The lectin level and the effect of abscisic acid on hemagglutinating activity during rye germination

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Abstract

As we have previously found, the embryo is the only source of lectin in the mature, dry rye seed. During early germination the lectin activity decreased and most of it was found in the coleoptile. Leaves were found to contain only a trace amount of this protein. A 5-7 fold increase in the amount of lectin was found in grains which had imbibed for 6-12 hours in the presence of 10^{-5} M and 10^{-4} M ABA. At an ABA concentration of 10^{-4} M, about 6 μ g of lectin per germ was found even in grains which had been germinating for 5 days. The immunological properties and molecular masses of both RGA and RGA-like lectin accumulated in the presence of ABA were found to be identical.

Key words: lectin, abscisic acid, germination, rye seedling

INTRODUCTION

The molecular structure and occurrence of lectins in the grains of *Gramineae* plants have been studied intensively and well documented (Peumans et al. 1982a, b, Peumans and Stinissen 1983, Mishkind et al. 1983a, b). Less is known about their presence in developing tissues and about the changes in their levels during this process. The biosynthesis and accumulation of lectins during embryogenesis and seed

Abbreviations: RGA — rye germ agglutinin, ABA — abscisic acid, IgG — immunoglobulin G, FITC — fluoresceine isothiocyanate, HRPO — horseradish peroxidase, SDS-PAGE — sodium dodecyl sulfate polyacrylamide gel electrophoresis, TGE — trypsinized glutarylated erythrocytes

formation has been confirmed (Peumans et al. 1982c, Triplett and Quatrano 1982, Peumans and Stinissen 1983, Quatrano et al. 1983, Raikhel and Quatrano 1986) as has the role of abscisic acid in these processes (Triplett and Quatrano 1982, Quatrano et al. 1983, Raikhel and Quatrano 1986). Less interest has been paid to the lectin level changes accompanying the early stages of imbibition and germination.

In our previous study we have reported that in rye grains, lectin is located in the embryo (Ferens and Morawiecka 1985b). In this study we have traced the occurrence of lectin in the developing rye seedling tissues and the effect of abscisic acid on the agglutinating activity.

MATERIAL AND METHODS

Plant material. Rye grains var. Dańkowskie Złote harvested in 1985 were obtained from Plant Breeding Station Rogaczewo. Plants were grown in Petri dishes on filter paper moistened with water, in natural day-and-night conditions at room temperature, for 5 to 8 days. Cultures containing ABA at concentration of 10^{-7} , 10^{-5} and 10^{-4} M were conducted simultaneously. Samples were collected after 6, 12 and 24 hours of imbibition and then after every 24 hrs. The samples consisted of 100 caryopses imbibed for 6 and 12 hrs, 50 1- and 2-day old plants and 25 older seedlings. Embryos were isolated from imbibed caryopses while leaves, roots and coleoptiles were taken for analysis from the seedlings. After weighing, the tissues were homogenized in a glass homogenizer in a 2-fold volume of 100 mM acetate buffer, pH 5.1. After 1 hour of extraction followed by centrifugation the clear supernatants were collected for further use.

Protein and lectin assays. The protein content was determined according to Lowry et al. (1951). Hemagglutination tests with trypsinized and glutarylated rabbit erythrocytes were carried out on microtiter plates as described previously (Ferens and Morawiecka 1985b).

The results are expressed in agglutination units. One unit is defined as the amount of lectin which agglutinates 20 mm³ of rabbit TGE under the conditions of the method and corresponds to 20 µg of purified RGA.

Electrophoretic methods. Electrophoresis in 15% polyacrylamide gel was performed according to Laemmli (1970). Gels were blotted to nitrocellulose for 16 hrs at 3V/cm, and then the lectin fraction was identified using its reactivity towards HRPO labeled antibodies. The method of Szopa and Rose (1986) was modified for direct use of goat-anti-RGA-IgG, labeled with HRPO according to Farr and Nakane (1981).

Immunodiffusion. The double immunodiffusion experiment was performed on agarose plates according to Ouchterlony (1968).

RESULTS

Lectin occurred only in the embryo in dry, mature caryopsis. Its amount reached 2.1 μg per embryo, which comprises about 2% of the total embryo protein. During imbibition and early germination the rapid increase in the protein content (Fig. 1) was followed by a slight increase in the lectin activity

Fig. 1. Fresh weight (A) and protein content (B) of rye seedlings germinating with or without ABA: $\circ$ — water culture, $\bullet$ — ABA 10^{-7} M, $\blacksquare$ — ABA 10^{-5} M, $\blacktriangle$ — ABA 10^{-4} M

after the first six hours and then, on the second day, by its rapid decrease to 30% of the initial value, leading to its disappearance on the 5th day of development (Fig. 2). The lectin activity was highest and remained present for the longest time in the coleoptile. Only trace hemagglutinating activity in the young leaves was found. The lectin was also degraded most rapidly in that tissue (Table 1).

Table 1

Lectin content in organs of young rye seedlings

Seedling age (days)	RGA-like lectin level (units per seedling)		
	roots	leaves	coleoptile
3	4.5	trace	7.0
4	4.0	0.0	3.5
5	0.0	0.0	trace

Rye seedlings exposed to 10^{-7} M ABA during their germination, although slightly inhibited in their growth and protein biosynthesis (Fig. 1) did not show any changes in lectin activity when compared with the control plants (Fig. 2).

Higher concentrations of the abscisic acid caused substantial inhibition of plant growth (Fig. 1) and concomitant rapid accumulation of lectin was observed (Fig. 2). ABA at a concentration of 10^{-5} M in the medium caused about an approximately 3-fold increase in lectin activity in the germs imbibed for six hours. The rise in the ABA concentration to 10^{-4} M caused a further increase of lectin accumulation during 6- and 12-hour long imbibition (Fig. 2). During the second day of germination, the lectin activity decreased and from the third day it stayed unchanged until at least the 5th day.

Fig. 2. Lectin activity in the rye seedlings germinating with or without ABA: ○ — water culture, ● — ABA 10^{-7} M, ■ — ABA 10^{-5} M, ▲ — ABA 10^{-4} M

Using the double diffusion test, we have compared the reaction with anti-RGA-IgG of the RGA and RGA-like lectin accumulated in the embryo under conditions of high ABA concentrations. The result indicates the identity of the antigenic determinants of both proteins (Fig. 3). SDS-PAGE results have shown that both lectins also have identical molecular masses and do not exhibit any heterogeneity (Fig. 4).

Fig. 3. Double immunodiffusion of RGA-like lectin accumulated in seedlings grown with endogenous ABA against anti-RGA immunoglobulins. Anti-RGA immunoglobulins were applied in well (a), well (b) contained RGA and well (c) the lectin isolated from embryos imbibed for twelve hours in 10^{-4} M ABA

Fig. 4. SDS-PAGE of RGA and lectin accumulated under ABA control (a) RGA purified from dry embryos, (b) RGA-like lectin isolated from embryos imbibed for twelve hours in 10^{-4} M ABA. Gels were blotted for nitrocellulose and stained for HRPO-activity after incubation with HRPO-labeled anti-RGA-IgG

DISCUSSION

The synthesis and accumulation of lectin during embryogenesis in cereals is well documented (Stinissen et al. 1981, Stinissen and Peumans 1983, Peumans et al. 1982c, Morris et al. 1985, Quatrano et al. 1983). In contrast data concerning the early germination stages are still controversial. Stinissen et al. (1981) have reported a considerable loss of lectin activity during imbibition as short as half an hour. On the other hand, Morris et al. (1985) have shown a slight increase in the lectin level during the first day of wheat and barley grain imbibition followed by its rapid decrease the second day.

In this study we have found that the increase in lectin activity starts earlier, after six hours of imbibition, and reaches 125% of the initial value. We have not found any lectin activity in 5-day old plants, although some authors have reported *de novo* synthesis of lectin in roots (Stinissen et al. 1985) and shoot

basis (Raikhel et al. 1986) of young cereal seedlings. Like Raikhel et al. (1984) in wheat, we have not found considerable hemagglutinating activity in young leaves. The presence of lectins in leaves of *Gramineae* plants was reported so far by only Cammue et al. (1985a, b) albeit this in mature plants grown in field conditions.

It has been considered for many years that abscisic acid influences the lectin level only during cereal seed formation (Stinissen et al. 1981, Peumans et al. 1982c, Morris et al. 1985). Recent years have brought some data concerning the ABA-related enhancement of lectin biosynthesis (Stinissen et al. 1984) and accumulation (Raikhel et al. 1986) during early germination. In our experiments, we have observed a high enhancement of the lectin level after 6 and 12-hours of imbibition, 2- or 3-times higher than reported by the authors mentioned above. As the lectin isolated from embryos imbibed in ABA-rich medium does not differ from RGA by immunological criteria and molecular mass, we can estimate its amount as 10-15 μg per embryo imbibed for 6-12 hrs. This amounts to about 7-10% of the total embryo protein, compared with 1.5% in control plants. In addition, we have found that lectin accumulation in 10^{-4}M ABA lasts for at least five days of growth. Its amount (6 μg per germ) exceeds 3-fold the level of RGA in the dry embryo and again equals 2% of total germ protein, in comparison with 0.4-0.2% in control plants.

In this study we are supplying further proof for the occurrence of lectin not only in mature grains, but also in young vegetative tissues of cereals and for the ABA-effect on their synthesis and accumulation. Although these facts together with the previously reported possibility of activation of plant hydrolases by lectins (Ferens and Morawiecka 1985a, b) do not explain the physiological role of plant lectins, they do suggest an active role for these proteins in the processes essential for plant metabolism and do not permit them to be treated simply as storage proteins.

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Poziom lektyny i wpływ kwasu abscysynowego na aktywność hemaglutynującą podczas kiełkowania żyta

Streszczenie

W naszych wcześniejszych badaniach stwierdziliśmy, że w dojrzałych, suchych nasionach lektyna występuje wyłącznie w zarodku. We wczesnych stadiach kiełkowania stopniowo zanika aktywność aglutynująca, największą stwierdza się w koleoptylach. Liście zawierają zaledwie śladowe ilości tego białka. W ziarniakach poddawanych 6-12-godzinnej imbibicji w obecności 10^{-5} M i 10^{-4} M ABA stwierdza się 5-7-krotny wzrost ilości lektyny. 5-Dniowe kiełki rosnące w obecności 10^{-4} M ABA zawierają około 6 μ g lektyny na siewkę. Stwierdzono, że RGA i lektyna kumulowana w obecności ABA są identyczne pod względem masy molekularnej i własności immunologicznych.