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Classics

A PAPER IN A SERIES REPRINTED TO CELEBRATE THE CENTENARY OF THE JBC IN 2005

JBC Centennial
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100 Years of Biochemistry and Molecular Biology

The Discovery of Ferredoxin and Its Role in Photosynthesis: the Work of Anthony San Pietro

Photosynthetic Pyridine Nucleotide Reductase. I. Partial Purification and Properties of the Enzyme from Spinach
(San Pietro, A., and Lang, H. M. (1958) *J. Biol. Chem.* 231, 211–229)

The light reactions of photosynthesis occur in the thylakoids and depend on the interplay of two photosystems. Photosystem I, excited by light of wavelength shorter than 700 nm, generates NADPH via the formation of reduced ferredoxin. Photosystem II, requiring light of wavelength shorter than 680 nm, produces a strong oxidant that leads to the formation of O_2. The resulting accumulation of protons in the thylakoid lumen causes the formation of a transmembrane pH gradient, which is then used to drive the synthesis of ATP.

These light reactions were first recognized in 1939 when Robert Hill demonstrated that isolated chloroplasts could simultaneously produce oxygen and reduce a high potential electron acceptor in the presence of light. Over the next 20 years a variety of substances were found to function as oxidants for the Hill reaction. The ability of chloroplasts to reduce pyridine nucleotides was first demonstrated in 1951, when three laboratories independently reported that illuminated chloroplasts were capable of generating oxygen and reducing NADP (1–3). This was the beginning of enzymatic research on NADP photoreduction in chloroplasts.

Within the next 7 years, three protein factors were isolated: methemoglobin-reducing factor, which is needed for utilizing methemoglobin as a Hill oxidant (4); triphosphopyridine nucleotide-reducing factor, which is a functional catalyst for photosynthetic phosphorylation (5); and photosynthetic pyridine nucleotide reductase (PPNR), which is catalytically active for pyridine nucleotide reduction in illuminated chloroplasts (6). The *Journal of Biological Chemistry* (JBC) Classic reprinted here reports on the isolation of the last protein factor, PPNR.

Anthony San Pietro, a young faculty member at Johns Hopkins University, and his research assistant, Helga M. Lang, had previously shown that when NADP was incubated with chloroplast grana, NADPH accumulated in the reaction mixture, provided that high concentrations of grana and NADP were used (6). Preliminary experiments also showed that reduction of pyridine nucleotides could occur at low grana concentrations if a soluble extract from chloroplasts was added. This Classic describes San Pietro and Lang's isolation of the enzyme from chloroplast extracts that was needed for the photochemical reduction of NADP.

Using chloroplasts from whole spinach leaves, San Pietro and Lang extracted the enzyme and precipitated it. They confirmed that the enzyme did indeed catalyze the transfer of hydrogen to pyridine nucleotides and determined some of its properties. Based on these properties and the role of the enzyme in photosynthesis, San Pietro and Lang suggested it be named "photosynthetic pyridine nucleotide reductase."

Subsequent studies showed that methemoglobin-reducing factor, triphosphopyridine nucleotide-reducing factor, and PPNR were the same chemical compound, and they were collectively referred to as PPNR. In 1962, Tagawa and Arnon compared PPNR and a new electron carrier called "ferredoxin," which was discovered in the hydrogenase system of *Clostridium pasteurianum*. They revealed that while one enzyme was involved in bacterial hydrogen generation and the other in higher plant NADP photoreduction, they were functionally similar and were interchangeable non-heme-iron proteins (7). Thus, PPNR became known as ferredoxin.

San Pietro continued his work on photosynthesis and later isolated transhydrogenase, the enzyme that transfers hydrogen from NADPH, formed by PPNR, to NAD (8). This was the basis of his Transhydrogenase Theory, which states that illuminated grana reduce NADP in the presence of PPNR and transhydrogenase then reduces NAD using reduced NADP.

In 1962, San Pietro joined the Charles F. Kettering Research Laboratory in Yellow Springs, Ohio. While at the Kettering lab, he extended his research to include chromatophores of photosynthetic bacteria. During his 6 years at the Kettering, San Pietro and his group published some 40 papers, and he organized three Symposia that attracted scientists from around the world who were working on photosynthesis and the synthesis of proteins during cellular differentiation. San Pietro moved to the University of Indiana at Bloomington in 1968 to chair the Department of Plant Sciences. While serving as Chairman, he maintained an active laboratory for investigating the mechanisms of the light-dependent reactions of photosynthesis and published over 160 papers. Currently San Pietro is an Emeritus Distinguished Professor of Biochemistry at Indiana University. He edited several of the photosynthetic volumes of *Methods in Enzymology* and also served on the Editorial Board of the JBC. He was honored for his outstanding scientific achievements by election to the National Academy of Sciences in 1983.[1]

Nicole Kresge, Robert D. Simoni, and Robert L. Hill

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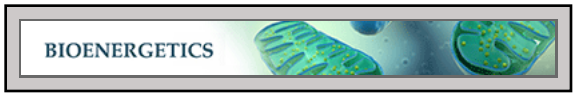
[1] All biographical information on Anthony San Pietro was taken from Refs. 9 and 10.

Classics:

The Discovery of Ferredoxin and Its Role in Photosynthesis: the Work of Anthony San Pietro



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