Lysine rich 18 kDa storage polypeptide from buckwheat seed

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Received December 20, 2001; accepted in revised form August 26, 2002

Key words: in vivo labeling, lysine rich, SDS PAGE, storage proteins

ABSTRACT

Lysine, which is a limited amino acid in cereals, has a remarkably high content in buckwheat seed proteins. We have analyzed the pattern of 14C lysine incorporation into buckwheat seed polypeptides during the mid-maturation stage of seed development. Based on this approach, an 18 kDa polypeptide could be assigned as being lysine rich.

INTRODUCTION

Proteins of buckwheat seed are generally recognized as the best known source of high biological value proteins in the plant kingdom, having 92.3% of the value of non-fat milk solids and 81.4% of that of whole egg solids (Pomeranz and Robins, 1972). According to net protein utilization (NPU) they are classified as being close to the proteins from animal sources (Hannan et al., 1996). The storage proteins of buckwheat seed, which make up a considerable part of the total proteins, are divided into two classes: salt-soluble globulins, making up 70% of the total seed proteins and water-soluble 2S albumins. The main storage protein of buckwheat, 13S globulin, resembles a legumin-like seed storage protein from other species. It is a hexamer of non-identical subunits, each consisting of one larger acidic, and one smaller basic polypeptide, linked by disulfide bonds. Fractionation of buckwheat seed proteins, on a sucrose density gradient, revealed the existence of a new minor class of globulins with a sedimentation constant of 8S (Radovic et al., 1996). The 8S globulin is a trimer, composed of subunits with MW of 57 kDa, the structure common to all vicilin-like storage proteins (Radovic, 1998). It was shown that a third fraction from the sucrose density gradient was composed of 2S albumins, as well as a few polypeptides which don't belong to the albumin fraction (Radovic et al., 1999). The most prominent polypeptide in that unclassified group of polypeptides was an 18 kDa polypeptide.

One of the important characteristics of the amino acid composition of buckwheat seed proteins is the high content of lysine (Pomeranz and Robins, 1972). Taking into account that cereals that are commonly used in human and animal nutrition are limited in this amino acid, determination of the protein fraction responsible for that specific feature, is of great interest. In this paper we attempted to estimate the distribution of lysine among the storage polypeptides and to ascertain those polypeptide(s), which could be lysine-rich and subsequently could be of interest in the cloning of their genes which then could be introduced into the cereals and other species that are limited in this essential amino acid.

MATERIALS AND METHODS

Plant material

Buckwheat (Fagopyrum esculentum Moench, cv. Darja) was field-grown at the Botanical Garden of the University of Belgrade. The maturation period of the buckwheat seed was approximately 30 days after flowering. Developing buckwheat seeds were classified according to size, fresh weight, seed morphology and liquid to starchy endosperm. Different stages of development were designated as 9-11, 14-17, 19-21, 23-25 days after flowering (DAF). Seeds were harvested at the desired times after flowering and used immediately or were stored at -70°C before being used for protein extraction.

Protein extraction

Buckwheat seeds in the mid-maturation stage of development (19-21 DAF) were frozen in liquid nitrogen and ground to a fine powder. The salt-soluble protein fraction was extracted in 5-10 volumes of buffer A (0.035 M K-phosphate pH 7.6, 0.4 M NaCl, 1 mM phenylmethylsulfonylfluoride) at room temperature for 1 hour. The resulting suspension was centrifuged at 10,000 g for 15 min. The supernatant was precipitated with 2 volumes of cold acetone. A globulin fraction was separated from the albumins by dialysis against water.

SDS PAGE

The protein samples were analyzed by electrophoresis in 12-15% SDS-polyacrylamide (SDS PAA) slab gels (Laemmli, 1970). Gels were stained with Coomassie Brilliant Blue R 250. Gels containing radioactive polypep-
RESULTS AND DISCUSSION

The main outstanding characteristic of the buckwheat seed proteins is the high content of the essential amino acid lysine (average of 6.1%), which is higher than in any of the cereal grains (Pomeranz and Robins, 1972). The high content of prolamin (alcohol-soluble fraction) in cereals accounts for the low content of the essential amino acids (de Lumen, 1990). In order to determine the distribution of lysine among the buckwheat seed polypeptides, we prepared in vivo labeling of buckwheat seeds with \(^{14}\text{C}\) lysine. Buckwheat seeds, at the mid-maturation stage of development, were harvested and isolated embryos were incubated, either in a \(^{14}\text{C}\) labeled amino acid mixture or in a solution of \(^{14}\text{C}\) lysine. After in vivo labeling, the proteins were extracted from the seeds and separated by SDS PAGE. The fluorogram of the in vivo labeled proteins is shown in Fig. 1. Comparing the fluorogram of \(^{14}\text{C}\) lysine labeled polypeptides with that obtained by amino acid mix labeling, shows that the \(^{14}\text{C}\) lysine has been incorporated, presumably into \(18\) kDa polypeptides as well as into \(16\) kDa albumin. The content of lysine in the \(16\) kDa albumin (5.6%) was also confirmed by the determination of the amino acid composition in our previous paper (Radović et al., 1999). Due to the same rate of biosynthesis of the storage polypeptides in the mid-maturation stage of seed development (Maksimović et al., 1996), the stage we used in presented the in vivo labeling experiments, we can consider these polypeptides as being lysine rich. When we compared the proportion of \(18\) kDa polypeptide to the total seed proteins (5%), with the distribution of \(^{14}\text{C}\) lysine incorporation, it could be concluded that the high content of a lysine is a remarkable feature of this unique polypeptide. As the distribution of the \(^{14}\text{C}\) labeled amino acid mixture resembled the protein pattern characteristic for the mid development stage under the same conditions of in vivo labeling, the high level of \(^{14}\text{C}\) lysine incorporation into the \(18\) kDa polypeptide could be a strong indicator of the seed lysine content.

According to data obtained previously (Radović, 1998), such as its abundance among the total seed proteins, its progressive accumulation during seed maturation, as well as its egradation during germination, the
18 kDa polypeptide could be considered as a storage fraction polypeptide. In order to classify the 18 kDa polypeptide according to its solubility, the total protein extract was dialyzed against water, and the water insoluble proteins were analyzed using SDS-PAGE. As is shown in Fig. 2, the 18 kDa polypeptide belonged to the globulin family of buckwheat seed storage proteins. In vivo labeling studies with pulse chase (Maksimović, 1997) also showed that this globulin polypeptide is a monomer, and was not a product of proteolytic processing of a larger precursor.

The high lysine content of the 18 kDa polypeptide should be considered when discussing the possibility of using its specific buckwheat gene for transfer to cereals in an effort to improve their nutritional quality. It is well known that lysine is the first nutritionally limiting essential amino acid in most cereals and that there have only been a few lysine-rich seed storage proteins identified so far. A high lysine content has been also reported for the 26 kDa basic subunit of buckwheat (Rout et al., 1997). However, that polypeptide was a part of the large hexameric globulin, whose structure and biosynthetic pathway are complex for the appropriate expression and storage in a heterologous system (Muntz et al., 1993). The individual 18 kDa polypeptide seems to be more suited to that purpose. In our future experiments, we are going to attempt to determine the amino acid composition of this specific polypeptide and, if the high lysine content is confirmed, we intend to clone the corresponding gene.

ACKNOWLEDGEMENTS

This work was supported by Ministry of Science, Development and Technology of Republic of Serbia (Grant 1451).

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