THE ENZYMATIC DEFICIENCY IN THE WAXY MUTANT OF MAIZE[1]

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Since 1943, it has been known that the endosperms of maize seeds homozygous for the waxy (wx) allele contain starch which is entirely amylopectin (Sprague, Brimhall, and Hixon). In contrast, seeds which are heterozygous or homozygous for the non-waxy (Wx) allele contain starch which has up to 25 percent amylose with the remainder being amylopectin.

The enzymatic basis for this difference has to date been elusive. Bliss and Naylor (1946) showed that developing seeds of the waxy mutant did contain phosphorylase. Fuwa (1957) found approximately equal activity in developing seeds of waxy and non-waxy maize for both phosphorylase and for the Q enzyme, making it unlikely that different proportions of these enzymes could explain the difference.

During the past summer, it has been found that waxy seeds 16 days after pollination completely lack a UDPG transferase activity which is present to a substantial degree in similar preparations from non-waxy seeds. The activity is localized in the starch granules. This is apparently the same enzyme which has been detected by Leloir, Rongine De Fekete, and Cardini (1961) in starch granules of potatoes, sweet corn, and beans and which transfers the glucose from UDPG to the non-reducing end of the acceptor. Recondo and Leloir (1961) have subsequently shown that the glucose is transferred from ADPG approximately 10 times as rapidly as from UDPG.

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METHODS AND RESULTS

Ears of comparable strains of waxy and non-waxy maize were self-pollinated, and then harvested and frozen 16 days after pollination. The kernels were then sliced from the ears and processed into acetone powders which were stored at -15°.

The isolation of the starch granules followed the procedure of Leloir et al. (1961) as did the measurement of enzymatic activity.

Reference to Fig. 1 will show the relative activities of the two preparations under the stated conditions. Subsequent preparations of starch granules from the two genetic types have shown that these results are reproducible. No starch granule preparation from the waxy strain has shown any UDPG transferase activity whatsoever. Mixtures of Wx and wx starch granules have an activity which corresponds closely with the activity attributable to the amount of Wx granules present.

The question next arises as to whether the lesion in the mutant might not be simply a release of the protein from the starch granule or a failure of the protein to attach to the starch granule. Two lines of evidence indicate that this is not so. First, a measurement of the protein content of the starch granules by the method of Lowry et al. (1951) using crystalline bovine serum albumin as a standard shows an average protein content of 5.6 μgs/mg. starch granules for the non-waxy preparations and 6.6 μgs/mg. starch granules for the waxy preparations. If there were a release or a failure of attachment, one might expect the waxy starch granules to have a substantially lower protein content.

Secondly, if there were a failure of attachment to the starch granule, one might expect to find UDPG transferase activity in the soluble fraction of the wx extracts where the expression of its activity would be dependent on the presence of a glucose acceptor. In our tests, the acceptor was a maltodextrin mixture having maltotetraose as the lowest member. This mixture was prepared by the partial hydrolysis of amylose and separation on a charcoal-Celite

column according to Whelan et al. (1953). The eluate coming off the column at between 20 and 50 percent ethanol was collected and dried.

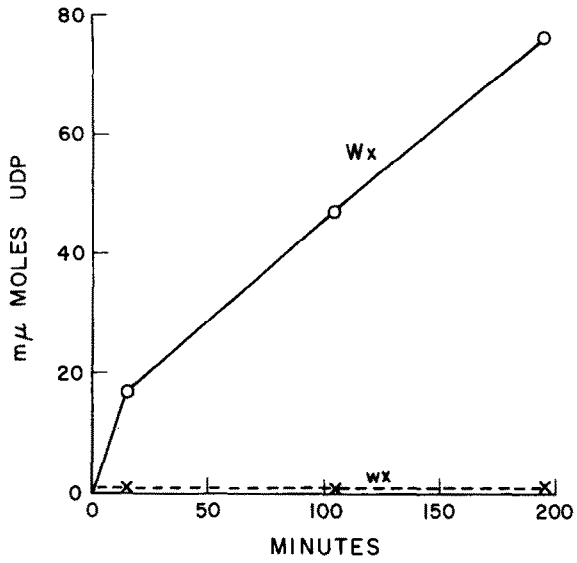

FIGURE 1. The amount of UDP released by starch granule preparations from wx and Wx seeds in reaction mixtures containing 5 mg. of starch granules and 0.3 micromoles of UDPG, 0.1 micromoles EDTA, and 4.0 micromoles glycine in a buffer solution (pH 8.4). The total liquid volume was 15 microliters. Incubation was at 37°. Fifteen minutes before the stated times 25 microliters of a 0.01 M phosphoenolpyruvate solution and 25 microliters of a pyruvate kinase solution containing approximately 26 enzyme units/ml. were added for the measurement of UDP according to the method of Leloir and Goldemberg (1960).

Various ammonium sulphate fractions of water extracts of the acetone powders from waxy and non-waxy seeds were tested after dialysis against distilled water for 22 hours for the ability to release UDP from UDPG in the presence and in the absence of the acceptor. In both genetic types, there was an appreciable release of UDP by the fractions which were from 25 to 50 percent and from 50 to 75 percent of ammonium sulphate saturation, but activity was nearly equal for both types. No effect from the presence of the glucose acceptor could be detected, and it is assumed that the activity observed is an enzymatic splitting of the glucose from UDPG since glucose is detectable after the incubation period.

The reaction seems essentially irreversible in the sense that Wx starch granules are unable to transfer glucose from starch in the granules to either UDP or ADP.

The activity of Wx starch granules is substantially reduced by grinding to a powder while dry. This same effect was noted by Leloir et al. (1961).

DISCUSSION

Since seeds homozygous for waxy contain as much starch as do non-waxy seeds in spite of lacking the UDPG transferase system, it is clear that the amylopectin of the waxy type cannot be formed by the branching of amylose formed by the UDPG system. This finding offers a clear indication of the presence of two different paths for starch formation, one leading to the branched chain polymer (amylopectin), and the other leading via the starch granule-bound UDPG transferase system to the straight chain polymer (amylose).

With the recognition of the metabolic lesion in the waxy mutant, it should now be possible to utilize the other mutants in maize effecting starch formation to work out the pathways of starch formation in the endosperms of cereals in considerable detail.

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