CHARACTERISATION OF RECEPTORS FOR THE ENDOGENOUS LECTINS OF SOYBEAN AND JACKBEAN SEEDS

Dianna J. BOWLES and Susan MARCUS
Department of Biochemistry, University of Leeds, 9 Hyde Terrace, Leeds, LS2 9LS, England

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1. Introduction

Soybean (*Glycine max*) and jackbean (*Canavalia ennisformis*) are species of legumes that are characterised by large quantities of water-soluble seed lectins [1]. The major lectin of soybean, soybean agglutinin (SBA) is a glycoprotein with a sugar specificity related to D-galactose. In jackbean, the major lectin, concanavalin A (con A) is a protein with a sugar specificity related to D-mannose. Immunological techniques and peptide sequencing data has demonstrated that seed lectins represent a highly conserved family of proteins in the plant kingdom [2–4]. As a way into studying the function of these lectins, we have investigated the nature of the glycoconjugates in the storage tissue of the seeds that the endogenous lectins can recognise.

2. Methods

Dry seeds were imbibed in water for 4 h max., after which the testa was removed and the embryonic axis was separated from the cotyledons. In general, 3 g tissue (fresh wt) were homogenized at 4°C in phosphate-buffered saline (PBS), 5 mM PMSF (pH 7.2) in a buffer:fresh wt ratio of 20:1 and the suspension was sonicated before centrifugation of the total homogenate at 150 000 × *g* for 1 h to obtain a soluble extract of the tissues. Protein estimations [5], (BSA as standard) of the homogenates and extracts indicated that for all extractions, >85% of the total protein had been solubilised. Pure SBA was prepared by affinity chromatography from the same variety of seeds [7] and con A was purchased from Sigma. These lectins were immobilised on Sepharose 4 B [7] and used as affinity systems to purify potential receptors from the soluble extracts of the cotyledons. When extracts of jackbean cotyledons were applied to SBA–Sepharose, the column was developed in PBS containing 0.1 M α-methyl-mannoside to reduce binding of con A in the cotyledon extract to the SBA–Sepharose. The capacity of the affinity columns was standardized using asialofetuin which had been radioactively-labelled with ^{125}I. After applying the extract to the columns (5 ml immobilised lectin–Sepharose) and washing off unbound material, possible receptors were eluted with 0.2 M in PBS of either galactose (SBA–Sepharose) or α-methyl-mannoside (con A–Sepharose). Protein was estimated in the eluants and after trichloroacetic acid-precipitation of the peak protein fractions, the receptors were analysed. To analyse total extracts and receptors, trichloroacetic acid-precipitated proteins were separated by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS–PAGE) on 10–15% gradient slab gels under reducing conditions [8]. M_r markers (Boehringer) were coelectrophoresed:soybean trypsin inhibitor (21 500); bovine serum albumin (68 000); RNA polymerase α-chain (39 000); β-chain (155 000); β′-chain (165 000). The M_r-values of the seed components were calculated from a log M_r/log % acrylamide plot of the standard markers.

3. Results and discussion

Channels A and B, of fig.1,2 show the solubilised components of the cotyledons and embryonic axes from soybean and jackbean. Channel C in each figure is the lectin characteristic of the seed: SBA in fig.1 and con A in fig.2. For either species, the pattern of components of the embryonic axis are clearly distinguishable from those of the cotyledon. Since the samples were reduced before analysis and electrophoresed

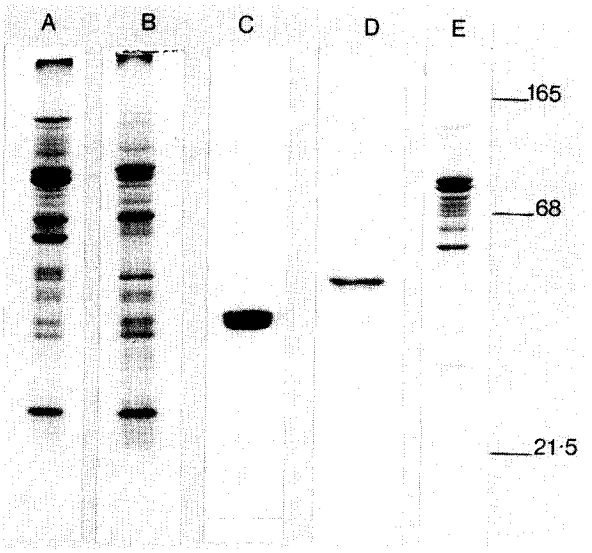

Fig.1. SDS–PAGE of soybean components: *Glycine max*. cv. Fiskeby V. Protein loading/channel was 50 μg in A, B, E and 20 μg in C, D. Protein was visualised using Coomassie blue. (A) Cotyledon; (B) embryonic axis; (C) SBA lectin; (D) SBA receptor; (E) con A receptors.

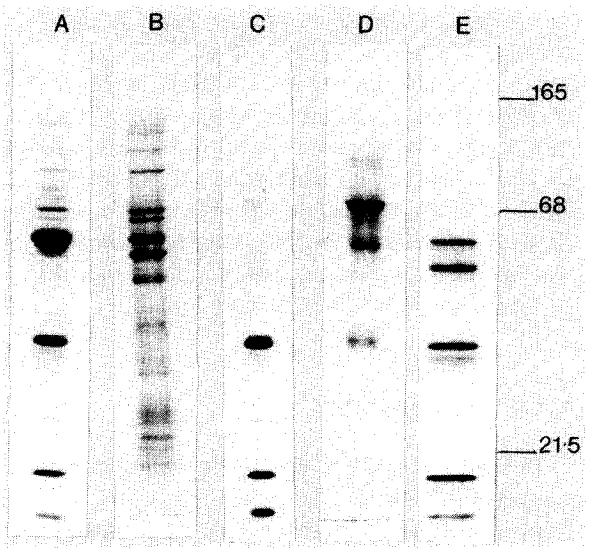

Fig.2. SDS–PAGE of jackbean components *Canavalia ensiformis* cv. unknown (Sigma). All amounts as for fig.1: (A) cotyledon; (B) embryonic axis; (C) con A lectin (Sigma); (D) con A receptors; (E) SBA receptors.

in the presence of SDS, the bands represent single polypeptide chains and no assessment of their arrangement into multimeric units can be made. In contrast to the components of soybean cotyledons, those of jackbean are resolved into a much less complex pattern. There is one major polypeptide of app. M_r 56 000, and 3 bands at 33 000, 19 000, and 17 000, which coincide with the pattern of bands shown for the Sigma Con A (channel 2C).

When soybean cotyledons were examined using affinity chromatography on immobilised lectins only one receptor to the endogenous lectin SBA was observed (fig.1D). The polypeptide of app. M_r 36 000, was not a major component of the storage tissue (compare fig.1A,D). However, receptors to con A in soybean cotyledons were numerous and as many as 16 polypeptides could be found (fig.1E). In contrast, when con A was used to isolate receptors from cotyledons of its own species jackbean, the endogenous lectin recognised only one receptor, a polypeptide of app. M_r 69 000 (fig.2D). Trace amounts of the major storage tissue polypeptide and the 33 000 M_r subunit of con A were also found in the preparation. Receptors to SBA in jackbean cotyledons were analysed and these were again more numerous: at least 7 polypeptides were found in addition to the characteristics subunits of con A (fig. 2E). Since SBA is a glycoprotein which contains mannose, it is probable that the con A present in the extract of jackbean cotyledons could interact weakly with the SBA–Sepharose. Although 0.1 M α-methyl-mannoside was included in the PBS buffer to reduce this possibility, the presence of con A subunits in the receptor preparation is suggestive that some con A–SBA binding still occurs.

These results demonstrate that the lectin of a particular species may exhibit a very narrow range of specificity towards endogenous glycoconjugates. This suggests that not only is there one major lectin in the cotyledons of a given plant, but also one soluble glycoprotein in the cotyledons that the lectin can recognise. The striking specificity shown between homologous lectin and receptor is not maintained when the lectin is used to isolate glycoproteins from the same tissue in other plant species. If the two species studied here are typical the specificity of the seed lectins would suggest that their functional role may well lie in some aspect of the physiology of cotyledons. The need to solubilise the cotyledon components in order to analyse them, allows no insight into the potential complexing of lectin and receptor in situ in the seed. Also,

if the soluble extract contains both lectin and glycoconjugates that the lectin can recognise, presumably complexing can occur in the extract. The situation may exist that the extract contains free lectin, lectin–receptor complexes and free receptors: the amounts of free lectin and free receptor depending on their relative quantities and the affinities between lectin and receptor(s). If this mixture is then applied to an immobilised lectin-column, what is purified by the use of the column will also depend on the relative quantities of the lectin and receptor, their affinities for each other, and the relative affinity of the receptor(s) for soluble lectin compared to immobilised lectin. It is possible that numerous receptors to the endogenous lectin may exist, but are of such high affinity that they remain complexed to soluble lectin, do not equilibrate with immobilised lectin and consequently are not purified. Experiments are in progress with antisera raised to the seed lectins to test these possibilities. At the onset of germination, both soybean and jackbean seeds have relatively vast quantities of their major lectins. In jackbean, the lectin is localised solely in the cotyledons, whereas in soybean the lectin can be purified from either cotyledons or embryonic axis, although it is present in higher quantities in the former tissue (unpublished). When the amount of affinity-purified glycoconjugate is calculated, and compared to an estimate of the quantity of lectin also present (table 1), interestingly the lectin is calculated to be in great excess over that of its potential receptor. However, it could be argued that the quantity of receptor purified by the affinity column is only that small proportion of total receptor which is uncomplexed to soluble lectin in the extract. That this possibility is unlikely at least in jackbean, is suggested by the pattern of protein bands in SDS–PAGE which shows that the affinity-purified con A receptor is a minor component of the cotyledons, whereas con A is a major component.

As yet, we have not purified large enough quantities of the specific receptors to the endogenous lectins to permit any analysis of potential carbohydrate content. However, when total trichloroacetic acid-precipitable material from soluble extracts of the cotyledons was analysed for carbohydrate using gas–liquid chromatography, a clear distinction in the patterns of glycosylation of proteins in the species was evident, in that substantially more mannose than galactose was found in soybean, whereas the reverse was shown in jackbean (Chaplin; S. M., D. B., in preparation). These observations, taken in conjunction with the data from the affinity systems, suggests that the biochemical situation in a fully-developed seed ensures specific and minimal complexing of the endogenous lectin to glycoproteins. It has been suggested that the function of lectins may lie in their reversible interaction with

Table 1
Quantitation of endogenous lectins and potential receptors

	HU/μg pure protein	HU/μg protein in soluble extract	μg lectin/ mg protein in soluble extract	μg receptor/ mg protein in soluble extract
Con A	400		550	0.71
Jackbean		220		
SBA	914			
Soybean		41	45	0.48

Rabbit erythrocytes were trypsin-treated and used in a standard haemagglutination assay to determine haemagglutination units/μg protein for pure samples of con A (Sigma) and SBA (purified as in section 2). The titre of soluble extracts was also determined using the haemagglutination assay and the same batch of erythrocytes. The values were then used to estimate the amount of lectin present in the extracts. The values for receptors were calculated from the amounts purified by the affinity systems as described and quantified using the Coomassie dye-binding procedure [5].

receptors: the interaction being used to allosterically regulate a wide range of activities [8]. In this context, it is significant that at the onset of germination the lectin may exhibit maximum complexing potential. The changes in populations of glycoprotein receptors during germination are currently under investigation.

Acknowledgements

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