di-iodotyrosine is present in higher concentration in the plasma of the immunized rabbits than in that of the normal ones. It is suggested that this is due to combination of the hapten with antibodies in the plasma of the immune animals.

3. During the same period inorganic iodide is also present in higher concentration in the plasma of the immunized rabbits than in that of the normal ones.

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The Mechanism of Carbohydrase Action

3. THE ACTION PATTERN OF β -AMYLASE*

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β -Amylase is a plant enzyme which hydrolyses α -1:4-linked polymers of D-glucopyranose by an endwise action. This action commences at the non-reducing end of the polymer, and the penultimate and subsequent alternate linkages are broken to yield maltose. These facts being established, there are two possible mechanisms for the enzyme action. These are a single-chain mechanism in which the enzyme completely hydrolyses one substrate molecule at a time or a multi-chain mechanism in which the enzyme attacks the ends of the polymer chain in a completely random fashion. *A priori* considerations suggest that single-chain action is unlikely, but the first investigations of the problem provided evidence in its favour (Swanson, 1948; Cleveland & Kerr, 1948). These authors' conclusions were criticized by Hopkins & Jelinek (1949) and by Bourne & Whelan (1950), both groups of authors

favouring multi-chain action. More recently, Kerr & Cleveland (1951) have provided further arguments in favour of single-chain action, while Hopkins & Jelinek (1954) and Bird & Hopkins (1954) take the contrary view.

There have been two methods of approach to the problem. The first is that used by Swanson (1948), Cleveland & Kerr (1948) and Kerr & Cleveland (1951). This employs native amylose as the enzyme substrate. The polysaccharide remaining at stages in the hydrolysis is isolated and its properties are compared with those of the original material. If the action is single-chain in character this residual polysaccharide should be unchanged amylose. In the second method, instituted by French, Levine, Pazur & Norberg (1950*b*), a much smaller substrate molecule is used in order to facilitate tests for the production of intermediate products. Using maltoheptaose, French *et al.* (1950*b*) observed that under optimum conditions for the enzyme action maltoheptaose was degraded to maltose and maltotriose without the intermediary formation of maltopentose, i.e. the action was apparently single-chain. At

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extremes of temperature and pH, however, maltopentaose formation was detected (French, Knapp & Pazur, 1950a). These results were interpreted to mean that 'either the enzyme-substrate affinity constants are seriously decreased at higher temperatures or unfavourable pH, or the rate at which the enzyme-substrate complex disintegrates into products is diminished, or possibly a combination of these two effects'. In the present investigation of β -amylase action we have used both short-chain dextrins (maltoheptaose and maltohexaose) and also a synthetic amylose of average chain length 49 units as substrates for the enzyme.

EXPERIMENTAL

Materials

Enzymes. Soya-bean β -amylase was prepared as by Peat, Pirt & Whelan (1952b). Crystalline sweet-potato β -amylase was the gift of Dr A. K. Balls. Potato phosphorylase was prepared as by Whelan & Bailey (1954).

Maltodextrins. The preparation of these substances from potato amylose is described by Whelan, Bailey & Roberts (1953).

Synthetic amylose. This was prepared from maltohexaose primer as by Whelan & Bailey (1954) and had an average degree of polymerization (DP) of 49 glucose units. Concentrations of the amylose solutions were determined by acid hydrolysis to glucose (Pirt & Whelan, 1951).

Buffers. The components of the four buffer systems used were sodium acetate-acetic acid, NaH_2PO_4 -sodium citrate (McIlvaine, 1921), NaH_2PO_4 - Na_2HPO_4 and HBO_3 - $\text{Na}_2\text{B}_4\text{O}_7$.

Methods

All concentrations of digest components given were those finally obtaining in the digests.

The method of Somogyi (1945) was used for determining reducing powers. β -Amylase activity was determined and units defined as by Hobson, Whelan & Peat (1950). The solution used for iodine staining contained 0.2% I_2 in 2% (w/v) KI. Light-absorption of stained solutions was measured in 1 cm. cells in a Unicam SP. 500 spectrophotometer.

Relative rates of β -amylolysis of starch and maltodextrins. Each digest (5.5 ml.) contained malto-tetraose, -pentaose, -hexaose or -heptaose (3.7 μ moles) or potato starch (14.9 mg.), 17 mM-acetate buffer (pH 4.8) and crystalline β -amylase (0.1 unit). Measurements of reducing power (1.5 ml. portions) were made after incubation at 35° for 5, 15 and 30 min. and the initial reducing power of each substrate was also determined. The reducing power/time curves were plotted and tangents drawn to the curves to determine the approximate initial relative rates of hydrolysis. Relative to potato starch (=100), these were malto-tetraose, 30; -pentaose, 30; -hexaose, 75; -heptaose, 85.

Small-scale fractionation of maltodextrin mixtures. A mixture of maltose hydrate (11.0 mg.), malto-triose (5.5 mg.), -pentaose (21.0 mg.) and -heptaose (14.5 mg.) was placed on a charcoal-Celite column (38 cm. $\times$ 1.5 cm.). The sugars were separated by successive elution with 7.5, 15, 22 and 28% (w/v) aqueous ethanol, fractions of 20 ml. being

collected. The optical rotation of each fraction was measured in a 4 dm. tube and the recovery of each sugar estimated in terms of its specific optical rotation (Whelan *et al.* 1953). The percentage recoveries were: maltose, 100; malto-triose, 87; -pentaose, 75; -heptaose, 70. The overall recovery on a weight basis was 81%.

Determination of extent of multi-chain action on maltodextrins. The following procedure was adopted in each experiment. A small-scale digest was used to determine the time necessary to attain the desired conversion, the range 50–70% being suitable for obtaining the maximum yield of intermediate. A larger-scale digestion was then carried out for the required time and the enzyme was rapidly inactivated by immersing the digest in a boiling-water bath. When the digestion was at 0.5°, HgCl_2 was added as an additional means of securing rapid inactivation.

The two maltoheptaose digestions (Table 1) were carried out at 35° as follows. The digests (50 ml. each) contained maltoheptaose (32 mg.), 20 mM-acetate buffer (pH 4.8) or McIlvaine buffer (pH 8.03, in fivefold dilution) and crystalline β -amylase (1 and 8 units respectively). The times of incubation were 32 and 10 min. respectively. After the enzyme had been inactivated, the reducing powers were measured on 2 ml. portions and the rest of the digests fractionated on charcoal-Celite as above. The respective overall recoveries of sugar were 81 and 87% (see Table 2).

The three maltohexaose digests contained substrate (for wt. see Table 2) and purified soya-bean β -amylase in 40 mM-acetate buffer (49 ml.). One digest (200 units of enzyme) was incubated at 0.5° for 19 min., when HgCl_2 (20 mg., 1 ml.) was added to inactivate the enzyme, followed by heating to 100°. The second digest (20 units of enzyme) was incubated for 20 min. at 35° followed by heat inactivation. The third (120 units of enzyme) was kept at 70° for 10 min. before the temperature was raised to 100°. A portion (1 ml.) was removed from each digest to determine the extent of conversion (Table 2) and the remainder was fractionated on charcoal as previously described. The overall recoveries of carbohydrate for the different experiments were 0.5°, 89%; 35°, 76%; 70°, 91%.

Calculation of proportion of multi-chain action during β -amylolysis of malto-heptaose and -hexaose. The following mathematical treatment requires that the reactions under consideration should obey first-order reaction kinetics. This was so for each of the substrates under the conditions employed.

The multi-chain action of β -amylase on maltoheptaose corresponds to a two-stage consecutive reaction:

where M=maltose, T=maltotriose and k_1 , k_2 are the respective rate constants for the β -amylolysis of maltoheptaose and -pentaose. If the initial amount of maltoheptaose is given by a , then the amount of unchanged maltoheptaose at time t is given by $ae^{-k_1 t}$ (denoted as x) and of maltopentaose by $ak_1(e^{-k_1 t} - e^{-k_2 t})/(k_2 - k_1)$ (denoted subsequently as y) (Daniels, 1948). At complete conversion of maltoheptaose 2 mol. prop. of maltose has been liberated. Therefore the measured increase in reducing power taking place during complete hydrolysis is proportional to $2a$. On the same basis the increase in reducing power at any intermediate stage is proportional to $2(a-x)-y$. The measured fractional conversion into maltose is therefore

equal to $[2(a-x) - y]/2a$. By using the known values of a (Table 2) and k_1/k_2 (see above), this last equation was solved graphically for t in terms of k_1 or k_2 . The value of t was then substituted in the expression for y (the k terms cancel) giving the amount of maltopentaose at time t , and hence at the measured degree of conversion. This is the amount theoretically obtainable on the basis of completely multi-chain action. It was compared with the actual amounts found by direct analysis of the digest mixture as already described. It is also possible to calculate the theoretical amount of unchanged maltoheptaose from x ; of maltose formed, from $2(a-x) - y$; and of maltotriose formed, from $a - x - y$. Similar calculations were applied in the experiments where maltohexaose was used.

Phosphorolytic degradation of synthetic amylose. Each digest (16 ml.) contained 0.06% of synthetic amylose, 63 mM-phosphate buffer (pH 7.0), 0.47% of freeze-dried potato phosphorylase, $\mu\text{M-HgCl}_2$ and 0.45% of ammonium molybdate [see Bailey, Thomas & Whelan (1951) concerning the use of HgCl_2 and molybdate as enzyme inhibitors]. The digests were incubated at 0°, 35° and 55° respectively, and at zero time and subsequent intervals, according to the rate of phosphorolysis, portions (1 ml.) of the digests were withdrawn and stained with iodine (0.5 ml.) in a total volume of 25 ml. The light-absorption in the range 500–640 $m\mu$ was determined for each iodine-stained solution. Figs. 1 and 2 show the patterns of light-absorption curves obtained during the synthesis of this polysaccharide and during phosphorolysis at 35°. The slope of the line joining the peaks of each family of curves was measured. The slope for each temperature is plotted in Fig. 5.

β -Amylolysis of synthetic amylose. Four types of experiment were performed.

(i) The digests contained 0.08% of amylose in 24 mM-acetate buffer (pH 4.8, 12.5 ml.). After equilibration at the desired temperature a portion (0.85 ml.) was stained with iodine (0.5 ml.) in 25 ml. and to the remainder of the digest was added purified soya-bean β -amylase. Portions (0.85 ml.) were subsequently removed for iodine staining. No correction was applied for the slight dilution of the digest caused by the addition of the enzyme. The light-absorption of each solution in the range 500–800 $m\mu$ was determined. Table 1 summarizes the details of the experiments and the results obtained are shown in Figs. 3 and 5.

(ii) A digestion was carried out as in (i) except that borate buffer (pH 8.1) replaced acetate. The digest contained 180 units (0.2 ml.) of enzyme. Eight observations were made during 43 min. (see Fig. 5).

(iii) In order to examine the reversibility of heat effects on

the enzyme, β -amylase solution (0.5 ml.; pH 4.8; 450 units) was heated at 70° for 5 min., cooled rapidly to 35° and 0.2 ml. of the solution used in an otherwise exact repetition of the first type of experiment at 35°. Six observations were made during 6 min. incubation (see Fig. 5).

(iv) The action of the enzyme at 35° was then examined in the presence of a 130-fold molar excess (over synthetic amylose) of maltotetraose. The only other difference in conditions in this latter experiment was that 6 units (0.1 ml.) of enzyme were added initially, followed by a further 30 units (0.1 ml.) after 4.2 min. Seven observations were made during 8.25 min. (see Fig. 5).

Optimum temperature of action of soya-bean β -amylase. Each of five digests (50 ml.) contained 0.6% soluble potato starch, 24 mM-acetate buffer (pH 4.8) and purified soya-bean β -amylase (30 units). The digests were brought to the incubation temperature before adding and mixing the enzyme (1 ml.). Portions (5 ml. each) were removed after 3 and 8 min., the enzyme action being arrested by mixing immediately with Somogyi (1945) copper reagent (5 ml.) for determination of reducing power (as for maltose) which values are expressed in Fig. 6 in terms of the 5 mM-thio-sulphate used in the Somogyi method.

Examination of the dialysable products of β -amylolysis. The detailed results of these experiments are not given since they were negative. The methods were as follows. Potato amylopectin was incubated with crystalline β -amylase at 35° and 70°, both at pH 4.8. At six intervals, during the time taken to attain limiting β -amylolysis of the amylopectin, inactivated portions of the digests were dialysed into water and the diffusible material was examined on paper chromatograms. No product other than maltose was detected.

RESULTS AND DISCUSSION

Outline of methods

We have used two types of low-molecular-weight substrate in this investigation. These are: (i) pure maltodextrins produced from amylose by acidic hydrolysis (Whelan *et al.* 1953); and (ii) synthetic amylose of known homogeneity and average degree of polymerization (DP), prepared from maltohexaose and glucose 1-phosphate by phosphorylase action. The preparation of such polymers of known properties has become possible as a result of the proof that potato phosphorylase functions by multi-chain action (Whelan & Bailey, 1954).

Table 1. *Experimental conditions in the β -amylolysis of synthetic amylose*

The measurements were made at irregular intervals during the stated period (see e.g. Fig. 3).

	Temp. of incubation							
	0°	5°	21°*	21°*	35°	55°	70°†	75°‡
β -Amylase solution (ml.)	0.1	0.2	0.1	0.1	0.1	0.1	0.012	0.024
Units of activity	90	180	9	45	9	14	6	4
Total no. of observations	12	4	11	7	10	6	12	7
Time of last observation (min.)	27.5	6.0	43	8.75	22.5	5.0	22.5	18.2

* Low and high enzyme: substrate ratios (Fig. 5).

† Because of heat inactivation a further portion of enzyme (0.012 ml.; 3 units) was added after 15 min.

‡ Because of heat inactivation the same amount of enzyme was added a further six times during incubation.

By using these two types of substrate it is possible to predict in advance the changes which would be observed during single-chain and multi-chain action when the course of reaction is followed by iodine staining (for synthetic amylose substrate) or by charcoal-chromatographic analysis (for maltodextrin substrate). During single-chain action the substances present at any stage will be maltose and unchanged substrate. Intermediary products in course of degradation may be neglected since the magnitude of the turnover number of β -amylase (England & Singer, 1950; England, Sorof & Singer, 1951) is such that in any β -amylolysis proceeding at a measurably slow rate the molar ratio of enzyme to substrate is negligibly small. Therefore in the course of the single-chain degradation of maltohexaose and -heptaose one would not expect to detect malto-tetraose or -pentaose respectively. During the single-chain β -amylolysis of a synthetic amylose the changes in iodine stain should correspond to those of increasingly dilute solutions of the original substrate, an important point being that the wavelength of maximum light-absorption ($\lambda_{\max}$) of the iodine complex should not change.

Whereas the patterns of single-chain action can be predicted, certain experimental measurements must be made to establish the characteristics of multi-chain action. For the achroic maltodextrins it is necessary to know the relative rates of β -amylolysis of the substrate and of substrate molecules containing 2, 4, etc. fewer glucose units. The simplest suitable substrates are malto-hexaose and -heptaose, from each of which only two molecules of maltose can be removed. Each can therefore give rise to only one multi-chain intermediate, namely malto-tetraose and -pentaose respectively. The relative rates of β -amylolysis of malto-tetraose, -pentaose, -hexaose and -heptaose were measured. The amount of intermediate theoretically obtainable by multi-chain action was then calculated for the particular degree of hydrolysis as shown in the Experimental section. The actual amounts of intermediate present in our experiments were determined by charcoal-chromatographic fractionation of the

products of partial degradation. In this way the enzyme action under given conditions was characterized in terms of the proportion of intermediate actually detected, as compared with that calculated on the basis of completely multi-chain action (Table 2).

The characteristics of multi-chain degradation of the synthetic amylose (DP 49) were established by subjecting it to phosphorolytic degradation with the potato enzyme and measuring the light-absorption of the iodine-stained intermediate products. As is mentioned above, it has been shown that potato phosphorylase functions by an exclusively multi-chain action pattern. The iodine-staining method is only suitable for affording a clear differentiation between single- and multi-chain action if the DP of the substrate is less than about 80 glucose units, since molecules of greater size show little difference in iodine-staining properties (Bailey, J. M. & Whelan, W. J. unpubl. work). Consequently if natural amylose (DP at least several hundred) is used (as by Swanson, 1948; Cleveland & Kerr, 1948; Kerr & Cleveland, 1951) it is impossible, except in the final stages of the enzymic degradation, to determine by iodine staining whether all the chain molecules are being degraded (multi-chain action) or are disappearing one at a time (single-chain action) (Bourne & Whelan, 1950).

Two specimens of β -amylase were used. One was a purified preparation from soya bean (Peat *et al.* 1952*b*), the other was the crystalline sweet-potato enzyme (Balls, Walden & Thompson, 1948). There appear to be no differences in the enzymic properties of the two preparations (Peat *et al.* 1952*a, b*) and they have therefore been used interchangeably.

β -Amylolysis of maltodextrins

β -Amylase was allowed to degrade maltoheptaose or -hexaose to intermediate stages under varying conditions of temperature and pH. The products were separated and the amounts of malto-pentaose or -tetraose intermediate obtained were compared with the amounts calculated for completely multi-chain action. The results are summarized in Table 2.

Table 2. *Action of β -amylase on malto-heptaose and -hexaose*

See Experimental section for details.

Expt. no.	Source of enzyme	Substrate	Wt. of substrate (mg.)	Temp.	pH	Conversion into maltose (%)	Recovery of intermediate (mg.) (A)	Calc. recovery* (mg.) (B)	Proportion of multi-chain action (100A/B)
1	Sweet potato	Maltoheptaose	32.0	35°	4.8	52	3.3	12.6	26
2			32.0	35°	8.0	58	4.1	11.7	35
3	Soya bean	Maltohexaose	92.7	35°	4.8	64.5	25.3	45.7	56
4			97.1	70°	4.8	48	27.1	48.8	56
5			92.7	0.5°	4.8	69.5	35.6	36.4	98

* Calculated on the assumption of completely multi-chain action.

These results show that even under optimum conditions there is an appreciable proportion of multi-chain action (Expts. 1 and 3). This finding conflicts with the results of French *et al.* (1950*b*), mentioned above, but the explanation is probably in our use of a more selective fractionation technique. French *et al.* employed electrophoretic fractionation of the hypoiodite-oxidized products. In agreement with French *et al.* (1950*a*), an unfavourable pH increased the proportion of multi-chain action over that obtaining at optimum pH (Expts. 1 and 2). Raising the temperature above the optimum did not increase the multi-chain action (Expts. 3 and 4) but at 0.5° (Expt. 5) the action on maltohexaose was almost completely multi-chain.

The results show that under optimum conditions the enzyme action displays characteristics intermediate between the extreme types and they provide further insight into the enzyme mechanism by demonstrating the dependence of the action pattern on environmental conditions. While these results alone might form the basis of deductions concerning the mechanism of the enzyme action, it was thought desirable to provide confirmatory evidence with a larger molecule as substrate for the following reasons: (a) Limiting substrates probably lead to an unusually high accumulation of intermediate (e.g. the removal of the second molecule of maltose from malto-heptaose and -hexaose is accomplished two to three times as slowly as that of the first molecule); (b) an increase of one glucose unit in the size of the substrate was accompanied by a fall from 56 to 26 in the percentage of multi-chain action (Expts. 1 and 3). Such 'end' effects should be much less prominent in larger molecules.

β -Amylolytic of synthetic amylose

A synthetic amylose (DP 49) was used as substrate for β -amylase and for phosphorylase under varying conditions of temperature and pH. The progress of degradation was measured by iodine-staining, the absorption curves of the coloured complexes being plotted in the region 500–700 $m\mu$. It was found that a straight line could be used to join the peaks (i.e. $\lambda_{\max}$ values) of the curves. Two values of wavelength were determined. These were $\lambda_{\max}$ at zero time, t_0 , and the wavelength at which the projection of the straight line cut the wavelength axis, i.e. at zero extinction, E (e.g. see Fig. 2). Since the same polysaccharide was used in all the experiments the slopes of the various straight lines could be compared in terms of ($\lambda_{\max}$ at $t_0 - \lambda$ at $E = 0$). The value of the slope was used to characterize the type of degradation under any given set of conditions. Four such patterns of absorption curves are shown, one for phosphorylase synthesis (Fig. 1), one for phosphorolytic (Fig. 2) and one for β -amylolytic degradation (Fig. 3) of the 49-unit

amylose. Fig. 4 shows the pattern of curves expected for single-chain degradation, and Fig. 5 summarizes the results of a number of experiments.

Fig. 1. Light-absorption curves of iodine-stained products during the phosphorylase-catalysed synthesis of amylose (DP 49) from maltohexaose primer and glucose 1-phosphate at 35°, pH 7.0. Equal volumes of the digest were stained during the reaction and the uppermost curve is that given by the final product when the reaction was terminated by heat inactivation of the enzyme. The numerals against the curves refer to the time of incubation (min.). The digest contained 0.18% freeze-dried potato phosphorylase. After 172 min. the concentration was increased to 0.22%. Experimental conditions are given by Whelan & Bailey (1954).

Fig. 2. Phosphorolysis of synthetic amylose (DP 49) at 35°, pH 7.0. Light-absorption curves of iodine-stained products. The numerals against the curves refer to the time of incubation (min.). Conditions are given in the Experimental section.

As explained above, single-chain degradation of the polysaccharide by β -amylase would give a pattern of curves with zero slope (see Fig. 4), whereas the slope for completely multi-chain degradation is given by the curves for phosphorylytic action (Fig. 2). The slope of the phosphorylase

Fig. 3. β -Amyloysis of synthetic amylose (DP 49) at 35°, pH 4.8. Light-absorption curves of iodine-stained products. The numerals against the curves refer to the time of incubation (min.). Conditions are given in the Experimental section.

Fig. 4. Family of curves representing the changes in iodine-staining properties of amylose which should occur during β -amyloysis by single-chain action. The curves actually refer to potato-amylose solutions of increasing dilution.

curves was found not to be subject to temperature variation (Fig. 5). In view of the comparative behaviour of β -amylase, discussed below, this is a strong indication that phosphorylase action is completely multi-chain at all temperatures (cf. Whelan & Bailey, 1954). The fact that the slope during phosphorylytic degradation is less than the slope during phosphorylase synthesis of the polysaccharide (Figs. 1 and 2) does not mean that there is any difference in mechanism between the two types of action. The reason for the change in slope has been discussed by us elsewhere (Whelan & Bailey, 1954).

As regards β -amylase, it is evident from Fig. 5 that under none of the conditions investigated does the enzyme act by completely multi-chain or completely single-chain action; the proportion of multi-chain action is greatest at extremes of temperature

Fig. 5. Effects of temperature on the action patterns of soya-bean β -amylase and potato phosphorylase. 'Slope of light-absorption curves' refers to the slope of the line joining the maxima of the light-absorption curves of the iodine-stained products of β -amyloysis and phosphorylysis of synthetic amylose (DP 49) (see Figs. 2, 3). The method of calculating the slope is explained in the Results section. The value would be zero for completely single-chain action. ●, Phosphorylase at pH 7.0; ○, β -amyloysis at pH 4.8; □, β -amyloysis at pH 8.1; ■, β -amyloysis in the presence of 130-fold molar excess of maltotetraose and β -amyloysis, with heat-treated enzyme, both at pH 4.8 (the two points are coincident). The experimental points at 21°, pH 4.8 for β -amyloysis at two different concentrations of enzyme are coincident. Details are given in the Experimental section.

Fig. 6. Activity/temperature relationship for soya-bean β -amylase acting on potato starch at pH 4.8. Details are given in the Experimental section. The curves show the extent of action after (a) 3 min. and (b) 8 min.

constants are not clear but alterations in the configuration of the enzyme or substrate are possible lines for exploration. The variations in action patterns bear a strong resemblance to the variation in rate of action of the enzyme with temperature (Figs. 5, 6), but although the increases in rate of action of the enzyme and in proportion of single-chain action at the lower temperatures are probably due to related causes, it is not considered that there is any such connexion at the higher temperatures. Although the extent of enzyme action is decreased at high temperatures the activity of an individual enzyme molecule should continue to increase with temperature for as long as it retains its catalytic power. It is shown in Fig. 5 that a five-fold variation in the concentration of enzyme did not influence the action pattern and a test was made for the possibility that at high temperatures the enzyme passes through a less active stage in the process of denaturation. If such a change were irreversible then the less active form could be prepared by heat treatment. It was observed, however, that a specimen of enzyme which had been heated to 70° had the same type of action at 35° as did an unheated specimen (Fig. 5).

The investigation now reported has been extended by an alternative method of approach using synthetic amyloses labelled with ^{14}C at the non-reducing chain ends (Bailey & French, 1957). Results with crystalline sweet-potato β -amylase indicate that at 35°, pH 4.8, the enzyme removes, on average, four units of maltose per enzyme-substrate encounter.

SUMMARY

1. The action pattern of β -amylase has been studied under a variety of conditions using as substrates two maltodextrins and a synthetic amylose of average chain length 49 units.

2. With all the substrates and under all conditions the action pattern was always intermediate between single- and multi-chain. With maltohexaose the highest proportion of multi-chain action (98 %) was at 0.5°, pH 4.8; the proportion was much less at 35° and was the same at 70° as at 35°. With synthetic amylose at optimum pH the proportion of multi-chain action decreased continuously between 0° and 20°, remained approximately constant between 20° and 60° and increased between 60° and 75°. The proportion was also increased when the pH was changed from the optimum.

3. The action pattern on synthetic amylose was not significantly influenced by the addition of a 130-fold molar excess of maltotetraose.

4. The results show that the enzyme removes several maltose units during each encounter with a substrate molecule. The data using maltohexaose and -heptaose indicate that this number is small. One explanation of the variation in action pattern induced by changes in temperature and pH is that it represents a change in the average number of maltose units removed per encounter. It is suggested either that the enzyme molecule contains several active centres or that multiple reactions can occur at a single active centre, but no conclusive distinction can at present be afforded between these hypotheses.

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