ENZYMATIC PATTERNS OF ADAPTATION TO FRUCTOSE, GLUCOSE, AND MANNOSE EXHIBITED BY *PSEUDOMONAS AERUGINOSA*[1]

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In a preliminary report (Eagon and Randles, 1956), it was demonstrated that *Pseudomonas aeruginosa* exhibits an apparent metabolic relation between fructose, glucose, and mannose as indicated by enzymatic adaptation experiments. The metabolic ability of whole cells adapted to the utilization of mannose or fructose suggested the schema:

Mannose → fructose → glucose

At that time, it was not determined whether the sugars or their phosphorylated derivatives were actual participants in these conversions.

This paper describes the occurrence of the enzymes involved in the metabolic relationship of these three hexoses when *P. aeruginosa* is adapted to the utilization of each sugar and delineates some of the properties of the adaptive systems.

EXPERIMENTAL METHODS AND RESULTS

Whole cell experiments. The rate and extent of oxidation of glucose, fructose, and mannose by whole cells of *P. aeruginosa* strain 64, when grown on a mineral-salts medium containing 0.1 per cent yeast extract and 1.0 per cent hexose, are indicated in figure 1. These data indicate that systems for fructose and mannose dissimilation are adaptive. Systems for the oxidation of glucose are constitutive in nature and these systems were always present irrespective of the substrate on which the organisms were cultivated. These data are also suggestive that mannose dissimilation proceeded through fructose since cells cultured on mannose show adaptation to fructose and mannose utilization.

Kinases, adaptive and constitutive. Extracts were prepared by sonic oscillation from *P. aeruginosa* grown on each of the three hexoses. These extracts were assayed for hexose kinases. The phosphorylation of the hexoses by adenosine triphosphate could be demonstrated manometrically as shown in table 1. The data of table 1 show that glucose and fructose are phosphorylated by extracts prepared from cells grown on all three hexoses. Phosphorylation of mannose, on the other hand, is carried out only by extracts from mannose-adapted cells.

The low rate of fructose and mannose phosphorylation could be increased 2-fold by the addition of 0.02 M cysteine or 0.001 M 2-mercaptoethanol. These reducing agents, on the other hand, had little or no effect on the phosphorylation of glucose by cells grown on any one of the three hexoses. Similar control experiments carried out in the presence of 100 μmoles NaF to inhibit adenosine triphosphatase in the extracts showed no fluoride sensitive adenosine triphosphatase. However, a slight fluoride inhibition of fructokinase and mannokinase activity was noted. Thus, NaF was omitted in all subsequent experiments.

Confirmation of the phosphorylation of fructose to fructose 6-phosphate was obtained by coupling the bacterial system to glucose 6-phosphate dehydrogenase in the presence of excess phosphoglucoisomerase from rabbit muscle. Data for the spectrophotometric reduction of triphosphopyridine nucleotide in this system are presented in table 2. Similar experiments using excess phosphoglucoisomerase and phosphomannoisomerase showed that mannose was phosphorylated to mannose 6-phosphate. These data are also presented in table 2.

Partial separation of kinases. The data in table 3 suggest that glucokinase and fructokinase can be partially separated. Two experiments are recorded in this table, the top row of figures from extracts of glucose-grown cells and the bottom row from extracts of fructose-grown cells. Inspection of the ratio of the phosphorylation of glucose and fructose observed for the crude extracts and the two ammonium sulfate fractions

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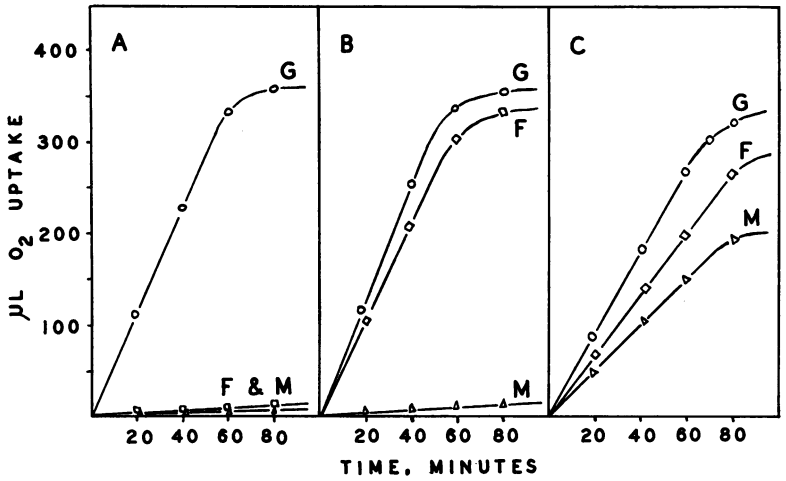

Figure 1. Rate and extent of oxidation of glucose (*G*), fructose (*F*), and mannose (*M*) by whole cells of *Pseudomonas aeruginosa* when grown on glucose (curve *A*), on fructose (curve *B*), and mannose (curve *C*). Four μmoles of hexose per Warburg vessel were used.

indicates partial separation of glucokinase and fructokinase. Activity is lost when further fractionation procedures are carried out irrespective of the type of fractionation procedures used. Once this activity is lost it cannot be restored either by recombination of the fractions or by the addition of a large variety of metal ions or coenzymes.

Mannokinase is distinct from glucokinase or fructokinase since mannose is phosphorylated only by extracts of mannose-grown cells.

Adaptive isomerases. Preliminary control experiments with extracts of cells cultured on glucose indicated that phosphomannoisomerase activity was either absent or present only in negligible amounts and that mannoisomerase was completely absent. At the same time the data suggested adaptive isomerases for mannose and its phosphate ester since extracts of mannose-grown cells phosphorylate mannose and since whole cell experiments showed a metabolic relation of mannose and fructose.

Thus, extracts from cells grown on each of the three hexoses were assayed for enzymes catalyzing the isomerization of mannose and mannose 6-phosphate. The results are shown in table 4. These data indicate: (a) that extracts of glucose-grown cells contain neither mannoisomerase or phosphomannoisomerase; (b) that extracts of fructose-grown cells contain mannoisomerase, which presumably interconverts mannose and fructose, while phosphomannoisomerase is absent; and, (c) that extracts of mannose-grown cells contain both mannoisomerase and phosphomannoisomerase, the latter enzyme interconverting mannose 6-phosphate and fructose 6-phosphate.

TABLE 1

Manometric determination of relative rates of phosphorylation of hexoses by extracts of cells grown on the different hexoses

Substrates	μL CO_2 Evolved/Hr/Mg Protein		
	Glucose	Fructose	Mannose
Glucose	32	5	0
Fructose	36	5	0
Mannose	45	19	8

Protocol: Warburg, 30 C, 5 per cent CO_2-95 per cent N_2 atmosphere. Main compartment: 100 μmoles $NaHCO_3$; 20 μmoles adenosine triphosphate; 10 μmoles substrate. Side arm: 0.5 ml cell-free extract prepared in 0.02 M cysteine or 0.001 M 2-mercaptoethanol. Total volume, 1.7 ml.

The mannoisomerase appeared to be specific for mannose and failed to catalyze the interconversion of other sugars. Those examined were D-glucose, D-galactose, D-lyxose, D-arabinose, L-xylose, and L-rhamnose.

Adaptive transport mechanisms. The experimental results have thus shown that glucose-grown cells are unable to oxidize fructose even

TABLE 2

Spectrophotometric determination of phosphorylation of hexoses by extracts of cells grown on the different hexoses

Substrates	Specific Activity		
	Glucose	Fructose	Mannose
Glucose	0.047	0.029	0
Fructose	0.039	0.028	0
Mannose	0.035	0.045	0.014

Protocol: Beckman spectrophotometer, 30 C, 100 μmoles tris(hydroxymethyl)aminomethane, pH 8; 0.5 μmole triphosphopyridine nucleotide; 20 μmoles adenosine triphosphate; 10 μmoles $MgCl_2$; 0.3 unit glucose-6-phosphate dehydrogenase (1600 Kornberg units per g); excess phosphoglucoisomerase and phosphomannoisomerase (rabbit muscle); 10 μmoles substrate; 0.1 ml dialyzed cell-free extract; total volume, 3.0 ml. Specific activity; ΔF_{340} per min per mg protein (corrected for enzyme blanks).

TABLE 3

Partial separation of fructokinase and glucokinase from extracts of Pseudomonas aeruginosa

Fraction	Specific Activity		Glucose/ Fructose
	Glucose	Fructose	
Crude dialyzed extract	87	69	1.26
	71	32	2.22
$(NH_4)_2SO_4$ precipitate (0–0.4)	44	12	3.67
	28	0	—
$(NH_4)_2SO_4$ precipitate (0.4–0.8)	14	17	0.82
	20	63	0.32

Protocol: The procedure for the estimation of kinases was based on a method suggested by Hers (1952*a*, *b*). The following reaction mixture was incubated at 30 C for 30 min: 100 μmoles tris(hydroxymethyl)aminomethane, pH 8; 6 μmoles substrate; 10 μmoles adenosine triphosphate; 10 μmoles $MgCl_2$; 0.1 ml dialyzed extract; total volume, 0.7 ml. Reaction stopped by addition of 0.3 ml of 5 per cent $ZnSO_4$ and 0.3 ml of 0.3 N $Ba(OH)_2$. Precipitate was removed by centrifugation and reducing sugar was determined by the Folin-Wu method. The control blanks reaction mixture did not contain adenosine triphosphate. Specific activity: $Klett_{420}$ units per hr per mg protein.

TABLE 4

Determination of mannoisomerase and phosphomannoisomerase in extracts of cells grown on the different hexoses

Substrates	Specific Activity	
	Mannoisomerase	Phosphomannoisomerase
Glucose	0	0
Fructose	135	0
Mannose	109	293

Protocol: Enzymatic reaction mixture: 0.05 ml cell-free extract; 2 μmoles substrate; 100 μmoles tris(hydroxymethyl)aminomethane, pH 7.5; 10 min incubation at 37 C; total volume, 1.4 ml. Fructose and fructose 6-phosphate determined by the cysteine-carbazole reaction (Dische and Borenfreund, 1951). Specific activity: $Klett_{540}$ units per hr per mg protein.

TABLE 5

Evidence for hexose transport mechanisms

Substrates	Specific Activity			
	Intact protoplasts		Protoplast lysates	
	Fructose	Mannose	Fructose	Mannose
Glucose	0	0	17	0
Fructose	24	0	15	18

Protocol: Protoplasts were prepared by the lysozyme-Versene technique described by Repaske (1956). The organisms as well as all reagents were prepared in 0.55 M sucrose in order to obtain intact protoplasts. The protoplasts were collected by centrifugation and osmotically lysed by suspending in 0.033 M phosphate buffer, pH 7. Specific activity: μL O_2 uptake per hr per mg protein.

though extracts of these same cells have been shown to contain a fructokinase. This suggested that the cells were impermeable to fructose. Extracts, however, should be able to oxidize fructose readily. This was shown to be the case and was demonstrated by using lysozme-prepared protoplasts. The results are indicated in table 5. Thus it is seen that intact protoplasts of glucose-grown cells indeed cannot oxidize fructose. On the other hand, an extract prepared by osmotically rupturing the protoplasts can oxidize fructose.

Protoplasts of fructose-grown cells, furthermore, readily oxidize fructose. This indicates that adaptation to fructose induces a transport mechanism for fructose which is located in the cell membrane.

By the same reasoning, since extracts of fructose-grown cells have been shown to contain a mannoisomerase, as well as fructokinase, the cells therefore must be unable to oxidize mannose due to impermeability to mannose. The data in table 5 indeed show that protoplasts of fructose-grown cells do not oxidize mannose but that osmotically ruptured protoplasts readily oxidize mannose. Therefore, adaptation to mannose must induce an adaptive mannose transport mechanism which is also located at the site of the cell membrane.

DISCUSSION

P. aeruginosa is able to utilize glucose irrespective of the culture substrate and contains an enzyme which phosphorylates glucose, presumably at the 6-carbon position. Claridge and Werkman (1954) reported a similar glucokinase and these authors further stated that the kinase was specific for glucose and inactive toward other hexoses and pentoses.

Extracts of glucose-grown cells also contain an enzyme which phosphorylates fructose and the evidence suggests that it is distinct from the glucokinase. Thus, cell-free extracts of glucose-grown cells can utilize fructose, but whole cells do not. Furthermore, lysozyme-prepared protoplasts of these cells are unable to utilize fructose, suggesting that the cell membrane is impermeable to fructose.

Whole fructose-grown cells, as well as their protoplasts, utilize fructose. This indicates that an adaptive transport mechanism has been induced by cultivation on fructose and that the transport mechanism is located at the cell membrane.

Adaptation of *P. aeruginosa* to fructose induces the appearance of a second adaptive enzyme, mannoisomerase. This enzyme interconverts mannose and fructose. A similar enzyme in *Pseudomonas saccharophila* was reported by Palleroni *et al.* (1956) and Palleroni and Doudoroff (1956). The enzyme reported by the latter workers was nonspecific for mannose and also interconverted D-lyxose and D-xylulose, D-rhamnose and D-rhamnulose, and D-glycero-D-manno-heptose and sedoheptulose. The mannoisomerase of *P. aeruginosa*, on the other hand, has thus far failed to interconvert other sugars.

Protoplasts of fructose-grown cells cannot utilize mannose but extracts of these protoplasts do utilize mannose. In this case, the evidence suggests that mannose is isomerized to fructose which in turn is phosphorylated and interconverted to glucose 6-phosphate which then is degraded by the aerobic mechanism of this organism.

Two additional adaptive enzymes plus an adaptive transport mechanism for mannose are induced by growing *P. aeruginosa* on mannose. First of all, an enzyme which phosphorylates mannose is induced. Secondly, phosphomannoisomerase, which interconverts mannose 6-phosphate and fructose 6-phosphate, is induced. At the same time, mannoisomerase and a transport mechanism for fructose are induced as well.

In summary, mannose-adapted *P. aeruginosa* can utilize the three hexoses by the following schema:

(1) Mannose $\xrightarrow{\text{mannokinase}}$ mannose 6-phosphate

(2) Mannose 6-phosphate $\xrightarrow{\text{phosphomannoisomerase}}$ fructose 6-phosphate

(3) Mannose $\xrightarrow{\text{mannoisomerase}}$ fructose

(4) Fructose $\xrightarrow{\text{fructokinase}}$ fructose 6-phosphate

(5) Fructose 6-phosphate $\xrightarrow{\text{phosphoglucoisomerase}}$ glucose 6-phosphate

(6) Glucose $\xrightarrow{\text{glucokinase}}$ glucose 6-phosphate

(7) Glucose 6-phosphate $\rightarrow$ aerobic pathway

SUMMARY

Glucose utilization by *Pseudomonas aeruginosa* is constitutive.

Extracts of glucose-grown cells contain fructokinase as well as glucokinase but whole cells are unable to utilize fructose due to permeability factors.

Adaptation of *P. aeruginosa* to fructose induces: (a) a transport mechanism for fructose; and, (b) mannoisomerase, which interconverts mannose and fructose.

Adaptation of *P. aeruginosa* to mannose induces: (a) a transport mechanism for mannose;

(b) mannokinase, which phosphorylates mannose at the 6-carbon position; (c) phosphomannoisomerase, which interconverts mannose 6-phosphate and fructose 6-phosphate; (d) mannoisomerase, which interconverts mannose and fructose; and, (e) apparently, a transport mechanism for fructose.

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