A receptor protein from a host plant, *Trigonella foenum-graecum*, which binds to *Rhizobium meliloti* strains

R. Apte and V.V. Modi

Department of Microbiology, Faculty of Science, M.S. University of Baroda, Baroda-390002 (India), May 11, 1982

Summary. The correlation between release of receptor protein from germinating seeds, competence factor released by *Rhizobium* and nodulation indicates that the period of strongest interaction lies between the 3rd and the 4th day.

Symbiosis between bacteria of the genus *Rhizobium* and legume plants involves nodulation of the plant roots and biological fixation of atmospheric nitrogen. The *Rhizobium* legume root symbiosis is a specific relationship, because, for example, *Rhizobium leguminosarum* (Pea symbiont) does not infect clover (Trifolium) or *Trigonella* and similarly *R. trifolii* and *R. meliloti* do not infect pea. Several scientists have suggested that lectin, a receptor protein, may be involved in the specific recognition of particular *Rhizobia* by legumes, before their entry into the root. Lectins have the capacity to bind to, or recognize specific carbohydrate structures, and various species of legumes produce lectins with different binding capacities[1-3]. Bohlool and Schmidt[4] found that soyabean lectin binds 22-25 strains of *R. japonicum* but not any other rhizobial species. A similar pattern has been observed in white clover[5]. However, concanavalin-A, a lectin from jackbean was reported to bind to all strains of rhizobia tested, irrespective of their ability to infect and nodulate jackbean[6]. Chen and Phillip[7] surveyed a variety of species, and found no correlation between lectin binding and infectivity. The existing literature about the hypothesis is confusing and more work is required for clarification; we undertook our study with this in view.

Receptor protein was extracted from seeds, roots and nodules of *Trigonella foenum-graecum* L. in 0.9% NaCl and was precipitated by ammonium sulfate (0.90% saturated). Several different strains of *Rhizobium meliloti*; RM-03, 13, 1,5,7,11,17, M4014 and M4015 were used as homologous strains; and *R. japonicum*-D211, Sb-16, *R. phaseolous* and *R. trifolii* from cowpea were used as heterologous strains. The total protein in all the plant extracts was measured by the method of Lowry et al.[8], using bovine serum albumin as standard. Protein profiles were carried out by the gel electrophoresis method using polyacrylamide gels stained with Coomassie blue[9]. The hemagglutination test was performed by the method of Tobiska[10] using equal volumes of receptor protein (70%) and red blood cells (3%). For the nonimmune agglutination test equal volumes of *Rhizobium* culture and receptor proteins were used. Liberation of receptor protein during seed germination was carried out using sterile plates containing sterile moist filter papers. FITC labeling was performed by the method of Bohlool and Schmidt[4]. Nodulation was carried out as described by Vincent[11]. Molecular weight was determined by the gel filtration technique using Sephadex-G-100, and standard proteins, bacitracin and ovalbumin. Protein estimation data indicate that seeds contain the maximum amount. It was confirmed by the hemagglutination test that the receptor protein is a lectin, and all the 3 sources show nonspecific hemagglutination for all the blood groups (table 1). For the nonimmune agglutination test, non-heated, heated and phenol treated cells were tried (table 2) and all showed positive agglutination, which indicates that the binding sites on *Rhizobium* cells may be polysaccharides. Our findings here differ from those of Dazzo and Hubbell[4]. On polyacrylamide gel electro-phoresis, 4 bands were obtained from seeds and 2 from roots and nodules (fig. 1). Two bands from seed receptor protein and both the bands from roots and nodules showed cell binding ability and hemag-

Table 1. Receptor protein from *Trigonella foenum-graecum* L.

Source of extract	Protein (μg/ml)	Hemagglutination (for all blood groups)
Seeds	393.0	+ +
Roots	117.2	+ + +
Nodules	93.9	+

Table 2. Nonimmune agglutination test

Rhizobium culture	Agglutination		
	Non-heated R. cells	Heated R. cells	Phenol-treated R. cells
Homologous strains	+	+	+
Heterologous strains	−	−	−

R. cells. 0.5 ml + receptor protein 0.5 ml + N saline 2.0 ml.

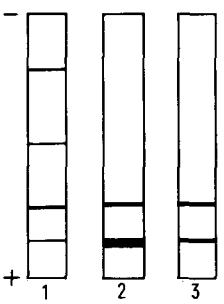

Figure 1. Zymograms of receptor protein obtained on gel electrophoresis. 1. Seed R.P.; 2. root R.P.; 3. nodule R.P.

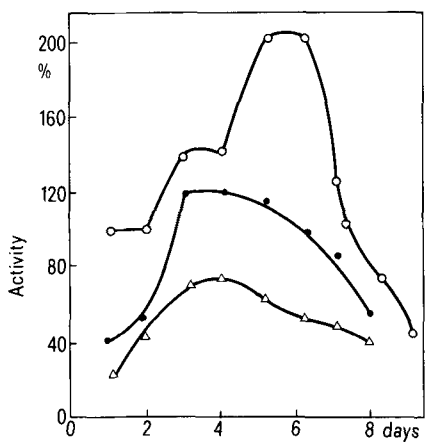

Figure 2. Correlation between release of receptor protein from germinating seeds and percent of RMO3 cells showing + ve binding and nodulation. ○, Liberation of receptor protein, μg/g of seeds. ●, Nodulation/10 plants. △, percent FITC binding. Days - i.e. Days after the treatment. 1. Liberation of R.P. from seeds kept for germination on each day. 2. Nodulation, days on which seeds were inoculated. 3. Days indicate age of *Rhizobium* strain RMO3.

glutination. By Schiff's staining it was found that the receptor protein is a glycoprotein. The molecular weight of the receptor protein as found by the gel filtration technique is approximately 40,000 daltons.

In the seed germination experiment, as the seeds germinate the receptor protein is liberated into the surrounding medium; there is a specific period, i.e. the 3rd and 4th day, when a moderate amount of lectin is available in the medium and the *Rhizobia* are in the competence stage, and at this particular time the binding takes place. Receptor protein released after this period is of no use, once the binding is over. The maximum amount is released on the 5th and 6th day, after which the leaching of receptor protein declines. In the nodulation test the highest nodulation obtained is in seeds inoculated on the 3rd and 4th day. Thus the correlation between release of receptor protein from germinating seeds, FITC cell binding of *Rhizobium* and nodulation indicates that the best period for interaction lies between the 3rd and 4th day (fig. 2). Nodulation and cell binding by the FITC technique were shown only by homologous *Rhizobia* the heterologous strains failed to show it (table 3). These results are in agreement with those of Bohlool and Schmidt[4], Bhuvaneswari et al.[3] and others, indicating that lectin receptor protein may have the role of controlling specific binding in *Rhizobium* - legume root nodule symbiosis.

Table 3. Nodulation and cell binding ability of different *Rhizobium* cultures

Rhizobium strain	Nodulation per plant	% R. cell binding by FITC technique
1. M-13	+	65
2. D211	−	0
3. M-03	+	54
4. R. phaseolus	−	0
5. M-4015	+	60
6. R. trifolii	−	5
7. M-1	+	44
8. M-4013	+	52
9. M-11	+	50
10. M-5	+	45
11. M-7	+	48
12. M-17	+	41
13. R. cowpea	−	0
14. Sb-16	−	0

1 Lis, H., and Sharon, N., A. Rev. Biochem. *42* (1973) 541.
2 Bauer, W.D., in: Genetic engineering for nitrogen fixation, p. 283. Ed. A. Hollander. Plenum Press, New York 1977.
3 Bhuvaneswari, T.V., Pueppke, S.G., and Bauer, W.D., Pl. Physiol. *60* (1977) 486.
4 Bohlool, B.B., and Schmidt, E.L., Science *185* (1974) 269.
5 Dazzo, F.B., and Hubbell, D.H., Appl. Microbiol. *30* (1975) 1017.
6 Dazzo, F.B., Pl. Soil *43* (1975) 713.
7 Chen, A.T., and Phillips D.A., Physiologia Pl. *38* (1976) 83.
8 Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., J. biol. Chem. *193* (1951) 265.
9 Shuster, L., Meth. Enzym. *22* (1971) 412.
10 Tobiska, J., Die phythamagglutinine Hämatologie und Blutransfusionswesen, vol. 3, p. 169. Adaemic Verlag, Berlin 1964.
11 Vincent, J.M., I.B.P. handbook No. 15. Blackwell, Oxford 1970.
12 Stacey, Gary, Paau, A.S., and Brill, W.J., Pl. Physiol. *65* (1980) 67.

0014-4754/83/050509-02$1.50 + 0.20/0

Monoamine oxidase A deficit in liver of germ-free rats

M. A. Reveley[1], V. Glover, M. Sandler[2] and M. E. Coates

Bernhard Baron Memorial Research Laboratories and Institute of Obstetrics and Gynaecology, Queen Charlotte's Hospital, Goldhawk Road, London W6 OXG (Great Britain), and Nutrition Department, National Institute for Research in Dairying, Shinfield, Reading RG2 9AT (Great Britain), September 27, 1982

Summary. Monoamine oxidase A but not B was found to be significantly decreased in the liver of germ-free compared with conventional rats. This change is the opposite of that found in germ-free chicks. Rat brain enzyme was not affected by the germ-free state.

Phillips et al.[3] reported significantly higher monoamine oxidase (EC 1.4.3.4; MAO) activity in the liver of the germ-free chicks compared with conventionally-bred controls. Böhm et al.[4] confirmed this difference for both the A and B forms of MAO in liver, but were unable to find any change in brain MAO activity between germ-free and conventional animals of this species. In the present study we have attempted to determine whether a similar pattern was present in the rat, where gross anatomical changes have been noted in the gut of the germ-free animal[5]. In the event, a striking difference in enzyme activity was observed between the 2 species.

Method. Male rats (268–383 g) of the Lister hooded strain were used. They were born and reared germ-free in Gustafsson stainless steel isolators[6] until weaning. Part of each litter was retained in the germ-free isolators and the rest of the animals were removed to a conventional environment where the conditions of lighting, humidity and temperature were matched to those obtaining in the isolators. In order to ensure efficient establishment of the indigenous gut microflora, droppings from healthy conventional rats were scattered on the food during the first day after removal from the isolators. The animals were housed similarly in both environments, in wire-mesh rat cages without bedding. They all received a cubed diet (Spiller's Small Animal Diet, Dalgety Ltd, Pangbourne, Berks, U.K.) sterilized by gamma-radiation, and weekly supplements of vitamin E, K and B_{12}. Sterile drinking water (Smedley's, Whiteleaf, Surrey) was supplied to the germ-free rats and tap water to their conventional controls.

The rats were reared to 8 weeks of age, during which time growth and physical condition were normal in both environments. Sterility checks, described by Fuller[7], were performed on the germ-free rats, which remained free from contamination throughout the experiment. At the end of the 8-week period the mean body weights of the 2 groups