

Short sequence paper

Purification and crystallization of coconut globulin cocosin from *Cocos nucifera*

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Abstract

Cocosin is a legume class reserve protein found in coconut endosperm. Using coconut endosperm, two methods of purification were done. Crystallization was achieved by vapor diffusion (hanging drop) method using MPD, PEG 3350 and PEG 4000 as precipitants. X-ray diffraction data to 3.5-Å resolution were collected using Mar345 image plate detector system. Crystals of cocosin grown under 20% MPD, are rhombohedral with space group *R*3 and cell dimensions $a=92.829$ Å, $b=92.829$ Å, $c=215.290$ Å.

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Keywords: Storage protein; Coconut endosperm; Cocosin; Purification; Crystallization; Space group *R*3

Plants store proteins in embryo and vegetative cells to provide carbon, nitrogen, and sulfur resources for subsequent growth and development. Storage proteins are a group of proteins generated mainly during seed production and are stored in the seed that serve as nitrogen sources during germination. The endosperm appears to function as a food reservoir for the development of embryo. It is obviously more effective for the plant to use proteins instead of secondary plant products for this purpose. Storage proteins are important for human nutrition (plant proteins) and therefore numerous studies evolved concerning their structure. The following properties are noted in storage proteins: (a) the proteins have no enzymatic activities; (b) they serve as nitrogen sources for the germinating seed; (c) they occur normally in an aggregated state within a membrane-surrounded vesicle (protein bodies, aleuron grains); (d) they are often built from a number of different polypeptide chains.

There are two major types of storage proteins in legume seeds, known as vicilins and legumins, which are distinguishable by their sedimentation coefficients (7S/11S), oligomeric organization (trimeric/hexameric) and polypeptide chain structure (single chain/disulfide linked pair of chains) [1–4]. The first X-ray structure of a storage protein to be reported was that of phaseolin obtained in the

cotyledons of the French bean *Phaseolus vulgaris* and belongs to vicilin or 7S class of legume storage proteins [5]. The 11S proteins are hexamers and their constituent monomers comprise a pair of disulfide-linked chains formed by cleavage of a single primary translational product.

Globulin (11S) is one of the major storage proteins of many legume and nonlegume seeds [6]. The coconut globulin cocosin [7] is a legume class reserve protein from coconut and belongs to the fourth group of proteins, having a molecular weight of about 208 kDa. In chemical composition, cocosin resembles excelsin, both having high sulfur content and, with respect to solubility, cocosin and edestin are almost identical. Cocosin is obtained in a definite crystalline state, which may be taken as an indication of its homogeneity. The purification protocol used is the same as reported earlier except for acetone precipitation [8].

The following two methods were adopted to purify the cocosin protein. The first method deals the purification of cocosin using ammonium sulfate precipitation: The nuts were freed from shells and skins and ground to a fine paste and suspended in 10% NaCl solution. Toluene was added to remove fat. The mixture was stirred at room temperature for 24 h. The solution was freed from oil and fat by filtering and centrifuging. Saturated ammonium sulfate was added to 60% saturation and the precipitate was filtered and washed with 50% saturated ammonium sulfate solution. The resulting protein precipitate was dissolved in 0.033 M sodium phosphate buffer, pH 6.7, containing 7% NaCl. The protein

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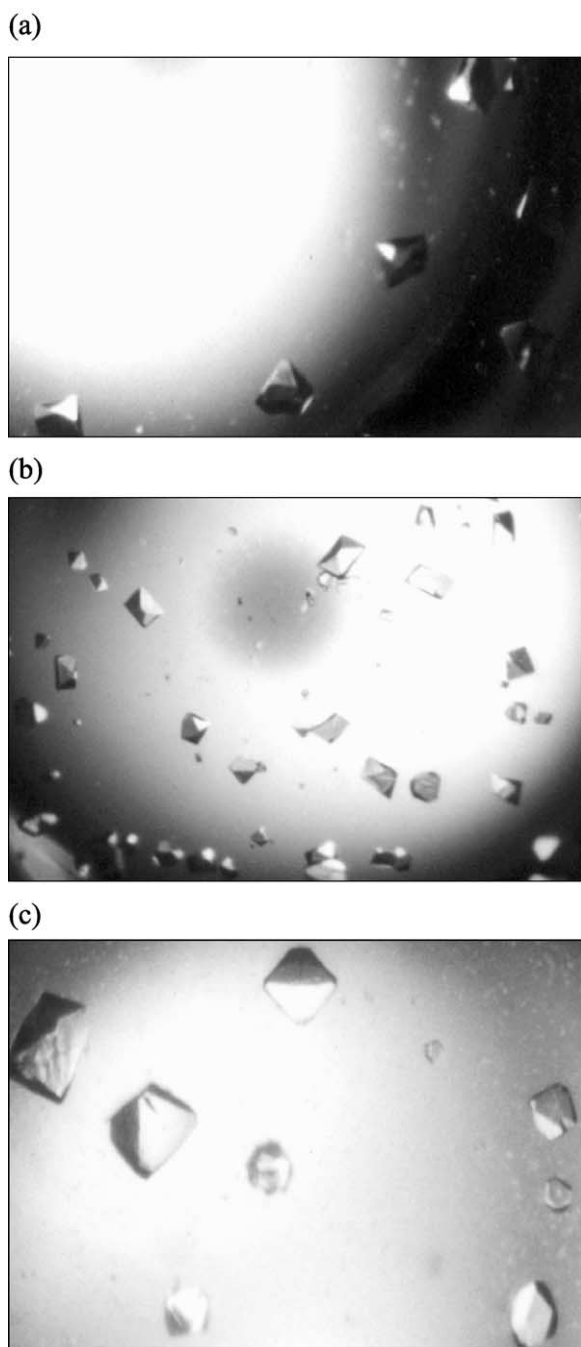


Fig. 1. (a) Crystals grown under 20% PEG 4000; (b) crystals grown under 15% PEG 3350; and (c) crystals grown under 20% MPD.

solution was then dialyzed against the same buffer for 6 days. The protein sample gives single zone upon electrophoresis.

The second one is the purification of cocosin using acetone precipitation: Coconut meal was taken and ground to a fine paste. The paste was dissolved in Na/K phosphate buffer containing 10% NaCl solution. Enough toluene was added to remove fat and stirred overnight for 24 h. The crude extract was filtered and centrifuged at $4500 \times g$ for 30 min to remove oil and fat and the supernatant collected. To

one volume of the supernatant solution, two volumes of acetone was added and the precipitate was collected after centrifugation. The precipitate was dissolved in Na/K phosphate buffer containing 7% NaCl and dialyzed for 10 days against the same buffer. The protein sample gives single zone upon electrophoresis. The sequence of the protein is not yet reported. We are aiming to sequence the protein at the moment.

Crystallization: (i) Cocosin (from Method I) was crystallized by vapor diffusion method (hanging drop) from drops containing 8- μ l protein solution (15–25 mg/ml in 33 mM phosphate buffer, pH 6.5–7.5) equilibrated against 1 ml of reservoir solution (10–20% PEG4000 and PEG3350). Good crystals were grown within a week as shown in Fig. 1a and b. (ii) Protein cocosin (from Method II) was crystallized by vapor diffusion method (hanging drop) from drops containing 8- μ l protein solution equilibrated against 1 ml of reservoir solution (10–20% PEG4000 and 10–20% MPD). Good crystals were grown within a week as shown in Fig. 1c. The crystals were of $0.25 \times 0.15 \times 0.02$ -mm size.

Data Collection: X-ray diffraction data up to 3.5-Å resolution were collected using Mar345 image plate detector system. Crystals of cocosin grown under 20% MPD are rhombohedral with space group R3 and cell dimensions $a=92.829$ Å, $b=92.829$ Å, $c=215.290$ Å.

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