Pyruvate uptake induced by a pH jump in mesophyll chloroplasts of maize and sorghum, NADP-malic enzyme type C_4 species

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Received 16 May 1990; revised version received 21 June 1990

A sudden pH decrease (pH jump) of the medium enhanced pyruvate uptake in the dark in mesophyll chloroplasts (MCp) of *Zea mays* and *Sorghum bicolor*, NADP-malic enzyme type C_4 plants, while it was reported that a Na^+ jump enhanced pyruvate uptake in MCp of *P. miliaceum*, a NAD-malic enzyme type [(1987) FEBS Lett. 219, 347]. The enhancement effect of the pH jump decayed completely in 5 min and the decay was accelerated by proton gradient-collapsing reagents. The results suggest that active pyruvate uptake into MCp of NADP-malic enzyme type C_4 species is primarily driven by the proton gradient across the envelope.

Pyruvate transport; Mesophyll chloroplast; C_4 photosynthesis; pH jump; Proton gradient; *Zea mays*

1. INTRODUCTION

In C_4 plants, pyruvate must be transported into MCp to regenerate PEP, the substrate of the initial carboxylation reaction of the C_4 pathway. Pyruvate transport into isolated MCp of C_4 plants was first demonstrated in the dark by Huber and Edwards [1]. Further studies have shown that isolated MCp of maize (a NADP-malic enzyme type) [2] and *Panicum miliaceum* (a NAD-malic enzyme type) [3] accumulate pyruvate actively in the light. In MCp of *P. miliaceum*, a Na^+ jump of the medium induced pyruvate uptake in the dark, mimicking the light effect [4]. In a further study using ^{22}Na, Na^+ and pyruvate when added together enhanced the uptake of each other in the dark, and concurrent uptake of Na^+ and pyruvate was seen in the light in MCp of *P. miliaceum* and two species from the PEP-carboxykinase type C_4 subgroup, *Panicum maximum* and *Chloris gayana* (Ohnishi, Flügge, Heldt and Kanai, submitted). However, such mutual effects of Na^+ and pyruvate were not found in maize or sorghum, NADP-malic enzyme type C_4 species in that study. We present here evidence that an artificial H^+ gradient across the envelope (pH jump) drives pyruvate uptake in the dark in MCp of these NADP-malic enzyme type species. Thus, light-induced pyruvate transport into C_4 MCp could be driven by either a Na^+ or a H^+ gradient across the envelope, depending on C_4 species.

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Abbreviations: CCCP, carbonylcyanide-*m*-chlorophenylhydrazone; MCp, mesophyll chloroplasts; PEP, phosphoenolpyruvate; $[Pyr]_{in}$, stromal concentration of pyruvate

2. MATERIALS AND METHODS

Mesophyll protoplasts were isolated enzymatically from young leaves of *Zea mays, Sorghum bicolor* and *Panicum miliaceum* [3,5]. Intact MCp obtained therefrom [3] were suspended in 0.35 M sorbitol, 5 mM EDTA and 25 mM Hepes-KOH (pH 7.8), unless otherwise indicated. When analyzed by flame photometry, the suspending medium contained about 100 μM Na^+ originating from impurities. Thus, the supernatant of chloroplast suspension contained about 150 μM Na^+ due to the carry-over by the chloroplasts.

[^{14}C]Pyruvate uptake into the 'sorbitol-impermeable space' of MCp was measured by silicone oil filtering centrifugation method [6] at 4°C using two different systems. One is the ordinary single-layer system [3,6] and the incubation was started by the addition of [^{14}C]pyruvate at 0.2 mM (0.2 μCi/tube) and a tracer amount of [^{3}H]sucrose (0.5 μCi/tube) to 200 μl of chloroplast suspension. The other is a modified double-layer system as originally developed by Howitz and McCarty [7]; the tube contained from the bottom, 20 μl 1 M $HClO_4$, 70 μl of normal silicone oil ($\varrho = 1.04$), 100 μl of uptake layer of suspending medium (containing radioisotopes and half of its sorbitol being replaced by sucrose), 30 μl of low density ($\varrho = 0.96$) silicone oil and 100 μl of chloroplast suspension. On centrifugation, the low density silicone oil layer floated to the top and chloroplasts passed through the uptake layer in very short time within 2 s [7,8]. Light incubation was done under an incandescent lamp at 100 μE m^{-2} s^{-1} (sufficient for light saturation, see [9]) and dark incubation under a dim green fluorescent light [3]. pH (or Na^+) jump in the single-layer system was accomplished by adding a small volume of 1 M HCl (or 200 mM or 1 M NaCl) to the chloroplast suspension, and that in the double-layer system (Fig. 2) by using low pH media as the uptake layer. The amount of [^{14}C]pyruvate in the chloroplast pellet after centrifugation was corrected in each tube for the contribution of the 'sorbitol-permeable space' (in this case measured as sucrose-permeable space) using the count of [^{3}H]sucrose in the pellet [6].

Chlorophyll was determined according to [10].

[1-^{14}C]pyruvate; ^3H_2O, [U-^{14}C]sorbitol, and [^{3}H]sucrose (for the measurements of 'sorbitol-permeable' and '-impermeable spaces' [6] were all obtained from Amersham Japan.

3. RESULTS AND DISCUSSION

Since a Na^+ jump had no effect on pyruvate uptake in MCp of maize and sorghum, we tried a pH jump in

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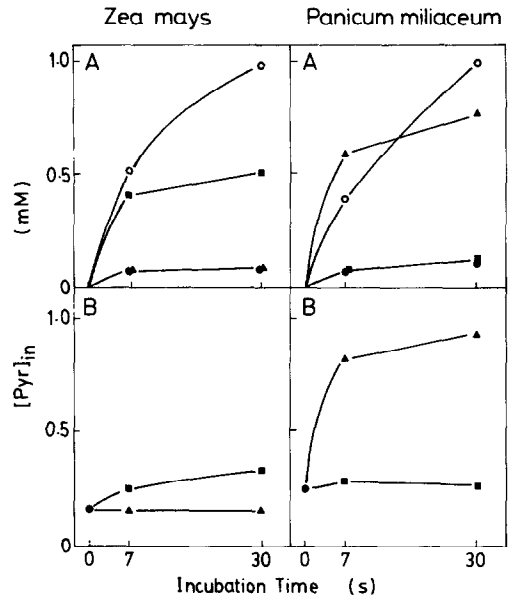

Fig. 1. Time course of [1-^{14}C]pyruvate uptake in MCp of maize (left) and *P. miliaceum* (right). (A) Pyruvate uptake in the light (○) and dark (●), and the effect of a Na^+ jump (2 mM, ▲) and a pH jump (7.8 to 6.8, ■) on the dark uptake. (B) Effects of Na^+ and pH jumps in the dark on the distribution of pyruvate in MCp preincubated with pyruvate for 10 min.

these NADP-malic enzyme type C_4 species. Fig. 1 shows the time courses of pyruvate uptake in MCp of maize (left) and *P. miliaceum* (right, species of positive Na^+ effect, as a control) in the light or darkness. The top panels (A) show the uptake of pyruvate added at zero time with or without a pH or a Na^+ jump and the bottom panels (B) $[Pyr]_{in}$ change on a pH or a Na^+ jump in MCp preincubated with pyruvate in the dark. MCp of the two C_4 species responded quite differently to Na^+ (+2 mM) and pH ($\Delta pH = -1$) jumps in both A and B. A pH jump, but not a Na^+ jump, drove pyruvate uptake in MCp of maize, while a Na^+ jump, but not a pH jump, did in MCp of *P. miliaceum*. Essentially the

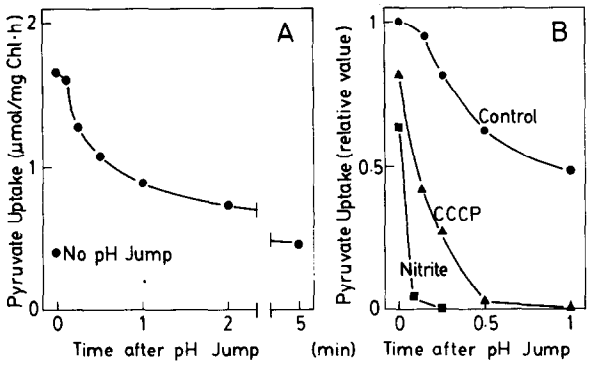

Fig. 3. Decay of the capacity of dark pyruvate uptake in MCp of maize after a pH jump. (A) HCl was added at zero time to make a pH jump (7.8 to 6.8) and at indicated times 7-s pyruvate uptake was determined. (B) MCp were preincubated with no addition (●), 10 μM CCCP (▲) or 4 mM Na-nitrite (■) and pyruvaate uptake was determined at indicated times after a pH jump. Pyruvate uptake was expressed as a relative value; controls with a pH jump (at 0 s) and without a pH jump were taken as 1 and 0, respectively.

same result as in maize was obtained also in sorghum (data not shown). Fig. 2 shows pyruvate uptake in MCp of maize induced by pH jumps of various ΔpH. The pH jumps larger than 0.4 were effective for pyruvate uptake and the enhancement increased with increasing ΔpH. The pH jump-induced uptake exceeded the light-induced uptake at ΔpH of 0.8. A large part of pH or Na^+ jump-induced pyruvate uptake was complete within the shortest incubation time (7 s), while light uptake continued further (Fig. 1). The decay of the pH jump-induced capacity for pyruvate uptake in maize MCp was rapid and completed within 5 min (Fig. 3A). The decay was accelerated by pH gradient collapsing reagents, protonophore CCCP and the weak acid Na-nitrite [11], the latter being more effective (Fig. 3B). (As noted below, the inclusion of 4 mM Na^+ in the medium had no effect on pyruvate uptake.) These results suggest that an artificial H^+ gradient across the envelope in the dark drives pyruvate uptake into MCp of maize and

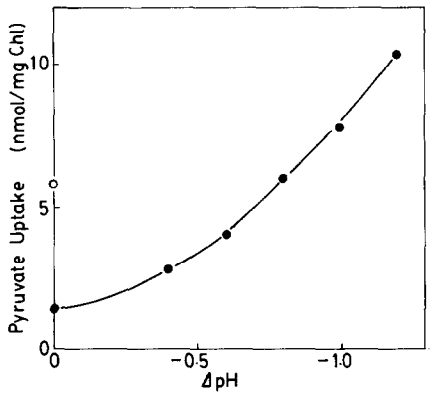

Fig. 2. Dependency of dark pyruvate uptake in MCp of maize on the extent of pH jump (ΔpH). The double-layer system was adopted using the uptake layer of various pH containing Hepes (>pH 7.0) or Mes (<pH 7.0) and chloroplasts suspended in a pH 8.1 medium. ○, light control without a pH jump.

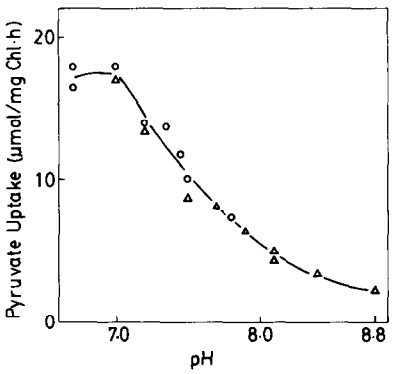

Fig. 4. pH dependency of pyruvate uptake in the light in MCp of maize. MCp were initially suspended in pH 7.3 (○) or 7.9 (△), then mixed with an equal volume of media of various pH's (containing Mes, Hepes or Tricine) and preincubated in the light for over 10 min at final pH values as indicated.

sorghum, while a Na^+ gradient does in MCp of *P. miliaceum*.

Light-induced pyruvate uptake into MCp of maize at various steady state pH of the medium was shown in Fig. 4. As the pH of the medium became lower from 8.8 to 7.0, the rate of pyruvate uptake became higher, and possibly gave an optimum at pH lower than 7. This is in sharp contrast with the results in *P. miliaceum*, which showed an optimum at pH 7.8, and the activity falling down steeply at lower pH (Fig. 4 of [3]). Furthermore, preequilibration of maize MCp with extra Na^+ (up to 10 mM) had no effect on pyruvate uptake both in the light and dark, while preequilibration of *P. miliaceum* MCp with extra Na^+ greatly enhanced pyruvate uptake in the light (5-fold enhancement with 5 mM NaCl) with no effect on dark uptake (data not shown). These results together with the above jump experiments in the dark suggest that the H^+ gradient across the envelope drives light-dependent pyruvate transport in MCp from maize and sorghum, NADP-malic enzyme type C_4 species, while the Na^+ gradient does in MCp from *P. miliaceum*, a NAD-malic enzyme type species (and also two species of PEP carboxykinase type as noted in Introduction).

Acknowledgements: This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan, and also by grants from the Ministry of Agriculture, Forestry and Fisheries and from Itoh Science Foundation.

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