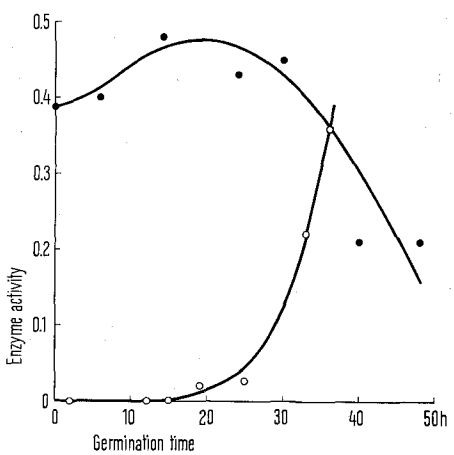
Glutamate Decarboxylase in Barley Aleurone and its Relationship to α-Amylase Development During Germination

Both the enzyme glutamate decarboxylase and its product γ-aminobutyric acid (GABA) have been reported to occur widely in plants[1]. Their physiological importance has not yet been clearly defined although tracer experiments have all shown a close relationship between GABA and Krebs cycle acids[1–3]. GALSKY and LIPPINCOTT[4] have recently reported that the synthesis of α-amylase is activated in embryoless barley seeds by a number of amino acids, including glutamic and aspartic acids. GABA did not induce α-amylase activity in these experiments but we considered it would be of interest to examine the effect of GABA on α-amylase which was induced by use of gibberellic acid (GA)[5].

The barley used was *Hordeum vulgare* L. var. Maris Otter, dehusked with 50% sulphuric acid and stored in a desiccator at room temperature. Studies of the interaction of GABA and GA were carried out using 2 mm endosperm slices, as previously described for similar studies with cyclic AMP and GA[6]. α-Amylase activity was assayed by the iodine-dextrin colour method of BRIGGS[7]. The results were expressed in arbitary units (AU)[8]. The effect of GABA on α-amylase activity was determined in the normal assay system. A fixed amount of enzyme was preincubated for 1 h with appropriate concentrations of GABA before determining the activity. Alternatively, GABA was added to the substrate to give a suitable concentration in the final assay mixture.

Aleurone was prepared from dehusked barley germinating at 25 °C using the method previously described by DUFFUS[9]. Samples of 50 grains showing optimum germination rates were taken and the aleurone was prepared from 2 mm slices cut from the central portion of each grain. The combined aleurone pieces were mixed with ground glass and 0.5 ml of water in a Potter type homogenizer and extracted at 4 °C. The crude homogenate was centrifuged at 1000 *g* and 4 °C for 10 min. The supernatant was assayed for glutamate decarboxylase activity. The assay system had a total volume of 0.3 ml and contained 75 m*M* potassium phosphate pH 6.2, 10 μ*M* pyridoxal phosphate, 40 m*M* sodium glutamate and 0.1 ml of aleurone extract. The solution minus glutamate was incubated at 25 °C for 10 min when glutamate was added to start the reaction. After 30 min further incubation at 25 °C, 0.03 ml of 5 *N* H_2SO_4 were added to stop the reaction. The GABA formed was estimated spectrophotometrically in a neutralised aliquot of the reaction solution using enzymes prepared from *Pseudomonas fluorescens*[10]. In the case of each extract, the GABA present in an assay to which no glutamate had been added was measured. The difference between the two GABA contents was then taken as a measure of glutamate decarboxylase activity.



Changes in activity of glutamate decarboxylase and α-amylase during germination at 25° C. α-amylase activity is in AU/grain and glutamate decarboxylase activity is expressed as μmoles GABA produced/h/aleurone slice.

glutamate decarboxylase ●
α-amylase ○

Initial experiments on the effect of GABA on α-amylase activity were carried out using the system designed to measure the production of α-amylase by barley aleurone under the influence of GA. The results appeared to indicate that concentrations of GABA of between $10^{-6}M$ and $10^{-3}M$ cause an approximately 30% decrease in α-amylase production. However, further experiments showed that GABA acts directly as an inhibitor of α-amylase in vitro rather than exerting an effect on the biosynthetic system. Whereas $2\times10^{-6}M$ GABA was without effect on α-amylase activity, a maximum inhibition of 25% was obtained at $5\times10^{-6}M$ GABA with no further increase at concentrations up to $10^{-3}M$. Inhibition by GABA was found only when preincubation of GABA and enzyme was carried out; addition of a mixture of GABA and substrate to the enzyme did not result in any detectable inhibition of α-amylase activity.

The glutamate decarboxylase and α-amylase activities in the aleurone of optimally germinating barley samples were followed for the first 48 h of germination (Figure). Glutamate decarboxylase showed an initial slight rise in activity followed by a steep decline. The onset of this decline appears to coincide with the appearance of α-amylase activity in the tissue.

Attempts were made to demonstrate the presence of GABA in barley aleurone using the specific dansylation and chromatographic method of SEILER and WIECHMANN[11] but the compound could not be detected at a sensitivity level of approximately 0.5 μmoles/g aleurone. If GABA is present at a level found to be effective against α-amylase in vitro, e.g. $5\times10^{-6}M$, calculation suggests that the aleurone from more than a kilogram of barley may be necessary for the extraction of sufficient GABA to enable identification with present techniques.

INATOMI and SLAUGHTER[1] reported that they could not detect glutamate decarboxylase activity in extracts of barley endosperm but in their case whole endosperm was used and it seems probable that significant disruption and extraction of the aleurone cells was not achieved by the

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technique used. In the present paper it has been shown that water extracts of separated aleurone layers contain glutamate decarboxylase activity in easily detectable amounts. Thus barley aleurone appears to have the capacity to produce GABA as does the embryo[1]. It does not follow, however, that the functions of GABA are the same in both tissues: in embryos the enzyme glutamate decarboxylase increases in activity as germination proceeds and considerable quantities of GABA are synthesised[1], whereas in aleurone cells the glutamate decarboxylase activity declines steeply after a few hours of germination, and GABA cannot at present be detected. It has been suggested that in the developing embryo glutamate decarboxylase and GABA are important in the build-up and functioning of the Krebs cycle[1,3] whereas in the aleurone cells it seems possible that GABA may serve a regulatory function.

The discovery of a possible control function for GABA in plants suggests that GABA may have the same two broad functions in plants as in animals[12–14]. Namely as an intermediary metabolite concerned with the functioning of the Krebs cycle and as part of a system controlling the development and functioning of the overall organism[15].

Résumé. L'enzyme glutamate décarboxylase se trouve dans les extraits d'eau de l'aleurone de l'orge. Au début de la germination, l'activité de cette enzyme augmente, mais après, quand apparait l'enzyme α-amylase, elle diminue. Quoiqu'au dessous d'une concentration de $2\times10^{-6}M$ l'acide γ-amino butyrique (GABA) n'affecte pas l'activité de l'α-amylase in vitro, la GABA à $5\times10^{-6}M$ peut provoquer une inhibition de 25% qui n'est pas modifiée par des concentrations de GABA comprises entre $5\times10^{-6}M$ et $10^{-3}M$.

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Characterization of Nuclei from Immature Barley Endosperm

The ultrastructure of the developing barley endosperm has been described by BUTTROSE[1]. Little is known, however, of the biochemical events following anthesis and, in particular, of the role of the nucleus in these events.

Few convenient methods for the isolation of nuclei from higher plants have been reported, mainly because of the presence in the selected tissues of chloroplasts, amyloplasts and mechanically resistant cell walls. In the cereal grain, however, immediately after anthesis, rapid division of the triploid endosperm nucleus takes place without accompanying cell wall formation[2]. The nuclei are close packed with only a few undifferentiated spherical bodies present. Thus a method could be easily devised for the isolation of relatively uncontaminated and intact nuclei. This paper then describes some of their properties in terms of chemical composition, metabolic activity and histone content.

Materials and methods. The 2 row barley, *Hordeum distichum* (L.) Lam. CV. MARIS BALDRIC, was used and was grown either in quantity on the University farms or in the greenhouse under lights.

100 grains, never older than 3 days after anthesis were individually squashed in an ice-cold agate mortar in 2 ml of M sorbitol containing 30% glycerol and $0.001\,M$ Ca^{2+}. The mixture was filtered through 2 layers of muslin on to 2.0 ml of $1.8\,M$ sorbitol, containing 30% glycerol and $0.001\,M$ Ca^{2+} in a 5 ml polypropylene centrifuge tube. Thus 2 layers were formed. A glass rod was passed once through the interface to give a slight mixing of the layers. This was centrifuged for 20 minutes at $200\times g$ and the nuclei accumulated in the lower layer. The top layer and interface were discarded. The lower layer was then transferred to another 5 ml centrifuge tube and centrifuged at $200\times g$ for 2 h. The pellet was further compacted by accelerating to $1{,}000\times g$ for 1 min at the end of the 2 h. This pellet was used without further treatment in the experiments to be described.

The purification was monitored routinely with a Vickers Patholux microscope using phase contrast lenses and a magnification of 1,000 diameters. A Leitz microscope was used to take phase contrast photomicrographs at a magnification of 700 diameters.

DNA, RNA and protein were determined in the nuclear pellet as described by ROZIJN and TONINO[3]. RNA polymerase was assayed as described by WEISS[4] using boiled nuclei as a control. The pH of assay was 7.8. NAD pyrophosphorylase was assayed by the method of GINSBURG-TIETZ et al.[5] and the NAD synthesized measured by the method of KLINGENBERG[6]. Fumarase was assayed by the method of PIERPOINT[7] and succinoxidase spectrophotometrically as described by VEEGER et al.[8].

Histones were extracted from the nuclear pellet by a modification of the method of MURRAY et al.[9]. The nuclei were extracted with 3.0 ml of a KCl-HCl buffer ionic strength, 0.1, pH 2.8, and the suspension left at 4 °C for 20 min before centrifuging at $5{,}000\times g$ for 10 min. The supernatant was discarded and the pellet washed once with 3.0 ml of the same buffer. The washed pellet was then

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