367. *The Enzymic Synthesis and Degradation of Starch. Part V. The Action of Q-Enzyme on Starch and its Components.*

By S. A. Barker, E. J. Bourne, and Stanley Peat.

The end-product of the action of Q-enzyme on amylose is a red-staining polysaccharide, having a ramified structure and an average chain-length less than that of amylose. This polysaccharide, like that produced from potato starch by Q-enzyme, is indistinguishable from natural amylopectin. The hypothesis of Bourne and Peat (*J.*, 1945, 877) that Q-enzyme catalyses the conversion of amylose into amylopectin is substantiated.

In the preceding paper (Part IV) we recalled that Bourne and Peat (*J.*, 1945, 877) ascribed a dual function to the Q-enzyme of the potato. Like the heat-labile enzyme of heart, liver, and yeast (Cori, Swanson, and Cori, *Federation Proc.*, 1945, **4**, 234), it catalyses, in association with P-enzyme (phosphorylase), the synthesis of amylopectin from glucose-1 phosphate. In addition to playing this rôle in polysaccharide synthesis, Q-enzyme effects the conversion of blue-staining amylose into a red-staining polysaccharide without the concomitant liberation of reducing sugar. When potato starch was used as a substrate for Q-enzyme, a polysaccharide was formed which was closely similar to natural amylopectin with respect to blue value (B.V.) and limiting conversion (57%) into maltose by β-amylase (Bourne, Macey, and Peat, *J.*, 1945, 882). Since little polysaccharide was lost during the experiment, it appeared highly probable that the amylose component of the starch had been converted by the enzyme into amylopectin. At that time no attempt had been made to isolate the red-staining polysaccharide produced by Q-enzyme from amylose itself, and the purpose of the present communication is to report an extension of the earlier work, whereby we have conclusively demonstrated that amylose is indeed transformed by Q-enzyme into amylopectin.

Experimental confirmation of the original hypothesis has had to wait upon the development of an improved method for the isolation of Q-enzyme, in which the main obstacles to be overcome were related to the labile nature of the enzyme and the presence of amylases. Details of the successful purification of Q-enzyme have been given in Part IV. Briefly, Q-enzyme is precipitated with lead acetate and eluted with sodium hydrogen carbonate solution in the presence of carbon dioxide. It is then precipitated (fraction Q1) with neutral ammonium sulphate (19 g./100 c.c.) and can be further purified by re-precipitation (fraction Q3). Both fractions, Q1 and Q3, have been employed in this work.

In Part IV it was shown that an average specimen of the enzyme at the Q1 stage of purification effects (at 20·5° and pH 7·04) a decrease of as much as 87% in the absorption value, A.V. (6800 A.), of amylose (B.V., 1·04) while the reducing power developed corresponds to only 2% conversion of maltose. That this represents the end-point of the reaction is evident from the progress curves in Fig. 1. With this particular Q1 sample the A.V. (6800 A.) decreased 86% in 2 hours and by only 3% more (to 89%) in 20 hours; the " apparent percentage conversion into maltose " was never greater than 4%. The changes in the iodine-staining properties of the polysaccharide are shown by the absorption curves in Fig. 2; the wave-length of peak absorption gradually moved from 6400 A. to 5200 A. as the enzymic reaction proceeded. The latter value is very close to that shown by waxy-maize starch.

The formation of a ramified structure from linear amylose chains was demonstrated by allowing Q-enzyme and β-amylase (from soya-bean) to act simultaneously on amylose. Whereas a highly active solution of β-amylase acting alone on amylose (B.V., 1·30) at pH 7·0 and 20·5° converted 90·2% of the polysaccharide into maltose in 210 minutes, a second portion of the same amylase solution acting together with Q-enzyme (fraction Q1) gave only 74·2% of maltose in the same time. Furthermore, incubation of the amylose with Q-enzyme before the addition of β-amylase resulted in the liberation of even smaller quantities of maltose. Thus, when the amylose was incubated with Q-enzyme for 30, 90, and 180 minutes (thereby decreasing the A.V. by 75, 83, and 87%, respectively) before the introduction of the β-amylase, the limiting conversions into maltose effected by the latter enzyme were 63·0, 57·0, and 55·8%, respectively.

Although these results were in full accord with the hypothesis that Q-enzyme had transformed the unbranched amylose chains into the branched chains of amylopectin, there remained the possibility that a ramified structure had been built up from intact amylose chains without a diminution in the length of the chains. To distinguish between these alternatives, the experiment was repeated with much weaker solutions of β-amylase and attention was directed not to the limiting conversions but rather to the initial rates of maltose production. For, if it is

recognised that β-amylase functions by the end-wise removal of maltose units from polyglucose chains, then the speed of hydrolysis will depend on the number of chain-ends available. It follows that any decrease in average chain-length due to fragmentation of the original amylose chains by Q-enzyme should be revealed by an increase in the initial rate of β-amylolysis. From Fig. 3 it will be seen that the simultaneous action of Q-enzyme and β-amylase increases considerably the rate of maltose production over that of β-amylase acting alone. Pre-treatment

FIG. 1.

Hydrolysis of amylose (B.V., 1·04) by Q-enzyme at 20·5° and pH 7·04.

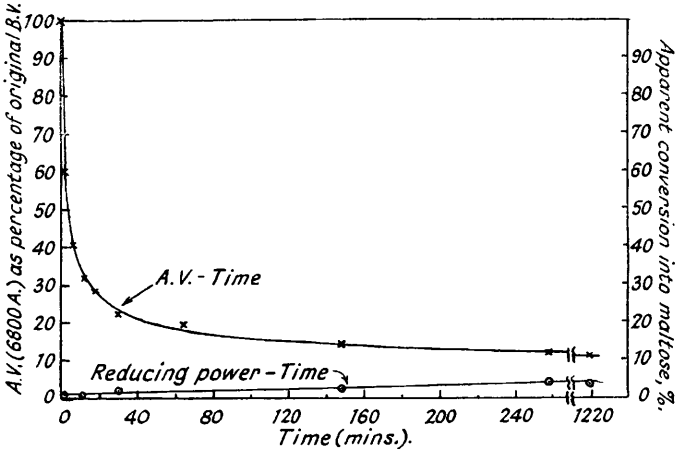
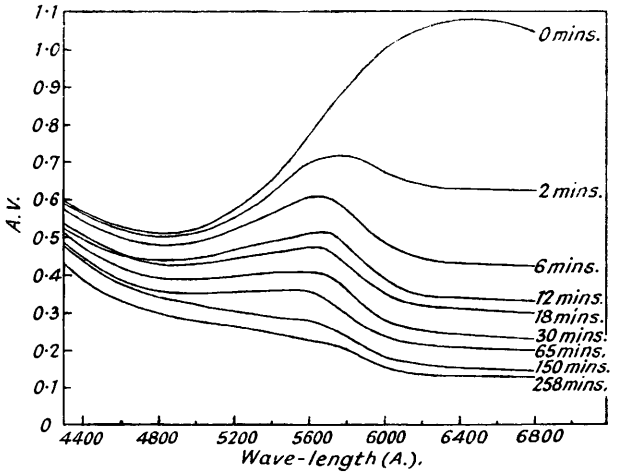


FIG. 2.

Light absorption curves obtained during treatment of amylose (B.V., 1·04) with Q-enzyme at 20·5° and pH 7·04.



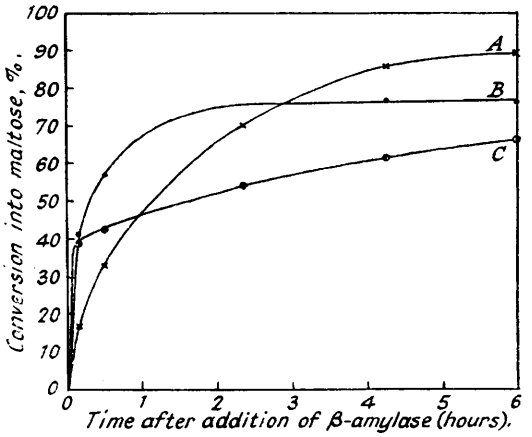
with Q-enzyme is even more effective. Nevertheless, this increase in the rate of β-amylolysis in the presence of Q-enzyme is accompanied by a decrease in the extent of the limiting conversion into maltose. Q-Enzyme is thus shown to have two actions on amylose, namely, scission of the chains and cross-linking of the fragments. These two functions of the enzyme are not necessarily independent and it may well be that scission of each 1 : 4-link in the original polysaccharide chain is accompanied by the simultaneous formation of a branching 1 : 6-link. Such a mechanism would be in keeping with the low reducing powers developed and also with energy requirements. In such circumstances it is not to be expected that appreciable concentrations of short unbranched chains (called pseudo-amylose by Bourne and Peat, *J.*, 1945, 877) would appear during the earlier stages of the incubation. Dr. Shlomo Hestrin, in

a personal communication (June 1948), suggested that a non-hydrolytic exchange of linkage without an intermediate hydrolysis might account for the experimental findings.

In order that a closer study of the products of Q-enzyme action might be made, red-staining polysaccharides (I—IV) were prepared from four different substrates, namely potato-starch, -amylose, and -amylopectin, and waxy-maize starch. The yield of polysaccharide was over 90% in every case except in that when amylose was used as substrate (polysaccharide I). A part of the product from amylose passed through the dialysis membrane and, in consequence,

FIG. 3.

Hydrolysis of amylose (B.V., 1·30) by β-amylase and Q-enzyme, acting simultaneously at pH 7·0 and 20·5°.



A.—β-Amylase alone.
B.—β-Amylase and Q-enzyme added together.
C.—β-Amylase added 1 hour after Q-enzyme.

the yield of polysaccharide I was only 74%. The properties of the four products are listed in Table I. They were readily soluble in hot water and were free from protein. Each showed more than 93% conversion into glucose when hydrolysed with acid and gave less than 0·7% ash. The reducing power of each corresponded to no more than 1·5% of that of maltose. With β-amylase, each gave a limit dextrin and maltose (52—57%), whereas the limiting con-

TABLE I.

The properties of polysaccharides prepared from starches and starch fractions by the agency of Q-enzyme.

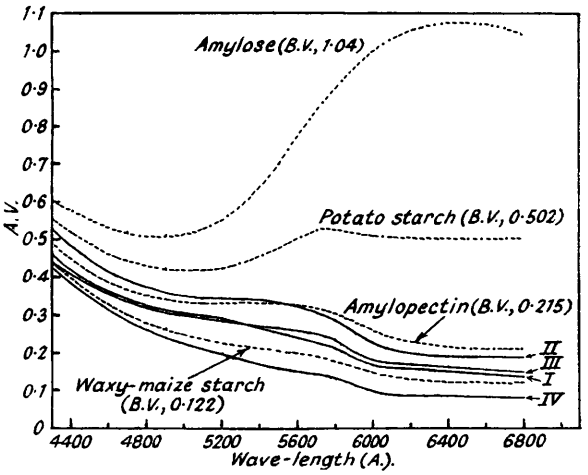
Property.	Potato amylose.	Polysaccharide I.	Potato starch.	Polysaccharide II.	Potato amylopectin.	Polysaccharide III.	Waxy-maize starch.	Polysaccharide IV.
Source	—	Potato amylose	—	Potato starch	—	Potato amylopectin	—	Waxy-maize starch
Yield (%)	—	74·2	—	90·0	—	91·2	—	92·9
Solubility in hot water	Sparing	Ready	—	Ready	Ready	Ready	—	Ready
Reducing power (as percentage of maltose)	—	1·5	—	0·4	—	0·2	—	0·1
Protein tests	—	Negative	—	Negative	—	Negative	—	Negative
Iodine-stain	Blue	Red	Blue	Reddish-purple	Reddish-purple	Red	Red	Red
B.V.	1·04	0·136	0·502	0·189	0·215	0·147	0·122	0·080
Approx. wave-length of peak absorption (A.) (Fig. 4)	6400	5200	5800	5400	5600	5300	5200	5100
Ash (%)	—	0·32	—	0·47	—	0·61	—	0·70
Glucose produced by acid hydrolysis (%)	—	97	—	96	—	>93	—	>93
Limiting amount of maltose produced by β-amylolysis (%)	90	55	69	57	56	52	50	53

versions of the polysaccharides from which they were derived were 90% (amylose), 69% (potato starch), 56% (amylopectin), and 50% (waxy-maize starch).

The blue value of the product was invariably lower than that of the parent polysaccharide, the difference being pronounced when amylose or potato starch was used, but small when amylopectin or waxy-maize starch was employed. The observed fall in B.V. of potato amylopectin and waxy-maize starch was probably due to the conversion into amylopectin of the small amounts of amylose which are known to be present in waxy-maize starch and in potato amylopectin prepared by the " thymol " method (Bourne, Donnison, Haworth, and Peat, *J.*, 1948, 1687; Bourne and Peat, this vol., p. 5). The light-absorption curves of the iodine complexes of polysaccharides I—IV closely follow the curve for natural amylopectin (see Fig. 4). The wave-lengths of peak absorption quoted in Table I were obtained, in the case of

FIG. 4.

Light absorption curves of polysaccharides isolated after Q-enzyme action.



red-staining polysaccharides, by using more concentrated polysaccharide–iodine solutions than are usually employed with amylose. Polysaccharides I—IV exhibited absorption peaks between 5100 and 5400 A., compared with 5600 A. for the sample of " thymol " amylopectin employed and 5200 A. for waxy-maize starch.

It is desirable to emphasise at this point that no claim is made that the polysaccharides I—IV are identical. Indeed Table I shows the contrary. For example, the blue values of the products of Q-action differ and are related to the blue value of the particular substrate from which each was derived. The results do prove, however, that all of these products, whatever their starch source, have the branched-chain amylopectin structure.

These studies, in which a stable, highly purified Q-enzyme has been used, fully substantiate the hypothesis put forward by us in 1945 (Bourne and Peat, *J.*, 1945,877), namely, that Q-enzyme plays a dual rôle in that it catalyses (i) the fragmentation of long amylose chains and (ii) the synthesis of the branched-chain structure of amylopectin, presumably by the formation of cross linkages between these fragments.

A practical consequence of this investigation is that there is now available, in stable powder form, an enzyme preparation which will effect the conversion not only of amylose, but of any natural starch or mixture of starches wholly into non-retrograding, water-soluble amylopectin.

Since the completion of this work Bernfeld and Meutémédian (*Nature*, 1948, **162**, 297, 618; *Helv. Chim. Acta*, 1948, **31**, 1724, 1735) have described the isolation, from potatoes, of an " isophosphorylase," which in some respects seems to fulfil the same function as Q-enzyme. These authors stipulate, however, that a phosphate transfer is involved in its action We intend to discuss the relation between the two enzymes in a later communication.

EXPERIMENTAL.

Analytical Methods.—The analytical methods employed in this work have already been described in the preceding paper (Part IV).

Fractionation of Starch.—The amylose and amylopectin fractions were derived from potato starch by selective precipitation of the former with thymol, according to the standard procedure of Bourne Donnison, Haworth, and Peat (*J.*, 1948, 1687). Several such fractionations were necessary in order to prepare the purer sample of amylose, which had B.V. 1·30.

Isolation of Q-Enzyme.—The Q1 fraction was isolated from potatoes by treatment with lead acetate, sodium bicarbonate–carbon dioxide, and ammonium sulphate, as described in the previous paper. It was stored at 0° as the powder obtained when a solution was freeze-dried. In some cases this powder was purified before used by reprecipitation with neutral ammonium sulphate (19 g./100 c.c.), giving fraction Q3.

Preparation of β-Amylase.—The β-amylase was isolated from soya-beans by the method of Bourne, Macey, and Peat (*J.*, 1945, 882). There was no increase in reducing power when the enzyme was incubated with maltose in acetate buffer at pH 5·3.

β-Amylolysis of Polysaccharides.—A solution of the dry polysaccharide (20 mg.) in 0·1N-sodium hydroxide (25 c.c.) was exactly neutralised with 0·5N-hydrochloric acid and diluted to 34 c.c. After the addition of acetate buffer (pH 5·3; 6 c.c.) and 0·2% β-amylase solution (10 c.c.), making a total volume of 50 c.c., the digest was incubated at room temperature. At intervals, the reducing power was determined, in terms of maltose, by the Shaffer–Hartmann method (*J. Biol. Chem.*, 1921, **45**, 377), a small correction being made, when necessary, for the reducing power of the enzyme.

Action of Fraction Q1 on Amylose (B.V., 1·04). Measurement of Iodine Stains and Reducing Powers.—A solution of fraction Q1 (20 c.c.; from 100 c.c. of potato juice) was incubated with amylose (B.V. 1·04), as in the standard digest described in the previous paper, except that three-fold quantities of the reagents were employed. The changes in the iodine stain of the polysaccharide and the reducing power of the digest are shown in Figs. 1 and 2.

Hydrolysis of Amylose (B.V. 1·30) by Q1 Fraction and β-Amylase Acting Simultaneously.—(a) *Using a 0·04% β-amylase solution.* A sample (1·6 g., equiv. to 236 c.c. of potato juice) of freeze-dried Q1 fraction was dissolved in water (40 c.c.), a small insoluble residue being removed by the centrifuge. Digests (total volume, 50 c.c.) were prepared, containing amylose (20 mg.; B.V. 1·30; dissolved as in previous paper), maltose (8 mg.), and M-acetate buffer (5 c.c.; pH 7·0), together with 0·2% β-amylase solution (10 c.c.) and/or Q1 solution (5 c.c.). The digests were incubated at 20·5° and, at intervals, aliquot portions were withdrawn for the determination of reducing power and for the measurement of A.V. (6800 A.). For the latter measurement 2·5 c.c. of digest solution (equiv. to 1 mg. of amylose) were stained with iodine (2 mg.) and potassium iodide (20 mg.) in a total volume of 100 c.c. A control sample from which the amylose was omitted was incubated simultaneously with each digest. These controls revealed that the Q1 preparation and β-amylase, acting separately, or together, showed no maltase activity. In each control there was a small, constant reducing power, for which a correction was applied in the corresponding main digest. The results are tabulated in Tables II and III.

TABLE II.

Conversion of amylose (B.V. 1·30) by Q-enzyme acting alone.

Time (mins.).	Apparent conversion into maltose (%).	Fall (%) in A.V. (6800 A.).
0	0	0
30	0·9	75·2
60	—	77·7
90	—	82·7
180	2·8	86·9

TABLE III.

Conversion of amylose (B.V. 1·30) by Q-enzyme and β-amylase, acting together.

Enzymes used.	Time of incubation with Q1 before addition of β-amylase (mins).	Conversion into maltose (%): 30 mins. after addition of β-amylase.	210 mins. after addition of β-amylase.
β-Amylase	—	63·0	90·2
β-Amylase + Q-enzyme ...	0	47·5	74·2
,, ,, ...	30	45·2	63·0
,, ,, ...	90	38·0	57·0
,, ,, ...	180	38·0	55·8

(b) *Using a 0·01% concentration of β-amylase.* The previous experiment was repeated under the same conditions, except that the final concentration of β-amylase was 0·01 instead of 0·04%. The enzyme and amylose specimens were the same as those previously employed. The reducing power was determined at more frequent intervals in order that the initial rates of β-amylolysis could be ascertained. The results are shown graphically in Fig. 3.

Method of Isolation of the Polysaccharides Produced from Starches and their Components by Treatment with Q-Enzyme.—The starch, or starch fraction (2·8 g.), was dissolved, by warming, in 0·5N-sodium hydroxide (150 c.c.). The solution was cooled, diluted with water to *ca.* 1300 c.c., and neutralised to phenolphthalein with N-sulphuric acid. After the introduction of maltose solution (equiv to 0·6 g. of maltose), M-acetate buffer (500 c.c.; pH, 7·04), and Q-enzyme solution (500 c.c.), the digest was diluted with water to 2800 c.c. and kept at room temperature. At intervals, aliquot portions were removed for the determination of reducing power (4 c.c.) and of A.V. (6800 A.). For the latter measurement, the

digest sample (1 c.c., equiv. to 1 mg. of original polysaccharide) was stained with iodine (2 mg.) and potassium iodide (20 mg.) in a total volume of 100 c.c.

When the A.V. (6800 A.) reached a constant value, the reaction was arrested by heating the digest in a boiling water-bath for 1 hour. The solution was cooled and the coagulated protein removed. In the case of polysaccharide I (from amylose), the crude polysaccharide was precipitated with alcohol (3 volumes), ground with alcohol and then with ether, and dried at 60° over phosphoric oxide in a vacuum.

In the other cases the protein-free supernatant liquid was concentrated under reduced pressure to 250 c.c. Each of these solutions, and also a solution of crude polysaccharide I, was dialysed in cellophane against frequent changes of distilled water. The dialysis was continued for 24 hours after the dialysate had ceased to give a precipitate with barium chloride solution. After the addition of a trace of sodium chloride, the polysaccharide was precipitated with alcohol (3 volumes), ground with alcohol and then with ether, and dried at 60° in a vacuum over phosphoric oxide.

(a) *Polysaccharide I from amylose* (*B.V.* 1·04). Fraction Q1, freshly prepared from 2,400 c.c. of potato juice, was dissolved in water (500 c.c.). Initially, half of this solution was added to the digest, the remainder being added after 4 hours. The reaction was terminated after 24 hours, by which time the A.V. (6800 A.) was 0·120 and the " apparent conversion into maltose " was 4·0%. Crude polysaccharide I (11·1 g.) contained 74% of ash, and had B.V. 0·107 (calculated on the assumption that there had been no loss of polysaccharide). During dialysis a small proportion of the polysaccharide passed through the membrane. Polysaccharide I (yield, 74·2%) had B.V. 0·136 and ash, 0·32%.

(b) *Polysaccharide II from potato starch* (*B.V.* 0·502). The Q-enzyme (500 c.c.) used in this digest was at the Q3-stage and had been isolated from 250 c.c. of potato juice and stored at 0° in the freeze-dried Q1-form for 9 weeks. When the digest was stopped after 520 minutes, the A.V. (6800 A.) was 0·195 and the " apparent conversion into maltose " was 2·1%. No iodine-staining material passed through the dialysis membrane. Polysaccharide II (yield, 90·0%) had B.V. 0·189.

(c) *Polysaccharide III from amylopectin* (*B.V.* 0·215). The Q3 fraction (500 c.c.) employed had been prepared from 210 c.c. of potato juice and stored at 0° as freeze-dried Q1 fraction for 14 weeks. The A.V. (6800 A.) and the " apparent conversion into maltose " were 0·135 and 0·7%, respectively, when the digest was inactivated after 7 hours. There was no detectable passage of iodine-staining material during dialysis. Polysaccharide III (yield, 91·2%) had B.V. 0·147.

(d) *Polysaccharide IV from waxy-maize starch* (*B.V.* 0·122). The waxy-maize starch had been defatted with aqueous dioxan and then with aqueous methanol. The Q3 preparation was the same as that used in (c). The digest was stopped after 345 minutes, the A.V. (6800 A.) being 0·107 and the " apparent conversion into maltose " 1·4%. No iodine-staining polysaccharide was lost during dialysis. Polysaccharide IV (yield, 92·9%) had B.V. 0·080.

The properties of these polysaccharides are summarised in Table I.

The authors are indebted to Professor Sir Norman Haworth, F.R.S., for his interest in this work.

UNIVERSITY COLLEGE OF NORTH WALES, BANGOR.
A. E. HILLS LABORATORIES, THE UNIVERSITY,
BIRMINGHAM 15.

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