# Agmatine iminohydrolase activity during development and germination of groundnut seeds[1]

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*Summary.* During the development of groundnut seeds agmatine iminohydrolase activity increased in the cotyledons but remained constant in the embryo. In seeds stored for 1 year, decreased activity of the enzyme was found in both cotyledons and embryo. During germination, the enzyme activity increased in the cotyledons, but in the embryo it increased up to day 3 and then decreased to the initial level.

It is now known that the polyamines spermidine and spermine and the related diamine putrescine are probably ubiquitous in animals and plants, and that at least 1 of these 3 amines is present in all microorganisms[3]. In plants, polyamine biosynthesis appears to proceed either by the decarboxylation of arginine to agmatine and its subsequent conversion to putrescine[4], or via ornithine which can be decarboxylated directly to putrescine[5,6]. The putrescine formed by either of these pathways may be converted to the polyamines spermidine and spermine[4]. Groundnut seeds accumulate large quantities of protein-bound arginine in their cotyledons[7]. During germination these storage proteins are hydrolyzed to give amino acids, which may either be utilized in situ or directly transported to the growing embryo. Arginine, apart from its role in the urea cycle, may be utilized for glutamate or proline formation[8], or serve as a precursor for polyamine biosynthesis. Agmatine iminohydrolase (EC 3.5.3.12), which converts agmatine to N-carbamylputrescine and ammonia, has been reported to be present in the leaves and seeds of maize and sunflower seedlings[9]. A survey of higher plants showed that this enzyme is particularly active in extracts of groundnut seeds. Results of studies on agmatine iminohydrolase activity during the development, storage and germination of groundnut seeds are reported in this communication.

*Materials and methods.* Plants of groundnut (*Arachis hypogea* L. var. Punjab-1) bearing pods were brought from a local farm. Seeds were removed and separated into different groups based on their size. 30–40 seeds were taken in each group and the average fresh weight of the seed was determined. The cotyledons and embryos were removed and a portion of these was used for dry weight determination. The remaining portion was used for the determination of the enzyme and protein content. Samples were collected and analyzed several times during the development of seeds over a period of 2 months. At least 3 independent determinations were made for each stage, and the values reported in the table are a mean of these determinations. The values for the development stage 1 and 2 are the whole seed values, since cotyledons and embryo could not be separated. In stage 3 and 4 also we could not get enough embryo material so only the cotyledons were analyzed. The pods after harvest were brought to the laboratory and stored at room temperature for a period of 1 year.

*Germination.* Seeds stored for 1 year were used for these studies. The seeds were surface sterilized with lysol, washed thoroughly with $H_2O$ and then soaked in $H_2O$ for a period of 16 h. The seeds were then kept for germination at 22 °C in the light in Petri dishes on moist filter papers. The time when seeds were kept for germination, after soaking for 16 h, was considered as day zero of germination.

*Enzyme extract.* At specified periods cotyledons and embryos were separated and a 10% (w/v) extract of the tissues was prepared by grinding in a chilled pestle-mortar using 10 mM Pi buffer (pH 7). The extract after passing through 2 layers of cheese cloth was used for enzyme assay.

Agmatine iminohydrolase activity was determined by estimating N-carbamylputrescine. The assay system consisted

Dry weight, protein content and agmatine iminohydrolase activity during development, storage and germination of groundnut seeds

| | Average fresh wt of seed (mg) | Dry wt (%) | | Protein (mg/g fresh tissue) | | Agmatine iminohydrolase (units/g fresh tissue) | |
|---|---|---|---|---|---|---|---|
| | | Cotyledon | Embryo | Cotyledon | Embryo | Cotyledon | Embryo |
| Development stage | | | | | | | |
| 1 | 6 | 8 | | 6 | | 0.7 | |
| 2 | 36 | 13 | | 12 | | 1.3 | |
| 3 | 75 | 15 | - | 13 | - | 2.1 | - |
| 4 | 146 | 21 | - | 21 | - | 2.4 | - |
| 5 | 234 | 27 | 49 | 28 | 39 | 2.8 | 5.3 |
| 6 | 338 | 33 | 58 | 69 | 52 | 3.7 | 6.0 |
| 7 | 454 | 64 | 57 | 101 | 81 | 3.8 | 5.8 |
| 8 | 569 | 69 | 58 | 123 | 84 | 4.0 | 5.5 |
| 9 | 706 | 72 | 67 | 120 | 88 | 4.0 | 5.5 |
| Storage (months) | | | | | | | |
| 6 | 485 | 76 | 70 | 135 | 89 | 4.6 | 4.7 |
| 12 | 420 | 78 | 72 | 138 | 93 | 2.1 | 1.9 |
| Germination (days) | | | | | | | |
| 0 | | 70 | 63 | 136 | 111 | 2.4 | 2.4 |
| 1 | | 64 | 47 | 121 | 89 | 2.6 | 2.8 |
| 2 | | 59 | 31 | 100 | 45 | 3.4 | 3.6 |
| 3 | | 58 | 29 | 93 | 26 | 4.3 | 4.6 |
| 4 | | 57 | 26 | 93 | 24 | 4.9 | 3.8 |
| 5 | | 56 | 25 | 86 | 20 | 6.2 | 3.0 |
| 6 | | 55 | 19 | 86 | 19 | 6.0 | 2.8 |
| 7 | | 52 | 9 | 80 | 19 | 6.0 | 2.7 |
| 8 | | 51 | 4 | 66 | 19 | 6.0 | 2.6 |

The values for development stage 1 and 2 are whole seed values.

of 25 mM Pi buffer (pH 7.5); 1.25 mM agmatine (Sigma), and enzyme (1 ml) in a total volume of 4 ml. After incubation at 37 °C for 1 h, the reaction was terminated by adding 0.5 ml of 10% TCA. In the blank, agmatine was added after incubation. The supernatant, after removal of proteins by centrifugation, was used for the estimation of N-carbamylputrescine by the method of Archibald[10]. The enzyme unit is defined as the amount of enzyme required to liberate 1 µmole of N-carbamylputrescine/h under the assay conditions. The protein content of the tissue was determined by the method of Lowry et al.[11] with bovine serum albumin as a standard. Dry weight of cotyledons and embryo was determined by drying fresh tissue at 60 °C to constant weight.

*Results and discussion.* Seeds were collected and grouped into different stages of development as described in the experimental section. The average fresh weight of the seed and dry weight of the cotyledons and embryo was determined. Stage 9 represents the fully mature seed, each weighing between 0.6 and 0.8 g. Storage of these seeds, over a period of 1 year, resulted in about 40% decrease in fresh weight (table). Dry weight of the cotyledons increased progressively up to stage 7 and was fairly constant after that. Storage resulted in a slight increase in dry weight. The dry weight of the embryo remained constant from stage 6 with some increase during storage. Protein content of the cotyledons and the embryo increased rapidly up to stage 7 and thereafter the increase was slow. It is suggested that during cotyledon development there are 2 phases of growth[12]; an initial one of cell division and a later one of cell expansion. Accumulation of storage material occurs mainly in the expansion phase. The final phase of seed development (maturation) is marked by a severe water loss, a gradual reduction of metabolic activity and a very small increase in the dry weight. The protein level of the kidney bean has been found to be relatively constant during development whereas it increases in the cases of pea, peanut and soybean[13]. The protein content of soybean increased throughout development, whereas the increase in peanut took place early, and that in pea took place later in development[13]. Our results are in agreement with the data reported for peanut[13]. During the germination of seeds, the protein content of both cotyledons and embryo decreased progressively over a period of 8 days. Agmatine iminohydrolase activity increased during development of the cotyledons but was constant in the embryo. In seeds stored for 1 year, decreased activity of the enzyme was found in both cotyledons and embryo. During germination, the enzyme activity increased further in the cotyledons. In the embryo, however, it increased up to day 3 but came back to its original level with further germination.

The studies reported above thus suggest that during the development of groundnut seeds, the reserves in the cotyledons are built up, as shown by an increase in dry weight and protein content associated with increased agmatine iminohydrolase activity. Germination of seeds represents a process where the reserve material of the cotyledons is being utilized, as shown by a decrease in protein content. The enzyme activity increased during the development of cotyledons, and also during germination, suggesting that a part of the arginine released from the reserve proteins of the cotyledons may be utilized through the agmatine iminohydrolase reaction.

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## Solubilization of human red cell membranes by lysolecithins of various chain lengths

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*Summary.* Red cells and membranes prepared from them were treated with graded amounts of palmitoyl, myristoyl and lauroyl lysolecithins. There was no release of phospholipids from the intact red cells in the absence of hemolysis. The solubilization pattern of lipid and protein from the red cell membranes by lysolecithins varies with their chain length.

Lysolecithins are amphiphatic compounds which, like the anesthetics[3], interact with the red cell membrane inducing concentration-dependent effects such as red cell crenation, sphering, protection against hypotonic lysis and finally, hemolysis[4,5]. At much higher concentrations lysolecithins act as detergents and solubilize membranes. The present study reports on the solubilization of red cell membrane components by lysolecithins of various chain lengths. Red cells were separated from fresh human blood, washed in isotonic buffered saline (pH 7.4) and resuspended at about 40% hematocrit. Red cell membranes were prepared from recently outdated blood, essentially according to Dodge et al.[6] by hemolyzing washed red cells in 30 vol. of 10 mM Tris-HCl buffer (pH 7.4) supplemented with the proteinase inhibitor Trasylol (19 KIU/ml), repeatedly washed and resuspended in the hemolysis buffer to give 50 µg lipid phosphate/ml. Palmitoyl (C 16:0), myristoyl (C 14:0) and lauroyl (C 12:0) lysolecithins were 10 µmole/ml in either isotonic buffered saline or in hemolysis buffer. Since the lysolecithins presented residual venom phospholipase A activity, both the red cell and the membrane suspensions were supplemented with 1 mg/ml EDTA prior to addition