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Plant basic 7 S globulin-like proteins have insulin and insulin-like growth factor binding activity

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Basic 7 S globulin (Bg) is a cysteine-rich glycoprotein present in soybean seeds. Mature Bg is composed of high- and low-kDa subunits linked by disulfide bonding. A ligand blotting experiment using [^{125}I]insulin and [^{125}I]insulin-like growth factor-I and -II showed that Bg subunits are able to bind not only to insulin but to insulin-like growth factors-I and -II. Bg-like proteins from other legume species cross-reacted with anti-Bg antibody also bind to insulin and insulin-like growth factors. Bg-like protein in carrot cells was found to have insulin binding activity. Bg-like proteins may be involved in an insulin-like regulatory mechanism in many plant species.

Legumes; Carrot; Seed protein; Basic 7 S globulin; Insulin binding protein; Insulin-like growth factor binding protein.

1. INTRODUCTION

Basic 7 S globulin (Bg) is a cysteine-rich glycoprotein in soybean seeds, consisting of 27 kDa and 16 kDa subunits linked by disulfide bonding [1]. The partial amino acid sequence of Bg was determined by actual protein sequencing [2] and the complete sequence was deduced from the nucleotide sequence of Bg cDNA [1]. When immersed in hot water (40–60°C), soybean seeds release a large amount of Bg. This suggests that Bg is a protein with important functions, and not simply a seed storage protein. Recently, a protein which is able to bind to insulin and insulin-like growth factor (IGF)-I was identified in the germinating seeds of soybean [3]. This protein consisted of 27 kDa and 16 kDa subunits linked together by disulfide bonding. The N-terminal amino acid sequences of these subunits were found to be highly homologous with those of Bg 27 kDa and 16 kDa subunits. Bg may thus be involved in an insulin-like regulatory mechanism in soybean.

Previously, we reported that Bg-like proteins cross-reacted with anti-Bg antibody are widely distributed in the seeds of legume species, such as azuki-bean, cowpea, French bean, lupin, mung bean and winged bean [4]. These Bg-like proteins are released from seeds in hot water. Recently, a Bg-like protein was found to be released from cultured carrot cells into the medium during the formation of somatic embryos [5]. In the present study, we found that these Bg-like proteins are capable of binding to insulin, IGF-I and IGF-II. Bg-like proteins may participate in insulin-like regulatory mechanism in many plant species.

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2. MATERIALS AND METHODS

2.1. *Protein extraction*

Dry mature seeds of soybean (*Glycine max* L. Merrill cv. Miyagishirome), azuki bean (*Vigna anglaris* L.), lupin (*Lupinus albus* L.), mung bean (*Vigna radiata* L.) and cowpea (*Vigna sinensis* Endl.) were used. They were immersed in water at 60°C for 2–3 h. The seeds released a large amount of Bg and Bg-like proteins. The water was dialyzed against deionized water, lyophilized and subjected to electrophoresis.

2.2. *Immunoblotting*

Proteins extracted were separated by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) [6] and electroblotted onto a PVDF membrane. The blotted bands which cross-reacted with antibodies raised against Bg subunits, were detected by peroxidase enzyme immunoassay [4].

2.3. *Detection of insulin and IGF binding proteins*

Blotted proteins capable of binding to radioiodinated insulin and IGFs were detected as described in [7]. After transfer of proteins to the PVDF membrane, the membrane was dried at 37°C for 5 min. It was then soaked at 4°C, first, for 30 min in saline (0.15 M NaCl, 0.01 M Tris-HCl, pH 7.4, 0.5 mg/ml sodium azide) supplemented with 3% Nonidet P-40; second, for 2 h in saline containing 1% bovine serum albumin (BSA); and finally, for 10 min in saline containing 0.1% Tween 20. The PVDF membrane was then sealed in a plastic bag with 400 000 cpm [^{125}I]insulin, [^{125}I]IGF-I or [^{125}I]IGF-II (Amersham, Buckinghamshire, UK) with 3 ml saline containing 1% BSA and 0.1% Tween 20. The PVDF membrane was incubated overnight at 4°C. The PVDF membrane was washed twice for 15 min at 4°C in saline containing 0.1% Tween 20, and then 3 times for 15 min in saline. The blot was dried and exposed for 7 days at −70°C to Fuji X-ray film.

2.4. *Competition binding assay*

Bg (200 pmol) electroblotted onto the PVDF membrane was incubated for 30 min at 4°C with increasing concentration insulin. Then the amount of [^{125}I]insulin bound to Bg was measured and the dissociation constant was calculated as described in [8].

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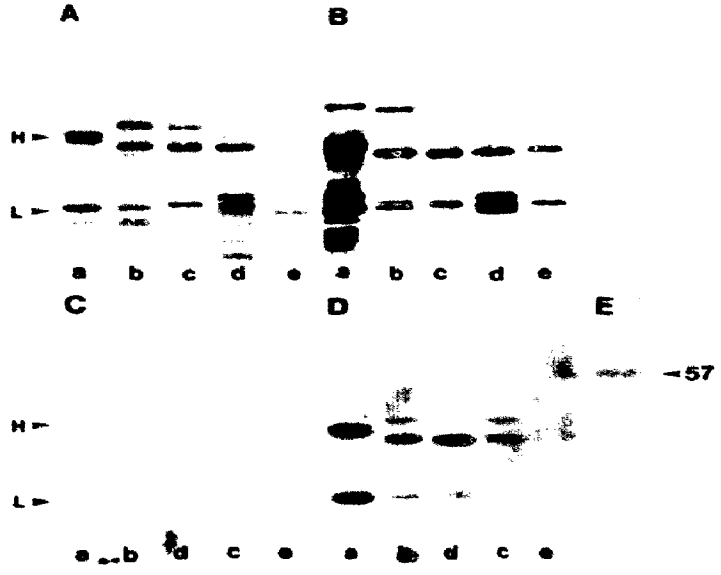

Fig. 1. Detection of proteins bound to insulin in legume species. (a) Soybean; (b) lupin; (c) cowpea; (d) mung bean; (e) azuki bean. (A) Polypeptides detected with Coomassie blue stain. (B) Immunoactive polypeptide with anti-Bg antibody. (C) Radioactive polypeptides with [^{125}I]insulin containing 10 μg/ml insulin. (D) radioactive polypeptides with [^{125}I]insulin. (E) Protein bound to [^{125}I]insulin in carrot cells. H and L show the positions of 27 kDa and 16 kDa.

3. RESULTS AND DISCUSSION

Bg released from mature seeds in water at 50°C was separated by SDS-PAGE and electroblotted onto a PVDF membrane. The blotted bands were reacted with [^{125}I]insulin, IGF-I and -II. The Bg was able to bind to insulin, IGF-I and -II (Figs. 1D$_a$, 2A$_a$ and 2B$_a$). The contribution of the nonspecific binding was determined by measuring [^{125}I]insulin binding in the presence of excess unlabeled insulin to saturate the specific binding sites. However, the nonspecific binding was not detected on the membrane (Fig. 1C$_a$). The preliminary competitive binding assay showed that the dissociation constants of Bg–insulin and Bg–IGF-I complexes are about 15 nM and 60 nM, respectively. Thus, specific binding was observed in different affinities for insulin and IGF-I.

Bg-like proteins released from the seeds of azuki bean, lupin, mung bean and cowpea in water for 2–3 h at 60°C, which were highly homologous in the N-terminal sequence to Bg [4], were isolated by SDS-PAGE and electroblotted. Their capability of binding to radiolabeled insulin, IGF-I and IGF-II was examined. Bg-like proteins in legume species were found to have binding activity to insulin and IGFs (Figs. 1D$_{bcde}$, 2A$_{bcde}$, 2B$_{bcde}$).

During the formation of somatic embryos in carrot, a glycoprotein having molecular mass of 57 kDa (GP57) was found to be released from the cultured cells into the medium [9]. This protein had a highly homologous sequence with Bg [5]. The carrot Bg-like protein was separated by SDS-PAGE and electroblotted, and its capability of binding to [^{125}I]insulin was examined. It was found to have binding activity to insulin (Fig. 1E).

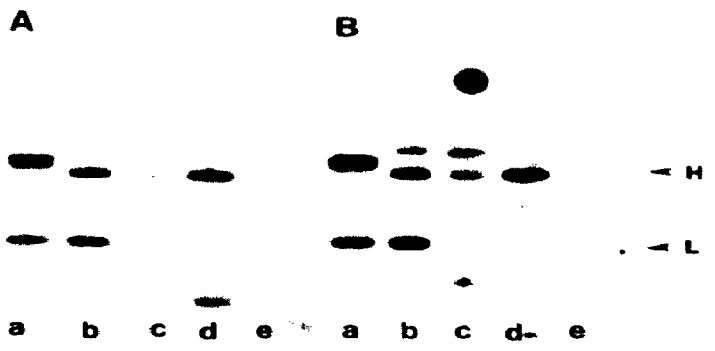

Fig. 2. Detection of proteins bound to IGF-I and IGF-II. (a) Soybean; (b) lupin; (c) cowpea; (d) mung bean; (e) azuki bean. (A) Radioactive polypeptides with [^{125}I]IGF-I. (B) radioactive polypeptides with [^{125}I]IGF-II. H and L show the positions of 27 kDa and 16 kDa.

From these results, it is concluded that all Bg-like proteins in plants have insulin, IGF-I and IGF-II binding activity. Bg-like proteins may thus possibly be involved in an insulin-like regulatory mechanism in plants. However, there is no evidence that Bg-like proteins function as insulin receptors or IGF receptors in plants. The structural characteristics of Bg [1] were compared with those of the insulin receptor [10], IGF-I receptor [11] and IGF-II receptor [12]. No homology in amino acid sequence was found among these proteins. However, there are structural similarities in glycosylation, the presence of a cysteine-rich domain and disulfide-bound α-β subunit structure between Bg and the insulin receptor or IGF-I receptor, and in glycosylation and the presence of cysteine-rich domain between Bg and IGF-II receptor. The insulin and IGF-I receptors are synthesized as a precursor polypeptide which is post-translationally cleaved at the N-terminal side of serine residue to generate α and β subunits. This resembles the post- translational processing scheme for Bg precursor polypeptide. The existence of proteins capable of binding to insulin, IGF-I and IGF-II suggests that there is a kind of insulin-like regulation in plants. These proteins may help to isolate a new group of regulatory compounds.

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