty of Medicine and Surgery of the University of Florence coincided with my definite choice to work for the Biochemistry Institute. In 1958 I married Gabriella. We have two sons, Luca and Nicola. Gabriella always accompanied me thoughout my career, even to foreign countries and in spite of difficulties. My first biochemistry study was of the acetylphosphatase activity in the colleteric glands of a particular locust, called *Acrida bicolor.* We captured these insects dur-

ing happy excursions in the meadows on the hills of Florence. The acetylphosphatase activity in liver homogenates had been discovered by Lipman, but both the physiological role and molecular characteristics of the enzymes involved were completely unknown. Thus began the study of this enzyme and after approximately forty years, it still has not ended! In my research I have always tried not to change research topic, not to follow the general trends, and to remain faithful to the phosphomonohydrolases and, in some cases, to various subjects related to this enzyme class.

The first problem was to obtain a purified protein and a method of fast and reliable enzymatic assay. It was the period of the continuous-optic enzymatic methods, that I learned to appreciate in Marburg (Germany) in the laboratory of Theodor Bucher, where I had gone in order to learn the secrets of protein purification. I returned to Florence having profited from my German experience, both from a scientific and gastronomic point of view. In turns I visited the numerous and delicious pastry shops and the equally inviting beer pubs along the pleasant canals of Marburg, also known as 'Venice of the North'. My experience in organic chemistry was useful to synthetize benzoyl-phosphate, an acylphosphate for a continuous optical method. With this enzymatic test I was able to assay numerous samples and follow their kinetics.

In that period I was fascinated by histones and their post-translational modifications. For this purpose I went to Edinburgh, where the school of Cruft had published optimal papers on this argument. John Liver received me in his laboratory in the Department of Biochemistry directed by Edward Fisher. I began to purify histones from the crustacean hepatopancreas, procured from the fishermen of the North Sea coast; the large and delicious crustaceans, after having sacrificed their hepatopancreas to science , finally ended up on our table.

In 1969 during a FEBS Meeting in Madrid, I got to know Fritz Lipman, who first studied the acylphosphatasic activity in pigeon liver. On that occasion he signed a manuscript of mine about the purification of the same enzyme from horse muscle. I also met Santiago Grisolia, an excellent scientist also interested in acylphosphatases, who invited me to Kansas City as a visiting professor. Grisolia, in whose laboratory I remained for ten months, was a second teacher for me, giving me a vision of experimental research which was completely different from what I had made by myself in Italy, together with managerial and organizational ability. Upon my return to Italy, these characteristics were decisive for the creation of a large department, an unthinkable operation without the precious collaboration of Guido Camici, Gianni Cappugi and Giampaolo Manao, young graduates at that time and now colleagues of great value. In Kansas City I dedicated myself to the development of the chemotropic theory of Grisolia, who had demonstrated that some compounds are non-enzymatically bound to proteins. Together we demonstrated that both carbamoylphosphate, and 1-3-diphosphoglycerate covalently bind to histone lysines. Upon my return to Italy I further contributed to these studies by demonstrating that acyl-AMP, as well as the above mentioned acyl-phosphates, behaved similarly in histone lysine acylation. In particular, carbamoyl-phosphate or cianate formed by its spontaneous hydrolysis at neutral pH, led to homocitrulline formation from lysines as the backbone of hystone proteins. These studies led to the identification of enzymes called acylphosphatases that, through hydrolysis of acylphosphates, prevent these protein modifications. Acylphosphatase (E.C. 3. 6. 1. 7) is a small cytosolic enzyme which my group and I have extensively studied since 1960. In 1969 my group achieved the complete purification of the enzyme [1], determining successively the complete amino acid sequence (the first one in Italy) [2]. Recently, to my great satisfaction, a similar reaction, again not enzymatic, between homocysteinyl-thiolactone and the $NH_2$ or SH groups of proteins has been demonstrated [3].

My pioneering studies preceded the spread of the present success of my schooling at the University of Florence. During the last decades I have extensively investigated essentially two experimental models, each involving phosphomonohydrolases: the acylphosphatases and the low molecular weight phosphotyrosinephosphatases.

## The Acylphosphatase model

Acylphosphatase [4] is one of the smallest enzymes known, containing only 98 amino acid residues. It is present in organs and tissues of vertebrate species as two isoenzymes sharing over 55% of sequence homology; these appear highly conserved in differing species. It catalyses the hydrolysis of acylphosphates, compounds containing a carboxylphosphate bond such as 1,3-*bis*phosphoglycerate, carbamoylphosphate, succinylphosphate, acetylphosphate and $\beta$-aspartylphosphate. These molecules play very important metabolic and physiological roles as intermediates in glycolysis, the tricarboxylic acid cycle, pyrimidine and urea biosynthesis and in the activity of membrane ion pumps. Acylphosphatase activity was first described in 1946 by Lipmann and subsequently investigated by my group. My work focused essentially on three directions: molecular evolution of acylphosphatase enzymes among different organisms, determination of a catalytic mechanism, and the clarification of the in vivo physiological role of acylphosphatase.

The solution and crystal structures of different isoenzymes have been elucidated by my group over the past decades, revealing a closely packed protein with a fold similar to that shown by other phosphate-binding proteins. The three-dimensional structure of both isoen-

zymes, determined by NMR and X-ray crystallography, shows a similar conformation, a typical $\alpha/\beta$ globular fold found in other phosphate-binding proteins. The structural data, together with an extended site-directed mutagenesis investigation, led to the identification of the residues involved in enzyme catalysis. Site-directed mutagenesis experiments suggest that Arg-23 and Asn-41 are essential residues, Arg-23 being involved in the binding of the substrate phosphate moiety.

Several reports from my group indicate that acylphosphatase is involved in controlling membrane ion pumps, because it displays hydrolytic activity against the aspartyl phosphate intermediate formed during the action of membrane $Na^+$, $K^+$- and $Ca^{2+}$-ATPases. The involvement of this enzyme in cell differentiation has been demonstrated during myogenesis and erythroid differentiation involving migration to the nucleus and the transcriptional regulation in response to T3 hormone. Finally, nuclear migration has been associated with deoxyribonucleic and ribonucleic hydrolytic activity of acylphosphatase and its ability to induce apoptosis in eukaryotic cells.

Recently, my colleagues and I have demonstrated that acylphosphatase is able to form protein aggregates morphologically similar to those involved in degenerative pathologies such as Alzheimer's disease and Parkinson's disease [5]. Due to these outstanding studies, at present acylphosphatase is considered a promising model system for studies of misfolding and aggregation.

## The low molecular weight phosphotyrosine phosphatase family

Low molecular weight protein tyrosine phosphatases (LMW-PTP) [6, 7] are a group of 18-kDa enzymes that are widely expressed. The idea that LMW-PTPs, originally called acid phosphatases, might be included in the phosphotyrosine phosphatase family, originated in 1989 after the completion of peptide sequencing of LMW-PTP by my group. Despite very limited sequence similarity to the PTP superfamily, they display a conserved signature motif in the catalytic site, containing the PTP CXXXXXR 'logo'.

The striking effort of my group on LMW-PTP focused on the determination of the catalytic mechanism and the in vivo biological function of the enzyme. The discovery of a key catalytic role of Cys12, Cys17, Arg18 and Asp-129 as essential residues in the catalytic mechanism of LMW-PTP, entirely thanks to my group, permits the merging of the LMW-PTP family in the large superfamily of PTPs and opens the door to elucidation of the crystal structure of LMW-PTP. [Su X. D., Taddei N., Stefani M., Ramponi G., Norlund P. (1994) Nature **370:** 575–578]. Afterwards, we investigated the in vivo function of LMW-PTP. The first studies demonstrated a well-defined role of this enzyme in PDGF-induced mitogenesis, showing that activated PDGF receptor is a substrate for LMW-PTP. The most relevant phenotypic effect of LMW-PTP overexpression in in vitro cell cultures is a strong reduction in cell growth rate in response to PDGF stimulation. Deeper investigations, prevalently performed by my group, showed that LMW-PTP associates and dephosphorylates many growth factor receptors, such as platelet-derived growth factor receptor (PDGF-r), insulin receptor and ephrin receptor, thus down-regulating many of the tyrosine kinase receptor functions that lead to cell division. In particular, LMW-PTP acts on both growth-factor-induced mitosis, through dephosphorylation of activated PDGF-r, and on cytoskeleton rearrangement, through dephosphorylation of p190RhoGAP and the consequent regulation of the small GTPase Rho. LMW-PTP activity is modulated by tyrosine phosphorylation on two specific residues, each of them with specific characteristics. LMW-PTP activity on specific substrates depends also on its localization, LMW-PTP being restricted predominantly to caveolae and dephosphorylating caveolin. Moreover, LMW-PTP is reversibly oxidized during growth factor signalling, leading to inhibition of its enzymatic activity. Recovery of phosphatase activity depends on the availability of reduced glutathione and involves the reduction of an S-S bridge between the two catalytic site cysteines. Furthermore, studies on the redox state of LMW-PTP in contact-inhibited cells and in mature myoblasts suggest that LMW-PTP is a general and versatile modulator of growth inhibition. Finally, my current studies have recognized LMW-PTP as a positive regulator of tumor growth in vivo, linking the biochemical and biological actions of LMW-PTP to ephrin A2 receptor dephosphorylation. These last findings have striking significance as they definitely dislodge LMW-PTP from the negative PTP family and propose this enzyme as a positive regulator [8].

I hope I am still far from a final report of my working activity: my greatest satisfaction is to have so many ideas to be investigated, to always be fascinated by experimental data, and to be in continuous and daily contact with the PhD students who carry out the experiments.

1 Ramponi G., Guerritore A., Treves C., Nassi P. and Baccari V. (1969) Arch. Biochem. Biophys. **130:** 362–369
2 Cappugi G., Manao G., Camici G. and Ramponi G. (1980) J. Biol. Chem. **255:** 6868–6874
3 Olszewski A. J. and McCully K. S. (1993) Free Radic. Biol. Med. **14:** 683–693
4 Stefani M., Taddei N. and Ramponi G. (1997) Cell Mol. Life Sci. **53:** 141–151
5 Chiti F., Webster P., Taddei N., Clark A., Stefani M., Ramponi G. and Dobson C. M. (1999) Proc. Natl. Acad. Sci. USA **96:** 3590–3594
6 Raugei G., Ramponi G. and Chiarugi P. (2002) Cell Mol. Life Sci. **59:** 941–949
7 Chiarugi P. (2001) IUBMB. Life **52:** 55–59
8 Chiarugi P., Taddei M. L., Schiavone N., Papucci L., Giannoni E., Fiaschi T., Capaccioli S., Raugei G. and Ramponi G. (2004) Oncogene **23**(22)**:** 3905–3914