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Research article

Respiratory complex I deficiency results in low nitric oxide levels, induction of hemoglobin and upregulation of fermentation pathways

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ABSTRACT

The cytoplasmic male-sterile (CMS) mutant of *Nicotiana sylvestris* which lacks NAD7, one of the subunits of respiratory complex I (NADH: ubiquinone oxidoreductase, EC 1.6.5.3), is characterized by very low (~10 times lower as compared to the wild type plants) emissions of nitric oxide (NO) under hypoxic conditions. The level of the non-symbiotic class 1 hemoglobin, as shown by Western blotting, is increased compared to the wild type plants not only under hypoxia but this protein reveals its marked expression in the CMS mutant even under normoxic conditions. The activity of aconitase (EC 4.2.1.3) is low in the CMS mutant, especially in the mitochondrial compartment, which indicates the suppression of the tricarboxylic acid cycle. The CMS mutant exhibits the severalfold higher activities of alcohol dehydrogenase (EC 1.1.1.1) and lactate dehydrogenase (EC 1.1.1.27) under the normoxic conditions as compared to the wild type plants. It is concluded that the lack of functional complex I results in upregulation of the pathways of hypoxic metabolism which include both fermentation of pyruvate and scavenging of NO by the non-symbiotic hemoglobin.

1. Introduction

The cytoplasmic male-sterile (CMS) mutant of *Nicotiana sylvestris* lacking functional complex I [1,2] is characterized by altered redox state, energy level [3–8] and elevated nitrogen content [9]. The mutant has increased respiration but decreased photosynthesis which is linked to modified intracellular redox recycling [3,8,10]. It is also characterized by enhanced rates of nitrogen assimilation and realignment of metabolite profiles to a nitrogen-rich state.

The impairment of complex I in the CMS mutant is partially compensated by the upregulation of the cytochrome pathway [2], by higher capacity of the alternative internal NADH dehydrogenase (NDin) and the alternative oxidase [10,11]. Total cellular ATP content was higher in both the light and the dark, with unchanged ATP/ADP ratio [9,10]. Generally, the mutant is characterized by the increased pool of pyridine nucleotides, increased mitochondrial NADH/NAD ratio and elevated cytosolic and chloroplast NADPH/NADP [9], although this may depend on light conditions [6,12] and carbon/nitrogen supply [13]. For a cucumber mutant with complex I deficiency both the decreased ATP/ADP and increased NAD(P)H/NAD(P) ratios were reported [7,14,15]. The elevated redox level may explain higher leaf superoxide content [6], while concentrations of H_2O_2 are lower or unchanged [3,6]. Altered redox level may also explain the higher superoxide content previously reported in the *fro* Complex I mutant of Arabidopsis [16,17].

The high nitrogen content reported for the CMS mutant of tobacco is reflected in elevated levels of several amino acids including alanine, asparagine, glutamine, arginine, glycine and serine, but decreased aspartate [9,13]. The elevation of alanine may indicate upregulation of the fermentation glycolytic pathway in conditions when the capacity of mitochondria for the respiratory flux is suppressed at the level of complex I while the total respiratory flux (via alternative NADH and NADPH dehydrogenases) is increased in order to maintain ATP production [2,11]. While the nitrogen status is increased but maximal extractable nitrate reductase activity is slightly decreased [9], it is interesting to determine possible changes in nitric oxide (NO) production in CMS plants which in plants are linked to mitochondria with the contribution of nitrate reductase that supplies nitrite [18]. In this study we show that NO production by CMS tobacco plants under

Abbreviations: ADH, alcohol dehydrogenase; AOX, alternative oxidase; CMS, cytoplasm male sterile; LDH, lactate dehydrogenase; NO, nitric oxide; NR, nitrate reductase.

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hypoxic conditions is extremely low, while the expression of the NO-scavenging non-symbiotic hemoglobin is elevated. This indicates that the CMS mutant plant exhibits some features of hypoxic metabolism in normal air, which is reflected in the expression of the non-symbiotic hemoglobin, the upregulation of lactate and alcohol dehydrogenases and the suppression of aconitase activity making the tricarboxylic acid cycle less operative.

2. Results

2.1. NO production

The maximum rate of NO production from leaves of the CMS mutant was approximately ten times lower than from the wild type (Fig. 1A). When the leaves were incubated in nitrate solution under anoxic conditions, after the 2 h lag period, leaves started to produce NO and in 5–6 h the rate of NO emission reached 200 nmol NO g^{-1} FW h^{-1} which corresponds to the values for higher plants reported in Ref. [19]. For the mutant the lag phase was longer (near 5 h) and the rate of NO emission was close to 20 nmol NO g^{-1} FW h^{-1}. Rotenone did not affect the rate and the shape of curve of NO emission not only in the mutant but also in the wild type (data not shown). The inhibition of nitrate reductase by tungstate resulted in threefold decrease of NO emission in the wild type, shortening of the lag period a faster approaching the maximum level, while in the CMS mutant it did not affect the rate and profile of NO emission (Fig. 1B).

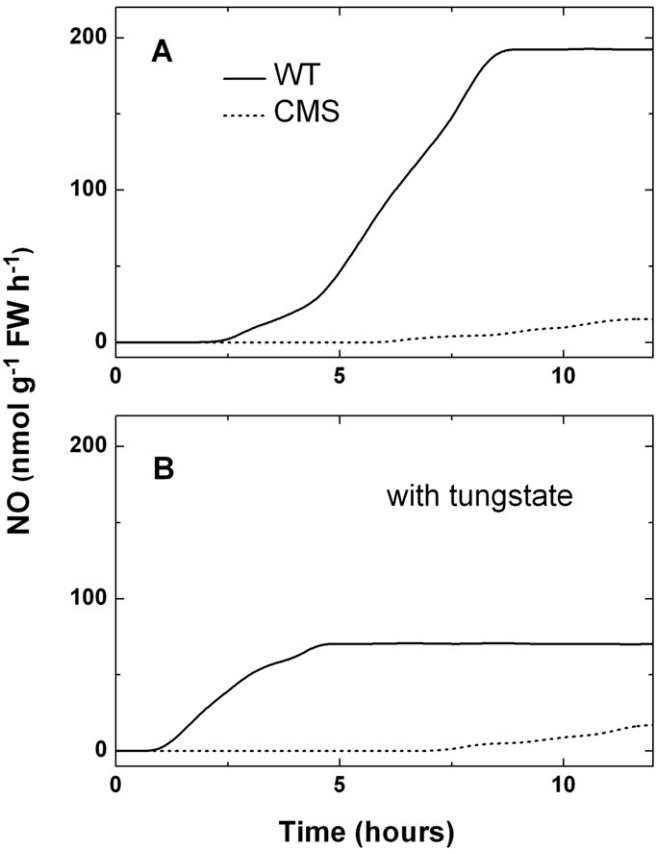

Fig. 1. Nitric oxide emissions from leaves of wild type (solid line) and CMS mutant (dashed line) under nitrogen atmosphere. Leaves were incubated in 50 mM $NaNO_3$ (A) and in 50 mM $NaNO_3$ plus 50 μM tungstate (B). The typical experiment is presented from 5 independent measurements showing similar results.

2.2. Hemoglobin blotting

Protein blotting of non-symbiotic hemoglobin revealed its higher expression in leaves of the CMS plant as compared to the wild type not only under hypoxic conditions when it is usually induced [20] but also under normoxia when its level was lower but still clearly detectable as compared to the wild type where its expression was negligible (Fig. 2). The expression of Hb protein was more than twice higher under hypoxia and under normoxia it was at a similar level in the CMS mutant as under hypoxia in the wild type. The experiment was repeated three times, the same pattern was obtained.

2.3. Aconitase

Aconitase activity was significantly (about threefold) lower in leaves of the CMS plants. In particular this low activity is related to strongly decreased level of aconitase in mitochondria (5–6 times lower than in wild type) than to the cytosol. This corresponded to a different distribution of the enzyme in CMS mutant and WT. In the WT, cytosol contained 35–40% of aconitase activity while mitochondria 60–65%. In CMS mutant, 65–70% of aconitase was in the cytosol and only 30–35% in the organellar fraction (Fig. 3).

2.4. Fermentation enzymes

Lactate and alcohol dehydrogenase were measured both under normoxic and hypoxic conditions in leaves and exhibited markedly higher activity in the CMS mutant in normoxia as compared to the wild type (Fig. 4). The ADH activity was more than 4 times higher in the CMS than in the wild type plants even under normoxia while the short-term (3 h) hypoxia is not sufficient to stimulate significantly the ADH activity in the wild type. In the CMS mutant, hypoxia resulted in some decrease of ADH activity which may be due to a decreased viability of these plants under stress conditions. LDH activity exhibited a threefold increase in the wild type plants under 3-h hypoxia and this corresponded to the observed level of LDH in the CMS mutant under normoxia and hypoxia (Fig. 4).

3. Discussion

3.1. NO emissions and hemoglobin content

Despite of its high nitrogen status, the CMS tobacco mutant shows very low rate of NO emission under anoxic conditions. The formation of NO in plants occurs mainly via reduction of nitrite that takes place anaerobically by mitochondria, nitrate reductase and possibly by the reductases of plasma membrane [18]. The NO emission takes place when leaves take nitrate [21] but this does not

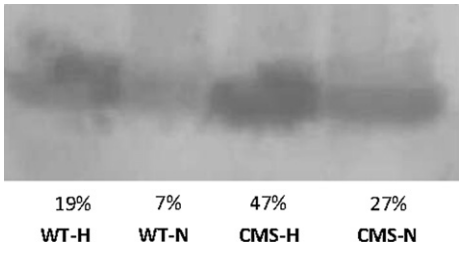

Fig. 2. The levels of non-symbiotic hemoglobin in wild type and CMS mutant under normoxic and hypoxic conditions. WT-H – wild type hypoxia, WT-N – wild type normoxia, CMS-H – CMS mutant hypoxia, CMS-N – CMS mutant normoxia. The result of a typical experiment is presented. Densitometry values for spots were determined after background subtraction and normalization by total spot intensity.