Hormonal Regulation of the Lectin Biosynthesis in Callus Culture of the *Phaseolus vulgaris* Plant

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ABSTRACT

Callus cultures established from *Phaseolus vulgaris* seedlings were used to investigate hormonal influence on lectin biosynthesis. The plant tissue cultures were initiated using defined levels of both a cytokinin (kinetin) and an auxin (2,4-dichlorophenoxyacetic acid) and were then transferred to media containing different amounts of these hormones. The lectin content of each callus culture was determined using an enzyme immunoassay specific for the seed lectin of the *P. vulgaris* plant. The lectin biosynthesis was directly affected by the levels of auxin and cytokinin in the culture media and no lectin was detected in hormone-free medium. This enabled us to compose culture media yielding a maximal or minimal lectin content of the callus cultures, illustrating the ability to induce an enhancement or suppression of the *in vitro* lectin biosynthesis. The lectin level of callus tissue during the growth cycle of a culture was, furthermore, related to the cellular growth rate which might indicate an involvement of the lectin in cellular events during rapid cell division.

The biological functions of leguminous plant lectins have been investigated for several years and many functions have been postulated (10) but no conclusive evidence has been presented for any of them. One approach to obtain information about the physiological role of this class of carbohydrate-binding proteins has been to study the distribution of lectin in various tissues throughout the life cycle of plants. Although lectins constitute a major part of the nitrogen content of the seeds, their existence in vegetative tissue of different adult plants has only recently been reported (1, 7, 15, 16). Lectins prepared from seeds and 5- to 6-week-old stems and roots of the *Phaseolus vulgaris* plant were shown to be identical (2), whereas the *Dolichos biflorus* plant contained two different lectins, one isolated from seeds (4) and the other from 5- to 6-week-old stems and leaves (17). These two lectins also exhibited a somewhat different carbohydrate specificity (5), which was in contrast to lectin isolated from various tissues of *P. vulgaris*. The seed lectins from *P. vulgaris* and *D. biflorus* were recently also shown to bind to adenine derivatives, e.g., kinetin via specific hydrophobic sites (14).

Lectins have also been isolated from plant tissue cultures where they have been found on the cell surface of soybean callus cells (3), in differentiated callus cells from *Psophocarpus tetragonolobus* (11) and in the cell wall and cytoplasm of *D. biflorus* callus cells (6). To further elucidate possible biological functions of the lectin in *P. vulgaris*, we have utilized callus cultures of this plant for the investigation of how the regulation of the lectin biosynthesis was influenced by plant hormones.

MATERIALS AND METHODS

Culture Media. MS (12), LS (9), M1 (8), and 67v (18) plant tissue culture media were used, with the hormone concentrations modified to 0.1 mg kinetin and 1.0 mg 2,4-D/L. The hormones were filter sterilized and then added to the autoclaved media. The media were solidified using 0.8% (MS, LS, M1) or 1.0% (67v) agar.

Callus and Suspension Cultures. Seeds from *Phaseolus vulgaris* (var red) (AB Sativa, Stockholm, Sweden) were surface sterilized in 70% ethanol and 5% NaOCl for 5 min and washed three times in sterile water. The seeds were then allowed to germinate and grow on humidified cotton in sterile flasks for 9 d. Explants were taken from hypocotyl of the seedlings and placed on solidified 67v or LS media containing 0.1 mg kinetin and 1.0 mg 2,4-D/L. The resulting calli were grown at 27°C, using a 24-h photoperiod of diffuse light, and subcultured every 3rd week.

Suspension cultures were established by disintegrating a callus culture grown on solidified 67v medium into small pieces and inoculating the pieces into liquid 67v medium. The suspension cultures were cultivated in 100 ml medium in 250 ml Erlenmeyer flasks on an orbital shaker (125 rpm) at 25°C and subcultured at 3-week intervals.

Growth Determination of Callus Cultures. The growth of callus cultures in media containing different hormone concentrations was determined in arbitrary units by estimating the size of the callus. No growth was set to 0, whereas 1 indicated the appearance of a few new cells, and 2 to 5 indicated approximately how many times the total cell volume had increased. In experiments where the culture medium was designed to yield maximum or minimum lectin synthesis (Max/Min media) the fresh weights of the cultures were measured in the beginning and end of each 3-week culture period. The lectin content of callus cultures was also measured as a function of the culture time and the growth was then determined by dry weight analysis of the cultures. The tissue was dried on filter paper at 60°C for 3 d and the weight was measured.

Extraction and Analysis of Callus Tissue. The different callus cultures were run in duplicates and extracted as described for plant tissue from *P. vulgaris* (1). Briefly, the tissue was frozen in liquid N2 and ground to a powder using a mortar and pestle. A 5-fold (v/w) excess of extraction buffer was then added. The extraction buffer was 0.1 M K-phosphate (pH 7.0), containing 0.1 M isocitric acid, 2 M sodium thioglycolic acid, and 0.1 M phenylmethylsulfonyl fluoride. The mixture was stirred for 20 min at 4°C, the solution was centrifuged at 27,000 g for 20

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Abbreviations: MS, Murashige and Skoog medium; PHA, phytohemagglutinin; LS, Linsmaier and Skoog medium; M1, see reference (8) for composition; 67v, see reference (18) for composition.
min at 4°C, and the resulting supernatant was dialyzed overnight against 50 mM sodium phosphate buffer (pH 7.2), containing 0.15 M NaCl.

The lectin content was analyzed using a sensitive enzyme immunoassay that we recently described (2) and all samples were run in triplicates. The protein concentration of callus extract was determined according to Read and Northcote (13).

RESULTS

The callus cultures used for this investigation were established using fragments of hypocotyl from 9-d-old antiseptically grown seedlings. To obtain optimal growth conditions MS, LS, M1, and 67v culture media, supplemented with 0.1 mg kinetin and 1 mg 2,4-D/L, were tested initially. MS medium and M1 medium, which contains 3% coconut milk, were shown to yield the highest growth rates and were used throughout the experiments. Newly established callus cultures on MS and M1 media contained detectable levels of lectin as determined by the enzyme immunoassay. These callus cultures were then utilized to study the hormonal influence on the lectin biosynthesis. Since the coconut milk, used to supplement M1 medium, contained endogenous hormones both M1 and MS media were used when the effect of various concentrations of an auxin and a cytokinin on the lectin levels was tested.

The auxin (2,4-D) level was set to 1 mg/L and the cytokinin (kinetin) concentration varied in the range of 0 to 10 mg/L. The callus cultures were grown for 3 weeks using the two different media before being tested for lectin content and further subcultured. This procedure was repeated for a second 3-week growth period. The dose-response maximum in MS medium occurred at a kinetin concentration of 0.5 mg/L and in M1 medium at 0 to 0.1 mg/L (Fig. 1). In the next set of experiments the kinetin concentration was held constant at 0.5 mg/L (MS medium) and at 0 mg/L (M1 medium) and the 2,4-D concentration was varied in the range of 0 to 10 mg/L. The calli were cultured in two 3-week growth periods and also under these conditions the lectin biosynthesis was greatly influenced by the hormonal variation (Fig. 2). The dose-response pattern looked similar in the two different media except that the maximal lectin content was shifted towards lower exogenous hormone concentrations in M1 medium.

Based on the results described above, two new media were composed which should give maximal or minimal lectin biosynthesis as well as an optimal cell growth. These media, designated Max and Min, were based on MS medium since this was a more defined medium than M1, which contained coconut milk. Max was MS medium containing 0.5 mg kinetin and 2.5 mg 2,4-D/L. A concentration of 2.5 mg 2,4-D/L was used since this gave the best growth in combination with a high lectin content (Fig. 2A). MS medium containing 0.1 mg kinetin and 0.5 mg 2,4-D mg/L was called Min. Callus cultures were started on Max and allowed to grow for two 3-week periods before they were inoculated on Min for three 3-week growth periods. The callus cultures were then finally reinoculated on Max and allowed to grow for one 3-week period. Samples of the callus were taken at each 3-week growth period when the cultures were reinoculated and analyzed for their lectin content using the enzyme immunoassay (Fig. 3). The lectin biosynthesis could be very clearly influenced by changing the hormone ratios in the culture medium.

The lectin levels during the growth cycle of a callus culture were, furthermore, determined; 1.5 g of callus inoculum was allowed to grow on Petri dishes using MS medium containing 0.1 mg kinetin and 1.0 mg 2,4-D/L. Calli were harvested from the Petri dishes five times during a 7-week period and taken to lectin and growth analysis (Fig. 4). The highest lectin levels were detected in tissue taken from cultures in a logarithmic growth phase.

DISCUSSION

The results described above clearly showed that plant tissue cultures could be utilized as an experimental model to determine how lectin synthesis and turnover were regulated. Both callus and suspension cultures derived from hypocotyls of P. vulgaris seedlings were shown to contain significant amounts of the lectin when cultured in media containing 0.1 mg kinetin and 1.0 mg 2,4-D/L. MS and M1 media were used when the effect of different amounts of exogenous auxin and cytokinin was tested. The hormonal influence on the lectin biosynthesis was similar in both media although the absolute levels of lectin were higher in M1 medium. This was attributed to the presence of 3%

![Fig. 1](image) Lectin concentration as a function of kinetin content in the culture medium. The callus was grown for 3-week culture periods in (A) MS medium containing 1 mg 2,4-D/L or in (B) M1 medium containing 1 mg 2,4-D/L. The lectin concentration was expressed as ng PHA/mg protein (●) or as ng PHA/g callus (■).
endogenous hormones. The callus was grown for 3-week culture periods in (A) MS medium containing 0.5 mg kinetin/L or in (B) M1 medium containing 0 mg kinetin/L. The lectin concentration was expressed as ng PHA/mg protein (●) or as ng PHA/g callus (■).

coconut milk in the M1 medium, since this supplement contained a variety of endogenous hormones. The lectin level of callus cultures in these experiments was tested at 3-week intervals. The concentration of lectin seemed in most cases to reach a constant level after the first 3-week period.

If the callus cultures were propagated in the absence of exogenous hormones the cells did not exhibit any growth which suggested that the cells did not produce sufficient levels of endogenous hormones. This was in contrast to what James and Etzler (6) recently reported using callus cultures of the Dolichos biflorus plant. When these callus cells were cultured in medium containing neither auxin nor cytokinin, significant levels of cross-reactive material were detected and the presence of exogenous hormones rather decreased the cellular expression of this plant lectin. The expression of lectin in D. biflorus cultures was suggested to be correlated to a rapid cell growth and greening of the cultures (6). This correlation between cell growth and lectin content was similar to the results obtained with P. vulgaris cultures where the lectin biosynthesis seemed to be directly correlated to the total cell growth (Fig. 4). There was a maximal lectin concentration in callus tissue at the early logarithmic growth phase of the culture which was followed by a rapid decrease in lectin levels when the callus cells reached their stationary phase. Although the correlation was not absolute, since the Max medium yielded high lectin content but not an optimal growth rate, this might indicate an involvement of the lectin in cell-cell contact during rapid cell division.

The lectin biosynthesis could, furthermore, be influenced by using the Max/Min media. There was a significant effect of the
two media on the lectin levels but the expression of lectin could not be completely abolished except when the media were completely devoid of exogenous hormones. If this regulation of the lectin synthesis and turnover was due to a direct hormonal action or to a secondary effect remains to be determined.

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