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DIRECT UTILIZATION OF MALTOSE BY ESCHERICHIA COLI*

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In the course of genetic studies, a mutant strain of *Escherichia coli* was developed which is characterized by rapid fermentation and oxidation of maltose but not of glucose. Since this mutant offered an excellent opportunity to investigate the so called direct utilization of disaccharides (1), a study of the enzyme systems involved in maltose decomposition was undertaken.

Experiments with dry cell preparations from the mutant strain led to the conclusion that maltose is initially transformed to polysaccharide and glucose. This reaction is followed by the phosphorolytic decomposition of the polysaccharide and the usual sequence of glycolytic steps. While the work was in progress, Monod and Torriani reported the discovery of a polysaccharide-forming enzyme in *E. coli* which they called "amylomaltase" and described a method of separating it from other enzymes involved in fermentation (2). They showed that neither phosphate nor glucose-1-phosphate is involved in the transformation of maltose to polysaccharide and that the enzyme is adaptive in nature, being formed only when maltose is used as substrate for the bacteria. They represent the action of "amylomaltase" on maltose by the following equation:

$$n \text{ maltose} \rightarrow n \text{ glucose} + [\text{glucose}]_n$$

Monod and Torriani further found that, if the glucose is removed as it is formed in the reaction, the polysaccharide produced is of the "starch" type, giving a blue complex with iodine. If, on the other hand, the glucose is allowed to accumulate, no product giving a blue color with iodine appears.

The work presented in this paper deals with the integration of the action of amylomaltase with other metabolic processes of the cell. We have demonstrated that the reaction catalyzed by amylomaltase is reversible

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and that the polysaccharide formed is phosphorolytically decomposed to glucose-1-phosphate. The reversibility of the reaction probably accounts for the low molecular weight of the polysaccharide formed in the presence of glucose. By taking advantage of this observation, short chain reducing dextrins have been produced from maltose, and maltose itself has been synthesized from glucose and glucose-1-phosphate.[1]

Amylomaltase appears to belong to the same group of enzymes for which we have proposed the name "transglycosidases" (3) and which includes the dextran- and levan-forming enzymes of certain bacteria and the sucrose phosphorylase of *Pseudomonas saccharophila*. It is probable that this type of enzyme plays an important rôle in the biological decomposition and synthesis of various carbohydrates.

Materials and Methods

The bacteria used in these experiments are all derived from *Escherichia coli* strain K-12. Strain Y-10 is a nutritional mutant requiring threonine, leucine, and thiamine, but fermentatively normal. Strain W-108 was isolated from populations of Y-10 treated with ultraviolet light on the indicator medium, eosin-methylene blue-lactose agar. This mutant is substantially unable to ferment lactose, glucose, or maltose. When large populations of W-108 were inoculated into a maltose synthetic medium, secondary mutations restoring the capacity to ferment this sugar were selected. For the most part, they proved to be reverse mutations to the Y-10 type, but one strain, W-327, was isolated which remained lactose- and glucose-negative, although maltose-positive. Genetic tests on W-327 showed that its ability to utilize maltose was derived from a mutation at a locus different from that involved in the mutation from Y-10 to W-108 (4).

The bacteria (strain W-327) were grown on a rotary shaker in a medium containing 1 per cent peptone, 0.3 per cent beef extract, 0.5 per cent NaCl, and 0.5 per cent maltose. Dry cell preparations were made according to the method of Lipmann (5), the bacteria being harvested after 12 to 18 hours of growth and dried *in vacuo* over P_2O_5. For enzymatic studies, suspensions of the dry cells were usually made in 0.1 M $NaHCO_3$ saturated with CO_2 and incubated in an atmosphere of CO_2 at 30°. In the present work, no attempts were made to separate or purify the various enzymes involved in maltose metabolism.

Determinations of reducing sugar were carried out with the method of Hassid (6). Glucose was estimated from the decrease in reducing value after fermentation with *Torula monosa*. Maltose was determined from the difference between reducing values obtained after fermenting first with

[1] Since the preparation of this manuscript, Torriani and Monod have shown the reversible nature of the amylomaltase reaction (14).

suspensions of *T. monosa* and then with *Saccharomyces cerevisiae*. For these tests *S. cerevisiae* was grown on yeast extract-maltose agar. Since there is some evidence that low molecular weight dextrins may be slowly attacked by *S. cerevisiae*, the fermentation was not allowed to proceed for more than 20 minutes after the rapid evolution of CO_2 had ceased. Even under these conditions, it is probable that the values for maltose when present together with dextrins may have been too high.

Phosphoric esters of sugars were identified and estimated quantitatively by the methods described by Umbreit *et al.* (7).

EXPERIMENTAL

Utilization of Maltose by Intact Resting Cells

The rates of oxidation and fermentation of maltose and glucose respectively were determined with resting cell suspensions in a Warburg respirometer. The bacteria were grown in yeast-maltose medium, and washed and suspended in 0.03 M phosphate buffer at pH 6.8 for experiments on respiration and in 0.1 M $NaHCO_3$ for studies on fermentation. Oxygen uptake was determined in air, while fermentation was measured as CO_2 production from bicarbonate in an atmosphere of CO_2.

In a typical experiment the following rates were observed:

	Fermentation	Oxygen uptake
Endogenous	3 c.mm. CO_2 per 5 min.	4 c.mm. per 5 min.
0.025 M glucose	4 " " " 5 "	7 " " 5 "
0.0125 " maltose	26 " " " 5 "	17 " " 5 "

To determine whether the entire maltose molecule is fermented, a heavy suspension of bacteria was allowed to ferment the sugar in a bicarbonate-phosphate mixture in an atmosphere of CO_2. Determinations of residual maltose and tests for glucose were made periodically. The rate of maltose disappearance was found to be quite constant and no appreciable quantity of glucose could be detected at any time in the medium. The results can be summarized as follows in mg. per ml.: maltose initially present, 3.6; maltose after 45 minutes at 30°, 2.4; maltose after 90 minutes at 30°, 1.2; maltose after 135 minutes at 30°, 0.1; maltose after 180 minutes at 30°, 0.0; and glucose, less than 0.1 mg. at any time.

Maltose Fermentation with Dry Cell Preparations

Dry cell preparations were found to carry out a vigorous fermentation of maltose. They also showed varying rates of endogenous fermentation, which presumably involves the decomposition of reserve carbohydrate. The rate of glucose fermentation was not appreciably higher than the endogenous rate. Fermentation was almost completely inhibited when either fluoride or iodoacetate was added. Since glucose-1-phosphate was thought to be a probable intermediate in the decomposition of maltose, the rate of

fermentation of this compound alone and together with glucose was also tested. The observed high rate of fermentation of glucose-1-phosphate was in agreement with the hypothesis that this phosphoric ester is involved in maltose fermentation. The results are shown in Table I. Glucose accumulated in the medium during the fermentation of maltose by dry cell preparations, a phenomenon which was not observed with intact cells.

TABLE I

Fermentation of Sugars by Dry Cell Preparations of E. coli W-327

1.4 ml. of 3 per cent dry cell preparation in 0.1 M bicarbonate, 0.01 M phosphate, at pH 6.8, incubated in an atmosphere of CO_2 at 30°. Experiments 1 and 2 were performed with different batches of dry cells.

Experiment No.	Substrate	Other additions	CO_2 evolved from bicarbonate per 5 min.
			c.mm.
1	None		8
	0.05 M glucose		11
	0.025 " maltose		49
	0.025 " "	0.001 M iodoacetate	2
	0.025 " "	0.05 " fluoride	6
2	None		2
	0.05 M glucose		5
	0.025 " maltose		31
	0.025 " glucose + 0.025 M glucose-1-phosphate		31
	0.05 M glucose-1-phosphate		50

In unpublished studies with *Lactobacillus bulgaricus* it had previously been shown that glucose was not fermented rapidly by dry cell preparations of bacteria grown with lactose as substrate. However, the addition of a small amount of lactose or galactose caused not only the fermentation of these sugars, but also a subsequent rapid fermentation of glucose. To learn whether a similar situation occurs in the fermentation of maltose by preparations of strain W-327, minute amounts of maltose were added together with a large quantity of glucose. In all cases, rapid fermentation was observed only for a short time and the amount of CO_2 evolved was proportional to the amount of maltose added. This indicates that maltose does not act as a "starter" for glucose utilization by such dried preparations.

It could be shown that phosphate is esterified during the fermentation of maltose. However, in the absence of metabolic inhibitors, the uptake of orthophosphate was not very great, and usually somewhat irregular. In the absence of substrate or in the presence of glucose, phosphate was also esterified but at a considerably lower rate. When fluoride, and es-

pecially when iodoacetate, was added together with maltose to the bacterial preparations, a very significant uptake of orthophosphate was observed. Under similar conditions, little or no esterification occurred without substrate or with glucose (see Table II).

In experiments in which glucose-1-phosphate was added to the dry cells it was found that this compound disappeared very rapidly, while glucose-6-phosphate, fructose-6-phosphate, and a polysaccharide which gave a brown to blue color with iodine appeared in the medium. This indicates that phosphoglucomutase, phosphohexoisomerase, and a phosphorylase similar to those found in muscle and potato are present in the bacteria. It is known that fluoride interferes with the enzyme phosphoglucomutase which converts glucose-1-phosphate into glucose-6-phosphate. It was therefore expected that the addition of fluoride, but not of iodoacetate, would decrease the rate of conversion of glucose-1-phosphate to other esters, and consequently increase polysaccharide formation. This was found to be the case with the dry cell preparations.

Polysaccharide Formation from Maltose

When dry cell preparations treated with iodoacetate to prevent fermentation were allowed to act on maltose in the absence of added phosphate, glucose and polysaccharide were produced rapidly. In some experiments, approximately 1 mole of glucose was formed per mole of maltose decomposed. This is in agreement with the postulated equation for amylomaltase action. In most experiments, however, the ratio was found to be somewhat less than unity, but in no case less than 0.76. It is possible that errors in the determination of maltose and of polysaccharide, some sources of which have already been mentioned, may have contributed to the low ratios. However, a perfectly reasonable explanation for such results lies in the nature of the polysaccharide produced in the presence of glucose. This will be discussed later in the paper.

The polysaccharide produced from maltose did not give a blue color with iodine. This was in agreement with Monod's observation that iodine-colored polysaccharide is not formed if glucose is allowed to accumulate during the reaction. The following experiment was carried out to elucidate the nature of the polysaccharide produced under these conditions. 40 ml. of a mixture containing approximately 3 per cent dry cells, 0.1 M $NaHCO_3$, 0.002 M sodium iodoacetate, and 0.2 M maltose were incubated at 30° for 180 minutes in an atmosphere of CO_2. The reaction was stopped by the addition of trichloroacetic acid to a concentration of 6 per cent. The precipitate was extracted with 6 per cent trichloroacetic acid and the supernatants combined. The supernatant solution was passed through ion exchange columns and concentrated by vacuum distillation. It was then analyzed for free glucose, maltose, and total glucose released by acid

hydrolysis. The following results were obtained in micromoles per ml.: initial maltose, 192 ± 2; maltose decomposed, 114 ± 3; free glucose produced, 112 ± 2; and glucose as polysaccharide, 116 ± 3.

The major portion of the solution was then fermented with *S. cerevisiae* to remove maltose and glucose, again passed through ion exchange columns, and evaporated to dryness under a vacuum. The remaining material was separated into three fractions on the basis of solubility in alcohol. A minor fraction (No. 1) (100 mg.), soluble in hot absolute alcohol, was syrupy and could not be properly characterized. Fraction 2, soluble in hot 95 per cent alcohol but not in absolute alcohol, and the remaining material, which was insoluble in 95 per cent alcohol but soluble in hot 85 per cent alcohol, contained most of the polysaccharide. 300 mg. were recovered in Fraction 2 and 220 mg. in Fraction 3. Both Fractions 2 and 3 were white solids and consisted almost entirely of short chain reducing dextrins. Neither substance formed an insoluble osazone.

Fraction 2 had a reducing value to alkaline ferricyanide corresponding to 44.7 per cent of an equivalent amount of glucose. Acid hydrolysis of this material yielded 87.5 per cent of the theoretical amount of glucose, while hydrolysis with β-amylase resulted in its breakdown chiefly to maltose (83.7 per cent of the theoretical reducing value). Glucose and maltose were identified by their characteristic osazones. Oxidation with hypoiodite indicated that, on the average, 1 out of 4 glucose units of this dextrin possessed a free carbonyl group. Assuming one reducing group per molecule, the average molecular weight of the substance was found to be 675 by this method. The theoretical value for an unbranched dextrin of 4 glucose units is 664.3. Its specific rotation (*c*, 2 per cent) in water was $[\alpha]_D = +145°$. The average molecular weight of the acetylated material as determined by Niederl and Niederl's modification of Rast's method (8) was found to be 1215 (theoretical for 4 glucose units, 1255).

Fraction 3 had a reducing value corresponding to 42 per cent of an equal amount of glucose and gave yields of 90.7 per cent of the theoretical amount of glucose or 89.4 per cent of maltose when hydrolyzed with acid or with β-amylase respectively. Its specific rotation in water (*c*, 2 per cent) was +162°. Oxidation of the compound with hypoiodite (9) indicated that it consists of 6 or 7 glucose residues having a molecular weight of 1110. The theoretical values for unbranched dextrins of 6 and 7 glucose units respectively are 990 and 1152. The average molecular weight of the acetylated product was found to be approximately 1400, with some decomposition occurring during the determination. The theoretical values for acetylated unbranched dextrins of 5 and 6 glucose units are 1543 and 1831 respectively.

Phosphate Esters Produced in Decomposition of Maltose

In the presence of maltose, added inorganic phosphate was esterified at a rate considerably lower than that of the decomposition of the disaccharide. The phosphorolytic nature of this esterification was indicated by the fact that the addition of fluoride and iodoacetate did not prevent the uptake of phosphate. To determine the nature of the phosphate esters, 20 ml. of a 3 per cent bacterial suspension containing 0.2 M maltose, 0.1 M $NaHCO_3$, 0.083 M Sørensen phosphate buffer at pH 6.8, and 0.002 M sodium iodoacetate were incubated for 240 minutes at 30° in an atmosphere of CO_2. Enzyme action was stopped by the addition of trichloroacetic acid and the phosphate esters precipitated with barium. 93 per cent of the esterified phosphorus was recovered in the barium-soluble fraction and was composed almost exclusively of glucose and fructose monophosphates. The amounts of the three principal products of the reaction, determined by the usual methods and corrected for values obtained in the absence of maltose, were found to be 3.4 μM per ml. of original digest for glucose-1-phosphate, 21.2 for glucose-6-phosphate, and 4.4 for fructose-6-phosphate.

Determinations of reducing values and of the rate of hydrolysis of the esters with acid were in agreement with these values. The formation of the three esters again indicated the presence of a phosphorylase, together with phosphoglucomutase and phosphohexoisomerase in the bacterial preparations. In experiments of short duration in which iodoacetate was employed to inhibit the fermentation of maltose, the addition of fluoride decreased the rate of phosphate uptake slightly but increased greatly the ratio of glucose-1-phosphate to the other esters.

Before Monod's successful separation of amylomaltase, it seemed possible that one or two phosphorolytic enzymes might be involved in the transformation of maltose to polysaccharide. Even after the demonstration that glucose-1-phosphate is not an essential intermediate in this transformation, the mechanism of the formation of the phosphate esters remained to be elucidated. Maltose, itself, or the polysaccharide, or both compounds might undergo phosphorolytic cleavage by one or more enzymes. Several lines of evidence, however, indicate that phosphorolysis is involved not in the primary decomposition of maltose but only in the decomposition of the polysaccharide produced from maltose. Briefly summarized, this evidence consists of the following observations.

1. The rate of breakdown of maltose and of the production of glucose and polysaccharide was not increased by the addition of phosphate to the bacterial preparations (see Table II). Even though a small amount of phosphate was present in the dry cells, one would expect a marked effect

of added phosphate on the rate of carbohydrate transformation if glucose-1-phosphate could serve as an intermediate between maltose and polysaccharide. If phosphorolysis of maltose were simultaneous with polysaccharide formation but independent of it, the addition of phosphate should increase both maltose decomposition and glucose production greatly.

2. The addition of arsenate did not prevent polysaccharide formation from maltose. Arsenate is known to replace phosphate with both sucrose and starch phosphorylases and to cause the decomposition of the carbohydrate substrates of these enzymes to their hexose components (10, 11). It

TABLE II

Maltose Decomposition with Dry Cell Preparation

3 per cent bacterial preparation in 0.1 M $NaHCO_3$, 0.002 M iodoacetate, incubated at 30° for 90 minutes in an atmosphere of CO_2. Maltose added to Tubes 2, 4, and 5; phosphate added to Tubes 3 and 4; arsenate to Tube 5.

	Micromoles per ml.				
	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5
(*a*) Initial phosphate	2	2	76	76	2
(*b*) " arsenate	0	0	0	0	67
(*c*) " maltose		76	0	76	76
(*d*) Phosphate esterified	0	1	0	18	*
(*e*) Glucose formed	0	47	0	45	65
(*f*) Maltose decomposed	0	55†	0	51†	61†
(*g*) Glucose units as polysaccharide (2(*f*) − (*d* + *e*))	0	62‡	0	39‡	57‡

* Not determined.

† The figures for maltose utilization may be somewhat high, for reasons explained earlier in the text.

‡ Since polysaccharide was determined by difference, the values given may be too high, the error in polysaccharide determination being double the error inherent in the maltose determination.

will be seen from Table II that a slight increase in maltose decomposition and a somewhat greater increase in glucose production were observed when arsenate was added to the enzyme preparation, together with maltose. These results support the view that arsenate participates directly only in the decomposition of polysaccharide. Maltose decomposition is presumably affected indirectly, since the removal of the polysaccharide can be expected to increase the extent of maltose decomposition.

3. Phosphate esterification with maltose was found to be partially or completely inhibited by the addition of saliva (α-amylase) to the bacterial preparations. Control experiments showed that traces of phosphatase, which may occur in saliva, could not account for this inhibition. Since

polysaccharide but not maltose is decomposed with saliva, this observation supports the conclusion that maltose is not phosphorolyzed.

4. The initial rate of phosphate esterification was found to be the same regardless of whether phosphate was added together with maltose or after the enzyme preparation had been allowed to decompose most of the maltose to polysaccharide and glucose. For instance, a 3 per cent bacterial preparation was found to esterify 6.5 μM of phosphate per ml. in the first 30 minutes after the addition of 100 μM of maltose per ml. Under similar conditions only 4.2 μM were esterified if the initial concentration of maltose was reduced to 50 μM per ml. However, when the phosphate was added 120 minutes after the addition of 100 μM of maltose, the phosphate uptake was found to be 6.6 μM in 30 minutes. At this time, less than 40 μM of maltose remained in the mixture.

Indirect Synthesis of Maltose from Glucose and Glucose-1-phosphate

It seemed very likely that the reaction catalyzed by amylomaltase would be reversible in nature, as are the phosphorolytic reactions involved in the breakdown of starch and sucrose. The occurrence of a reverse reaction would explain Monod's observation that the polysaccharide produced from maltose in the presence of glucose does not form a blue complex with iodine, since long polysaccharide chains would be broken down to dextrins of relatively low molecular weight, in accordance with the equation

$$(C_6H_{10}O_5)_n + m\ C_6H_{12}O_6 \rightarrow (C_6H_{10}O_5)_{n-m} + m\ C_{12}H_{22}O_{11}$$

The reversibility of both maltose decomposition and the phosphorolytic reaction of the mutant strain was clearly demonstrated by the following observations.

1. The addition of glucose, together with glucose-1-phosphate, prevented the formation of a polysaccharide giving a blue complex with iodine. That this was not due to a simple inhibition of phosphorylase activity was shown by the fact that glucose had only a slight inhibitory effect on the non-hydrolytic deesterification of glucose-1-phosphate. It is of interest to record that D-xylose and, to a slight extent, D-mannose also inhibited the formation of the starch-like polysaccharide. D-Fructose, D-galactose, D-arabinose, and L-arabinose had no significant effect. It seems possible that D-xylose and perhaps D-mannose may react with the polysaccharide in the presence of amylomaltase to yield disaccharides analogous to maltose.

2. The addition of glucose to bacterial preparations which had synthesized polysaccharide from glucose-1-phosphate caused the rapid transformation of the polysaccharide to a form which no longer gave a blue-colored complex with iodine. The experiment was conducted as follows: A 3 per cent dry cell preparation was incubated with 0.2 M glucose-1-

phosphate at pH 6.8 for 30 minutes at 30°. This mixture was then boiled and incubated with an equal volume of 6 per cent active dry cell preparation in the presence and in the absence of 0.25 M glucose. Before incubation the mixture gave a deep brown color with iodine which changed quickly to blue on standing. In the absence of glucose, the material still gave a dark brown color with iodine after 30 minutes of incubation and a reddish brown color after 60 minutes. This slow color change is due either to a slow hydrolytic cleavage of the polysaccharide by bacterial amylase or to the

TABLE III

Synthesis of Maltose and Polysaccharide with Dry Cell Preparation

3 per cent bacterial preparation in 0.1 M $NaHCO_3$, 0.002 M iodoacetate, 0.05 M NaF, incubated for 30 minutes at 30° in an atmosphere of CO_2. Glucose added to Tubes 2 and 3; glucose-1-phosphate added to Tubes 1 and 3 (solution adjusted to pH 6.8). About 2 μM of inorganic phosphate per ml. of preparation were present initially.

	Micromoles per ml.		
	Tube 1	Tube 2	Tube 3
Initial glucose	0	174	174
" glucose-1-phosphate	75	0	75
Total disappearance of glucose-1-phosphate*	63	0	52
Inorganic phosphate produced	49 ± 1	0	49 ± 1
Disappearance of free glucose†	−2 ± 1	2 ± 3	20 ± 3
Maltose formed	0	0	17 ± 1
Color with iodine	Blue	None	None

* Disappearance of ester due to transformation to other phosphate esters, formation of polysaccharide, and of maltose.

† Slight production of glucose from glucose-1-phosphate evident in Tube 1.

phosphorolytic decomposition due to the gradual conversion of glucose-1-phosphate to other esters. In the presence of glucose, on the other hand, the color with iodine changed to pale reddish brown after 7 minutes and disappeared after 10 minutes of incubation.

3. When glucose was added together with glucose-1-phosphate to the bacterial preparations, the production of maltose and reducing dextrins was observed. As in the previous experiments, maltose was estimated as sugar fermentable with *S. cerevisiae* but not with *T. monosa*. In addition, it was identified by the microscopic examination of its osazone. Maltose was not formed when glucose-1-phosphate alone or glucose alone was added to the enzyme preparations (see Table III).

Experiments with Wild Type Parent Strain

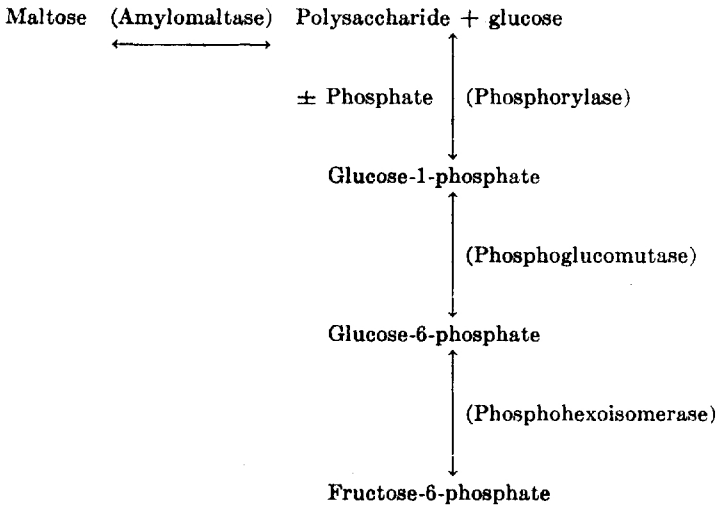
It was of interest to check whether the enzymes involved in the metabolism of maltose by the mutant strain W-327 are also present in the wild type strain K-12, from which the mutant had been derived indirectly. For this purpose, a dry cell preparation of *E. coli* K-12 was made from a culture grown with maltose. This preparation caused an esterification of inorganic phosphate when maltose was added together with iodoacetate. No phosphate was taken up by iodoacetate-treated cells in the absence of substrate or in the presence of glucose. A polysaccharide giving a blue color with iodine was formed when the preparation was allowed to act on glucose-1-phosphate in the presence of iodoacetate and fluoride. The addition of glucose prevented the production of the iodine-colored compound.

These observations were accepted as evidence that the wild type strain adapted to maltose does possess the same enzymes as the mutant strain.

Strain W-327 presumably differs from strain W-108, from which it was obtained directly, in the restoration of the original wild type metabolism of maltose but not of glucose or lactose.

DISCUSSION

From the above experiments, it seems reasonable to conclude that the initial stages of the fermentation of maltose by dry cell preparations of *E. coli* W-327 involve the set of reversible reactions shown in the accompanying scheme.



This scheme, however, fails to account for the observation that intact cells produce no glucose in the metabolism of maltose and yet possess practically no ability to ferment or oxidize glucose when this sugar is supplied in the medium. The situation is analogous to that found in the oxidation of sucrose, trehalose, and melibiose by *P. saccharophila* (12, 13). When grown with sucrose, this organism possesses a sucrose phosphorylase and an invertase, both of which decompose sucrose with the production of fructose. Yet fructose supplied in the medium remains practically unattacked by the intact cells, while the entire sucrose molecule is rapidly metabolized. Bacteria grown in the presence of trehalose possess an active trehalase which hydrolyzes the disaccharide to glucose. No evidence for either a phosphorylase or a special kinase could be found in experiments with dry cell preparations and intact cells. Yet adapted intact cells are capable of oxidizing trehalose at a much greater rate than glucose. Similarly, bacteria grown with raffinose or melibiose as substrate can oxidize melibiose at a much greater rate than the constituent monosaccharides glucose and galactose, provided that all these sugars are supplied in high concentration. As with trehalose, only a hydrolytic cleavage of melibiose appears to take place. In all of the above cases, the demonstration of enzymes responsible for the primary decomposition of disaccharides fails to explain the discrepancy in the rates utilization by intact cells of the disaccharides on the one hand and of hexoses on the other.

If the proposed course of maltose breakdown by *E. coli* W-327 is accepted, an explanation must be sought for the feeble metabolism of glucose by this strain. Although no study of hexokinase activity in either the mutant or the wild type strain has as yet been made, it seems unlikely that the absence of this enzyme would account for the difference between the mutant and parent cultures. If hexokinase were lacking, glucose should accumulate in the medium during the metabolism of maltose. This is not the case. A different impairment of the phosphate metabolism of the organism might offer a possible explanation for the anomalous behavior of the mutant.

Regardless of the nature of the difference between the parent and mutant strain of *E. coli*, it seems clear that amylomaltase plays an important rôle in the metabolism of this species. It is of particular interest that this enzyme is similar to the "sucrose phosphorylase" of *P. saccharophila*, and to the bacterial enzymes responsible for the formation of dextran and levan from sucrose, in that all of these enzymes catalyze the exchange of glycosidic linkages (3). Unlike levan production, the formation of polysaccharide from maltose is readily reversible. This is probably due largely to a lower energy content of the glycosidic bond in maltose than in sucrose.

In addition, it seems likely that the rate of the reverse reaction is greater with the polysaccharide of *E. coli* than with levan because the latter compound has a very high molecular weight and is therefore normally present in very low molar concentrations.

Many important details of the mechanism of polysaccharide formation from maltose remain to be elucidated through studies with purified amylomaltase. The experiments described to date do not show whether a polysaccharide nucleus is necessary for the initiation of polysaccharide synthesis. In comparable reactions catalyzed by muscle and potato phosphorylases it is known that catalytic amounts of polysaccharide are required. If no "starter" is needed, the initial reaction would involve 2 molecules of maltose which would be converted to 1 molecule of trisaccharide and 1 of glucose. It has been found experimentally that less than 1 mole of glucose is usually formed for each mole of maltose decomposed. This was to be expected, since the polysaccharide produced from maltose was found to be of low molecular weight. For instance, in the formation of a reducing tetrasaccharide possessing the formula $(C_6H_{10}O_5)_3C_6H_{12}O_6$, only 2 moles of glucose should be evolved from 3 moles of maltose, in accordance with the equation $3C_{12}H_{22}O_{11} \rightarrow (C_6H_{10}O_5)_3C_6H_{12}O_6 + 2C_6H_{12}O_6$. Another important question which has not been answered so far is whether catalysis by amylomaltase is limited to a transformation of disaccharide to polysaccharide or whether it also involves the condensation of polysaccharide molecules such as the following.

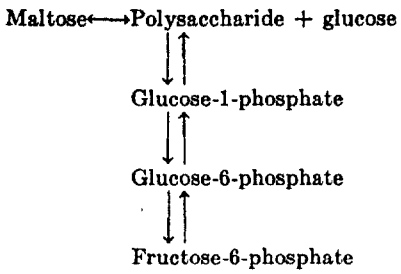
$$(C_6H_{10}O_5)_nC_6H_{12}O_6 + (C_6H_{10}O_5)_mC_6H_{12}O_6 \leftrightarrow (C_6H_{10}O_5)_{n+m}C_6H_{12}O_6 + C_6H_{12}O_6$$

Finally, the bearing of these studies on gene action may be mentioned. As far as they go, these experiments suggest that a single gene mutation, as in W-108, may interfere with the action or formation of several enzymes: in this case lactase, amylomaltase, and possibly hexokinase. It cannot yet be said whether these are primary or secondary effects of the mutation. On the other hand, a "suppressor" mutation, as in W-327, may undo part of these effects, restoring the amylomaltase function.

SUMMARY

1. A mutant of *Escherichia coli* was found capable of carrying out rapid oxidation and fermentation of maltose but not of glucose.

2. Studies with dry cell preparations indicated that both the mutant and the wild type parent strains contain the enzymes amylomaltase, phosphorylase, phosphoglucomutase, and phosphohexoisomerase. The first steps in maltose decomposition could be postulated as shown in the accompanying scheme.



3. When glucose is allowed to accumulate during the decomposition of maltose, the polysaccharide produced by amylomaltase was found to consist of reducing dextrins composed, on the average, of from 4 to 6 glucose units.

4. Due to the reversible nature of the above reactions, maltose and reducing dextrins were produced when glucose-1-phosphate and glucose were added together to the preparations. In the absence of glucose, only a starch-like polysaccharide was formed from glucose-1-phosphate.

5. The proposed mechanism for a "direct" utilization of maltose fails to explain the impaired ability of the mutant to utilize glucose.

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