

The function of the oxide anion in the bioenergetics of pyrophosphate

Manuel Losada

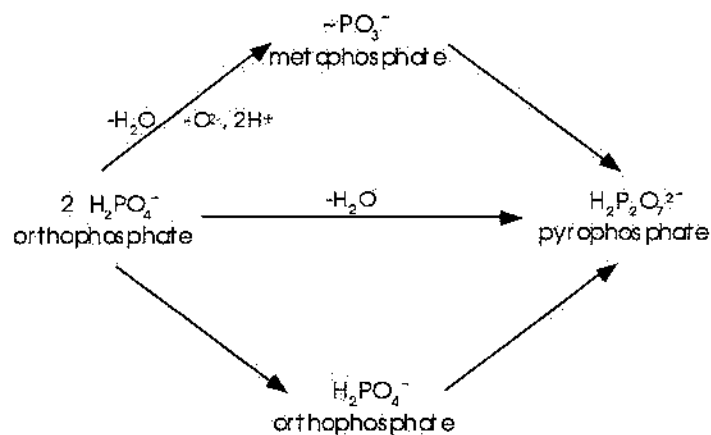
Instituto de Bioquímica Vegetal y Fotosíntesis, Universidad de Sevilla y CSIC, Sevilla, Spain

In a classical memoir entitled *General considerations on the nature of acids and on the principles composing them*, Lavoisier suggested for the “vital air”, or “dephlogisticated air” of Priestley, the name “principe oxygine”, or “acidifying principle”, which he later changed in his famous *Traité élémentaire de Chimie* (1789) to *oxygen*, from the Greek words meaning “to form an acid”, the “acid-maker”. Thus, Lavoisier, the brilliant founder of modern chemistry, who overthrew the phlogiston theory and understood clearly the role of oxygen in the processes of calcination, combustion and respiration, established the foundation of this new science on the erroneous belief that the gas he named oxygen was the “acid part” of air and that it entered the composition of all acids. Actually, he showed himself perplexed because no acid resulted from the combustion of *hydrogen*, the “water-maker”. The demonstration that water —*hydrogen oxide*, according to the new nomenclature— is composed of hydrogen and oxygen was certainly one of the earliest and greatest successes of the newly-born chemistry. Lavoisier was also the first who wrote the forerunner of a modern chemical equation:

$$\text{must of grapes} = \text{carbonic acid} + \text{alcohol}$$

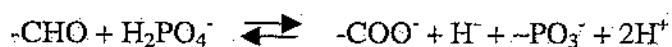
Modern biochemistry and enzymology were born one century after, when the German chemist Buchner achieved in 1897 the cell-free fermentation of sugar by extracts of yeast. The British biochemist Harden continued the work of Buchner on alcoholic fermentation and discovered not only that coenzymes (“cozymase”) were necessary for the successful action of the thermolabile enzymes (“zymase”), but that the gradual loss of

activity with time of the yeast enzymes could be reversed by addition of phosphate and that sugar phosphates were formed during fermentation as intermediates. One of the greatest achievements in modern biology was the elucidation by Warburg in 1937-38 of how *phosphorylation*, i.e., the generation of an energy-rich pyrophosphate bond, is eventually coupled during glycolysis to the dehydrogenation of glyceraldehyde-3-phosphate through the intermediate formation of its corresponding acyl-phosphate. The existence of three “acids hydrates” of phosphoric acid —“meta”, “pyro” and “ortho”— had already being proved in 1833 by the Scottish chemist Graham. According to Kornberg, it was Ochoa who in 1940 made the striking observation that inorganic pyrophosphate, a previously unknown constituent of cellular material, is a product of cell metabolism. Inorganic pyrophosphate can be also chemically obtained by fusing two orthophosphate molecules through dehydration in an oven at 400 °C, the chemical energy trapped in inorganic pyrophosphate being comparable to that trapped in ATP from the fusion, also through dehydration, of ADP with orthophosphate. The following scheme shows how *pyrophosphate* can originate from *orthophosphate*, either directly or indirectly, via *metaphosphate*, as it seems to be the case in bioenergetics:

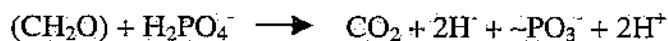


Most scientists agree today that life on earth arose from less-complex, non-living chemical systems, at a time when the atmosphere was reducing in character, not oxidizing. Photosynthesis and respiration, involving molecular oxygen evolution and consumption, respectively, developed later. Most probably, some protocells evolved glycolysis, when no

free oxygen was yet present in the atmosphere, as a means of degrading by transhydrogenation organic molecules and synthesizing metaphosphate ($\sim\text{PO}_3^-$), the energy-rich derivative of orthophosphate ($\text{H}_2\text{PO}_4^{3-}$), which results from its dehydration ($-\text{H}_2\text{O}$) by transfer of an oxide anion (O^{2-}) to the pertinent acylium cation and dissociation to the medium of its two protons (2H^+):

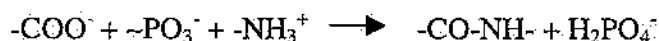


The unstable metaphosphate group preserves its energy content by keeping itself bound either to the corresponding carboxylate group (acyl-phosphate) or to another orthophosphate group (pyrophosphate) through an oxygen bridge. This pattern of anaerobic metabolism became so advantageous that only those organisms that possessed it survived to the present. What is important to underline in this mechanism of glycolytic phosphorylation is that oxygen in its reduced state of oxide anion plays a fundamental acid-base (not redox) role in the process, as well as in other key-steps of energy metabolism (see below). In fermentation, as well as in respiration, CO_2 is the product of carbon dissimilation during the operation of glycolysis and the Krebs cycle, typical anaerobic processes by themselves which do involve dehydrogenation but not free molecular oxygen. Paradoxically, one of the two oxygen atoms of CO_2 resulting from the dehydrogenation of sugar comes mainly from an oxide anion of orthophosphate, which becomes concomitantly energized to metaphosphate:



As a matter of fact, the enzymatic transfer of an oxide anion seems to be crucial for energy-transduction in the acid-base processes of phosphorylation, both at the substrate level just discussed and at the membrane level (photophosphorylation and oxidative phosphorylation). Fermentation, photosynthesis and respiration are energy-conversion processes in which chemical energy or light energy is sequentially transduced into redox energy, acid-base energy and eventually into metaphosphate energy. Most of the

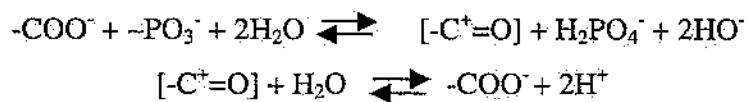
metaphosphate formed is continuously used as an energy carrier for endergonic processes, such as the synthesis of the amide or peptide bond (-CO-NH-) by removal of an oxide anion from a basic carboxylate group (-COO⁻) and two protons from an acidic amino group (-NH₃⁺), with metaphosphate deenergizing itself back to orthophosphate by its concomitant hydration:



The great founder of biochemistry Meyerhof stated that phosphorylation is necessary for the biological degradation of carbohydrates in all cells. In a review article he strengthened that “non-phosphorylating glycolysis is inexistent, except in the imagination of a few persons”. However, we know now that, in addition to the well-known phosphorylating NAD(P)-dependent glyceraldehyde-3-phosphate dehydrogenases, there exists an enigmatic non-phosphorylating NADP-dependent glyceraldehyde-3-phosphate dehydrogenase which, without the requirement of inorganic phosphate, catalyzes the direct anaerobic oxidation of 3-phosphoglyceraldehyde to 3-phosphoglycerate, water being in this case both the source of the oxide anion, which deenergizes the corresponding acylium cation to its carboxylate anion, and of the two protons, which are released to the medium:



Using a reconstituted system with these phosphorylating and non-phosphorylating enzymes, our group has been able to demonstrate that the energy of the pyrophosphate bond can be used for the endergonic ionization of water, which leads to the generation of a proton gradient:



Membrane-bound pyrophosphatases might also reversibly act through a similar mechanism, involving likewise the participation of the acylium cation-carboxylate anion acid-base pair from some crucial aminoacids of the protein chain, like aspartate.

The great American physical chemist Lewis, who in 1923 formulated his general theory of acids and bases by stating that an acid is a substance which can accept an electron pair and a base one which can donate a pair of electrons, made also a far-reaching prediction that I think is completely justified and relevant at the present time: "The cult of the proton has hindered the understanding and extension of the acid concept". No wonder, therefore, that the definition of the German chemist Lux of an acid as a substance, (e.g., CO_2 , $\sim\text{PO}_3$) that tends to gain an oxide anion (O^{2-}), and of a base as a substance (e.g., CO_3^{2-} , PO_4^{3-}) that tends to lose an oxide anion, has remained ignored by biochemists in spite of its fundamental significance in bioenergetics, both at the soluble and at the membrane levels. It should be firmly stressed that, besides the well-known strong oxidizing character of molecular oxygen (O_2), this element is characterized by the strong basic character of its reduced form, the oxide anion (O^{2-}). These two intimately linked properties (redox and acid-base) are the most significant and genuine qualities of oxygen in bioenergetics.

Water, constituted by one oxide anion and two protons, is the primary redox and acid-base substrate in energy metabolism, since it can either act as an amphoteric, an amphiprotic or an amphioxidic compound, that is to say, it can both donate and accept either electrons, protons or oxide anions in endergonic redox or acid-base reactions and, in this way, undergo autoelectrolysis, autoprotolysis or autooxidolysis, respectively. *Phosphate* is the second most important acid-base substrate in energy metabolism, since in its unenergized form of orthophosphate it is an amphoteric compound that can both donate an oxide anion and two protons, and in its energized form of metaphosphate it can act in the opposite direction and accept both an oxide anion and two protons, closing thus its energetic cycle. According to Boyer, phosphate participates in more chemical reactions than any other compound on the Earth's-surface except water. *Carboxylate anions*, and

their acylium cations counterparts, may well be the third most relevant acid-base groups in bioenergetics.

Most biochemists identify the chemiosmotic theory of Mitchell with protons. However, in what was probably his last written sentence on the phosphorylation mechanism in bioenergetics he proclaimed: "The chemistry of phosphorous-oxygen bond formation and cleavage in the reversible phosphorylation of ADP must involve the reversible withdrawal of an oxide group from inorganic phosphate". Time will say whether carboxylate groups and the transfer of oxide anion are critical for the reversible phosphorylation of orthophosphate and ADP at the membrane level, as it has been shown to be the case at the substrate level.

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