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SHORT COMMUNICATION

COLD LABILITY OF THE NITROGEN FIXATION SYSTEM IN EXCISED LEGUMINOUS NODULES

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Abstract—The rate of acetylene reduction by excised root nodules of five leguminous species decreased more rapidly when the nodules were kept at temperatures near 0° than when they were kept at 18–20°; this effect was not observed when nodules were given the cold treatment prior to excision. The cold lability is dependent on both time of storage and temperature. Freezing the excised nodules destroyed more than 90 per cent of the acetylene reduction activity.

INTRODUCTION

THE NITROGEN fixation system in *Clostridium pasteurianum* is cold labile[1,2] because of the cold inactivation of azoferredoxin,[3] one of the components of nitrogenase. Root nodules of leguminous plants lose the ability to catalyse nitrogen fixation after excision from the host plant.[4] This communication reports the instability of the nitrogen fixation system in root nodules at temperatures near or below 0°. Acetylene reduction was used as a measure of nitrogen fixation because of the high sensitivity of measurement of ethylene and since data[5,6] indicate that it is a true measurement of nitrogen fixation activity in root nodules.

RESULTS AND DISCUSSION

Root nodules from *Lotus pedunculatus* Cav., *Trifolium repens* L., *Lupinus angustifolius* L., *Galega officinalis* L. and *Vicia angustifolia* L., respectively, were kept in one layer in a pre-cooled beaker immersed in ice (0–1°) for 30 min then transferred to a beaker at room temperature (18–20°) for 15 min. These unfrozen nodules catalysed acetylene reduction at a lower rate than did nodules which had been kept at room temperature (18–20°) for 45 min (Table 1). The decline in acetylene reduction activity during cold storage occurred in air, argon or nitrogen.

The extent of the decrease in acetylene reduction capacity at 0–1° is proportional to the time of storage. Nodules from *Lotus pedunculatus* kept in ice at 0–1° for 5, 10, 20 and 30 min respectively catalysed acetylene reduction at rates which were 86, 76, 34 and 10 per cent of

[1] R. D. DUA and R. H. BURRIS, *Proc. Natl Acad. Sci. U.S.* **50**, 169 (1963).
[2] R. D. DUA and R. H. BURRIS, *Biochim. Biophys. Acta* **99**, 504 (1965).
[3] E. MOUSTAFA and L. E. MORTENSON, *Anal. Biochem.* **24**, 226 (1968).
[4] M. H. APRISON and R. H. BURRIS, *Science* **115**, 265 (1952).
[5] B. KOCH and H. J. EVANS, *Plant Physiol.* **41**, 1748 (1966).
[6] R. W. F. HARDY, R. D. HOLSTEN, E. K. JACKSON and R. C. BURNS, *Plant Physiol.* **43**, 1185 (1968).

those observed in nodules which had been stored at room temperature for corresponding periods of time.

The extent of the decrease in acetylene reduction capacity is also affected by the temperature at which the nodules were kept. Nodules from *G. officinalis* kept for 30 min at 1, 8, and 12° respectively catalysed acetylene reduction at rates which were 12, 38 and 71 per cent of those observed in nodules which had been kept at 25°.

The decline in the rate of acetylene reduction at 0–1° does not occur in unexcised nodules. This was shown by the results obtained when *L. pedunculatus* plants were carefully removed from the sand in which they were grown and their root systems immediately immersed in a nitrogen-free nutrient solution at 0–1° for 1 hr. The rate of acetylene reduction in nodules harvested from these plants was 1·48 μmoles/hr/g whereas a rate of 1·58 μmoles/hr/g was obtained with nodules harvested from plants which were kept for the same period of time with their root systems immersed in a nutrient solution at 18–20°. Pretreatment of the root system at 0–1° does not confer any protection on nodules maintained at this temperature after excision.

TABLE 1. STABILITY OF ACETYLENE REDUCTION SYSTEM IN ROOT NODULES

Plant source of root nodules	Rate of acetylene reduction (μmole/hr/g nodules)*			
	Freshly harvested	Stored at 18–20°	Stored at 0–1°	Frozen
Galega officinalis	9·00±0·25	5·00±0·57	2·00±0·19	0·07±0·01
Lotus pedunculatus	1·20±0·14	0·80±0·12	0·10±0·01	0·00
Lupinus angustifolius	7·50±0·40	5·60±0·22	2·40±0·24	0·02
Trifolium repens	14·00±0·57	11·20±0·58	4·20±0·38	0·12±0·01
Vicia angustifolia	13·00±0·54	10·50±0·59	0·96±0·11	0·10±0·01

* Average, three experiments.

When nodules were quick frozen, in a precooled tube immersed either in a dry ice–alcohol mixture or in liquid nitrogen, kept frozen for 30 min and then kept at 18–20° for 20 min after thawing, the rate of acetylene reduction was less than 5 per cent of the rate obtained with nodules stored at 18–20° for 50 min (Table 1). Later it was found that a similar loss in acetylene reduction activity occurred in nodules which had been frozen at liquid nitrogen temperature and thawed immediately.

The effect of cold storage on the rate of acetylene reduction could be due either to the cold inactivation of one of the nitrogenase components or to the lability of the phosphorylation process which leads to the formation of the ATP needed for acetylene reduction.[7]

EXPERIMENTAL

Nodules of *Lotus pedunculatus* and *Lupinus angustifolius* were harvested from plants grown in sand from seed which had been heavily inoculated with *Rhizobium* strain NZP 2021 and NZP 5087 respectively. The plants were watered with a nitrogen-free nutrient solution based on Hoagland's solution.[8] Nodules of

[7] E. MOUSTAFA and L. E. MORTENSON, *Nature* **216**, 1241 (1967).
[8] D. R. HOAGLAND and D. ARNON, *Circ. Calif. Agric. Exp. Stn.* 347 (1939).

Trifolium repens, *Galega officinalis* and *Vicia angustifolia* were obtained from plants collected in the field. Nodules (1–2 g) were harvested within 15 min of collecting the plants and were mixed well before samples were taken for assay.

The method of determination of the rate of acetylene reduction was essentially that described by Koch and Evans.[5] A batch of small nodules (0·1 g) such as those of *Lotus pedunculatus* or 0·2 g of the large nodules such as those of *Lupinus angustifolius* were placed in 11-ml vials fitted with serum stoppers. The vials were evacuated and filled with argon five times, then filled with 0·65 atm argon, 0·25 atm oxygen and 0·1 atm acetylene. The vials were shaken in a water bath at 25° and gas samples were taken using a gas-tight syringe; the samples were assayed for ethylene and acetylene by gas chromatography as described previously.[3]

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