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On the Specificity of Branching Enzymes[1]

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Branching enzymes prepared from rat liver, potato, broad bean and wrinkled pea have been tested with maltose, isomaltose and panose as co-substrates. No stimulation of branching has been noted in these experiments.

Introduction

The chain length specificity of branching enzymes of the starch–glycogen class (amylo-1,4 → 1,6-transglucosidases) has been the subject of several previous reports. Larner[2] using a sensitive labeling technique, has shown that the liver branching enzyme (amylo-1,4 → 1,6-transglucosidase) requires glycogen with an average outer chain length of between 6 to 11 glucose residues for activity. With the same method it was shown that Q enzyme from potato readily branched a synthetic glycogen of average outer chain length 13–14. The experiments of Peat, Whelan and Bailey[3] indicate that potato Q enzyme converting amylose requires a chain length of about 40 glucose units for maximum activity. Q will branch smaller linear chains (twenty-eight glucose residues) at a slower rate.

Barker, Bebbington and Bourne[4] have demonstrated that chain transfer as indicated by iodine color change catalyzed by Q enzyme from *Polytomella coeca*, is accelerated by the addition of various poly and oligosaccharides, including maltose. C^{14}-labeled maltose was incorporated into polysaccharide during this reaction. This experiment demonstrated that chain transfer could take place intermolecularly.

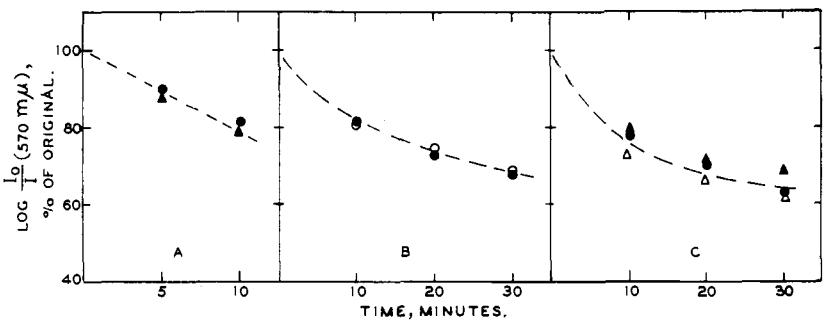

Fig. 1.—Activity of amylo 1,4 → 1,6-transglucosidase in presence of saccharides: (symbols apply to all figures): ●, control; ▲, maltose added; O, isomaltose added; △, panose added; A, reaction mixture contained amylopectin 3 mg.; maltose 1 mg.; B, reaction mixture contained amylopectin 4 mg.; isomaltose 2 mg.; C, reaction mixture contained: amylopectin 4 mg.; maltose 2 mg.; panose 2 mg.

In order to determine whether stimulation of branching by oligosaccharides was a general property of branching enzymes and also whether α-1,6-linked oligosaccharides could act in a similar manner, the branching enzymes from liver, potato, broad bean and wrinkled pea have been tested with maltose, isomaltose and panose as co-substrates. In as much as it has been shown[5] that intestinal extracts hydrolyzed isomaltotriose, it was of interest to test the incorporation of α-1,6-linked oligosaccharides during branching since such a reaction could lead to the formation of "double" branch points in starch. With none of the four branching enzymes tested could any stimulation be detected. The results are described in this report, and clearly distinguish these enzymes from the branching enzyme of *Polytomella coeca*.

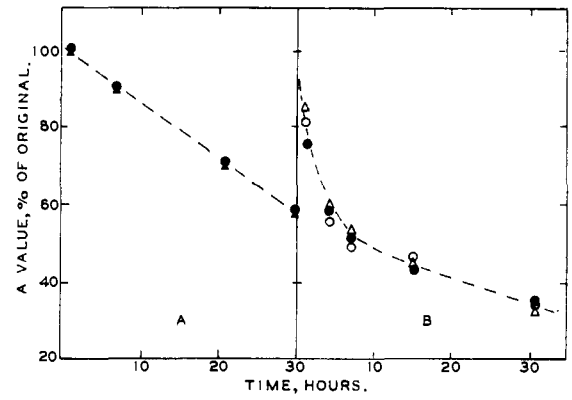

Fig. 2.—Activity of potato Q enzyme in presence of saccharides: A, reaction mixture contained amylose 4 mg.; maltose 2.5 mg.; B, reaction mixture contained amylose 4 mg.; isomaltose 2 mg.; panose 5 mg.

Material and Methods

Enzymes.—Amylo-1,4 → 1,6-transglucosidase was prepared from rat livers of fasted animals.[6] The extract after two adsorptions with commercial corn starch (Argo) to remove α-amylase was used without further fractionation.

Q enzyme from potato was prepared from mixed potatoes obtained locally according to the procedure of Gilbert and Patrick.[7] Preparations corresponding to precipitates I and III were used. The Q enzymes from wrinkled pea (Burpeana Early Dwarf #6222), and broad bean (Catalogue number 5083 W. Atlee Burpee Co.) were prepared by the method of Hobson, Whelan and Peat.[8] Peas were soaked for 48 hours at 3° in distilled water prior to grinding in preparation of the flour. Beans were ground directly in a plate mill and sieved through a number 40 mesh screen.[9]

(1) Supported in part by a grant from the National Science Foundation. Some of the experiments were done in collaboration with A. A. Strauss and C. Y. Karnes.

(2) J. Larner, *J. Biol. Chem.*, **202**, 491 (1953).

(3) S. Peat, W. J. Whelan and J. M. Bailey, *J. Chem. Soc.*, 1422 (1953).

(4) S. A. Barker, A. Bebbington and E. J. Bourne, *ibid.*, 4051 (1953).

(5) J. Larner, THIS JOURNAL, **77**, 6385 (1955).

(6) J. Larner, *Methods of Enzymology*, **1**, 222 (1955).

(7) G. A. Gilbert and A. D. Patrick, *Biochem. J.*, **51**, 181, 186 (1949).

(8) P. N. Hobson, W. J. Whelan and S. Peat, *J. Chem. Soc.*, 3566 (1950).

(9) This preparation of QI was supplied by L. M. Henderson, H. L. Pote and K. Chutikul.

In both cases it was found necessary to precipitate the QI fractions with an ammonium sulfate concentration of 20 g. per 100 ml. instead of 19 as recommended.

Substrates.—Maltose was a commercial product. Corn amylose (Blue Value 1.18) and amylopectin were kindly supplied by Dr. R. W. Kerr. Isomaltose was supplied by Dr. Allene Jeanes or was prepared by the acid hydrolysis of dextran essentially as described by Bacon and Bacon.[10] Panose was a gift of Dr. M. Killey.

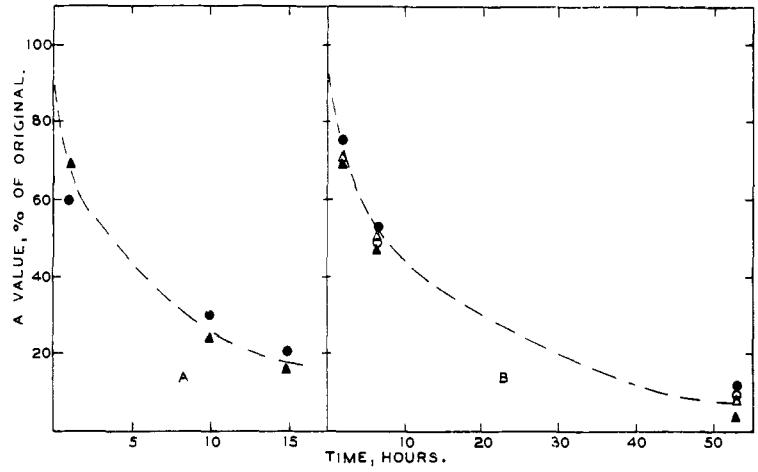

Fig. 3.—Activity of broad bean Q enzyme in presence of saccharides: A, reaction mixture contained amylose 4 mg.; maltose 2 mg.; B, reaction mixture contained amylose 4 mg.; maltose 2 mg.; isomaltose 2 mg.; panose 5 mg.

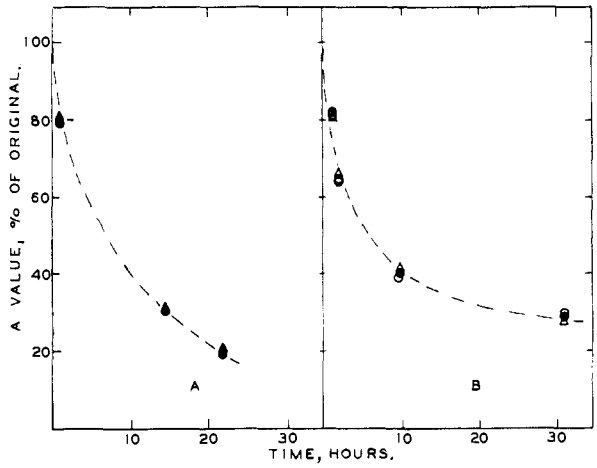

Fig. 4.—Activity of wrinkled pea Q enzyme in presence of saccharides: A, reaction mixture contained amylose 4 mg.; maltose 2 mg.; B, reaction mixture contained amylose 4 mg.; isomaltose 2 mg.; panose 5 mg.

Analytical.—Branching reactions were followed by change in iodine color. Amylo-1,4 → 1,6-transglucosidase activity was determined as described,[6] modified by omitting the neutralization of the perchloric acid filtrate. Q enzyme activity was followed essentially by the iodine color determination of Hassid and McCready.[11] Enzymatic reaction mixtures contained 0.025 *M* citrate buffer *p*H 7, amylose, enzyme and saccharides in amounts indicated, in final volumes of from 15 to 20 ml. Incubation was at room temperature. Aliquots (2 ml.) were pipetted into 50-ml. volumetric flasks, 0.5 ml. of I_2 + KI and 2 drops of *N* acetic acid added. After dilution, readings were made against blanks containing no polysaccharide in the Klett–Summerson colorimeter using the 660 mμ filter. Results are reported as *A* values in per cent. of the zero time readings. Reducing power was determined by the method of Nelson[12] with the modifications previously outlined.[5]

Results and Discussion

Data collected with the four branching enzymes are presented in Figs. 1 through 4. In these experiments, 2–5 mg. of oligosaccharide was tested with 4 mg. of amylose or amylopectin (except for the experiment of Fig. 1A) whereas in the experiments of Barker, Bebbington and Bourne 9 mg. of amylose and 5 mg. of disaccharide per 10 ml. of reaction mixture were used in the routine assay. Under these conditions (weaker enzymatic activity), in no case was a consistent stimulation of branching by a saccharide observed. That the observed changes in iodine color were due to branching (chain transfer) rather than hydrolytic reactions was indicated by determination of reducing power increase during the reaction. In an experiment with potato Q enzyme (not shown here) after 23 hours when the iodine color had decreased to 52.5% of its initial value, the increase in reducing power (as maltose) was only 3.2%. Similarly with a liver extract during a 16-minute incubation at 30°, when the iodine color had decreased to 71% of its initial value the increase in reducing power was 1% (as maltose).

Under these conditions the branching enzymes tested from potato, liver, broad bean and wrinkled pea were not significantly stimulated by maltose, isomaltose or panose. These results indicate a difference in the action of these enzymes as contrasted to branching enzyme from *Polytomella coeca*. A difference in chain length specificity of acceptor or co-substrate would seem a reasonable explanation of this difference in activity.

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(11) W. Z. Hassid and R. M. McCready, THIS JOURNAL, **65**, 1154 (1943).

(12) N. Nelson, *J. Biol. Chem.*, **152**, 375 (1944).