separated from *TMV* by treatment with sodium dodecyl sulphate was unstable, and other proteins also are more easily denatured after separation from their prosthetic groups. The presence of undegraded nucleic acid in solution with serum albumin has been found (Greenstein & Hoyer, 1950) to increase the thermal stability of the latter.

Many anomalies appear when the kinetics of the fission of *TMV* by alkali and the subsequent destruction of the liberated nucleic acid are studied. The results were partly systematized by Grégoire (1950), who found that 0·033N sodium hydroxide acting for 4 min. at 18° split *TMV* into at least three products: protein that could be precipitated at pH 5·2 along with a little nucleic acid, nucleic acid which could be precipitated by hydrochloric acid along with a little protein, and a substance, which remained in the acid solution, that could catalyse the conversion of *TMV* nucleic acid into material not precipitated by hydrochloric acid. This action takes place in 0·033N sodium hydroxide during a few hours and the advantage of using 0·5N sodium hydroxide in the conventional methods for making nucleic acid is not that this strength of alkali is needed for the fission but that it is needed to suppress the secondary loss of acid precipitability. The high concentrations of strontium nitrate that are present when it is used to bring about fission would make it difficult to recognize any similar mechanism if it were playing a part here. But the possibility that *TMV* undergoes fission more readily than the other nucleoproteins because of the presence of other catalytic substance in it must be kept in mind in further work.

SUMMARY

1. Tobacco mosaic virus is split at room temperature into denatured protein and free nucleic acid by solutions of strontium nitrate if the concentration of the latter is greater than molar.

2. Other nucleoproteins are less easily split in this way and other related salts are not so efficient as strontium nitrate.

3. Nucleic acid is decomposed by more intense treatment.

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The Action of Some α-Amylases on Amylose

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The action of α-amylase is due to the fission of the α-1:4-glucosidic linkage in amylose and amylopectin. By confining the study to amylose and its fission products, complications arising from the presence of 1:6-glucosidic linkages are avoided. It is well known that the action of this enzyme is at first relatively rapid until the iodine colour disappears (achroic stage), this being usually attained in the case of amylose when about 20% of the linkages have been split. The mixture now consists of short-chain fragments including maltose and glucose (Myrbäck, 1948; Bernfeld, 1951). A much slower reaction, in some cases only one-hundredth as fast, overlaps and succeeds the first one. In this the short-chain fragments are successively split until only maltose and glucose or sometimes maltotriose remain. According to Myrbäck (1948), Bernfeld (1951), Meyer & Bernfeld (1941), Meyer & Gonon (1951), Alfin & Caldwell (1949) and Roberts & Whelan (1951) all except the terminal linkages in

the substrate molecule are attacked. So far as malt α-amylase is concerned Myrbäck & Sillen (1944) concluded that six linkages are less readily attacked than the remaining ones, at least one of these six being at one end of the chain in question. Thus when the chain has been reduced to one of seven glucosidic units, all of the linkages will now be less readily attacked than those of a large molecule and only the slow stage of the action can proceed. Myrbäck (1948) takes the view that, so far as malt α-amylase is concerned, glucose formation starts early in the reaction, and is split from large as well as small molecules. He cites results which support this opinion, although he states that much of the glucose ultimately formed is very slowly split from short-chain products. Meyer & Bernfeld (1941) and Meyer & Gonon (1951), on the other hand, consider that glucose owes its origin solely to the splitting of maltotriose, a reaction which can take place only after fission of amylose has proceeded far enough to produce some maltotriose. Admittedly some of this maltotriose fission must overlap the rapid (dextrinization) reaction, but the origin of glucose, in the view of these authors, is the fission of maltotriose. They further state that potato or maize amylose, acted upon by malt or pancreatic α-amylase, will yield essentially the same fission products at a given stage or percentage of linkages split. These fission products depend on stage of reaction rather than on the source of amylose or α-amylase. In contrast to these cases, Roberts & Whelan (1951) state that salivary amylase forms no glucose from amylose.

From the above summary it appears that (1) the stage at which formation of glucose can take place is uncertain and (2) in view of the overlapping of the two phases of the reaction it is uncertain what the first phase achieves, and whether even maltose is split from the original molecule. It becomes of importance to investigate the action of α-amylases on large molecules and to minimize the overlapping hydrolysis of short-chain fission products. Somogyi (1940), using starch as substrate, concluded that glucose is not directly split by α-amylase from starch or its high-molecular fission products, but is formed from lower-molecular (achroic) dextrins. The more dilute the reaction mixture the lower was the percentage of glucose in the reaction products at a given stage of hydrolysis or percentage of linkages split. He pictured these various fission products as competing for the enzyme, and dilution as enhancing the dissociation of the enzyme-substrate complexes, especially when the substrate molecules are small. Possibly diffusion plays a part here. The value of the Michaelis constant for starch components and the α-amylases of pancreas, malt and *Bacillus subtilis* appears to be less than 0·1% of substrate, whereas that of certain achroic fission products has been determined in this Department as upwards of 1·0% in the case of the malt enzyme.

If then amylose at, say 0·01–0·05% concentration, were hydrolysed by addition of these α-amylases we should maintain conditions for a relatively fast reaction for the breakdown of amylose and its higher molecular fission products whilst rendering that of the short-chain fission products extremely slow. Overlap of the former by the latter reaction would be negligible. One would expect the achroic stage (maximum chain length, 8 units) to be attained with a lower percentage of linkages split than is usual at this stage.

It is reported here (Tables 1–3) that this expectation was fulfilled, but that maltose and often glucose were already present, suggesting that the fission of these sugars had already taken place in the very dilute reaction mixture prior to the achroic stage.

To ascertain whether these fissions occurred at the reducing or non-reducing end, similar experiments were performed with amylopectin, all the end groups of which at the outset of the reaction would be non-reducing. Amylopectin (from waxy maize) contains one non-reducing end group/20 glucose units (Brown, Halsall, Hirst & Jones, 1948). At a certain stage of α-amylolysis amylose would contain the same proportion of non-reducing end groups as unhydrolysed amylopectin, but, in addition, an equal number of reducing ends, in this respect differing from amylopectin. The next phase of α-amylolysis of these two products (Tables 4 and 5) revealed differences. The appearance of, for example, maltose in the fission products of amylose and its non-appearance in the early products of amylopectin was attributed to an attack by the α-amylases on the second linkage from the reducing end of the chain and to their failure to split the second linkage from the non-reducing end. In the case of the α-amylases of malt and *B. subtilis*, other sugars such as maltotriose were absent from the early amylopectin fission products, but were present when salivary amylase was used.

In view of the need to detect relatively small amounts of these sugars in the reaction products and the need to work with very dilute reaction mixtures, paper chromatography was used, by means of which a few micrograms can be detected. In cases where sufficient quantities of the individual sugars were formed their presence was confirmed by other methods such as chromatographic separation on a charcoal/Celite column (Whistler & Durso, 1950; Bailey, Whelan & Peat, 1950) or measurement of optical rotation and reducing power. It was also considered desirable to confirm the nature of the final fission products of the α-amylases of malt and *B. subtilis* (Tables 8 and 9) by the latter methods since, with the exception of Roberts & Whelan (1951), who used a charcoal/Celite column in their

work on salivary amylase, the workers mentioned above used fermentation methods to determine glucose and maltose. There are objections to such methods, e.g. errors in the determination of small proportions of glucose and failure to distinguish between maltose and maltotriose.

The actions of the α-amylases of malt, *B. subtilis*, saliva and *Aspergillus oryzae* on certain amylose fission products (Tables 6 and 7) revealed minor differences from their actions on amylose itself, and the results as a whole enabled a picture of the pattern of these actions to be formed. The *A. oryzae* enzyme, however, was so contaminated with maltase and, apparently, a group-transferring enzyme, that the results are reported without making specific deductions.

EXPERIMENTAL

Preparation of substrates. Amylose was prepared from potato starch by the method of Schoch (1942), and recrystallized and stored as described by Hopkins & Jelinek (1948). We are indebted to Dr Schoch for the gift of waxy maize amylopectin.

The hexasaccharide (maltohexaose) and other achroic fission products of amylose were prepared by hydrolysis of the latter with *B. subtilis* α-amylase until colourless with iodine, boiling, and then concentration and chromatographic separation by the method of Whistler & Durso (1950), which has been applied to similar products by Bailey *et al.* (1950). The hydrolystate was passed through a charcoal/Celite column (170 × 34 mm.) and the constituents were eluted with successive aqueous solutions of ethanol of increasing concentration:

Constituent eluted	Concentration of aqueous ethanol (%)	Volume used (ml.)
Glucose	0	800
Maltose	5	900
Trisaccharide	15	500
Tetrasaccharide	20	200
Pentasaccharide	25	200
Hexasaccharide	30	350

The volumes are average values. The elution of an individual constituent was carried out until no further material came off the column. The concentrated eluate was tested by chromatography and by optical rotation.

Glucose, maltose and maltotriose were effectively separated by a single application of this procedure. The products used as substrates in Tables 6 and 7, precipitated with acetone and dried *in vacuo*, had the following properties: hexasaccharide, $[\alpha]_D$ (equilibrium in water) +176°, R_m 33, chain length (periodate) 6·1; maltotriose, $[\alpha]_D$ (equilibrium in water) +157°, R_m 64, chain length (periodate) 3·0.

In the preparation of amylose dextrin, α-amylolytic action was stopped when the iodine reaction was quite red; the dextrin was precipitated with acetone and dialysed for 2 days.

α-Amylases. We are indebted to Dr Schwimmer for the gift of a specimen of crystalline malt α-amylase, prepared by the method of Schwimmer & Balls (1949). Malt α-amylase was also prepared by the method of Schwimmer & Balls (1948). We are also indebted for commercial and purified preparations of bacterial amylases, prepared from cultures of *B. subtilis*, to Wallerstein Laboratories, New York, and to Norman Evans and Rais Ltd., who also supplied a fungal amylase 'amylozyme' (*A. oryzae*). Saliva, in which little or no maltase activity could be detected, was often used directly after having been diluted and centrifuged. However, the method of purification described by Meyer, Fischer, Staub & Bernfeld (1948) was also employed.

Determinations. R_m was determined by the method of Blom & Rosted (1947). This quantity is the reducing power calculated as maltose and expressed as percentage of the maltose equivalent of carbohydrate present. The value of R_m is twice the percentage of glucosidic linkages (of all kinds) split.

Periodate oxidations were performed by the method of Potter & Hassid (1948).

Absorption values (A.V.) were measured by running 1 ml. or other suitable volume into 50 ml. solution containing 10 mg. I_2 and 100 mg. KI, diluting to 100 ml., and reading in a Spekker absorptiometer, after it had stood for 1 hr. at 18°. Late readings, when hydrolysis was well advanced, were made on larger volumes of reaction mixture but the results were calculated to that volume corresponding to the initial withdrawal. During α-amylolysis the values of A.V. at all wavelengths when plotted against R_m fell rapidly at first, but later asymptotically approached zero. The fall is slower the shorter the wavelength. The achroic point is therefore vague, because R_m increases appreciably, while A.V. (at 470–490 mμ.) approaches zero.

Paper-partition chromatography was performed essentially as described by Partridge (1948). Volumes (5–20 μl.) of hydrolysate or control sugars were applied with a graduated capillary pipette at intervals along a line 10 cm. from the top of the paper (Whatman no. 1) and dried. A mixture of 10 ml. glacial acetic acid and 90 ml. of 75% (v/v) aqueous *iso*propanol as solvent gave the best movement and separation. Irrigation was at 26° for 18 hr. in which time the solvent had moved 25–30 cm. Development was by spraying with aniline hydrogen phthalate (Partridge, 1949) and heating to 100° for 5–15 min. Dextrins of 3–6 glucose units length required a longer heating time. The bacterial enzymes gave dark patches usually just above the spots corresponding to trisaccharide. It should be clearly understood that the absence of a spot does not necessarily mean the complete absence of the corresponding sugar, but that the quantity present was less than a certain amount. This, in the standard development procedure used, was about 2, 10 and 20 μg. for glucose, maltose and maltotriose, respectively.

It must also be made clear that, while the depth of a spot is indicated in the tables by a suitable number of '+' units, these numbers only afford a satisfactory basis for comparison on any one chromatogram series when they refer to the same sugar. Even then, when a certain depth of colour is exceeded, further increase becomes difficult to detect. In estimating the relative quantities of different sugars greater error is possible since, the longer the saccharide chain, the less the depth of colour. Thus whilst 20 μg. of sugar gave a pronounced spot in the case of glucose, with maltose it was much fainter and with maltotriose just detectable. However, bearing these points in mind, and with experience, it was quite possible to form rough estimates of the relative quantities of different sugars.

Reaction mixtures. Reaction mixtures were set up as stated in the tables, often without added buffers if paper chromatographic methods were to be employed. Antiseptics, both toluene and nitrobenzene, were always added if the reaction was to last for more than a few hours. Portions withdrawn at suitable intervals were boiled to stop enzymic action, concentrated as necessary, and portions of the concentrate used for the determinations required. Rapid arrest of enzyme action, when desired, was achieved by the addition of one or two drops of acetic acid and raising to boiling point as quickly as possible.

RESULTS

Action of α-amylases on amylose

Achroic point of amylose at medium and low concentrations. Table 1 shows the R_m values. The dilute (0·022 %) solutions were achroic at R_m 26–27, equivalent to a mean chain length of 7–8. Further fission of some of these chains, which took place in the 0·44 % reaction mixtures, was minimized by the use of very dilute solutions.

Table 1. *Achroic point of amylose hydrolysed at medium and low concentrations*

(Three reaction mixtures containing 0·44 % amylose were divided immediately after addition of the α-amylase. One half was at once diluted to 20 vol., and the reaction allowed to proceed in parallel.)

	R_m value at the achroic stage	
Enzyme	0·44 % reaction mixture	0·022 % reaction mixture
Malt (stock)	41·5	26·4
Malt (crystalline)	37	26·1
Bacterial	37·5	27·6

Hydrolysis of amylose at various concentrations by malt α-amylase. As in Table 1 the approximate achroic point was reached in dilute solution (0·025 %) at a much lower R_m value than at higher concentrations. This is indicated by the results shown in Table 2.

The chromatograms indicated large amounts of dextrins in the range of 6–8 units chain length, but much smaller amounts (often traces) just below 6 units chain length. Only in the case of 0·025 % concentration at R_m 22·8 and 30·2 was the glucose so faint as to be only just recognizable (in this case less than 0·2 % of the carbohydrate present).

Maltose and maltotriose were present in appreciable quantities even at R_m 22·8.

Hydrolysis of amylose at low concentration by bacterial amylase. Separate reaction mixtures were set up and were stopped at the stages indicated in Table 3.

Chromatograms were carried out on concentrates sufficient to detect as little glucose as 0·1 % of total solids. The chromatograms from *C* indicated not more than 0·5 % glucose and *D* more than 1 %. Both maltose and maltotriose had appeared much earlier (at R_m 7·8) and increased with R_m as was to be expected.

No glucose was seen on the chromatogram of 0·041 % amylose at R_m 18, which was run on a concentrate such that glucose equivalent to 0·04 % of the carbohydrate present would have been detected.

In Tables 4–7 crystalline malt and Wallerstein bacterial amylases were used, the latter giving chromatograms comparatively free from foreign patches.

Table 2. *Hydrolysis of various concentrations of amylose by crystalline malt α-amylase*

(Temperature, 33°. Maltose and maltotriose present on all chromatograms.)

Amylose concn. in reaction mixture (%)	R_m value	Loss in A.V. (%) at 680 mμ.	600 mμ.	490 mμ.	470 mμ.	Chromatographic detection of glucose
0·46	40	99	99	93	91	+ +
0·25	38	100	99	93	91	+ +
0·077	33	99	99	94	93	+ +
0·025	22·8	99	98	80	75	Trace
0·025	30·2	–	–	–	98	Trace
0·025	35·7	100	100	99	99	+

Table 3. *Action of bacterial α-amylase on amylose*

(*A–E* are five separate experiments. *A–D*, amylase, 0·84 %, pH 6; reactions stopped at stages shown. *E*, amylose 0·041 %. The reaction ceased at R_m 18. The reaction mixtures were unbuffered and incubated at 33°. Maltose and maltotriose present on all chromatograms.)

	R_m	Loss in A.V. (%) at 680 mμ.	490 mμ.	Chromatographic detection of glucose
A	7·8	39	26	–
B	9·8	74	31	–
C	30·6	99	85	+
D	47·6	100	100	+
E	18	–	–	–

Fission products of dilute amylose near the achroic point. Table 4 shows the chromatograms. The control standards correspond to yields of 1·6 and 0·16 % of the amylose, respectively.

In the case of malt and bacterial α-amylases, dextrins of 6–7 units chain length, maltotriose and maltose predominated, but only slight traces of tetra- and penta-saccharides and of glucose were present. In the case of salivary and fungal amylases little material of 6–8 units chain length remained. Whereas in the case of malt and salivary amylases maltose appeared in greater yield than maltotriose, in that of bacterial and fungal enzymes the reverse was the case. Maltase, however, may be responsible for this in the case of the fungal enzyme.

Separation of the products in a charcoal/Celite column confirmed the presence of these fragments and, in particular, the presence of dextrins of both 6 and 7 units chain length in the malt and bacterial amylolytic products. The first fractionation gave a mixture of 6 and 7 units chain length. This, partly hydrolysed by crystalline β-amylase at pH 3·6, yielded maltose plus tetra- and penta-saccharide as shown by paper chromatograms, still later products being maltose and maltotriose.

Table 4. *Fission products of* 0·025 % *amylose near the achroic point*

(0·025 % amylose and α-amylase were incubated at 33° and the reaction was stopped as nearly as possible at the achroic point. The chain lengths of the fission products were determined by control chromatogram. The symbols '+', '2+', etc. give rough estimates of the relative quantities of the different sugars as shown on the chromatograms. For further data see Experimental section.)

	α-Amylase				Standards (as % of substrate)	
Chain length	Malt	Bacterial	Salivary	Fungal	1·6	0·16
8	2+	+	+	+	–	–
7–6	3+	4+	–	+	–	–
5	–	+	–	3+	–	–
4	–	–	2+	2+	–	–
3	+	5+	5+	4+	–	–
2	3+	2+	8+	+	Very faint	–
1	Very faint	Very faint	–	8+	4+	+
	R_m 30	40	35	46		
Loss in A.V. (470 mμ.) (%)	98	100	89	99		

Table 5. *Early fission products of* 0·027 % *amylopectin*

(Enzyme and substrate were incubated at 33°; reactions were stopped at an early stage. The chain lengths of the fission products were determined by control chromatogram. The symbols '+', '2+', etc. give rough estimates of the relative quantities of the different sugars as shown on the chromatograms. For further data see Experimental section.)

	α-Amylase					Standards (as % of substrate)	
			Salivary				
Chain length	Malt	Bacterial	(1)	(2)	Fungal	1·0	0·1
Undefined but large	10+	6+	+	2+	6+	–	–
6	+	+	+	4+	8+	–	–
5	–	–	+	4+	8+	–	–
4	–	–	2+	6+	8+	–	–
3	–	–	4+	12+	8+	–	–
2	–	–	–	+	3+	4+	–
1	–	–	–	?+	12+	16+	2+
Loss in A.V. (470 mμ.) (%)	30	11	7	11	57		

Early fission products of α-amylase on dilute amylopectin

Amylopectin (0·027 %) and α-amylase were incubated at 33° under conditions similar to those in Table 4 except that the reactions were stopped at a much earlier stage for reasons already discussed. Table 5 shows the chromatograms. The control standards correspond to 1 and 0·1 % of amylopectin, respectively.

Very little material of chain length less than 6 units was formed by the malt and bacterial enzymes, but considerable amounts of tri-, tetra-, penta- and hexa-saccharide were formed by the salivary and fungal enzymes. The latter as usual also formed much glucose but it had carried the reaction to a 57 % fall in A.V. One important point is that maltotriose made its appearance at a very early stage of fission by salivary amylase, another is the comparative absence of maltose from the products of malt and salivary amylases.

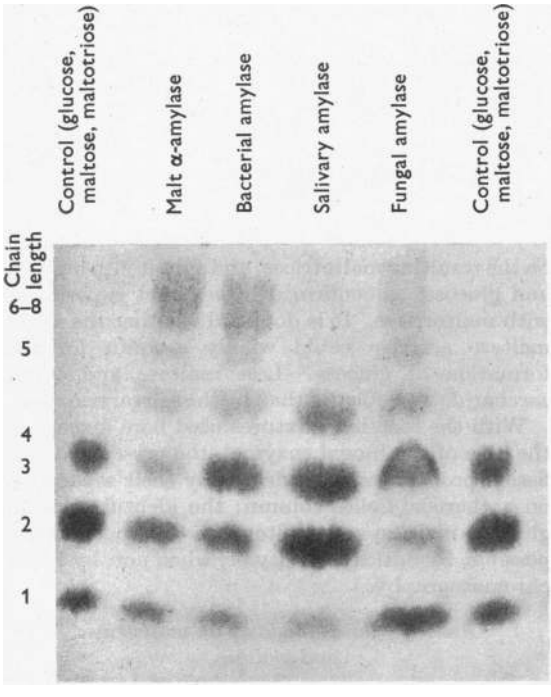

Fig. 1. Fission products of amylose dextrin of mean chain length approximately 20 units, near the achroic point.

	% A.V. lost		Glucose (%) from chromatogram
	680 mμ.	490 mμ.	
Malt (crystalline)	97	88	0·5
Bacterial	98	94	0·05
Salivary	97	92	0·01
Fungal	95	87	5·0

Fission products of amylose dextrin of mean chain length approximately 20 units

Dextrin (0·82 %) and α-amylase were incubated at 33° under conditions similar to those in Table 4. Chromatograms and absorption values were read at intervals on samples withdrawn without concentration. Whereas using salivary amylase under these conditions maltose, maltotriose and tetraose had appeared before the A.V. (490 mμ.) had fallen by 40 %, when using malt amylase these fission products had not appeared by 80% fall in A.V. Using the bacterial enzyme, maltotriose had appeared at 60 % fall in A.V. However when samples taken near the achroic stage were concentrated to one-tenth volume, the chromatograms appeared as in Fig. 1.

These indicate that the main fission products at the achroic stage were, in the case of salivary amylase, maltose, maltotriose and tetraose, and in the case of the malt and bacterial amylases, dextrins of 6–7 units chain length. All of these were also the first products to appear on the chromatograms run on the earlier withdrawals. It is concluded that the malt and bacterial amylases are better able to split linkages 6 or more units from the end of a chain than within this distance. The presence of some fission products of less than 6 units chain length, however, in these and the amylose reaction mixtures of Table 4, and their absence from the amylopectin early hydrolytic products suggest that they are split by these two enzymes from the reducing ends of chains. Salivary amylase however split linkages nearer to the ends much more readily than the other enzymes, but failed to split the second link from the non-reducing end or to split glucose from either end.

The fungal enzyme yielded products intermediate between those of the bacterial and salivary amylases, but with additional glucose, presumably due to maltase activity.

Fission products of linear hexasaccharide

The results are shown in Table 6.

Malt α-amylase. The first fission products were maltose plus tetrasaccharide, with smaller amounts of glucose plus pentasaccharide, the glucose appearing too soon for it to be a product of fission of trisaccharide. The latter however appeared soon. Tetrasaccharide did not diminish until hexasaccharide had disappeared. The final products were glucose, maltose and maltotriose.

It was clear that the fission products most readily formed were maltose and maltotetraose. The maltotriose spots were not only lighter but represent both the product split off and the residue in the reaction hexasaccharide→2 trisaccharide.

Bacterial α-amylase. Much less maltose but more glucose were formed than with malt α-amylase, which it otherwise resembled. With the glucose,

Table 6. *Progress of action of α-amylase on linear hexasaccharide*

(Hexasaccharide (0·78%) and α-amylase were incubated at 33°. A maltase control was run under strictly parallel conditions in the salivary amylase experiment. Chain length of saccharide and fission product as indicated by control chromatogram. The first column in the table represents a chromatogram run on the substrate alone. Times refer, in the case of salivary amylase, to actions on both substrates. The symbols ' + ', '2 + ', etc. give rough estimates of the relative quantities of the different sugars as shown on the chromatograms. For further data see Experimental section.)

Chain length	Malt α-amylase (crystalline)									Bacterial α-amylase (Wallerstein)				
6	4+	4+	4+	3+	3+	2+	+	–	–	3+	2+	+	–	–
5	–	–	–	+	+	+	–	–	–	+	2+	3+	3+	+
4	–	–	+	+	2+	2+	3+	2+	+	+	+	+	+	+
3	–	–	–	–	+	+	2+	2+	+	–	+	2+	2+	3+
2	–	+	+	2+	2+	3+	3+	4+	4+	+	+	2+	2+	3+
1	–	+	+	+	+	2+	2+	2+	2+	+	2+	3+	3+	4+
R_m	33	40	–	–	46	62	77	87	87	34	44	54	75	85
Time (hr.)		0·2	–	–	–	1	2	3	–	–	0·25	0·5	1	2
	Salivary α-amylase									Fungal α-amylase (*Aspergillus*)				
6	4+	+	+	–	–	–	–			3+	2+	–	–	–
5	–	–	–	–	–	–	–			–	–	–	–	–
4	–	+	+	–	–	–	–			–	–	+	+	–
3	–	+	+	+	3+	3+	2+			+	2+	2+	3+	4+
2	–	+	2+	3+	4+	6+	6+			–	–	+	2+	3+
1	–	–	–	–	–	+	2+			–	–	+	3+	4+
R_m	33	–	35	39	–	75	–			–	34	38	77	–
Time (min.)	–	2	6	25	120	180	300			Maltose control for salivary amylase				
2	2+				2+		2+							
1	–				–		–							

pentasaccharide appeared and increased for a time. The glucose was therefore split directly from the hexasaccharide. This enzyme readily split the end linkage of the hexasaccharide, in contrast to its action on longer chains (Table 4 and Fig. 1). The final products were glucose, maltose and maltotriose.

Salivary α-amylase. This enzyme split no glucose directly from a hexasaccharide but mainly split it into maltose and tetrasaccharide. Maltotriose also was formed from the outset, but in view of the consistently deeper colour of the maltose spots and having regard to the fact that two molecules of maltotriose are formed by one fission, it was concluded that the second linkage from one end of the maltohexaose was more readily attacked than the third. In view of the results of Table 5 this linkage cannot be the second from the non-reducing end. The very late, small yield of glucose seems to be accounted for by the action of the enzyme on maltotriose, confirmed by a later direct experiment, especially as the maltose was simultaneously increasing and the maltotriose decreasing. Maltase activity was negligible and was not detected in this experiment, but may be responsible for some of the glucose.

Fungal α-amylase. Fungal α-amylase resembled the salivary enzyme, but had a more marked action on the resulting maltotriose, and split it into maltose and glucose, as confirmed by a direct experiment with maltotriose. It is doubtful whether the slight maltase activity could wholly account for the formation of glucose. Less maltose and tetrasaccharide were formed than by the salivary enzyme.

With the reaction mixtures used here (except in the case of the fungal enzyme), the presence of the fission products was confirmed by their separation on a charcoal/Celite column; the identification of glucose, maltose and maltotriose was made, when possible, by optical activity or, when not, by paper chromatography.

Action of the α-amylases on maltotriose and maltose

All of the four enzymes in this experiment (Table 7) were able to split maltotriose: the feeble maltase activity of the salivary enzyme and stronger one of *Aspergillus* were quite inadequate to account for the rate of fission of maltotriose. Many samples of human saliva from different sources were tested for maltase against control runs with maltose alone. By this rigorous test a slight and variable maltase activity was always found, except in the instance recorded in Table 6.

Table 7. *Action of α-amylases on maltotriose and maltose*

(Maltotriose (0·4%) or maltose (0·26%) incubated with α-amylase at 33°. Chain length of substrate or fission product as indicated by control chromatogram. The symbols '+', '2+', etc. give rough estimates of the relative quantities of the different sugars as shown on the chromatograms. For further data see Experimental section.)

	Substrate						Substrate			
	Maltose			Maltotriose			Maltose		Maltotriose	
	Malt α-amylase (crystalline)						Bacterial α-amylase (Wallerstein)			
Chain length										
3	–	–	–	4+	3+	2+	–	–	4+	3+
2	4+	4+	4+	–	+	2+	4+	3+	–	+
1	–	–	–	–	+	+	–	–	–	+
	Salivary α-amylase						Fungal α-amylase (*Aspergillus*)			
3	–	–	–	4+	2+	–	–	–	+	–
2	4+	4+	4+	–	2+	4+	4+	2+	2+	+
1	–	–	+	–	2+	4+	–	2+	3+	4+
Time (hr.)	0	4	22	0	4	22	4	22	4	22

Table 8. *Fission products of action of malt α-amylase on amylose to 97 R_m*

(Amylose (0·72%) and malt α-amylase were allowed to react at pH 5·8 and 60° in the presence of antiseptics with subsequent additions of enzyme for 6 days. After filtration and concentration, the syrup was precipitated with acetone.)

	Percentage of total hydrolytic products		
	Precipitated with acetone to 67% (v/v)	Filtrate from acetone	Total
Glucose	Trace	5	5
Maltose	25	55	80
Maltotriose (including a trace of tetrasaccharide not determined separately)	15	Trace	15
Chromatograms			
Glucose	Slight trace	+	
Maltose	+	+	
Maltotriose	+	Slight trace	
Tetrasaccharide	Trace	—	

Prolonged action of malt α-amylase on amylose

Amylose (0·72%) and malt α-amylase were allowed to react at pH 5·8 and 60° in the presence of antiseptics with subsequent additions of enzyme until, after 6 days, reducing power corresponded to R_m 97·0. After separation of the small amount of flocculum and concentration to a syrup, precipitation with 2 vol. of hot acetone was followed by reprecipitation of a solution of the precipitate under the same conditions. The clear supernatant portions and washings were collected, freed from acetone and analysed, while the reprecipitated fraction was similarly treated. Analysis by paper chromatography indicated which constituents (two in each case) were present in the respective fractions. Calculation of these amounts from specific rotation and reducing power was made by the usual methods; the results are given in Table 8. A little of the reaction mixture at R_m 97·0 was fractionated on a charcoal/Celite column. Glucose, maltose and maltotriose were recovered in quantity, and identified by specific rotation, thus further confirming the results of paper chromatography.

The acetone precipitate (11·8 mg. maltotriose and 19·7 mg. maltose/ml.) was treated with such a quantity of malt α-amylase as would be equivalent (per unit of carbohydrate present) to 4 times the total used in the original hydrolysis. After some days the reaction appeared to have ceased. Analysis now gave (per ml.): 4·2 mg. maltotriose, 24·3 mg. maltose, 2·7 mg. glucose. Action of the amylase on the maltotriose had been exceedingly slow.

In a control experiment, maltose and enzyme were incubated under the same conditions. No maltase activity could be detected by change in either α_D or reducing power, but a correction for the small optical activity of the enzyme preparation was obtained from the readings.

Prolonged action of bacterial α-amylase on amylose

The previous experiment was repeated at pH 5·8 and 45° using bacterial instead of malt α-amylase. At the achroic stage, R_m 37, a portion of the reaction mixture was concentrated and fractionated on a charcoal/Celite column. Glucose (trace), maltose and maltotriose were separated and identified, and higher saccharides, less completely separated, were also present.

Table 9. *Fission products of action of bacterial α-amylase on amylose to completion*

(Amylose (0·72%) and bacterial α-amylase were allowed to react at pH 5·8 and 45° in the presence of antiseptics with subsequent additions of enzyme for 4 days. At R_m 74 a portion was precipitated with acetone (67%, v/v), the precipitate redissolved in water and treated with more enzyme for 6 days.)

	Percentage of total hydrolytic products		
	Filtrate separated by precipitation with acetone at R_m 74	Remainder (formed in complete hydrolysis) of precipitated portion	Total
Glucose	2	6·4	8·4
Maltose	18	73·6	91·6
Maltotriose	—	Trace	Trace

	Chromatograms (taken from whole reaction mixture)	
	At R_m 82	At R_m 90
Glucose	+	+
Maltose	+	+
Maltotriose	+	+
Tetrasaccharide	+	+ (very faint)

The reaction was continued as far as R_m 74 (4 days) when analysis of a portion by the same procedure as in Table 8 revealed the presence of 2% of glucose in the reaction products at this stage. The portion precipitated by acetone amounted to about 80% of the whole. This was not analysed at this stage but was redissolved in water, treated with more enzyme until action seemed to be complete (10 days, R_m 107) and analysed as in Table 8. The results are shown in Table 9.

The final products were glucose, maltose and a trace (undetermined) of maltotriose.

Portions of the total reaction mixture at the stages R_m 82 and 90 gave paper chromatograms as described in Table 9.

A maltase control was performed but no maltase activity could be detected.

DISCUSSION

Action of α-amylases on terminal and adjacent linkages

It will be convenient to refer to the rapid phase of the reaction which is terminated at about the achroic stage as 'dextrinization', and the subsequent fission of the achroic products as 'secondary fission'. It is concluded from the results of Tables 1 and 2 that the α-amylases of malt and *B. subtilis* split the amylose molecule in very dilute solution into chains of about 8 or 7 units length, i.e. short enough to be achroic, but very little further since R_m was 27, equivalent to a mean chain length of 7·4. It is true that a relatively small proportion of dextrins giving a faint-brown colour with iodine was still present, and to minimize secondary fission the reaction was arrested before these had quite disappeared. Although little or no fission of short chains could have occurred, maltose, maltotriose and slight traces of glucose were already present and had presumably been split off from longer chains. There were indications that less glucose is split from amylose by the bacterial than by the malt enzyme (Tables 2–4, Fig. 1) and none by salivary amylase, but all split off maltose.

To study the effects of the amylases on the outermost chains of amylopectin it was necessary to employ dilute solutions and stop the reactions early to prevent or minimize further action on the fission products (Table 5). The corresponding hydrolyses of amylose (Table 4) had to be carried further to be comparable, since the attainment of R_m 10 is necessary as a preliminary to bring the concentration of non-reducing end groups to the same value as that in the unhydrolysed amylopectin. Any marked differences between the low-molecular fission products in the two experiments in the next stages will be attributable to the action of the amylase on the linkages near the reducing ends. Such differences are most marked in the case of salivary amylase, which liberated much maltose from amylose and very little from amylopectin. The same effect was observed with malt α-amylase. The bacterial enzyme split maltotriose the most readily from early fission products of amylose but not from amylopectin. The conclusion is drawn that the salivary, malt and bacterial amylases readily attack the second, second and third linkages, respectively, from the reducing end. Linkages further from the end are also attacked (Tables 2 and 3, Fig. 1), unless they are within the proscribed distance from the non-reducing end, as is discussed below.

Features common to the hydrolysis of both substrates and, in particular, observed in the early stages with amylopectin, can well be attributed to enzyme action on linkages within a few units of the non-reducing end. Such a feature is the formation of tri- and tetra-saccharide by salivary amylase. It is noteworthy that malt and bacterial amylases split off from amylopectin only negligible traces of saccharides of less than 6 units length. It is clear, however, that the salivary enzyme can split the third linkage from the non-reducing end and other linkages further on, unless within the proscribed distance of the reducing end. The second linkage from the non-reducing end is split very much more slowly as in the fission of maltotetraose. Fission products containing α-1:6 linkages could not be smaller than 5 glucose units (Whelan & Roberts, 1952) and would not be represented on the chromatograms in the positions occupied by tri- and tetra-

saccharide. Even in the pentasaccharide position a branched compound is unlikely in the very early reaction products. Furthermore, while the proximity of α-1:6-linkages may influence the susceptibility of certain α-1:4-linkages to fission, the latter will be those which are furthest from the non-reducing ends. Failure of salivary amylase to split the second, and malt and bacterial amylases the second to fifth linkages from the non-reducing ends in amylopectin cannot be ascribed to the presence of 1:6-linkages situated several units along the chain.

The linear dextrin used in Fig. 1 resembles amylopectin in some respects such as percentage of non-reducing end groups. Its fission products with the respective enzymes support the conclusions drawn above.

In contrast to the salivary enzyme, the malt and bacterial amylases in Table 5 split off only negligible traces of saccharides of less than 6 units length. It is concluded that they are only able to attack the first five linkages from the non-reducing end very slightly or slowly. That these enzymes split the second and third links from the non-reducing end with difficulty is confirmed by the slow disappearance of maltotetraose and triose (Table 6). While resembling one another in this respect, malt and bacterial amylases differ in their attack on the linkages near the reducing end, malt readily attacking the second but bacterial amylase the third linkage (Table 4, Fig. 1). Similarly, malt α-amylase splits the first with much less ease than the second, while the bacterial enzyme splits the second linkage much less readily than the third, and the first even less easily. This has the effect that whereas five linkages at the non-reducing end and one at the reducing end, i.e. in all six linkages, are relatively immune to fission by malt α-amylase, five and two, or seven linkages, are correspondingly immune to the bacterial α-amylase. Thus, the bacterial amylase enters the slow, purely saccharification phase, 'secondary fission', at an earlier stage than is the case with the malt enzyme, i.e. when the substrate has been split to chains of seven linkages (chain length 8) as against six (chain length 7). (Cf. Hopkins & Kulka (1942) who used an enzyme from the same source.) But the minimum length of chain which results in any quantity from the dextrinization phase by these enzymes is 6, for the last fission by malt has usually removed not less than two and bacterial amylase not less than three units.

Although the bacterial amylase splits glucose from chains of longer lengths less readily than does malt α-amylase, yet relatively more glucose appears to be formed in its action on the hexasaccharide (Table 6). This, however, is because the bacterial enzyme is in this case unable to exercise freely its function of splitting off trisaccharide owing to the proximity to the non-reducing end of the linkage involved. The result is that relatively less maltotriose and maltose are formed and more of the enzyme action is forced on to the linkage next to the reducing end, yielding glucose. To a certain extent the same influence is seen in the fission of the hexasaccharide by malt α-amylase. This provides an additional reason why glucose formation becomes noticeable in the advanced stages of amylose hydrolysis. As the substrate chains become shorter, glucose constitutes an increasing proportion of the fission products. Glucose makes an earlier appearance in the fission of amylopectin or starch than of amylose (Myrbäck, 1948; Alfin & Caldwell, 1949). This also is due to the earlier appearance of short-chain fission products.

The possibility that β-glucosidic residues in amylose (Peat, Thomas & Whelan, 1952) may give rise to traces of glucose has been considered. Such residues could not be present in sufficient quantity to account for the yields of glucose liberated, especially those from the short-chain substrates, as in Fig. 1 and Table 6. β-Glucosidase activity was not found in the malt α-amylase, which yielded more glucose from amylose than did the bacterial enzyme. The fungal enzyme was admittedly far from pure, whilst the salivary enzyme split no detectable quantity of glucose except from the hexasaccharide and maltotriose.

Salivary amylase differs from the malt and bacterial enzymes in the readiness with which it splits the third linkage from the non-reducing end and the two linkages beyond. It also differs from malt α-amylase in its incapacity to split the first linkage from the reducing end, but resembles it in that it readily attacks the second linkage from the reducing end. With salivary amylase, chains of 5 units or more can be readily split at the second linkage from the reducing end, but a chain of 4 units will be only slowly split at its middle linkage (cf. Fig. 1).

The calculation of Meyer & Gonon (1951) indicates that salivary amylase should split amylose finally yielding 2·35 molecules of maltose to each one of maltotriose, or approximately seven to three (Roberts & Whelan, 1951). If, before the end of the reaction, some of the seven molecules of maltose were still present as tetrasaccharide undergoing slow fission, we could well imagine the reaction as entering on the slow secondary phase with two tetrasaccharide and three maltose to every three maltotriose molecules. This would correspond to R_m 70. The relative quantities of the oligosaccharides in the imaginary case correspond quite well with those which can be deduced from the chromatograms (Table 4, Fig. 1). It is to be expected that when amylopectin is the substrate, the increased ability to split off maltotriose in the early stages

would influence the final yield of this sugar, and the ratio would become less than 2·35:1, as was actually found by Whelan & Roberts (1952).

No glucose was ever detected in actions of salivary amylase on amylose, and only in those cases where the reaction conditions permitted the prolonged exposure of maltotriose to the action of the enzyme was any glucose formed. In Table 7 there was significantly more action on maltotriose than on maltose. The faint maltase activity could scarcely account for the complete fission of the maltotriose into maltose and glucose. The further fission of the resulting maltose was not accomplished. It is, however, possible that for some reason, the maltase acts on maltotriose much more rapidly than on maltose.

Here again we see the influence of the non-reducing end. When no available linkage remains save the one next to the reducing end, the salivary amylase is forced to act on this linkage. In maltotriose, the proximity of the non-reducing end is equivalent in its effect on salivary amylase to its effect on the bacterial amylase when the hexasaccharide is attacked, as discussed above. If pancreatic amylase resembles our salivary amylase in this respect, the conclusion of Meyer & Gonon (1951) that glucose is formed solely by fission of maltotriose would be justified so far as this enzyme is concerned.

Specific action of α-amylase. General picture

Before proceeding further, it seems desirable to form a general picture of the specific union of α-amylases with their substrates. For simplicity, no attempt has been made to depict the linear glucosidic chain as helical in form.

Let us represent the α-1:4-glucosidic chain by arrows joined as in Fig. 2, where each arrow represents a glucosidic residue, and the arrow head the reducing carbon atom. The α-amylase may be regarded as having a specific zone *A–B* enabling it to unite with the substrate molecule along, say (as in the case of bacterial amylase) 9 glucosidic units of its length, as shown in the diagram at *C*. Fission follows at the point indicated, the 'tension' necessary to effect this being easily brought to bear if the full number of glucosidic units possible had been united to the enzyme. When the chain overlaps the specific zone of the enzyme (as in *C*) this will always happen, union will be easy, affinity constant high and fission rapid.

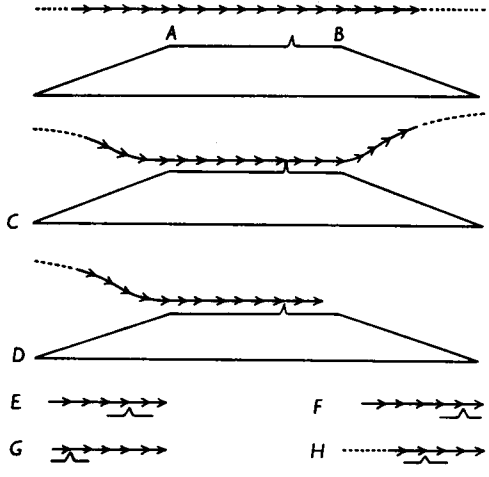

Fig. 2. Diagram to illustrate the union of α-amylase to its substrate.

But when a chain of less than 9 units has to be split it is forced to 'engage' only a part of the specific zone. Also the end of a long chain may fall by chance over the specific zone and unite as in the diagram at *D* without occupying all of the 9 available spaces. Union in such cases is assumed to be less easy, and fission to be much slower than in the previous case. With less glucosidic units engaged on either side of the point of fission there results a weakening of the hold and a lessening of the 'tension' which can be brought to bear.

We have assumed the point of fission to be three units from the one end of the specific zone of the enzyme, which in the case of our bacterial α-amylase would best explain the facts. In this case a fission of one glucosidic unit would be more difficult than that of two, and two more difficult than that of three units or more. A short chain of, for example, six units might (but would not necessarily) unite more readily as at *E* than as at *F* since in the position *E* a hold of two units on the one side of the fission point is secured even though this entails sacrifice of a one-unit hold on the other side. When a long chain is united in this way, but overlaps the specific zone at the other end, as at *D*, this sacrifice would not be incurred. In this case the chance of union and speed of fission as at *E* may exceed those of union *F* even more than they do with the short chain, since the long chain, while gaining an additional hold on one side, loses nothing on the other because all holds there are taken up in both cases. Thus the fission of one unit from the reducing end of a chain of 9 or more than 9 units length would be relatively rare.

On this hypothesis, fission of the links in long chains would be random, except for a limited number of links at each end. But fragments of 8 units and less tend to select their position on the enzyme and in consequence undergo fission in a more planned manner, depending on the source of the enzyme. For instance in the case of malt α-amylase, action as at *E* is the most common. However, fission at other links may take place excepting, of course, the link next to the non-reducing end, which is always sacrosanct to the amylases. Fission such

as G can never take place. The glucose group at the non-reducing end apparently cannot 'engage'. The exceedingly slow fission of maltotriose and non-fission of maltose follow from the above hypothesis. With salivary amylase the fission point would also be as at E, because there are holding places for two glucose units between the fission point and the end as against three such places with the bacterial amylase. However, with salivary amylase the number of units engaged on the other side of the fission point is only three (possibly four) as against six for bacterial and malt α-amylases.

It will be remembered that the shape of the chain is helical and that the six units to the left of the fission point (at AB) would be arranged in this way, occupying about one coil of the helix. The relative immunity from fission by the malt and bacterial amylases shown by the linkages in this coil is presumably associated with the need for the whole coil to unite with the receptive part of the enzyme to effect a firm union. Even in the case of salivary amylase five units (possibly six) in all are involved, amounting almost to one coil.

This hypothesis involves a random fission with exceptions in a number of linkages, especially at the non-reducing end, and consideration must now be given to the bearing of the experiments on this.

Action of the bacterial and malt α-amylases on amylose

To arrive at the achroic stage (at which no chains have greater length than 8 units) by the most economical fission of amylose, a planned attack by the enzyme on every eighth link would be needed, and the product would be achroic at R_m 25. By a purely random fission this would not be achieved at 3 times this value of R_m. In Tables 1 and 2 the achroic stage was reached in dilute solution at R_m 27–30, corresponding to mean chain length of about 7. Chromatograms (Table 4, Fig. 1) confirmed the relative abundance of dextrins of chain lengths 6, 7 and 8. However, this does not necessarily mean that the enzyme had primarily split the helical chains at regular intervals, say one turn or one coil apart. If the enzyme splits each coil at the corresponding link we should expect fission products equal to one coil each and all having the same chain length (either 6 or 7). The specific union of enzyme and substrate envisaged for this must essentially be associated with the helical structure of the substrate. But the same enzyme is also able to combine with a short saccharide chain such as hexasaccharide (also curved or part of a coil in shape) and to split from it glucose, maltose and maltotriose. For the latter purpose the enzyme needs to combine with the substrate molecule at all or most of the points along the chain. Such union and fission, if applied to the long helical chain, would be expected to result in the latter being split at various points, not necessarily one per coil, at uniform intervals and a more random type of fission would be expected. The variability in the chain length of the achroic dextrins (Table 4) also is more compatible with a random fission of the links in a long chain. There are, however, the important qualifications already discussed, namely, that in the case of the malt and bacterial amylases five links at one end and one or more at the other end of a chain are less readily split than the remainder. Assuming such qualification, it can be seen how it comes about that the fission products at the achroic stage are mostly of 6–8 units chain length. During the dextrinization phase with bacterial α-amylase, chains of 12 units or more can be split into parts of 6 or more. As the reaction progresses, chains of 6–11 units chain length increasingly appear. These are too short to give rise to two fission products of 6 units or more. Those of 9–11 units chain length are now split into, e.g. a hexasaccharide, and a short chain. Those of 6–8 units chain length are already achroic and are only split very slowly (secondary fission) as already discussed. In this example, from 6 chains, one each of 6–11 units chain length, respectively, are thus formed, six of chain length 6–8 and one each of chain length 2, 3 and perhaps 4 units each. Thus, at the end of the dextrinization phase, fission products of 6, 7 and 8 units chain length are the prevailing ones, and shorter chains of 2, 3 and perhaps 4 units chain length form a minority. The dextrin used in Fig. 1, itself a bacterial amylase fission product, yielded fragments of unit chain length 6, 7 and greater. The achroic fission products of amylose by the malt and bacterial enzymes were shown to contain both 6 and 7 units chain length by separation on a charcoal column followed by the action of β-amylase (Table 4).

The next point to be noted is that small but significant amounts of maltotriose, maltose and even glucose are formed during the dextrinization period even when secondary fission is minimized by the use of very dilute reaction mixtures (Tables 2–4). This indicates that the three linkages at one end of a long chain, if not both ends, are susceptible to fission even if this occurs less frequently than fission of the linkages further in the interior. As such linkages can be attacked when hexasaccharide is the substrate this is not surprising. Nevertheless, such linkages are split relatively slowly by the malt and bacterial amylases. From long chains the fission of glucose, for instance, is very much less than would be the yield by random fission. This is still true even if we take half the latter, because it is apparent that no splitting off of glucose takes place at the non-reducing ends. When bacterial α-amylase had split 0·041 % amylose to R_m 18 no evidence of glucose could be obtained by paper chromatography, indicating that less than

0·04 % had been formed (Table 3). Random fission would have yielded 0·8 % by R_m 18. Even in 0·84 % amylose, at R_m 30·6 (Table 3), glucose formed at most 0·5 % against 2·25 % theoretically producible by random fission. Malt α-amylase produced less than 0·2 % glucose in splitting 0·025 % amylose to R_m 22·8 (theoretical for random fission, 1·3 %). This was formed well before the achroic stage and could not have been split from short-chain fission products, but was derived from longer chains.

The fission of maltose and maltotriose from amylose cannot begin or is negligible until some reducing chain ends have been formed. There must be a lag, but as more primary fissions occur to render reducing ends available, the splitting off of maltotriose, maltose and, on a smaller scale, glucose begins. The rate of liberation of these sugars would start from zero and increase continuously. This increase would be roughly in arithmetic progression if the reaction as a whole (i.e. fission of all linkages attacked) were of zero order. Somogyi's (1940) results for starch as substrate and urinary amylase are in general agreement with this, but whereas Somogyi considered glucose to be split only from short-chain fission products, it is here contended that glucose can be split from the reducing ends of all fission products, whether short or long chains. Such fission, however, is slower than that of the linkages which are split at random, while fission of the end link at the non-reducing end never takes place with the α- and β-amylases. The very slow fission of maltotriose is illustrated by Tables 8 and 9. Inhibition of amylolysis by maltose was a contributory factor. Chromatograms have also shown that the stages of secondary fission by the malt and bacterial α-amylases at about R_m 90 include that of the tetraose.

The malt and bacterial amylases used here may therefore be taken only to attack with great difficulty the second to the fifth linkages from the non-reducing end, and the first or first two linkages as the case may be at the reducing end. Within these points other linkages are attacked at random.

Action of salivary amylase on amylose

Salivary amylase exhibits many features in marked contrast to malt and bacterial α-amylases. With it, fission of the first-formed achroic products proceeds relatively quickly and much maltose, maltotriose and tetraose are formed before the achroic stage is reached. On the other hand, less material of chain length 6–8 units is present at this stage than with malt and bacterial amylases (Table 4, Fig. 1). Whereas with bacterial amylase the minimum length of chain normally split from a non-reducing end is 6, with salivary amylase it is 3. The maximum lengths for resistance to fast fission are 8 and 4, respectively. Just as the prevalence of saccharides of 6–8 units chain length at the achroic stage is consistent with random fission by the bacterial amylase, so is the prevalence of 3–4 units chain length in the case of salivary amylase, in each case with the reservations postulated.

Roberts & Whelan (1951) and Whelan & Roberts (1952) have concluded that all the linkages in the amylose chain except one at each end are equally liable to fission by salivary amylase. Their final yields of maltose and maltotriose from amylose were in good agreement with the relative quantities (in moles, 2·35 : 1) calculated on this assumption. Our observations conflict with this conclusion so far as the second linkage from the non-reducing end is concerned. But if this is split very much more slowly than the others, while the others are split at random, the final yield of maltose would be less than that found by Roberts & Whelan. If, however, the third linkage, although split much faster than the second, is also split more slowly than the remaining susceptible linkages, the yields obtained could still be accounted for. This would mean that not only the end linkages but the second and third from the non-reducing end would be exceptions to the random attack.

General

Meyer & Gonon (1951) state that when a given fraction of linkages in amylose have been split, the proportions of the various fission products depend, not on the source of the α-amylase or of the amylose, but on the degree of fission. They cite results with the malt and pancreatic enzymes, the latter of which acts like salivary amylase (Bernfeld, Staub & Fischer, 1948). However, Table 4 and Fig. 1 clearly show that malt and salivary amylases differ in this respect near the achroic stage. The identity of action among α-amylases which has been claimed by these workers on the basis of identical ratios, saccharifying power/dextrinization power, may indeed be misleading. The early phases in which the necessary determinations were made are presumably sufficiently similar for the various α-amylases to give this effect because most of the fission is at random. But with increasing numbers of non-reducing ends, the number of linkages which are relatively immune to this or that enzyme becomes a controlling factor in determining where fission will take place.

SUMMARY

1. The α-amylases of malt and *Bacillus subtilis* hydrolyse amylose ultimately to maltose and glucose. The glucose is not derived wholly by fission of maltotriose.

2. At sufficient dilution the action of these amylases can be almost confined to dextrinization, with negligible fission of the achroic dextrins, which are of chain length 6–8 units.

3. Nevertheless, under these conditions, some maltotriose, maltose and glucose are formed by fission of longer chains. The yield of glucose is far below that expected from a random fission.

4. The action of these enzymes on dilute amylopectin yielded, in the early stages, negligible traces of fission products of shorter chain length than 6–8 units. Salivary and fungal (*Aspergillus oryzae*) amylases under the same conditions yielded products of chain length of 3 units and upwards.

5. Dilute amylose in corresponding reactions yielded appreciable quantities of fission products of chain length of 2 units and upwards (malt and salivary) or 3 units and upwards (bacterial and fungal).

6. Salivary amylase does not readily attack the first two, and the bacterial and malt α-amylases the first five linkages from the non-reducing end.

7. Salivary and malt α-amylases readily attack the second and bacterial amylase the third linkage from the reducing end, other linkages nearer this end with increasing difficulty. It is concluded that linkages other than those near the ends here specified are attacked at random.

8. The action of the α-amylases on shorter linear chains, such as maltohexaose and maltotriose, is restricted as regards linkages near the non-reducing end so that fission must often take place nearer to the reducing end than is normal, and more glucose may be formed.

9. A hypothesis is suggested to explain the actions of the α-amylases.

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The Purification of Aconitase

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The enzyme which catalyses the reaction citric acid ⇌ *cis*-aconitic acid ⇌ *iso*citric acid was discovered by Martius & Knoop (1936), and named aconitase by Breusch (1937). Although this enzyme has been known for some 16 years, it has not been isolated in the pure state. Ochoa (1948) was able to concentrate the enzyme by ammonium sulphate fractionation, but there was no increase in the specific acitivity and no further attempts were made to purify it. Buchanan & Anfinsen (1949) obtained a 23-fold purification of aconitase by low-temperature ethanol and ammonium sulphate fractionation. Electrophoretic analysis showed that the preparation consisted of 'three non-homogeneous components', and the purity of the enzyme was estimated to be 30%.

The purification of aconitase has been greatly hampered by its apparent instability. Krebs & Eggleston (1944) found that glycerol stabilized crude enzyme extracts, but Buchanan & Anfinsen (1949) reported that glycerol was without effect in stabilizing purified preparations. They also found

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