Plant Physiol. (1990) 92, 262–264
0032-0889/90/92/0262/03/$01.00/0

Received for publication July 21, 1989
and in revised form August 5, 1989

Communication

Binding of Isolated Plant Lectin by Rhizobia during Episodes of Reduced Gravity Obtained by Parabolic Flight[1]

Ralph L. Henry, Pat D. Green, Peter P. Wong, and James A. Guikema*

BioServe Space Technologies, Division of Biology, Kansas State University, Manhattan, Kansas 66506

ABSTRACT

Development of a legume root nodule is a complex process culminating in a plant/bacterial symbiosis possessing the capacity for biological dinitrogen fixation. Formation of root nodules is initiated by the binding and stabilization of rhizobia to plant root hairs, mediated in part by a receptor/ligand recognition system composed of lectins on the plant root surface and lectin-binding sites on the rhizobial cell surface. The dinitrogen fixation activity of these root nodules may be an important feature of enclosed, space-based life support systems, and may provide an ecological method to recycle nitrogen for amino acid production. However, the effects on nodule development of varied gravitational fields, or of root nutrient delivery hardware, remain unknown. We have investigated the effects of microgravity on root nodule formation, with preliminary experiments focused upon the receptor/ligand component. Microgravity, obtained during parabolic flight aboard NASA 930, has no apparent effect on the binding of purified lectin to rhizobia, a result that will facilitate forthcoming experiments using intact root tissues.

Regulation of cellular development requires precise binding interactions between signaling molecules (or ligands) and receptor molecules on cell surfaces. Development of legume root nodules is a particularly intriguing example of this interactive process, since nodule formation culminates in a plant/bacterium symbiosis of both biological interest and economic importance. In the first place, the bacterium and the plant contribute in a symbiotic manner to the fixation of dinitrogen into ammonia and, subsequently, into amino acids. As such, legume root nodules play a crucial role in the ecological recycling of nitrogen and have profound agronomic importance. This feature of root nodule activity will also prove useful in the design of CELSS[2], since it will reduce the requirement for resupply of nitrogen fertilizers to space-based CELSS programs (3). In the second place, root nodule development occurs with a precise pattern of bacterial/plant communication (4), initiated in part by the binding of the bacterium to the cell surface of legume root hairs (2).

Despite the predicted importance of legume root nodules in CELSS applications, virtually nothing is known about the effects of microgravity, or of nutrient delivery systems needed because microgravity prohibits traditional soil-based root-nurturing techniques, on nodule development. Investigations on biosatellites of the Cosmos series (6), as well as studies aboard Shuttle (5, 8–10), indicate that the space environment might alter the rates of plant cell division, induce chromosomal abnormalities, and change the morphological features of certain cell types within plant tissues. An investigation of microgravity effects on root nodule development will, within one experimental system, offer the advantages of (a) economic/agronomic relevance, (b) an interaction between prokaryotic and eukaryotic organisms, and (c) a method to evaluate root-nurturing technologies designed to provide root tissue with nutrients in environments of near weightlessness.

Formation of root nodules is initiated by the binding and stabilization of rhizobia to plant root hairs, and is mediated in part by a receptor/ligand recognition system composed of lectins on the plant root surface and lectin-binding sites on the rhizobial cell surface (2, 7). It seemed, therefore, that the plant/rhizobial recognition system represented a suitable starting point for our studies of microgravity effects on legume nodule development.

MATERIALS AND METHODS

Lectin was isolated and purified from *Phaseolis vulgaris* L. (cv Kentucky Wonder) seeds by affinity chromatography, as described earlier (14). Polyclonal antibodies against purified lectin were raised in rabbits (14), and an antibody fraction was collected from serum using Protein A affinity chromatography. This antibody fraction (1 mg protein/mL), in 0.5 M $NaHCO_3$ (pH 7), was incubated for 10 min (room temperature) in the presence of 2 mg/mL alkaline phosphatase plus 0.05% glutaraldehyde. After 10 min, the conjugation of enzyme to antibody was quenched by addition of 50 mM glycine. The antibody fraction was again collected by Protein A affinity chromatography, and was used without further purification. *Rhizobium leguminosarum* biovar phaseoli strain 127K14 was cultured in yeast extract/mannitol medium to mid-log phase (14), and was used for the binding experiments.

Binding experiments using lectin and rhizobia were performed by Kansas State University Division of Biology's BioServe Space Technologies research team on May 31, 1989, aboard NASA 930. This modified KC-135 aircraft, as a part of NASA's Reduced Gravity Research Program, achieves short episodes of reduced gravity by flying parabolic arcs

[1] This work was supported in part by the National Aeronautics and Space Administration (NAGW-1197) and represents contribution 90-23-J of the Kansas Agricultural Experiment Station.

[2] Abbreviation: CELSS: controlled ecological life support system.

between 7,900 and 10,400 meters. In all, 40 such arcs were flown on this flight, grouped into four sets of 10. Parabola numbers 21 to 31 were devoted to the lectin binding experiment, permitting four replicates of each data point. Reduced gravity was obtained when the aircraft pushed 'over the top' of each parabola. For parabolas 11 to 21, the gravitational environment (with the body of the aircraft as the frame of reference) was below 1*g* for 25.9 (±1.4) s per parabola, and less than 0.05*g* for 16.8 (±1.1) s per parabola. During this interval, the gravitational vector generally ranged ±0.015*g* around zero-gravity and we timed our experiments to take the maximum advantage of this low-gravity period.

In-flight tests were conducted by a pair of investigators, seated facing each other, with sample tubes and a Microfuge B between them, and with both investigators, and equipment, secured to the aircraft cabin floor. The two investigators each filled a preset positive displacement micropipette with 100 μL of lectin solution and, upon entry into microgravity, added the lectin solution to the rhizobia in a microcentrifuge tube. This addition was made in synchrony, and the tubes were rapidly capped and placed in the Microfuge B. At the end of 20 s of timed reduced gravity, the tubes were centrifuged (10,000 rpm for approximately 30 s), the supernatant was removed using a wick constructed of absorbent KimWipe tissue, and the tubes, with rhizobial pellets, were stored. Binding was quantitated as follows. Each tube with rhizobial pellet was washed three times with Tris-buffered saline (pH 7.5), incubated for 10 min with 100 μL of alkaline phosphatase-conjugated antibody. Following incubation, the tube was centrifuged, washed, and recentrifuged three times. Thus, the final pellet of rhizobia contained lectin, which bound to the receptor site on the rhizobial cell surface during reduced gravity, and alkaline phosphatase, which bound to the lectin via the conjugated antibody. Alkaline phosphatase reaction mixture (1 mg/mL nitrophenyl-P, 10 mM $MgCl_2$, 0.1 M Tris-NaOH, pH 9.5) was added to the tubes, the pellet was dispersed, and, after 15 min, the reaction was stopped with 100 μL 3.0 N NaOH. Following centrifugation, *A* of the supernatant was determined at 410 nm.

RESULTS AND DISCUSSION

Preflight experiments established the feasibility of this model receptor-ligand binding assay as a quantitative tool. Because of the short duration of microgravity, we limited our incubation times to 20 s to ensure that the incubation period would be completed before the aircraft finished the 'controlled free-fall' of parabolic flight. We found that we could obtain quantitative, reproducible binding data during this short incubation period (data not shown).

Figure 1 shows the amount of lectin bound to rhizobia during reduced gravity as a function of lectin concentration. The extent of binding increases in a nearly linear manner between 0.2 and 1.0 mg lectin/mL. Also shown are the values obtained at unit gravity, performed by the same investigators on the aircraft upon its return. These results, along with the error bars for both the reduced gravity and the unit gravity measurements, demonstrate that lectin binding to cell surface receptors is not affected by microgravity.

A nearly weightless environment has detrimental consequences for certain aspects of plant cell development. Often, the limited information we have is confusing. For example, studies aboard Cosmos suggest that plant roots are longer, and imply an enhanced rate of cell division in root tissue (6). Converse results have been obtained in Shuttle experiments (5). Furthermore, both Cosmos and Shuttle experiments have shown that morphological characteristics of some root cells are notably different (5, 6, 12, 13). There are suggestions that chromosomal aberrations may result from the space environment (8–10), which may in part account for a decline in cell multiplication rates observed in both cultured and root cells. Although limited, this array of information also suggests that the plant root may be a major target of space-environmental effects. Furthermore, reduced gravity interferes with traditional mechanisms of root nurturing, aeration, and nutrient delivery, making it difficult to pinpoint the underlying causes of aberrant root cell development.

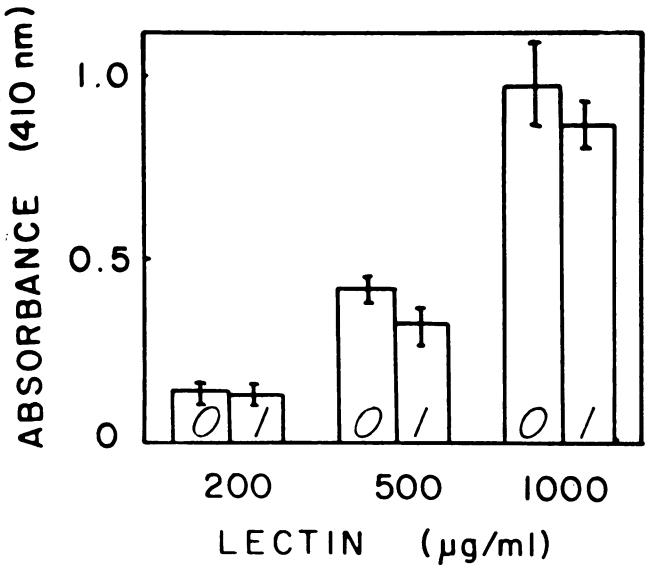

Figure 1. Extent of lectin binding to the rhizobial cell surface during microgravity (0) and unit gravity (1). Each experimental point shows the average of four trials, with error bars to show the standard deviation values.

Our experiments accomplish two important goals. First, we have documented that the association of a ligand (lectin) with a cell surface receptor is impervious to the effects of microgravity. Cell surface-mediated events are known to play a crucial role in development. When complex perturbations in development are observed, the underlying causal mechanisms often remain unclear. There are theoretical grounds for predicting that gravity is not a consideration for molecular interactions in solution (11); yet, the predictions are less clear when interacting molecules are associated with membranes or are immobilized on a cellular surface (11). Thus, it is important to document noneffects of microgravity at such levels. Our results are consistent with the recent demonstration that a different membrane-dependent model, the liposome-reconstituted cardiac gap junction, exhibits unimpaired channeling activity during reduced gravity (1).

Second, our results suggest that the first steps in legume

root nodule formation may occur in a reduced gravity environment. Nodule development, which integrates legume root growth, microbe/plant root cell communication, and tissue differentiation, may therefore be an intriguing model system for discriminating the cause(s) of aberrant root development in the space environment.

ACKNOWLEDGMENTS

Data, referred to in "Materials and Methods," concerning the gravitational environment during the May 31, 1989, flight were obtained by Drs. Bjarni Tryggvason and Bob Thirsk, National Research Council of Canada. Special acknowledgments to Mr. Bob Williams and Ms. Linda White of the NASA Reduced Gravity Research Program, Houston, TX, for their assistance during flight, and to Dr. Brian S. Spooner for assistance in experimental design, inflight operations, and for critical review of this manuscript.

LITERATURE CITED

1. **Claassen DE, Spooner BS** (1989) Effects of microgravity on liposome-reconstituted cardiac gap junction channeling activity. Biochem Biophys Res Commun **161:** 358–362
2. **Dazzo FB, Truchet GL, Sherwood JE, Hrabek EM, Abe M, Pankratz SH** (1984) Specific phases of root hair attachment in the *Rhizobium trifolii*-clover symbiosis. Appl Environ Microbiol **48:** 1140–1150
3. **Easterwood GW, Street JJ, Sartain JB, Hubbel DH, Bobitaille HA** (1988) Lunar base CELSS—A bioregenerative approach. *In* Lunar Bases & Space Activities in the 21st Century. NASA LBS-88-053. U.S. Government Printing Office, Washington, DC
4. **Halverson LJ, Stacey G** (1986) Signal exchange in plant-microbe interactions. Microbiol Rev **50:** 193–225
5. **Halstead TW, Dutcher FR** (1986) Plants in space. Annu Rev Plant Physiol **38:** 317–345
6. **Ilyin AE** (1983) Investigations on biosatellites of the Cosmos series. Aviat Space Environ Med Supp **54:** S9–S15
7. **Keen NT, Staskawicz B** (1988) Host range determinants in plant pathogens and symbionts. Annu Rev Microbiol **42:** 421–440
8. **Krikorian AD** (1988) Chromosomes and plant cell division in space. Am Soc Gravitational Space Biol Bull **1:** 13
9. **Krikorian AD, O'Connor SA** (1984) Experiments on plants grown in space: Karyological observations. Ann Bot **54:** Suppl 3, 49–63
10. **Krikorian AD, O'Connor SA, Smith DL** (1985) Space flight, chromosomes, and organized development in aseptically cultured plant cells (abstract). Physiologist **28:** 299
11. **Pollard EC** (1965) Theoretical studies on living systems in the absence of mechanical stress. J Theor Biol **8:** 113–123
12. **Tairbekov MG, Parfyonov GP** (1981) Cellular aspects of gravitational biology. Physiologist (supplement) **24:** 69–72
13. **Tairbekov MG, Parfyonov GP** (1981) Biological investigations aboard the "Biosatellite Cosmos-1129." Adv Space Res **1:** 89–94
14. **Wong PP, Shantharam S** (1984) Binding of pole bean (*Phaseolus vulgaris* L.) lectin and rhizobia that do or do not nodulate the pole bean. Can J Microbiol **30:** 1349–1356