ADSORPTION OF ADPG-STARCH TRANSGLUCOSYLASE BY AMYLOSE[1/]

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The inheritance of the glutinous (waxy) character in cereal grains is governed by a single recessive gene, wx, and the starch molecules in such cereals consist essentially of a branched type of polysaccharide called amylopectin. On the other hand, the starch of non-glutinous cereal grains is composed of 70-80% amylopectin, the rest being amylose, a linear type polysaccharide. Although there is considerable evidence available concerning the role of ADPG (UDPG)-starch transglucosylase in the formation of amylose molecules, this enzyme is completely absent in starch granules derived from waxy maize seeds (Nelson and Rines, 1962), and it has been found to be true also for glutinous rice grains (Murata et al., 1964, b). A genetic implication was put forward by the former workers for the different mechanism of amylose synthesis from that of amylopectin. Recent experiments in this laboratory (Murata et al., 1964, b) have shown, however, that the ADPG-starch transglucosylase activity in developing glutinous rice grains is almost exclusively found in the soluble fraction of the centrifugate of grain extracts, as sharply contrasted to the particulate enzyme nature in non-glutinous rice grains. A possible reason for such a marked difference in the distribution of transglucosylase activity between genetically distinct varieties can be sought in the physical structure of the enzyme molecules in grain cells.

The classical study of Rundle et al., (1944) disclosed the helical structure of the amylose molecule by X-ray diffraction analysis. Subsequently, this molecular configuration of starch formed the

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basis for the explanation of the mechanism of the iodine coloration of starches and the selective precipitability of the amylose molecule from amylopectin by certain aliphatic alcohols (see Bonner, 1950). This concept has prompted us to examine the possibility of the role of amylose molecules in the starch granules of non-glutinous cereals as an adsorbent of the ADPG-starch transglucosylase. That is, the effect of amylose and starch granules prepared from both glutinous and non-glutinous rice varieties on the soluble ADPG-starch transglucosylase was tested.

Soluble ADPG-starch transglucosylase was prepared after the method of Murata et al., (1964, b) using freshly harvested glutinous rice grains (variety Pah Leaud). The supernatant fraction of the 14,000 × g centrifugation containing the transglucosylase was treated with purified starch granules from glutinous and non-glutinous rice and also with pure amylose (G.B.I.). After shaking the individual whole mixtures gently at room temperature (23°C) for 60 minutes and duplicate samples for 180 minutes, aliquots of the suspensions were taken immediately for the assay of the transglucosylase activity. Simultaneously, the suspensions were spun down and the enzymic activities in the supernatant fractions were determined. Figure one shows that the simple incubation itself with starch samples from both glutinous and non-glutinous rice grains caused a slight decline in the enzymic activity of the whole suspension for some unknown reason, and the effect appeared to be more marked in the treatment with non-glutinous rice starch as compared with glutinous rice starch. However, a more significant loss of the enzyme activity occurred in the supernatant fraction, and the longer the incubation the lower the enzyme activity. The effect of the starch from non-glutinous rice was greater than from glutinous rice. The effect of amylose was most marked, with only about 10% of the original enzyme activity remaining in the supernatant fraction after 60 minutes of incubation. In order to demonstrate whether the decrease in the transglucosylase activity in the supernatant could be accounted for by the adsorption of the enzyme protein by the starches, the enzymic activities of the individual starch precipitates were measured after each washing step. As can be seen clearly from the results in Table I, the transglucosylase activity of the precipitated starch granules from glutinous rice was very weak throughout the washing period, and practically no enzyme activity remained in the final acetone powder. In contrast, a rather strong enzyme activity was detected in the similarly prepared starch precipitate from non-glutinous rice. There was a slight

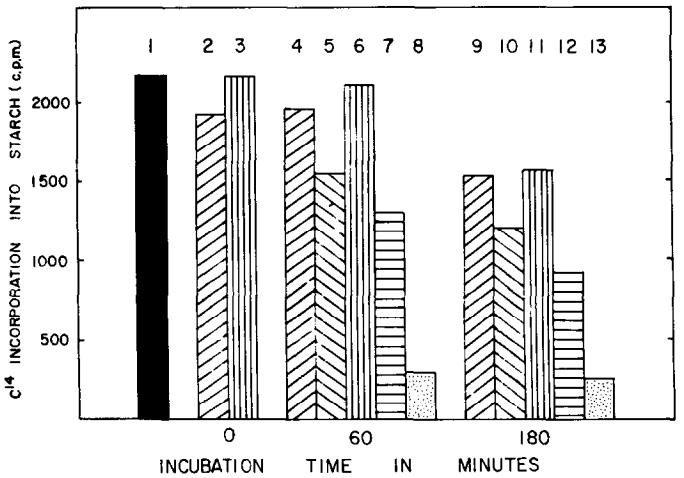

Fig. 1. Effect of various types of starch on ADPG-starch transglucosylase in glutinous rice grains

Six g of glutinous rice grains were ground with 3.6 ml of 0.07 M phosphate buffer solution (pH 7.5) containing 0.035 M glutathione and 0.07 M EDTA, and the whole homogenate was centrifuged first at 6,400 × g for 5 minutes, then at 14,000 × g for 20 minutes to get 3.1 ml of the liquid. The transglucosylase activity of this original supernatant was measured (1). To a 0.4 ml aliquot of this fraction was added 60 mg each of pure starch granules from both glutinous and non-glutinous rice and pure amylose. A 20 µl aliquot of the suspension withdrawn from the initial two systems was immediately used for the enzyme assay (2 and 3). Technically, the enzyme activity could not be measured in the amylose system. After incubating the remainder of the whole suspensions for 60 and 180 minutes respectively at room temperature (23°C), the enzyme activities were again determined in aliquot from the suspension of the two rice starch systems (4,5 and 9,10). The remainder of the individual suspensions were centrifuged at 6,400 × g for 5 minutes, and a 20 µl aliquot of the supernatant was assayed for the enzyme activity in the order of kinds of starch added (6,7,8 and 11,12,13). The reaction mixture contained (in µmoles), glycine buffer (pH 8.4), 4.0; ADP-glucose-C^{14}, 0.219 (5,700 c.p.m.); NaF, 3; 20 µl each of either whole suspension or supernatant fraction as explained above in a total volume of 30 µl. In the system of the supernatant fraction, 3 mg of starch granules prepared from glutinous rice was added as an acceptor molecule. Incubation was at 37°C and the radioactivity incorporation into the starch was determined after the method of Akazawa et al., (1964) and Murata et al., (1964, a).

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decline in the enzyme activity during the washing period, but the overall trend reflects the enzyme-adsorbing effect of the amylose molecule which is a characteristic constituent in non-glutinous rice starch. Indeed, the effect of amylose was the highest, and the transglucosylase activity of the acetone powder amylose precipitate showed a 13% glucose transfer from ADP-glucose-C^{14} to the starch mole-

Table I. Adsorption of soluble ADPG-starch transglucosylase by various types of starch

Expt.	Type of starch precipitate	Incubation time (minutes)	C^{14} incorporation into starch	
			Total c.p.m.	%
I	Glutinous rice starch precipitate (Washed once with H_2O)	60	40	0.7
		180	89	1.5
	" (Washed three times with H_2O	60	14	0.2
		180	55	0.9
	" (Acetone powder)	60	6	0.1
		180	6	0.1
II	Non-glutinous rice starch precipitate (Washed once with H_2O)	60	271	4.6
		180	346	5.9
	" (Washed three times with H_2O)	60	151	2.6
		180	241	4.1
	" (Acetone powder)	60	137	2.3
		180	197	3.3
III	Acetone powder amylose precipitate	60	753	12.8
		180	1,700	30.5
	" (Heated in a dry state, 100°C, 10 min.)	60	590	10.0
	" (Heated with buffer, 100°C, 10 min.)	60	93	1.6
	" (+ α-iodoacetamide, 2.3×10^{-3} M)	60	212	3.6
		180	219	3.7

To a 1.0 ml aliquot of the original supernatant (1.25 mg protein-N/ml), as explained in Fig. 1, was added 150 mg each of starch granules from both glutinous and non-glutinous rice and pure amylose. After the incubation for 60 minutes, the whole mixture was centrifuged at 6,400 × g for 5 minutes to get the individual starch precipitates, which were then washed three times with cold distilled H_2O and three more times with cold acetone (-15°C). The final acetone powder starch precipitates were dried *in vacuo* at room temperature for 60 minutes. In the case of the amylose treatment, the enzyme assay during the washing steps was omitted. Reaction mixture contained (in μmoles), glycine buffer (pH 8.4), 4.0; glutathione, 0.5; EDTA, 0.5; ADP-glucose-C^{14}, 0.34 (5,900 c.p.m.); and either 9 mg each of rice starch precipitate or 6 mg of amylose precipitate in a total volume of 44 μl. Glutathione was omitted in the reaction system containing α-monoiodoacetamide. Incubation was at 37°C.

cules in 60 minutes of incubation. This was about one third of that present in the original enzyme fraction. An interesting fact was that the enzyme activity disappeared on heating the acetone powder with a buffer solution, but practically no loss of activity occurred on heating in a dry state. This finding, which was in good agreement with the reported properties of granular starch synthetase (Leloir et al., 1961, Akazawa et al., 1964), can be interpreted by the fact that the enzyme was stabilized by amylose molecule. The enzyme action was also strongly inhibited by the addition of α-monoiodoacetamide.

An experiment was carried out next to examine any specificity which might exist in the enzyme-adsorbing effect of amylose. The results in Table II show that UDPG-sucrose transglucosylase, a soluble enzyme prepared from rice grains, was not effected by the three different starch samples, including the pure amylose. Thus it appears that the amylose effect is quite specific to starch synthetase. It should be recalled in this connection that acetone powder starch gra-

Table II. Effect of various types of starch on UDPG-sucrose transglucosylase activity

Expt.	Type of starch added to the original supernatant	Incubation time (minutes)	Sucrose formed (μmole)
I	None	10	0.430
		30	0.700
II	Glutinous rice starch	10	0.435
		30	0.700
III	Non-glutinous rice starch	10	0.420
		30	0.720
IV	Amylose	10	0.395
		30	0.710

Four g of rice grains of a non-glutinous variety (Peta) were ground in the same way as explained for the experiment portrayed in Fig. 1, and the original supernatant fraction was dialyzed against 0.01 M phosphate buffer (pH 7.5) for 3 hours at 2°C. The dialyzate (0.84 mg protein-N/ml) was used as a starting enzyme source, and was treated with three kinds of starch in the identical way as described in experiments reported in Table I. After incubation for 60 minutes, a 0.1 ml aliquot of the centrifugate (6,400 × g for 5 minutes) was used for the assay of sucrose synthesis. The reaction mixture contained (in μmoles), Tris buffer (pH 8.4), 100; fructose, 10; UDPG, 1.52; and 0.1 ml each of enzyme preparation in a total volume of 0.8 ml. Incubation was at 37°C, and the sucrose formed was assayed after the method of Roe (1934). Amount of sucrose synthesized by the original dialyzed supernatant under the identical conditions was (in μmoles), 0.425 (10 minutes) and 0.730 (30 minutes) respectively.

nules of rice exhibited a rather high activity of UDPG (ADPG)-pyrophosphorylase (Murata et al., 1964, a), which is one of the component enzymes engaged in the conversion of sucrose to starch.

It is truly an interesting matter for future elucidation to see what physical and chemical factors are involved in the formation of a complex of amylose and ADPG-starch transglucosylase. It is not certain at present whether the helical structure of the amylose molecule is indeed responsible for the enzyme adsorption or whether the mechanism is somewhat similar to that of dextran gel filtration, which is governed by the three-dimensional network of the polysaccharide-hydroxyl groups. Nonetheless, the selective adsorption and stabilization of the enzyme protein with its substrate pose an interesting opportunity for studying the mechanism of enzyme action, with particular reference to the biogenesis of the polysaccharide molecules. A clue will be sought also to the mechanism of the amylopectin biosynthesis in plant cells through this finding.

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References

Akazawa, T., Minamikawa, T. and Murata, T., Plant Physiol. 39, 371 (1964).

Bonner, J., "Plant Biochemistry" Academic Press, New York. (1950).

Leloir, L. F., De Fekete, M. A. R., and Cardini, C. E., J. Biol. Chem. 236, 636 (1961).

Murata, T., Sugiyama, T., and Akazawa, T., Arch. Biochem. Biophys. 107, 92 (1964, a).

Murata, T., Sugiyama, T., and Akazawa, T., Biochem. Biophys. Res. Comm. in press (1964, b).

Nelson, O. E., and Rines, H. W., Biochem. Biophys. Res. Comm. 9, 297 (1962).

Roe, J. H., J. Biol. Chem. 107, 15 (1934).