127. *The Enzymic Synthesis and Degradation of Starch. Part XII. The Mechanism of Synthesis of Amylopectin.*

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Evidence is presented which necessitates a modification of the hypothesis proposed earlier (*J.*, 1945, 877) whereby the synthesis of amylopectin from glucose-1 phosphate was pictured as involving the intermediate formation of short unbranched chains of glucose units (pseudoamylose). It is now shown that the synthesis proceeds as follows :

$$\text{Glucose-1 phosphate} \underset{\text{Phosphorylase}}{\rightleftharpoons} \text{Amylose} \xrightarrow{\text{Q-enzyme}} \text{Amylopectin}$$

The synthesis of starch from glucose-1 phosphate is brought about by at least two enzymes, phosphorylase and Q-enzyme. The former when acting alone synthesises the amylose component of starch, whilst the combined action of both enzymes leads to the synthesis of the ramified component, amylopectin. In addition Q-enzyme, acting in the absence of other enzymes or of phosphates, has the property of converting amylose into amylopectin (see Barker, Bourne, Wilkinson, and Peat, *J.*, 1950, 93, and earlier papers of this series). The interrelationships of these polysaccharides and enzymes were discussed by Bourne and Peat in 1945 (*J.*, p. 877) when it was suggested, as a working hypothesis, that the synthesis of amylopectin involved the formation of an intermediate polysaccharide (pseudoamylose) consisting of unbranched chains averaging *ca.* 20 glucose units in length and therefore equivalent to the basal chains of amylopectin. In the synthesis of amylopectin from glucose-1 phosphate, Q-enzyme was thought to establish 1 : 6-links between the short chains of " pseudoamylose," the latter being synthesised by the phosphorylase. For the conversion of amylose into amylopectin, the hypothesis requires Q-enzyme to possess a second function, namely, the fragmentation of amylose (by scission of 1 : 4-links) with the formation of the short chains of " pseudoamylose."

This hypothesis does not, however, cover all the facts of starch synthesis. In the first place, it assumes that both functions of Q-enzyme are operative in the conversion of amylose into amylopectin but that only one, the branching function, operates in the synthesis of amylopectin from the Cori ester. Again, in the case where the pseudoamylose chains are formed from glucose-1 phosphate by phosphorylase, it is difficult to see what could be the source of the energy required for the mutual combination of these chains by Q-enzyme. Evidence is now presented which favours a modified form of the original, admittedly tentative, hypothesis.

A series of dextrins were prepared *in situ* by fragmentation of the chains of potato amylose by salivary α-amylase. The digests were boiled to destroy the α-amylase and then treated with potato Q-enzyme. After incubation at 21° (for the same time as was necessary for the formation of amylopectin from the original amylose) the Q-enzyme was inactivated by heat, and the limits of conversion of the residual polysaccharides into maltose by soya-bean β-amylase were determined at pH 4·8. These values were compared with the β-amylolysis limits of the same α-dextrins before treatment with Q-enzyme. The difference in these conversion limits before and after Q-enzyme action is a measure of the extent to which the particular α-dextrin can serve as substrate for the branching action of Q-enzyme. At the same time the blue values of the α-dextrins were estimated.

The results, summarised in the Table, show that the ability of Q-enzyme to utilise the α-dextrins in the synthesis of branched polysaccharides diminishes as the blue value, and

therefore as the average chain-length, decreases. Dextrins with blue values of less than 0·45 are incapable of acting as substrates for the synthetic action of Q-enzyme, the β-amylolysis limits indicating that no additional branching is introduced into these dextrins by Q-enzyme.

α-Dextrins as substrates for Q-enzyme.

Blue value of the α-dextrin.	Limiting conversion by β-amylase (%): before Q-action.	after Q-action.	difference.	Blue value of the α-dextrin.	Limiting conversion by β-amylase (%): before Q-action.	after Q-action.	difference.
1·20 *	85·0	55·0	30·0	0·449	83·1	81·7	1·4
1·01	84·2	62·7	21·5	0·340	86·2	87·4	−1·2
0·847	82·2	69·7	12·5	0·137	86·8	86·8	0·0
0·544	84·4	79·9	4·5				

* Untreated amylose.

Measurement of the reducing power of the dextrins of blue value 0·45 suggests that the average chain-length is not less than 25 glucose units and is probably much greater.

The inference from these results is that Q-enzyme cannot link together the chains of " pseudoamylose." This supposition has been confirmed by making use of the " debranching " R-enzyme recently discovered in the broad bean and potato by Hobson, Whelan, and Peat (*Biochem. J.*, 1950, **47**, xxxix). R-Enzyme hydrolyses the branch-linkages of amylopectin, and the product is therefore akin to " pseudoamylose." Q-Enzyme is without action on this product, as is indicated by the negligible change in degree of β-amylolysis after Q-enzyme action. It therefore appears that the synthesis of a 1 : 6-link by Q-enzyme must be preceded by the scission of a 1 : 4-link, in which case the action of Q-enzyme is that of a transglycosidase and the energy needed for the synthesis of the 1 : 6-link is provided by that liberated in the scission of the 1 : 4-link. Furthermore, it is clear that scission and synthesis are interdependent and probably occur simultaneously.

Experiments are now in progress to determine the minimum length of chain which will serve as a substrate for Q-enzyme action. There are indications that this chain-length is not far short of that of natural amylose. In this case the path of synthesis of amylopectin from glucose-1 phosphate would be :

$$\text{Glucose-1 phosphate} \underset{\longleftarrow}{\xrightarrow{\text{Phosphorylase}}} \text{Amylose} \xrightarrow{\text{Q-enzyme}} \text{Amylopectin}$$

We are satisfied that the action of Q-enzyme is irreversible in a practical sense and that no conversion of amylopectin into amylose occurs by its agency (see Part VII, *J.*, 1950, 93). The question therefore arises as to why amylose is found in any plant in which a " branching " enzyme is present. It may be that the physical environment of synthesis is such that part of the amylose formed from the Cori ester is not susceptible to attack by Q-enzyme.

Experimental.

Analytical Methods.—For the methods used in measurement of the iodine stains of polysaccharides and in determination of reducing sugar and β-amylase activity see Part X (*J.*, 1950, 3566).

Preparation of Enzymes.—(*a*) Salivary α-amylase was prepared by diluting saliva one hundred times and centrifuging.

(*b*) Soya-bean β-amylase was prepared as in Part II (*J.*, 1945, 882). It was dissolved in 0·2M-acetate buffer, pH 4·8, to give an activity of 440 units/c.c., the insoluble residue being removed on a centrifuge.

(*c*) Potato Q-enzyme (Q3-fraction) was isolated as a freeze-dried powder by the method given in Part IV (*J.*, 1949, 1705).

(*d*) R-Enzyme was isolated as an acetone-dried powder from broad bean (Suttons' " Prolific Longpod ") by the method given in Part XIV (*J.*, to be published).

Fractionation of Potato Starch.—Amylose and amylopectin were separated by the thymol method of Bourne, Donnison, Haworth, and Peat (*J.*, 1948, 1687). The amylopectin was further fractionated by using the procedure of Part XIII (*J.*, to be published). An amylose of high blue value, prepared as in Part XIII, could not be used in the following experiments because of the partial precipitation of the polysaccharide which occurs on the addition of either Q-enzyme or α-amylase.

Successive Actions of α-Amylase, Q-Enzyme, and β-Amylase on Amylose.—Amylose (B.V., 1·20; 52·0 mg.), contained in a 50-c.c. standard flask, was dissolved in 0·1N-sodium hydroxide solution (20 c.c.) by warming the mixture gently with shaking. The solution was neutralised with 0·2N-H_2SO_4, 0·2M-acetate buffer (pH 7·0; 5 c.c.) and salivary α-amylase (3 c.c.) were added, and the solution was diluted

to 50 c.c. The digest was kept at 21° and at intervals portions were removed for determination of blue value. At chosen times two portions (10 c.c. each) were transferred to dry 25-c.c. standard flasks and heated for 10 minutes in a boiling water-bath. After cooling, 0·2M-acetate buffer (pH 7·0; 2 c.c. each) was added to the digests and to one was added water (3 c.c.), to the other Q-enzyme solution (3 c.c.; 67 mg.). After incubation at 21° for 2 hours the enzyme was heat-inactivated as above, and after being cooled, N-acetic acid (0·35 c.c., to adjust the pH to 4·8), β-amylase solution (2 c.c.), and water (to 25 c.c.) were added. The digests were incubated at 35·5° and the reducing powers, as maltose, were determined at intervals; the values became constant after 6 hours. Corresponding blank digests containing enzymes and buffer but without the polysaccharide were incubated at the same time in order to determine the reducing powers of the enzymes. Each digest with α-amylase provided two samples of α-dextrin. Three determinations were made by the procedure described above and hence the values for six α-dextrins are recorded in the Table. The results for the original amylose were obtained by the same procedure except that no α-amylase was used.

Successive Actions of R-Enzyme, Q-Enzyme, and β-Amylase on Amylopectin.—Digests were prepared, all of which contained amylopectin (10 c.c.; 18·7 mg.), 0·2M-acetate buffer (pH 7·0; 3 c.c.), and water (3·7 c.c.). In addition digest (1) contained water (2 c.c.), and digests (2) and (3) R-enzyme solution (2 c.c., 56 mg.). The digests were incubated at 21° for 21 hours, then portions (0·5 c.c.) were removed for determination of blue value, and the enzyme was destroyed by heat. To each, after it had been cooled, was added 0·2M-acetate buffer (pH 7·0; 1 c.c.); in addition, to digest (2) was added Q-enzyme solution (2 c.c.; 49 mg.), and to each water to 25 c.c. These digests were incubated at 21° for 6 hours and, after removal of portions (0·69 c.c.) for determinations of blue value, the digests were heated in boiling water for 10 minutes. The β-amylolysis limits of portions (20 c.c. each) were determined by the addition of acetic acid and β-amylase as described above.

We thank the Colonial Products Research Council for financial support and the Department of Scientific and Industrial Research for a maintenance grant to one of us (P. N. H.).

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