This Week's Citation Classic®

Cockburn W, Walker D A & Baldry C W. The isolation of spinach chloroplasts in pyrophosphate media. *Plant Physiol.* **43**:1415-18, 1968.
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The paper describes a simple method for using inorganic pyrophosphate as buffer in the isolation of intact spinach chloroplasts capable of rates of photosynthesis comparable to those of the whole leaf. [The *SCI*® indicates that this paper has been cited in over 140 publications since 1968.]

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In 1967, when I had the good fortune to be working on photosynthesis in isolated chloroplasts with David Walker and the late Carl Baldry at Imperial College, London, there was a contention that it was pointless to do experiments with isolated chloroplasts unless *in vitro* and *in vivo* rates of photosynthesis were comparable. In the "rate-race" that ensued, a very significant improvement resulted from the replacement of orthophosphate, TRIS, and Tricine buffers by the zwitterionic buffers introduced by N.E. Good.[1,2] However, the new buffers were expensive, and cost was not an insignificant consideration since we were isolating chloroplasts on a grand scale. Such was our use of spinach that I became known at Covent Garden Vegetable Market as a specialist who, regardless of the discount offered, would not take mushrooms or carrots instead of spinach.

Largely on the grounds of economy, we searched for an alternative to the Good buffers and ultimately came up with inorganic pyrophosphate (PP_i), which was very cheap and very effective indeed. So straightforward was the PP_i isolation method that we decided that the art of isolating intact photosynthetically competent chloroplasts, at that time the exclusive preserve of a handful of laboratories, was ready for general exploitation, and we published the work in that spirit. The number of citations attracted perhaps indicates that we achieved some success in our aim of widening the use of isolated chloroplasts as an experimental tool. Another component of the popularity of this paper lies in the explanation of the efficacy of PP_i in chloroplast isolation. The suggestion that it might strengthen chloroplast envelopes in the same way as it apparently strengthens sausage skins received little support! In fact, the success of PP_i turned out to be linked to the chloroplast-envelope phosphate-translocator mechanism then being elucidated.[3] It was shown that inorganic phosphate (P_i), through the phosphate translocator, plays a crucial role in the intracellular distribution of assimilates. A high external P_i level favours export that, if extreme, leads to inhibition of photosynthesis through depletion of chloroplast intermediates, whilst a low external level favours retention of assimilate within the plastid.

The concept of the phosphate translocator immediately explained why chloroplasts isolated in P_i buffers required supplementation with intermediates of the carbon reduction cycle and also why the P_i levels required by chloroplasts isolated in the Good buffers were so critical. Still unexplained, however, was the finding that PP_i even at very high levels allowed maximal rates of photosynthesis, and furthermore, that PP_i could prevent inhibition by high P_i. Eventually it was shown that PP_i participated in a kind of "P_i-stat" that by chance maintains a more or less optimal P_i level inside the chloroplast.[4] This rather complex involvement of PP_i took a good deal of time and effort to elucidate, and since the paper on PP_i as isolation buffer was on the fringes of this interesting and important work on the relationship of the chloroplast to the rest of the photosynthetic cell, this too has contributed to its popularity.

1. **Good N E, Winget C D, Winter W, Conolly T N, Izawa S & Singh R M M.** Hydrogen ion buffers for biological research. *Biochemistry—USA* 5:467-77, 1966. [See also: **Good N E.** Citation Classic. *Current Contents/Life Sciences* **26**(40):23, 3 October 1983.]
2. **Jensen R G & Bassham J A.** Photosynthesis by isolated chloroplasts. *Proc. Nat. Acad. Sci. US* **56**:1095-101, 1966. [See also: **Jensen R G.** Citation Classic. *Current Contents/Life Sciences* **26**(34):24, 22 August 1983.]
3. **Werdan K & Heldt H W.** The phosphate translocator of spinach chloroplasts. (Forti G, Avron M & Melandri A, eds.) *Photosynthesis, two centuries after its discovery by Joseph Priestley: proceedings of the 2nd International Congress on Photosynthesis Research.* The Hague: Junk, 1972. Vol. 2. p. 1337-44.
4. **Edwards G & Walker D A.** *C3, C4: mechanisms, and cellular and environmental regulation, of photosynthesis.* Oxford: Blackwell Scientific, 1983. p. 221-5.

