Preliminary Communication

Formate-forming Fungal Catabolic Pathway to Supply Electrons to Nitrate Respiration

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Pyruvate was catabolized anaerobically by resting cells of Fusarium oxysporum to form formate and acetate. Addition of nitrate decreased the accumulation of formate in the medium with concomitant formation of nitrite and N2O. The results suggested a unique metabolic pathway that occurs in fungi mediated by pyruvate-formate lyase to supply electrons via formate to fungal denitrification.

Key words: pyruvate-formate lyase; formate dehydrogenase; nitrate reductase; denitrification; Fusarium oxysporum

Most fungi have long been thought to be aerobic organisms. However, recent findings on fungal denitrification,1–3) codenitrification,2,4) and ammonia fermentation5) have revealed that many soil fungi have a variety of anaerobic energy metabolisms to rapidly adapt to changes in aerating conditions. This means that they are facultative anaerobes but not obligatory aerobes as previously thought. We recently showed that ubiquinone (UQ)-dependent formate dehydrogenase (Fdh) is involved in nitrate (NO3-) respiration of the denitrifying fungus Fusarium oxysporum, the reaction of which is coupled to ubiquinol (UQH2)-dependent, membrane-bound nitrate reductase (Nar).6) The fungal Fdh-Nar couple is similar to that involved in the non-denitrifying NO3- respiration (ammonification) system of enterobacteria such as Escherichia coli.7) By contrast, UQ-dependent Fdh has never been known to occur among denitrifying bacteria.8,9) Thus the fungal denitrifying system contains unique components such as Fdh and P450nor,6,9–12) showing that denitrifying fungi such as F. oxysporum have constructed during evolution a unique anaerobic respiration system that is distinct from the NO3- respiration system of denitrifying bacteria or enteric bacteria. Finding of the novel respiration system in the fungal mitochondria11,12) suggests presence of an unknown catabolic metabolism that provides formate to Fdh. Formate is formed in E. coli from pyruvate by the reaction of pyruvate-formate lyase (Pfl).7)

Here we examined the possible catabolic pathway via Pfl to form formate by incubating F. oxysporum cells with pyruvate in the presence or absence of NO3-. F. oxysporum MT-811 (JCM11502, Japan Collection of Microorganisms) was cultured anaerobically at 30°C for 3 days in 3 liters of the medium (containing 1.7% ethanol, 5 mM NaNO3, 10 mM KH2PO4, 0.2 g l-1 MgSO47H2O, and trace metal;1–6) pH 8.2) in a 5-liter Erlenmeyer flask. The conditions are expected to induce the activity of ammonia fermentation.9) The cells were then collected by centrifugation, washed with 0.6% NaCl solution, and resuspended in 300 ml of the NaCl solution. A portion (50 ml) of the cell suspension was mixed with 100 ml of 100 mM potassium phosphate buffer (pH 7.2) containing 20 mM sodium pyruvate in 500-ml Erlenmeyer flask. After inoculation the flask was sealed with a rubber stopper after filling the head space with argon gas, and then incubated on a rotary shaker (120 rpm) at 30°C. This incubation should have made the fungal cells rest, since other mineral and nitrogen sources were omitted. After incubation for 36 h, pyruvate (2 mmol) added to the medium completely disappeared and instead, comparable amounts of formate (1.2 mmol) and acetate (1.5 mmol) were accumulated (Fig. 1). Pfl of E. coli forms formate and acetyl CoA from pyruvate and CoA with a stoichiometry between all the reactants and products of 1:1.7) And we showed in the previous paper that the anoxic cells of F. oxysporum performing ammonia fermentation have acetate kinase (Ack) activity that metabolized acetyl CoA to acetate with concomitant production of ATP.9) The formation of acetate concomitantly with formate (Fig. 1) is highly indicative that F. oxysporum metabolized pyruvate under these anoxic conditions to form both catabolites (formate and acetate) by using the couple of Pfl.
and Ack enzyme systems.

Next, we examined the effects of NO\textsubscript{3} on the resting, anoxic incubation of \textit{F. oxysporum} cells with pyruvate. As shown in Table 1A, formate and acetate were yielded with concomitant consumption of pyruvate in both resting experiments (with or without NO\textsubscript{3}). The stoichiometry between the amounts of formed acetate and consumed pyruvate was near 1:1. And further, both formate and acetate were not formed at all when the cells were incubated without pyruvate (data not shown). The results support strongly our assumption that the catabolic degradation of pyruvate provided NADH in addition to formate. This metabolism of pyruvate to give both formate and NADH can be done when the two alternative, possible pathways to form acetyl CoA from pyruvate work simultaneously. One is mediated by pyruvate dehydrogenase, the major way in eukaryotes, which forms acetyl CoA and CO\textsubscript{2} with concomitant reduction of NAD\textsuperscript{+} to NADH, and the other depends on PfI as noted above. Acetyl CoA is formed from both enzymatic reactions that should be further metabolized to acetate as noted above. Therefore, the simultaneous functioning of the alternative routes to degrade pyruvate agrees with the results that the amount of formate formed was lower than that of acetate (without NO\textsubscript{3}) and that NADH was provided to form N\textsubscript{2}O from NO\textsubscript{3} (Fig. 1A).

Here we presented the first evidence for a possible catabolic pathway due to PfI to provide formate that is used as an electron donor for nitrate respiration (denitrification) by \textit{F. oxysporum}.

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