

There seems to be some relationship between methionine and crude protein content. The studies are in progress with inbred lines which might give some information regarding the protein content and the pattern of these essential amino acids.

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1. Alpha-amylase in developing maize endosperm.

An amylase-like enzyme was detected in developing maize endosperm during investigations of the phytoglycogen-forming branching enzyme. Investigations were then initiated to develop a reliable technique of extraction and fractionation of the enzyme, to determine the activity, nature and classification of the enzyme, and to determine the activity of this enzyme in several genetic endosperm mutants.

Fractionation and partial purification of the enzyme was accomplished through gradient elution with NaCl in Tris-maleate buffer from a DEAE-cellulose column. The products of enzyme action on amylose, glycogen and beta-limit glycogen were examined by thin-layer chromatography. The enzyme reaction was measured by the decrease in iodine-staining ability of an amylose solution that was being degraded by the enzyme. The enzyme activity was calculated by the rate of decrease in color of the iodine-starch complex.

The enzyme was found to degrade amylose, glycogen and beta-limit glycogen. Maltose, maltotriose and maltotetraose were produced in about equal amounts when amylose was used as a substrate. Action on glycogen and beta-limit glycogen gave less maltose than maltotriose and maltotetraose and the rate was slower than on amylose. No isomaltose or glucose were ever detected, even after extended periods of incubation, on linear or branched substrates.

Copper, iron, lead, mercury and p-chloromercuribenzoate inhibit enzyme activity. The enzyme was active after extended periods of dialysis against EDTA; therefore, a requirement for calcium was not demonstrated. The enzyme was very stable in water at 10°C for several weeks.

The enzyme was found to have a pH optimum near 6.8 in Tris-maleate buffer, which is higher than most amylases of plants. The pH of alpha-amylase in germinating corn kernels has been reported to be about 4.6-5.4; therefore, the amylase in developing maize endosperm was much higher. The Michaelis-Menten constant was calculated to be 0.08 per cent amylose.

Enzyme was detected in normal dent and the mutants ae, bt₁, bt₂, du, fl₁, o₂, sh₁, sh₂, su₁, su₂ and wx. Results of analyses of kernels at 16, 20, 24 and 28 days after pollination and at maturity (air dry) indicated that activity increased from 16 to 24 days and then began to drop. Very little activity was detected in dry kernels.

The enzyme may play an important role in starch synthesis even though it is a degradative enzyme. Possibly, the enzyme degrades the long molecules formed by ADPG- and/or UDPG-transferase to form more acceptors for glucose transfer, thereby increasing the efficiency of the transferases. The enzyme was not demonstrated to synthesize higher polymers. It was concluded that the enzyme is an alpha-amylase because it cleaved the glucosidic linkages of amylose and cleaved beyond the branch points of beta-limit glycogen to produce small molecular weight oligosaccharides. The other characteristics of the enzyme are similar to alpha-amylases. This is the first time that alpha-amylase has been characterized in developing maize endosperm.

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1. Modification of Anderson's method for transferring genes via trans-locations.

Anderson's method (see Brookhaven Symposia in Biology No. 9 pp. 30, 31) involves three main steps as follows: Finding a translocation with a breakpoint close to the locus of the gene to be transferred, backcrossing the translocation into the inbred to be improved, and backcrossing the desirable gene into the inbred that temporarily carries the translocation. Two complete backcrossing programs are necessary to obtain the recovered inbred that contains the desired trait, a normal chromosome complement, and a minimum amount of foreign chromosome.

With the advent of an increasing proportion of single cross commercial hybrids, it might be advantageous to permanently place the translocation into the converted inbred along with the desired trait. This would reduce the number of backcross programs from two to one per line.

The main points of the modified scheme might be summarized as follows: Cross the suitable translocation (one with a breakpoint near the desired gene) to the stock carrying the desirable gene; then make the appropriate backcross in order that both the translocation and the desirable gene are segregating; identify and maintain crossover plants that link the desirable gene to the translocation and the desirable gene. Once the desirable gene translocation stock has been made, it may be used as a source for backcross programs to inbred lines; selection may be for the desirable gene and/or semi-sterility due to the translocation.