Acid and Alkaline Invertases in Roots and Nodules of *Lupinus angustifolius* Infected with *Rhizobium lupini*

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**Summary.** Both acid and alkaline invertase activity were found in tips and cortical tissue of *Lupinus angustifolius* L. roots infected with *Rhizobium lupini* NZP 2257. Only the alkaline invertase was detected in the nodule cytoplasm. Weak invertase activity found in the bacteroids was probably a contamination from plant invertase. The alkaline invertase activity in the nodule cytoplasm was 250 times that detected in the bacteroids and 8 times that detected in cortical tissue. No intracellular or extracellular invertase was detected in *R. lupini* cultured in liquid medium containing sucrose.

**Introduction**

Following infection of the legume plant by rhizobia the metabolism of the bacterium becomes dependent upon substrates supplied by the host. Of the metabolites translocated from the leaves to the roots, sucrose appears to be of major importance in most higher plants (Zimmerman, 1960; Bach *et al.*, 1958). It might be predicted therefore that infection of root tissue by rhizobia could lead to changes in the level of invertase as a reflection of the higher rate of metabolic activity of nodules in comparison with cortical tissue.

The extent of sucrose metabolism by bacteroids *in vivo* has not been clearly established. Both nitrogen fixation (Bergersen and Turner, 1967) and respiration (Bergersen, 1958; Tuzimura and Meguro, 1960) are stimulated *in vitro* by organic acids but not by sucrose. However, sucrose has been shown to stimulate nitrogen fixation in nodule slices (Bach *et al.*, 1958).

In the present study the activities of acid and alkaline invertase in fractions from the roots of both infected and non-infected plants have been examined. Tests for the presence of invertase in cultures of *R. lupini* have also been carried out.

**Materials and Methods**

*Plant Material and Bacterial Cultures.* Lupins (*Lupinus angustifolius* L.) inoculated with a non-acid producing, slow-growing strain of rhizobia (*Rhizobium lupini* Schroeter NZP 2257) were grown under glass-house conditions in stainless-
steel troughs containing sterile pumice. Plants were supplied with a sterile, nitrogen-free nutrient solution (Hoagland and Arnon, 1939). Non-inoculated lupin plants were grown in the same way with the addition of inorganic nitrogen.

*R. lupini* NZP 2257 was maintained on yeast-extract-mannitol slopes at 2°C. Cultures were grown under aseptic conditions in liquid medium containing, per l; 0.50 g KH₂PO₄, 0.20 g MgSO₄·7H₂O, 0.10 g NaCl, 0.50 g Difco Yeast Extract, 10.0 g sucrose or mannitol. The medium was adjusted to pH 6.8 with 5 M KOH.

**Preparation of Extracts for Enzyme Assay.** Plants were harvested approximately 6–8 weeks after germination and the nodules, root tips, and cortical tissue were removed. *Nodules*, which were confined to a short section (3–4 cm) on the main root, just below the surface of the pumice, were picked directly into ice-cold buffer. The buffer, which was based on that of Stokes *et al.* (1968), contained 0.35 M sucrose, 0.35 M mannitol, 50 mM Na-phosphate, 1 mM EDTA, 2 mM β-mercaptoethanol, 2 mM K-metabisulphite (pH 7.0). This buffer system prevented leakage of enzymes from the bacteroids which were found to be susceptible to osmotic shock. After weighing, the nodules were macerated at 2°C in 2 volumes (w/v) of the same buffer using pestle and mortar. All tissue extracts were maintained at 2°C throughout the fractionation procedure. The macerated material was squeezed through two layers of Miracloth and centrifuged at 300 × g for 5 min. The pellet, containing starch granules and large organelles of plant origin, was discarded. The supernatant was centrifuged at 6000 × g for 5 min to give the crude bacteroid pellet and a supernatant which was recentrifuged at 30000 × g for 10 min to remove plant organelles. The supernatant from this step was used to assay levels of enzymes in the plant cytoplasm from the nodules. The crude bacteroid pellet was resuspended three times in the sucrose mannitol buffer, with recentrifugation at 6000 × g for 5 min. The final centrifugation was at 30000 × g for 10 min. The supernatant from this step was used for the assay of invertase in the final bacteroid wash. The pellet containing bacteroids, free from plant organelles as determined by electron microscopy, was resuspended in 5 ml of phosphate buffer (10 mM Na-phosphate, 1 mM EDTA, 2 mM β-mercaptoethanol; pH 7.0). After brief sonication for 3 min and centrifugation at 30000 × g for 10 min the supernatant was assayed to determine levels of enzymes in the bacteroids.

**Root tips** were obtained by excising the roots 2–3 cm from the tips. *Cortical tissue* was peeled away from the stele of non-infected plants in the region equivalent to that in which the nodules occurred in infected plants. Portions of mature root from infected plants were obtained by cutting pieces of root (3–4 cm in length) from immediately below the nodules. Root tip, mature root and cortex preparations were macerated in 1 part (w/v) sucrose-mannitol buffer. The macerate was filtered through Miracloth and the filtrate centrifuged at 30000 × g for 10 min. Supernatants were used for enzyme assays; these were carried out as soon as possible after maceration of the tissue.

The supernatants obtained after centrifuging root extracts at 30000 × g for 10 min were almost completely free of mitochondria as determined by electron microscopy. Mitochondria pelleted at 15000 × g for 10 min.

*R. lupini* was harvested at 12000 × g for 10 min and samples of the supernatant were taken for assay of extracellular invertase, sucrose and reducing sugar. The pellet was washed by resuspension in 10 mM Na-phosphate, 1 mM EDTA, 2 mM β-mercaptoethanol at pH 7.0. After sonication and centrifugation at 30000 × g for 10 min, samples of supernatant were taken for assay of intracellular invertase.

**Enzyme Assays.** Invertase was assayed using the method of Nelson (1944) for determining the rate of production of reducing sugars. Assays were carried out at 25°C for 45 min for the nodule cytoplasm and for 3–5 h for root-tip, cortex and bacteroid extracts. The times of assays were the minimum to allow accurate estimation of the rate of sucrose hydrolysis at each pH value. The incubation systems contained 5% sucrose. To avoid breaks in the curve of invertase activity over a