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Stimulation of adhesiveness, infectivity, and competitiveness for nodulation of *Bradyrhizobium japonicum* by its pretreatment with soybean seed lectin

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Abstract

Soybean seed lectin stimulates adsorption of *Bradyrhizobium japonicum* to its host roots. Pretreatment of the rhizobia with soybean seed lectin for at least 6–12 h previous to their interaction with the plants was required to detect the stimulatory effect. This activity could be observed with as few as 1000 soybean seed lectin molecules per bacterium, and required specific carbohydrate binding. Infectivity and competitiveness for nodulation were also stimulated by preincubation of the rhizobia either with soybean seed meal extract or soybean seed lectin, the extract being more effective in enhancing competitiveness. © 2000 Published by Elsevier Science B.V. on behalf of the Federation of European Microbiological Societies.

Keywords: Rhizobium; Soybean seed lectin; Infectivity

1. Introduction

The soil bacterial species *Bradyrhizobium japonicum* forms nitrogen-fixing nodules on the roots of soybean (*Glycine max*). Nodule development is achieved through a complex infection process, which expresses the mutual specific recognition among the symbiotic partners. Lectins from plants [1] and from bacteria [2] were reported to be involved in key steps of this process, such as the rhizobial adsorption to the root surfaces [2–4] and root hair infection [5,6].

Adsorption of rhizobia to host legume roots occurs in specific and non-specific modes, as was demonstrated by Favelukes and co-workers in *B. japonicum* [4] as well as in the fast-growing *Sinorhizobium meliloti* [7] and *Rhizobium etli* [8] systems. Specific and non-specific modes of adsorption have differential properties: specific adsorption is resistant to the presence of a 10^4 excess of heterologous competitor rhizobia [4,7,8], and requires the presence of the divalent cations Ca^{2+} and Mg^{2+} in the fast-growing but not in the slow-growing systems [4,8,9]. In *B. japonicum*, specific adsorption could be associated with polar binding of the rhizobia to emergent root hairs [3], and is prevented by the hapten of the bacterial lectin BJ38, while non-specific adsorption is resistant to the presence of this sugar [3,4]. Moreover, a role for BJ38 in the nodulation process was suggested by the delay in nodule formation displayed by *B. japonicum* chemical mutants in BJ38 [3]. *B. japonicum* attachment to soybean roots was shown to be insensitive to the presence of *N*-acetyl-D-galactosamine (D-GalNAc) [4,10], the known hapten for the soybean seed lectin (SBL) [11], thus suggesting no role for the sugar-binding activity of this plant lectin during specific or non-specific adsorption.

Other reports indicate that the plant lectins SBL [5] and common bean phytohemagglutinin (PHA) [6] stimulate early events of the respective root infection, although it was not possible to attribute the stimulation of infectivity to stimulation of an earlier step, such as adsorption [5]. However, a root-exuded protein that stimulates *S. meliloti* adhesiveness was described [12] and a preliminary indication suggested that this might be the alfalfa agglutinin

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(AAG) [13]. Both the alfalfa root-exuded protein, and SBL are present at very low concentration in the respective root exudates [12–15] and thus a dilute rhizobial population as well as a prolonged preincubation time before exposure of rhizobia to plants are required in order to observe a significant stimulatory effect on adhesiveness [12] or infectivity [5,14]. Moreover, stimulation of infectivity by SBL required de novo RNA and protein synthesis in the rhizobia during the preincubation time [5]. Hence, the above failures to demonstrate an effect of the plant lectin activity on soybean adsorption by *B. japonicum* [4,5,10] could be due on the one hand to the use of high rhizobial concentrations [5,10] and on the other, to the omission of pretreatment of rhizobia with SBL before their incubation with the plants [4,10].

The involvement of seed lectin activities in symbiosis was clearly demonstrated in other experiments, where transgenic legumes carrying a heterologous lectin gene were shown to extend their symbiotic range to the rhizobia specific for the lectin-donor plant species [15–17]. Although lectins are highly selective sugar-binding proteins, neither the role of the D-GalNAc-binding SBL nor that of the mannose-binding pea seed lectin (PSL) in extending the host range in transgenic plants is related to the binding of lipochitooligosaccharide Nod factors [15,17]. In addition, red clover hairy roots expressing the *psl* gene became sensitive to a range of heterologous rhizobial species and Nod factors, which normally do not trigger any response in pea or in red clover, suggesting that the presence of this foreign lectin removes a barrier that normally precludes a mitotic response to the heterologous signals [17]. This activity was abolished by altering the carbohydrate-binding sites in both PSL and SBL [15,17].

Although the symbiotic role of plant lectins is not yet clear, an important clue was obtained when it was shown that adsorption of *B. japonicum* to transgenic *Lotus corniculatus* roots harboring the *Le 1* SBL-encoding gene was greater than to control wild-type plants [15]. However, in this artificial system, the host lectin was also present, and interactions between both lectins could lead to unknown side effects. Thus, it remains to be determined how these plant proteins are involved in adsorption of *B. japonicum* to wild-type soybeans. Moreover, stimulation of infectivity could, in principle, give rise to better competitiveness for nodule occupation, which is a very desirable trait for the development of more efficient rhizobial inoculants. Although the SBL enhancement of *B. japonicum* infectivity has been previously shown, research into possible improvement of rhizobial competitiveness by plant lectins has scarcely been investigated.

In the present work, we address the above questions by studying both the activity of SBL in stimulating *B. japonicum* for adhesiveness, and the impact of this protein on rhizobial infectivity as well as on competitiveness for nodule occupation.

2. Materials and methods

2.1. Plant and rhizobial strain growth and maintenance

B. japonicum LP 3001 (Sp^r Sm^s), and LP 3004 (Sp^s Sm^r) are Nod^+ Fix^+ spontaneous derivatives of USDA 110. Both strains have the same competitiveness for nodule occupation as the original USDA 110 (A. Lodeiro, Ph.D. Thesis, UNLP). Rhizobia were grown in yeast extract–mannitol broth (YMB) or yeast extract–mannitol agar 1.5% (YMA) [18] with 100 mg l^{-1} antibiotics. For plant experiments, YMB cultures were harvested at late exponential phase [4].

Soybean Federada INTA was provided by INTA, Argentina. Seeds were surface-sterilized and germinated as described [8].

2.2. Agglutinins, hapten and antibodies

Common bean phytohemagglutinin PHA-P (PHA), which contains both E- and L-type subunits, was purchased from Sigma. AAG (purified and tested for agglutination of *S. meliloti* cells as already described [19]) was kindly provided by Dr. L.G. Wall. D-GalNAc, ε-aminocaproyl-β-D-galactosamine agarose, anti-SBL antibody, and alkaline phosphatase-labeled anti-rabbit IgG were purchased from Sigma.

2.3. Preparation of soybean seed meal extracts

Soybean seeds were ground and sieved through a 0.84-mm mesh. This powder was suspended in *N*-hexane for 1 h at −20°C, filtered and air-dried. Then it was suspended in N-free Fåhraeus solution [20] for 2 h at 4°C with stirring, and centrifuged at $10\,000 \times g$ for 20 min. The supernatant was the soybean meal extract (SME).

2.4. Preparation of soybean seed lectin

The SME was fractioned with ammonium sulfate between 30 and 70% saturation, and after resuspension and desalting, it was loaded into an ε-aminocaproyl-β-D-galactosamine agarose affinity column [21] at a rate of 1 ml h^{-1} at 4°C. The non-retained fraction of protein material was pooled and the column washed until 280 nm absorbance reached the blank value. Afterwards, SBL was eluted with 0.1 M galactose, and finally SBL was pooled, desalted, and lyophilized. The lectin-containing fractions were identified by their ability to agglutinate 2% human group A erythrocytes, and by electrophoretic analysis (see below). All the fractions were maintained at −20°C. Protein concentrations were determined as described [22].

2.5. Protein electrophoresis and immunoblotting

Non-denaturing PAGE was performed with 7.5% poly-

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acrylamide at pH 8.8 [21], while SDS–PAGE was performed as previously described [23]. Gels were silver-stained [24] and molecular masses were calculated with a Dalton Mark VII-L kit from Sigma. Lectins were detected in Western blots with rabbit anti-SBL antibody and with alkaline phosphatase-labeled anti-rabbit IgG.

2.6. Plant experiments

YMB-grown rhizobia were pretreated for 72 h (unless otherwise indicated) in N-free Fåhraeus solution at 28°C with no shaking at a density of ca. 10^7 CFU ml^{-1} – according to the protocol of Halverson and Stacey [5,14] – in the presence of either different lectins or samples from the SBL purification procedure; control rhizobia were always pretreated in the same conditions, without protein. After this pretreatment period, rhizobia were diluted about 1×10^3 into fresh Fåhraeus solution to study their adhesiveness, infectivity, or competitiveness.

Adsorption was measured as described [4]. Briefly, 1–5×10^4 rhizobia were incubated for 1 h in 50 ml Fåhraeus solution with ten 4-day-old soybean plants. After incubation, the roots were washed to remove loosely adsorbed rhizobia. Next, the roots were embedded in YMA to allow the firmly adsorbed rhizobia to develop microcolonies, which were counted with the aid of a dissecting microscope. Total counts of visible microcolonies on all primary roots, expressed as percentage of the total number of colony forming units present during the incubation, represent the adsorption index (%*A*). Stimulation of adsorption is the difference in %*A* between protein-pretreated and control rhizobia, as percentage of %*A* of control rhizobia [12].

Infectivity was studied by inoculating 42 plants with 5×10^4 rhizobia $plant^{-1}$ in plastic growth pouches. The infection rate was obtained from the mean distance of the uppermost nodule to the root infectible zone at inoc-

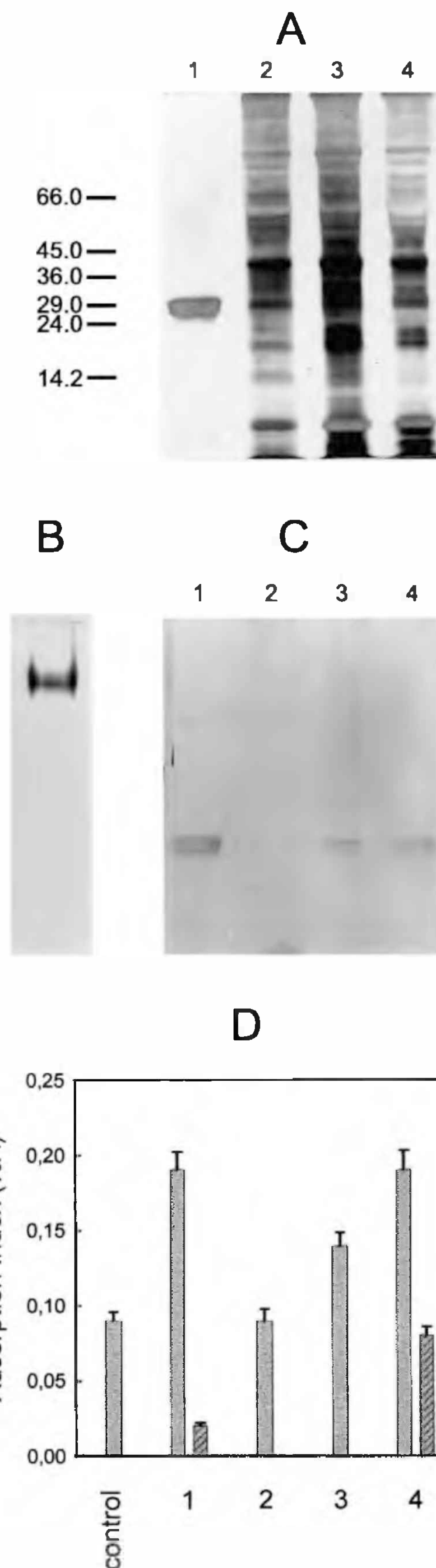

Fig. 1. Electrophoretic analysis and *B. japonicum* adhesiveness stimulation activity of samples from different SBL purification steps. (A) SDS–PAGE showing the purified SBL (lane 1), the non-retained fraction from the affinity column (lane 2), the ammonium sulfate precipitate (lane 3), and the SME (lane 4). twenty nanograms of protein were applied to lane 1, and 30 μg of protein to each one of lanes 2–4. The position of molecular mass standards is shown at the left. (B) Native PAGE of purified SBL (7.5% acrylamide, pH 8.8). (C) Western blot of the same protein samples as in A, incubated with a rabbit anti-SBL antibody, and alkaline phosphatase-labeled anti-rabbit IgG. (D) Stimulation of adsorption of *B. japonicum* LP 3001 to soybean roots. The rhizobia were preincubated in Fåhraeus solution at a final density of 2×10^6 CFU ml^{-1} for 72 h at 28° without shaking. Preincubations were done with no additions (control) or else with the addition of 2 μg ml^{-1} purified SBL (1), or 3 mg ml^{-1} of protein from either the non-retained fraction from the affinity column (2), the ammonium sulfate precipitate (3), or the SME (4). In some treatments, preincubations were also carried out in the presence of 45 μM D-GalNAc (crossed bars). After preincubations, the rhizobia were diluted 1:1000 in 50 ml fresh Fåhraeus solution and the adsorption index (%*A*) to 10 soybean rootlets was determined. Error bars indicate 95% confidence intervals.

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ulation, with an estimated root growth rate of 2.4 mm h^{-1} [25].

Competitiveness was assayed using 1:1 mixtures of LP 3001 and either LP 3004 or USDA 110 containing approximately 10^4 rhizobia ml^{-1} of each strain, prepared in bottles containing 800 ml of N-free Fåhraeus plant nutrient solution. The rhizobia-containing solution was added to 1.5-litter vermiculite pots. Next, three soybean plantlets were aseptically transferred to each pot and left for 25 days in the greenhouse at 26/18°C day/night temperature, with watering as required. Then, nodules were excised and surface-sterilized for 5 min with 20% v/v commercial bleach followed by six washes with sterile distilled water. Surface-sterilized nodules were crushed individually, and their contents plated onto YMA replica plates with selective antibiotics.

3. Results

3.1. Stimulation of adsorption of B. japonicum to soybean roots

We obtained SBL from Federada INTA soybean seeds following an already established affinity purification procedure. The electrophoretic analysis of relevant samples is shown in Fig. 1A–C. The SME, the ammonium sulfate fraction, and the non-retained fraction from the affinity column all gave complex banding patterns on SDS–PAGE, whereas the SBL eluted from the affinity column with 0.1 M galactose gave a single, wide band of ca. 30 kDa, as expected for the glycosylated SBL subunit (Fig. 1A). We further assessed the purity of our SBL preparation on non-denaturing PAGE, where again a single band was observed (Fig. 1B). The SBL obtained was also able

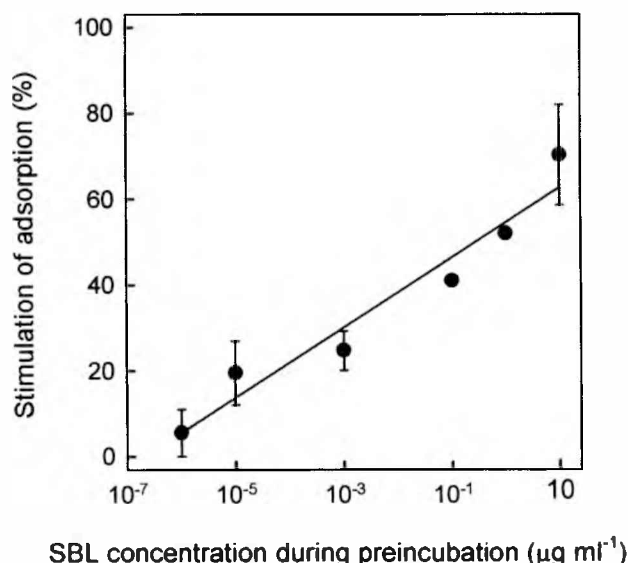

Fig. 2. Stimulation of adsorption of *B. japonicum* LP 3001 to soybean roots by pretreatment with different SBL concentrations. The rhizobia were preincubated in Fåhraeus solution at a final density of 1.5×10^7 CFU ml^{-1} with the addition of serially diluted SBL during 72 h at 28°C without shaking. Finally, the rhizobia were diluted 1:1000 in 50 ml fresh Fåhraeus solution and the adsorption index (%*A*) to 10 soybean rootlets was determined. Stimulation of adsorption was then calculated with reference to a control pretreated in the same conditions, without SBL ($\%A = 0.10 \pm 0.01$). Error bars indicate 95% confidence intervals; when not shown, they were smaller than the symbol.

to agglutinate a 2% suspension of group A human erythrocytes (not shown). All fractions, except the fraction not retained by the affinity column, gave a signal on a Western blot at the position expected for the SBL subunit (Fig. 1C). Pretreatment of *B. japonicum* in the SBL-containing fractions stimulated rhizobial adsorption to soybean roots up to 100%, while pretreatment in the SME containing all

Table 1
Competition for nodulation in 1:1 mixtures (inoculum concentration range: 1.0–1.5 10^4 rhizobia ml^{-1}) of *B. japonicum* LP 3001 (Sp^r Sm^s) pretreated in different protein solutions, and *B. japonicum* USDA 110 (Sp^s Sm^s, Expt. 1) or LP 3004 (Sp^s Sm^r, Expt. 2), pretreated in Fåhraeus solution without any addition, in two independent experiments

Addition to Fåhraeus solution	Percentage of nodules occupied by rhizobia with the following antibiotic resistance marks[a]:			Nodules analyzed
	Sp^r	Sp^s		
Expt. 1. LP 3001:USDA 110				
None	66*	34*		173
SBL 10 μg ml^{-1}	75*	25*		182
SME 4 mg protein ml^{-1}	85**	15**		182
	Sp^r	Sm^r	Sp^r+Sm^r	
Expt. 2. LP 3001:LP 3004				
None	46	32	22	184
SBL 10 μg ml^{-1}	55*	11*	34	150
SME 4 mg protein ml^{-1}	85**	3**	12	174

The percentages of nodules occupied by each strain were statistically analyzed with ANOVA. Statistical significance of deviations from 50%:50% occupation (into each row) is shown.
*Statistically different with $P < 0.05$.
**Statistically different with $P < 0.01$.
[a] Sp^r+Sm^r represent nodules occupied by both strains. In Expt. 1, Sp^r include nodules occupied either by LP 3001 only or by both strains.

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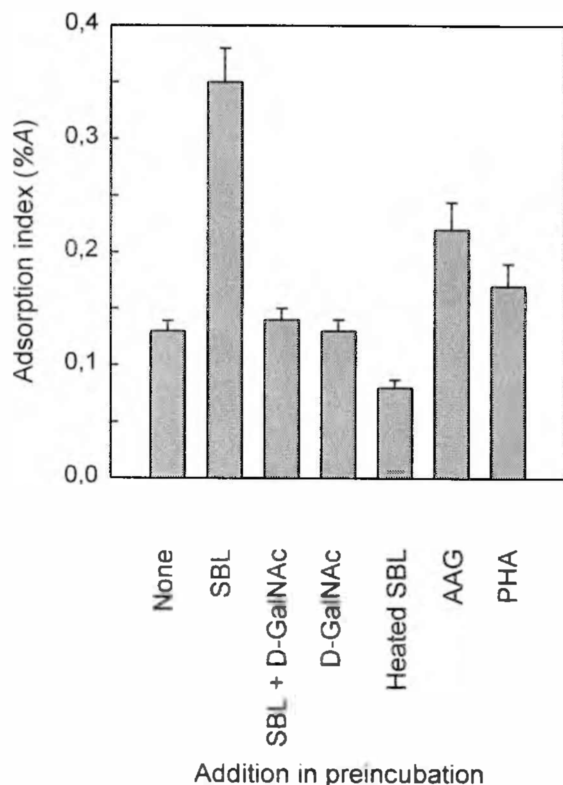

Fig. 3. Stimulation of adsorption of *B. japonicum* to soybean roots by pretreatment with different lectins, all at a concentration of 10 μg ml^{-1}. The rhizobia were pretreated in Fåhraeus solution at a final density of 1.6×10^7 CFU ml^{-1} in the presence of different lectins for 72 h at 28°C without shaking. Finally, the rhizobia were diluted 1:1000 in 50 ml fresh Fåhraeus solution and the adsorption index (%*A*) to 10 soybean rootlets was determined. Heat treatment of SBL was 100°C 30 min. Error bars indicate 95% confidence intervals.

the proteins except SBL did not (compare lane 2 with the others in Fig. 1A,C,D). In addition, the presence of 45 μM D-GalNAc in the SME and SBL pretreatments blocked the stimulatory effect (Fig. 1D). No loss of viability was observed throughout the pretreatments (data not shown).

In Fig. 2, we show the effect of SBL concentration during rhizobia pretreatment on stimulation of adsorption. We obtained a linear increase of stimulation with increasing log-concentrations of SBL. Very low SBL concentrations were enough to obtain detectable effects; with 360 ng ml^{-1} SBL (ca. 10^5 SBL molecules per bacterium) 50% stimulation of adsorption could be obtained, whereas only 4 ng ml^{-1} SBL (ca. 1000 SBL molecules per bacterium) is enough to observe a statistically significant difference from the control without SBL.

The inhibition of the SBL effect by a micromolar concentration of D-GalNAc strongly suggested that carbohydrate-binding is required for such an effect. To assess the specificity of the carbohydrate binding requirement, we pretreated the rhizobia with heat-denatured SBL, or two heterologous agglutinins, in comparison with 10 μg ml^{-1} SBL either in the presence or absence of 45 μM D-GalNAc. As observed in Fig. 3, we confirmed that the lectin stimulatory effect is blocked by the SBL hapten, and that the native protein is required. The heterologous lectins gave a much lesser, but yet statistically significant, stimulatory effect.

Finally, to study the time course of the stimulatory effect, we suspended the LP 3001 rhizobia in the Fåhraeus solution with or without 10 μg ml^{-1} SBL and pretreated them for 0, 6, 12, 24, or 72 h in the conditions already described. After pretreatment and dilution, we measured %*A* as before, and observed that stimulation of adsorption sharply increased until 12 h of pretreatment, while from 24 h on it remained relatively constant (Fig. 4A). The increase in adsorption stimulation until 12 h was due to an increase in the net number of adsorbed rhizobia to the roots without a parallel growth of free rhizobia (Fig. 4B). After this lag – likely to reflect the period of adaptation of rhizobia to the diluted medium – rhizobia started to divide; thereafter, the adsorption index diminished either with or without SBL (Fig. 4B) even when the number of adsorbed rhizobia continued to increase. Adhesiveness of rhizobia pretreated in Fåhraeus solution without SBL also increased during the 12-h lag of pretreatment, but less than with SBL (Fig. 4B).

3.2. *Effects of SME and SBL on nodulation*

Soybean plants inoculated with rhizobia pretreated either in Fåhraeus solution, SME, or SBL, as described above, were similar in their nodule, shoot or root masses (not shown), thus suggesting that lectin pretreatment has no long-term effect on nodulation or nitrogen fixation.

In order to search for short-term effects, we studied infectivity in plastic growth pouches. Mean distances of uppermost nodules elicited by LP 3001 rhizobia pretreated for 72 h either in SME (containing 4 mg protein ml^{-1}) or 10 μg ml^{-1} SBL to the root tip (RT) mark made at inoculation were -7.5 ± 5.5 and -7.5 ± 4.9 mm, respectively; negative numbers indicate that the positions referred to are in an area of the root zone that is younger than the area between the RT and the smallest emergent root hairs (SERH) marks; for further details see [25]. Since the mean distance between the SERH and the RT marks was 7 ± 0.45 mm, these results indicated that infections were initiated within 3–6 h after inoculation. In a control pretreated in Fåhraeus solution without SBL, and inoculated in the same conditions, the mean distance of the uppermost nodule to the RT mark was -19.0 ± 5.0 mm, demonstrating that infections started at about 8–11 h after inoculation.

The above result suggested that pretreatment with SME or SBL could also enhance competitiveness for nodule occupation. Therefore, we inoculated 10 soybean plants per treatment with 1:1 mixtures of LP 3001 and USDA 110 (Table 1, Expt. 1), or LP 3001 and LP 3004 (Table 1, Expt. 2) to examine nodule occupation by each strain. In both experiments, LP 3001 was suspended for 72 h at 28°C

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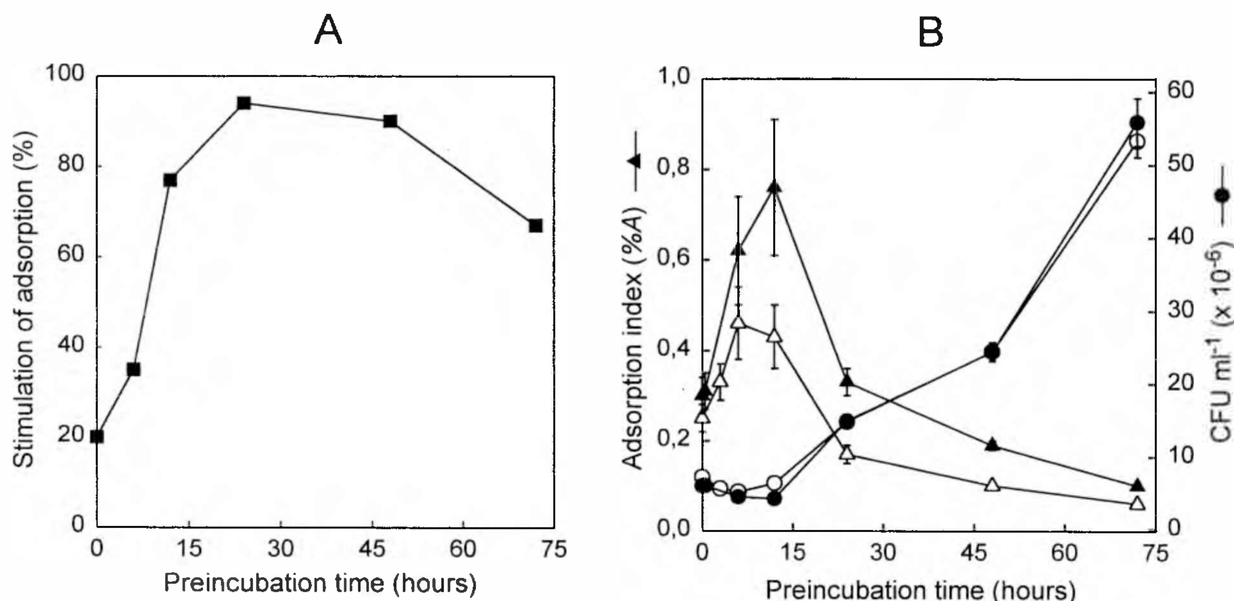

Fig. 4. Time course of expression of the SBL adhesiveness stimulation activity. The rhizobia were pretreated in Fåhraeus solution at an initial density of 3.0×10^6 CFU ml^{-1} with or without 10 μg ml^{-1} SBL for 0, 6, 12, 24, or 72 h at 28°C without shaking. At the end of each pretreatment period, the rhizobia were diluted 1:1000 in 50 ml fresh Fåhraeus solution and the adsorption index (%*A*) to 10 soybean rootlets was determined. (A) Stimulation of adsorption of the SBL-pretreated rhizobia with respect to the controls without SBL at the indicated times. (B) adsorption index (triangles) and CFU ml^{-1} (circles) of rhizobia pretreated with (filled symbols) or without (open symbols) SBL for the indicated times.

in Fåhraeus solution containing either SME or SBL, or no supplementation at all. Competitor USDA 110 and LP 3004 strains were pretreated in Fåhraeus solution with no supplementation, in the same conditions as LP 3001. A clear increase in competitiveness for the strain LP 3001 was observed when it was pretreated in SME or SBL (Table 1).

4. Discussion

SBL was the only soluble protein from soybean seeds that stimulated adsorption of *B. japonicum* to its host roots (Fig. 1D). The effect of stimulation of adsorption could be observed at very low concentrations of the lectin (Fig. 2), required the native protein, and was reversed by 45 μM concentration of its specific sugar hapten D-GalNAc (Figs. 1D and 3). This inhibitory activity of D-GalNAc at such a low concentration agrees with the concentration range at which the specific inhibition of erythrocyte agglutination was observed [11], but is three orders of magnitude lower than the concentrations used previously in adsorption assays, where it had no activity either in specific or in non-specific adsorption of *B. japonicum* to soybean roots [4,10]. This suggests that stimulation of adsorption by SBL is mediated by specific carbohydrate binding, and that preincubation of rhizobia in the SBL is an absolute prerequisite for obtaining a measurable effect. Stimulation was also assayed in the presence of two heterologous agglutinins: PHA and AAG, for which preliminary reports exist on their stimulatory activities for adsorption and early infection in their respective symbiotic systems (Lodeiro, 1994 Ph.D. Thesis, UNLP and [6,13]). PHA is a heterotetramer, while SBL is a homotetramer, both lectins being similar in subunit fold, quaternary structure, and each having one carbohydrate-binding site per subunit [26]. AAG has a subunit molecular mass of 14 kDa [19], lower than the mean molecular mass of legume lectin subunits [27], and its subunit fold, quaternary structure, and carbohydrate-binding sites have not yet been described. For this reason, the experiment in Fig. 3 was conducted with similar mass:volume protein ratios. Assuming a linear stimulation response with log-increases of concentration for PHA as shown for SBL in Fig. 2, the difference in molar concentration of PHA due to its 13% higher molecular mass with respect to SBL leads to a negligible difference in the theoretical %*A* value that would have been reached if PHA were at the same molar concentration as SBL. Although the %*A* values obtained by preincubation in PHA or AAG were significantly higher than for control or SBL+D-GalNAc preincubations (Fig. 3), the above calculations suggest that these low levels of enhanced adsorption caused by PHA or AAG could be due to improper sugar-binding and not to a difference in the number of available sugar-binding sites.

Stimulation of adsorption required a period of pretreatment of the rhizobia of at least 6 h in the absence of the plants, and was fully observed only after 12 h preincubation, coincident with the lag period before the onset of cell division in the Fåhraeus solution (Fig. 4). The increase in %*A* with preincubation was observed either with or without SBL, but to a lesser extent in its absence. Since the

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early infection activities of rhizobia are stimulated by incubation in nutrient-limited media [28,29] this result suggests that SBL stabilizes or enhances a process that already takes place as a consequence of the transfer of the rhizobia from the richer YMB to the C- and N-free Fåhraeus mineral solution. This time requirement could also explain why no SBL effect could be observed directly in the plant-rhizobia incubation medium [4,10]. The minimum pretreatment period that gave rise to a significant stimulatory effect for adsorption (6–12 h) was similar to that of reduction in the time required for the earliest infections (5 h) with the same rhizobial concentrations, suggesting that stimulation of adsorption and infection might be coupled.

SBL is released into the soybean root exudate [14,15], as AAG [13] and PHA (A. Lodeiro, 1994, Ph.D. Thesis, UNLP). However, a role for this lectin in establishing a bridge between the plant and bacterial surfaces [30] seems unlikely, since: (1) stimulation occurred with a very small amount of SBL molecules per bacterium (see Fig. 2 and also [5]), well below the amount of lectin molecules required to cover all the bacterial receptors [5]; (2) the rhizobial SBL receptors are located opposite to the cell pole where the bacterial lectin BJ38 accumulates, which indeed attaches to the root hair surface and plays a key role in determining symbiotic specificity in the process of adsorption [4]; and (3) the delay in time observed between the first contact of the rhizobia with the lectin and the expression of the stimulatory effect (Fig. 4) was much longer than the time required for bacterial binding of SBL [5]. Moreover, stimulation of infectivity by SBL also requires de novo protein synthesis in the rhizobia [5] and we have preliminary indications that this is also the case for adsorption (not shown). It was suggested that lectin-promoted rhizobial accumulation at the root hair tips surface might enhance Nod factor delivery to the plant membrane [15]. Even though the present results, together with our previous study [4], agree with this proposal, they suggest that rhizobial anchoring in the root hair surface is mediated by the bacterial lectin, while the plant lectin appears to act indirectly, by promoting in rhizobia unknown physiological changes in a long-term process which affect the level of adsorption.

We furthermore observed that rhizobia preincubated either in SBL or in lectin-containing SME were significantly more competitive than control rhizobia. Competitiveness of SME-preincubated rhizobia was reproducibly higher than that of SBL-preincubated rhizobia (Table 1). However, stimulation of adsorption was similar for both preincubations (Fig. 1D). This suggests that an additional component in the SME could enhance nodulation efficiency independently of the stimulation of adsorption. Flavonoids present in the SME could have accelerated the lipochitooligosaccharide synthesis and release by the rhizobia and this factor in turn could have stimulated nodule formation in parallel with stimulation of adsorption by SBL. Therefore, the addition of SME to rhizobial inoculants might improve their competitiveness by enhancing rhizobial adhesiveness and infectivity, and by promoting nodulation efficiency.

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