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Transhydrogenation in Root Tissue: Mediation by Carbon Dioxide

Abstract. *The $C^{14}O_2$ incorporated into organic acids by excised corn roots is released with an estimated half-time of 3 hours. Homogenates of root tissue can mediate these reactions by coupling, in series, phosphoenolpyruvate carboxylase, diphosphopyridine nucleotide malic dehydrogenase, and triphosphopyridine nucleotide malic dehydrogenase. An important consequence is the transfer of hydrogen from reduced diphosphopyridine nucleotide to triphosphopyridine nucleotide which is then reduced.*

During investigations concerning nonautotrophic CO_2 metabolism in succulent plants, we observed that the subsequent release of the incorporated CO_2 is relatively rapid (*1*). This finding, as well as our observations regarding the rapid loss of labeled malic acid after incubation in $C^{14}O_2$ (*2*), led us to postulate a CO_2 cycle which generates the reduced form of triphosphopyridine nucleotide (TPNH) by transhydrogenation from reduced diphosphopyridine nucleotide (DPNH) to TPN. The initial fixation of the CO_2 is by way of phosphoenolpyruvate (PEP) carboxylase, an essentially irreversible reaction (*3*), with formation of oxaloacetate. Nucleotide cofactors are not required, and the reaction generates inorganic phosphate (*3*). The oxaloacetate is immediately reduced to malate by DPN malic dehydrogenase (*4*) with the generation of DPN. Since the products of CO_2 fixation do not take part in the citric acid cycle (*5*) and the fixation reaction is not reversible, steady-state release in vivo is quite likely by way of TPN malic dehydrogenase. Under physiological conditions of malate concentration, the equilibrium of the TPN malic dehydrogenase is in favor of decarboxylation (*6*). We now present evidence that the capacity for such a cycle is present in corn roots and, in fact, that such a cycle probably is operative. The hypothesis is not entirely without precedent since Young *et al.* (*7*) reported the coupling of DPN malic dehydrogenase with TPN malic dehydrogenase in mammalian adipose and liver tissue and stressed its importance in lipogenesis. The proposed CO_2 cycle as envisioned for corn root tissue is shown in Fig. 1.

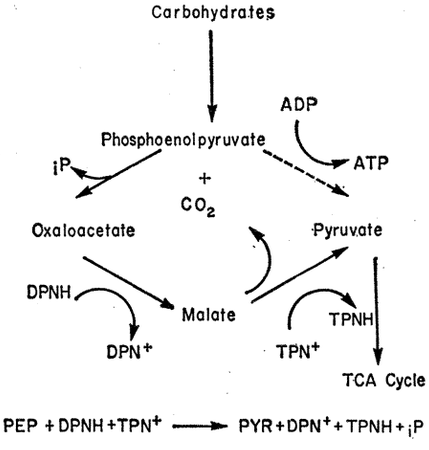

Fig. 1. The proposed cycle of the utilization of CO_2 with the net transfer of hydrogen from DPNH to TPN to form TPNH. The dashed arrow indicates the direct pathway for the utilization of PEP. TCA, tricarboxylic acid; iP, inorganic phosphorus.

About 0.3 g (from the first 2 cm) of 3-day-old corn roots (Burpee Golden Bantam) was incubated in 50 μc of $HC^{14}O_3^-$ (2.2 μc/μmole) in tris buffer (0.05*M*, *p*H 7.4) for 2 hours. After the incubation, the roots were quickly washed with distilled water and transferred to a 100-ml flask containing nonradioactive buffer. The flask was attached to a closed circulating system which was monitored with a 250-ml Cary-Tolbert ion chamber and Cary Model 31 vibrating-reed electrometer. Therefore continuous recording of the released $C^{14}O_2$ was obtained. Both the first and second incubations were conducted at 30°C. In agreement with our findings with *Opuntia* roots (*1*) and stem tissue (*2*), the release was biphasic and described adequately by two first-order equations (Fig. 2). The lower curve (phase 1) of Fig. 2 was obtained by extrapolating the linear portion of the upper curve (phase 2) to the vertical axis and subtracting the estimated values from the actual datum points. The first-order rate constant for phase 1 was 89.2 × 10^{-3} min^{-1} with a half-

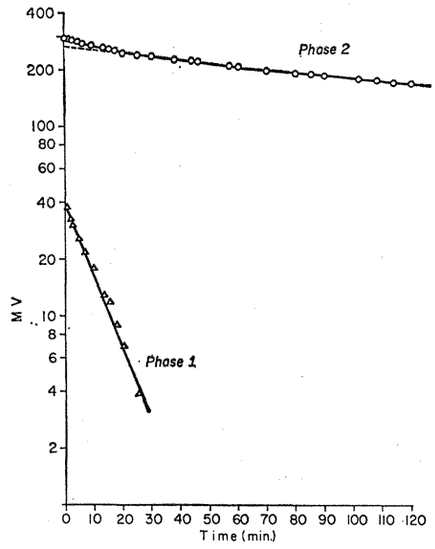

Fig. 2. Release of $C^{14}O_2$ by corn root tips after a 2-hour fixation period. Phase 2 represents steady state decarboxylation of the $C^{14}O_2$ fixation metabolites. MV, millivolts; 3300 dpm are equivalent to 1 mv.

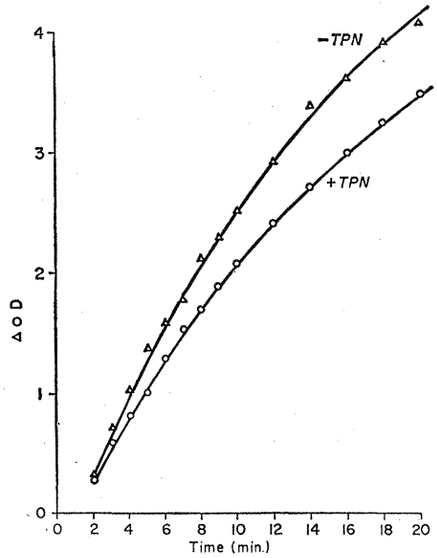

Fig. 3. Change in optical density (O.D.) per gram of tissue (fresh weight) for complete coupling reaction mixture (2 μmole PEP, 10 μmole $NaHCO_3$, 10 μmole $MgCl_2$, 0.256 μmole DPNH, 0.4 ml of enzyme preparation; with and without 0.37 μmole TPN). It was assumed that the slower apparent rate of DPNH oxidation in the presence of TPN was due to reduction of TPN. The reduction of TPN was due to the TPN-mediated enzymic decarboxylation of the initial product, malic acid, formed by PEP carboxylase coupled with DPN malic dehydrogenase.

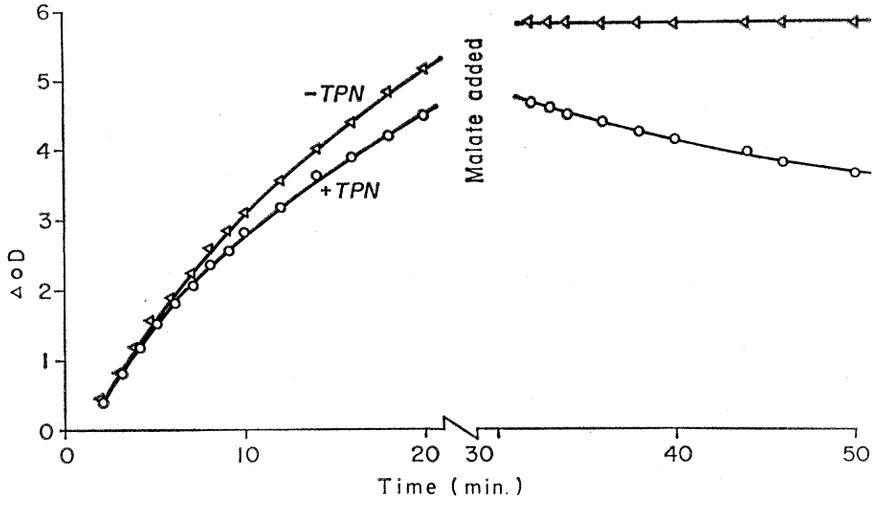

Fig. 4. Effect of adding malate (10 μmole) to complete reaction mixture (Fig. 3) after DPNH oxidation. Only the mixture containing TPN showed a subsequent change in optical density (per gram of fresh tissue) indicating the enzymic conversion of malate to pyruvate by way of TPN malic dehydrogenase rather than to oxaloacetate by way of DPN malic dehydrogenase.

time ($T_{1/2}$) of 7.77 minutes. Constants for phase 2 were 3.85×10^{-3} min^{-1} and 180 minutes. Analogous with our previous findings (*1*), both curves represented actual decarboxylations; the rapid release (phase 1) was explained by the change in partial pressure of CO_2 as the tissue was transferred from the first- to the second-incubation flasks. Phase 2, therefore, apparently represented steady-state CO_2 release with a $T_{1/2}$ of about 3 hours. Of the 3,262,000 disintegrations per minute per gram (dpm/g), in the tissue (0.306 g, Fig. 2) fixed during the initial 2-hour period, 1,815,000 dpm/g remained after the 2-hour second incubation. Thus, the loss during the second incubation was 44.5 percent.

In a subsequent experiment, root tissue was incubated in 50 μc of $HC^{14}O_3^-$ (58 μc/μmole) for 2 hours and fractionated for product analysis (*8*). The total 6,115,600 dpm/g present in the roots were distributed as follows (percent): lipid, 1.4; organic acids, 63.8; amino acids, 32.7; neutral components, 0.0; and insoluble residues, 1.8. Thin-layer chromatography analysis (*8*) indicated as the primary metabolites (in percent): malic acid, 25.6; succinic acid, 29.8; citric acid, 7.8; glutamic acid, 21.5; aspartic acid, 7.7; and alanine, 3.0. Much of the C^{14} activity was in malic acid or it was in pools directly convertible to malic acid.

Crude enzyme homogenates were made from about 6 g of root tissue by centrifuging at 20,000*g* for 15 minutes and dialyzing for 12 hours against two changes of tris buffer (*9*). Assay of TPN malic dehydrogenase was conducted with malic acid (10 μmole), TPN (0.37 μmole), $MgCl_2$ (10 μmole), 0.4 ml of enzyme homogenate, and tris buffer (0.05*M*, *p*H 7.4). The reaction was followed at 340 mμ in a Zeiss PMQ II spectrophotometer. On a fresh weight basis the calculated rate of oxidation was 52.7 nmole min^{-1} g^{-1}. The PEP carboxylase was assayed by coupling with DPN malic dehydrogenase and following the oxidation of DPNH with the spectrophotometer. The dehydrogenase was extremely active in the crude homogenates; 5 μmole of oxaloacetatic acid, 10 μmole of $MgCl_2$, 0.4 ml of enzyme homogenate, and 0.256 μmole of DPNH resulted in the complete oxidation of DPNH in less than 2 minutes. The assay of PEP carboxylase was conducted with PEP (2 μmole), $NaHCO_3$ (10 μmole), $MgCl_2$ (10 μmole), DPNH (0.256 μmole), and 0.4 ml of enzyme homogenate; with and without TPN (0.37 μmole). The assumption was that the apparent rate of DPNH oxidation would be less in the presence of TPN if some of the malate formed were decarboxylated by way of TPN malic dehydrogenase. On a fresh-weight basis initial linear rates were 110 nmole min^{-1} g^{-1} with TPN and 140 nmole min^{-1} g^{-1} without TPN (Fig. 3). The ratio of the initial rates was 0.79, corresponding to an apparent turnover for CO_2 of 21 percent. From these data we estimated a $T_{1/2}$ for the CO_2 of 2.34 hours. If malate (10 μmole) was added to the reaction mixture just prior to completion, only the reaction mixture containing TPN showed a subsequent change in optical density (Fig. 4). These results were important since they indicated that DPN malic dehydrogenase was not reversing (the reaction without TPN did not result in an optical-density change and therefore DPNH was not regenerated), but TPN malic dehydrogenase did decarboxylate malate and generated TPNH. Thus a series coupling of the reactions was indicated in vitro and the CO_2 was released by way of TPN malic dehydrogenase.

From these data obtained with intact root sections and crude enzyme homogenates we conclude that a possible consequence of nonautotrophic CO_2 metabolism in root tissue (and succulent stem tissue) is a transhydrogenation from DPNH to TPN to form TPNH. The cycle is driven by the enzymic hydrolysis of PEP and by the accumulation of malate with the result that one equivalent of adenosine triphosphate is lost. An additional source of synthetic reducing power (TPNH) in nongreen tissues (or succulent tissues in the dark) may be physiologically important in subsequent TPNH-requiring reactions such as lipogenesis (*7*), nitrate reduction, sulfate reduction, and indoleacetic acid metabolism (*11*) among others.

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