The homology of the major storage protein of jack bean (Canavalia ensiformis) to pea vicilin and its separation from α-mannosidase

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Abstract. The major storage protein of jackbean (Canavalia ensiformis) has been purified by a protocol involving ammonium-sulphate precipitation, gel filtration and ion-exchange chromatography. The protein was shown by partial amino-acid-sequence data to be homologous to vicilin, a major storage protein of pea (Pisum sativum), and is thus a member of the family of legume 7S proteins exemplified by pea vicilin. This protein is thus referred to as jack-bean vicilin rather than “canavalin” or “precanavalin” as previously used. Other properties of the jack-bean vicilin (e.g. subunit relative molecular mass (Mr) and structure, resistance to proteolysis) show similarity to phaseolin, the major 7S storage protein of Phaseolus vulgaris. Jack-bean vicilin contained no detectable α-mannosidase activity, either as isolated from mature or germinating seeds, or after proteolytic treatment. α-Mannosidase was also purified from jackbeans, and was shown to have a subunit Mr of approx. 120,000; it was separated completely from jack-bean vicilin by a similar protocol to that used for purifying the latter. The α-mannosidase was proteolytically cleaved after seed germination, but did not give polypeptides of the same Mr as jack-bean vicilin. It was concluded that α-mannosidase and jack-bean vicilin are not related proteins.

Key words: Canavalia (storage protein) – Canavalin – α-Mannosidase – Storage proteins (homology) – Vicilin.

Introduction

The major storage protein of the jack bean, Canavalia ensiformis, was described initially as an amorphous protein (Sumner 1919), but it was later shown that it could be isolated in a crystalline form if treated with proteolytic enzymes (Sumner and Howell 1936). Both these preparations were termed ‘canavalin’. Subsequent investigations using X-ray crystallographic techniques on ‘canavalin’ preparations crystallised by trypsin treatment (McPherson and Rich 1973) have indicated that the protein contained six identical or nearly identical subunits, Mr 24,000, arranged in a hexamer (McPherson and Spencer 1973); this model was subsequently refined to state that the ‘canavalin’ molecule contained three identical pairs of similar but non-identical subunits, each pair of subunits being derived from a 49,000 Mr “precursor” by proteolytic cleavage near the middle of the polypeptide chain (Smith et al. 1982). Proteolytic cleavage of the precursor, called “precanavalin”, which could be isolated from jack-bean meal, was demonstrated to take place during seed germination, and canavalin could be crystallised from germinating seeds. Concomitantly with these results, it was claimed that “canavalin” possessed α-mannosidase activity, or was α-mannosidase (Smith et al. 1982); “precanavalin” was initially regarded as an inactive precursor of α-mannosidase (McPherson and Smith 1980), but was subsequently claimed to possess this enzymic activity also (Smith et al. 1982). The protein, through its enzymic properties, was supposed to have some (unspecified) important functional role in the germinating seedling besides that of a storage compound. This accumulated body of knowledge on the major storage protein of jack beans contains a number of misinterpretations. The use of “canavalin” to describe a proteolytic cleavage product of “precanavalin”, the storage-protein polypeptide found in mature jack-bean seeds, is confusing since the proteolysis of “precanavalin” is in no way analogous to that of preproteins in animal systems where this terminology is used to describe cotranslational “leader” sequence removal (Blobel and Dobberstein 1975). In this
paper, the major storage protein of mature jack-bean seeds is referred to as jack-bean vicilin, since sequence data are presented showing its homology to vicilin, the 7S storage protein of pea seeds, which in its turn has been shown to be homologous to the 7S vicilin storage proteins of soyabean (conglycinin) and Phaseolus vulgaris (phaseolin) (Gatehouse et al. 1984). Other data to support this conclusion are also included. Evidence that jack-bean vicilin possesses no α-mannosidase activity, and that α-mannosidase is a separate and unrelated protein is also presented.

Material and methods

Plant material and chemicals

Jack beans (Canavalia ensiformis) were obtained as dry mature seeds from Sigma Chemical Co., Poole, Dorset, UK. When germinated jack beans were required, seeds were imbibed for 6 h at room temperature, then grown in the dark in a spray room at 25°C for 4 d (radicles were 5–10 mm in length). Seed cotyledons were then freeze-dried. Sephacryl S-300 was obtained from Pharmacia, London, UK, and diethylaminoethyl (DE) 52-cellulose was from Whatman Biochemicals, Maidstone, Kent, UK. Standard proteins, p-nitrophenyl-β-D-mannoside, 2-aminomethyl-1,3-propanediol (Tris) base (Trizma, reagent grade), p-nitrophenol standard solution, 4-chloro-1-naphthol and cyanogen bromide were from Sigma: other chemicals were from British Drug House Chemicals, Poole, Dorset, UK and were of analytical grade whenever possible. Nitro-cellulose (Type BA85) was from Schleicher and Schull, Dassel, F.R.G., and peroxidase-coupled goat [anti-rabbit immunoglobulin G (IgG), heavy and light chains] antiserum was obtained from Biogenzia Lemaria SA, Lausanne, Switzerland.

Electrophoretic methods

Polyacrylamide-gel electrophoresis (PAGE) was carried out in 17, 12.5 and 10% acrylamide-gel slabs according to Laemmli (1970). The sample buffer contained 2% (w/v) 2-mercaptoethanol (2-ME) for sodium dodecyl sulphate (SDS)-PAGE under reducing conditions, but 2-ME was omitted for SDS-PAGE under non-reducing conditions. Protein extracts were prepared by dissolving lyophilised protein samples, or by extracting seed meal with sample buffer (10 mg ml⁻¹ buffer) overnight at 4°C. Denaturation was carried out by incubating samples at room temperature for 45 min, unless otherwise mentioned.

Two-dimensional SDS-PAGE with non-reducing conditions in the first dimension and reducing conditions in the second dimension was carried out by equilibrating a stained strip of first-dimension slab for 1 h in the sample buffer containing 2% (w/v) 2-ME and transferring to a second gel slab as described by Matta et al. (1981).

Ammonium-sulphate fractionation

Finely ground meals of dry jack beans from which the testa had been removed were extracted twice for 30 min at 4°C with hexane (10 ml g⁻¹), which was decanted off. The meal was then air-dried. A portion (15 g) of defatted meal was extracted with 0.05 M sodium-borate buffer (10 ml of buffer/g of meal) pH 8.0, at 4°C for 2 h, centrifuged at 10,000 g for 30 min and the precipitate discarded. Finely ground solid (NH₄)₂SO₄ was added slowly to the stirred supernatant at 4°C to 50% saturation (291 g l⁻¹) and after 2 h the precipitate was removed by centrifugation (10,000 g for 30 min); (NH₄)₂SO₄ was added to the supernatant solution to 70% saturation (125 g l⁻¹) and the precipitate collected after 2 h as above; further (NH₄)₂SO₄ was added to the supernatant to a final concentration of 90% saturation (134 g l⁻¹) and after 2 h both the supernatant and precipitate were collected. The pellets were dissolved in a minimal volume of 50 mM sodium-borate buffer pH 8.0, and supernatant and pellets were freed of (NH₄)₂SO₄ by exhaustive dialysis against 5 mM sodium-borate buffer, pH 8.0, at 4°C and then freeze-dried.

Purification of vicilin of jack beans

A. Gel filtration (Sephacryl S-300). The material precipitating at 90% relative ammonium-sulphate saturation was dissolved in a minimal volume of 0.1 M Tris-HCl, pH 8.0, containing 0.25 M NaCl and 1% sodium azide (equilibrating buffer) and applied to a column (2.2 cm diameter, 320 ml vol.) of Sephacryl S-300 equilibrated and eluted with the same buffer, at a flow rate 16 ml h⁻¹, collecting 8-ml fractions. Fractions of each peak were pooled, dialysed, freeze-dried and analysed by SDS-PAGE.

B. Ion exchange (DE52 Cellulose). The freeze-dried protein of the peak containing jack-bean vicilin was redisolved in 10 ml of 50 mM Tris-HCl, pH 8.0 and applied to a column (1.6 cm diameter, 80 ml vol.) of DE52-cellulose that had been equilibrated with 50 mM Tris-HCl buffer, pH 8.0. The DE52-cellulose column was washed, then eluted with a linear salt concentration gradient (0 to 250 mM NaCl) at a flow rate of 32 ml h⁻¹, 8 ml fractions being collected. The fractions of each peak were pooled, dialysed, and freeze-dried.

Determination of molecular weight

Subunit molecular weight was determined in 17% and 12.5% polyacrylamide-gel slabs. The following standard proteins (Mr) were run on the same slabs: phosphorylase b (97,400), transferrin (76,600), bovine serum albumin BSA (67,000), catalase (60,000), ovalbumin (43,000), ferritin (18,000) and myoglobin (16,200). Pea vicilin and convicilin subunits (Mr, 71,000, 50,000, 33,000, 19,000, 16,000, 13,500 and 12,500) were also used as standards.

The Mr of vicilin in total protein extracts and of the purified protein was determined on a calibrated column of Sephacryl S-300 superfine (2.2 cm diameter, 520 ml vol.) equilibrated with 0.1 M Tris-HCl, pH 8.0, containing 0.25 M NaCl, 1.0% sodium azide as described by Croy et al. (1980).

The following standard proteins were used: ferritin (440,000), pea legumin (400,000), transferrin (76,600), ovalbumin (43,000) and myoglobin (18,000).

Cleavage with CNBr

Jack-bean vicilin dissolved in 70% (w/v) formic acid was reacted with 100-fold molar excess of CNBr (2g ml⁻¹) solution in acetoneitrile for various times (Kasper 1970). The solutions were evaporated to dryness under vacuum and the residues were analysed by SDS-PAGE.

Cleavage with proteases

Jack-bean vicilin (1 mg) was dissolved in 10 mM Tris-HCl, pH 7.5 (500 μl) and was digested separately by trypsin (as used