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METABOLISM OF PENTOSES AND PENTITOLS BY *AEROBACTER AEROGENES*

II. MECHANISM OF ACQUISITION OF KINASE, ISOMERASE, AND DEHYDROGENASE ACTIVITY[1]

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ABSTRACT

MORTLOCK, R. P. (Michigan State University, East Lansing), AND W. A. WOOD. Metabolism of pentoses by *Aerobacter aerogenes*. II. Mechanism of acquisition of kinase, isomerase, and dehydrogenase activity. J. Bacteriol. **88**:845-849. 1964.—*Aerobacter aerogenes* PRL-R3 possesses the genetic information to synthesize, in the presence of the appropriate inducer, at least three members of the family of ketopentokinases, two members of the family of pentitol dehydrogenases, and two members of the family of aldopentose isomerases. That is, D-xylulokinase, D-ribulokinase, L-ribulokinase, ribitol dehydrogenase, D-arabitol dehydrogenase, D-xylose (→ D-xylulose) isomerase, and L-arabinose (→ L-ribulose) isomerase activities were detectable within 4 hr after addition of inducer. The possibility that mutation and selection are involved in the formation of L-xylulokinase, L-arabitol dehydrogenase, D-arabinose (→ D-ribulose) isomerase and D-lyxose (→ D-xylulose) isomerase could not be eliminated, because 11 hr or more of incubation after the addition of inducer were required before the appearance of these enzyme activities. D-Xylulokinase activity was induced in less than 2 hr when D-xylose or D-arabitol were inducers, but 45 hr were required for the appearance of activity when xylitol was the inducer, and 83 hr were required when D-lyxose was the inducer. Likewise, the time required for induction of ribitol dehydrogenase was 2 hr for ribitol, 12 hr for D-arabinose, and 45 hr for xylitol. The time required for the appearance of enzyme activity correlated with the time required for the beginning of cell growth and substrate utilization.

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In a previous publication (Mortlock and Wood, 1964), the growth of *Aerobacter aerogenes* PRL-R3 on all of the aldopentoses except L-ribose and on all of the pentitols was demonstrated. In addition, the necessary isomerases or dehydrogenases, pentulokinases, and epimerases required for conversion of all of these to D-xylulose-5-phosphate have been demonstrated (Mortlock and Wood, 1964). The time required for growth varied from less than 24 hr to 3 to 4 weeks, depending upon the aldopentose or pentitol involved. Preliminary experiments indicated that the pentose and pentitols can be separated into two groups consisting of (i) those inducing relevant enzyme activities in a short time (several hours or less), and (ii) those requiring a lag of 10 hr or more and proliferating conditions before activity could be detected. These groups correspond roughly to the groups eliciting either rapid growth or growth after an extended lag. Thus, rapid growth on some of these compounds most probably results from induction of the necessary enzymes, whereas delayed growth on others may be attributed to the selection of mutants with an ability to produce the enzyme(s) required for utilization of these C_5 substrates.

The experiments were performed to further define the pre-existent genetic capacity of *A. aerogenes* PRL-R3 to synthesize the various members of the isomerase, pentitol dehydrogenase, and pentulokinase families needed for dissimulation of the aldopentoses and pentitols.

MATERIALS AND METHODS

Reagents, carbohydrates, media, and growth conditions were similar to those described previously (Mortlock and Wood, 1964). Oxygen utilization was followed by standard manometric techniques. Protein was determined by the method of Lowry et al. (1951), and direct cell

TABLE 1. *Behavior of Aerobacter aerogenes PRL-R3 in the absence of energy source**

Time	Direct cell count × 10⁴	Dry wt	Oxidation rate†	D-Glucokinase‡
hr		*mg/ml*		
0	2.5	0.75	39	1.3
12	4.8	0.60	0	0
17	3.4	0.58	0	0
35	3.1	0.58	0	0
41	3.1	0.58	0	0
60	3.0	0.51	0	0

* The culture was inoculated with cells grown in D-glucose which had been harvested and washed in sterile salts solution. Incubation was carried out in the salts solution at 26 C with shaking. Samples were removed at intervals for analysis.

† Expressed as microliters of O per milligram of cells per hour.

‡ Expressed as units per milligram of protein.

counts and dry weight estimations were carried out as described previously (Mortlock and Wood, 1964). For determination of enzyme activities, cell samples were collected by centrifugation, washed once with water, and the cells were broken by treatment for 6 min with a 10-kc oscillator. Whole cells and cell particles were removed by centrifugation for 10 min at 12,000 × *g*. Pentulokinases, pentitol dehydrogenases, and aldopentose isomerases were assayed as described by Anderson and Wood (1962).

Enzyme induction. Cells were grown on a D-glucose-salts medium (Anderson and Wood, 1962), harvested by centrifugation, washed once with 0.2 volume of sterile culture medium without glucose (salts solution), and resuspended in salts solution to 16% the original volume. The cells were then shaken at 30 C for 2 days prior to use as inoculum. Induction experiments were then performed in two ways. For method A, the induction system consisted of 0.4 volume of the starved cell suspension, 0.5 volume of double-strength salts, 0.1 volume of vitamin-free casein hydrolysate (acid digest, Nutritional Biochemicals Corp., Cleveland, Ohio), and 0.5% (w/v) of the inducer which was added at 0 time. For method B, the same cell suspension was diluted in salts solution to about 2×10^9 cells per ml, and the carbohydrate (inducer) was added at 0 time. In both cases, the suspensions were shaken in flasks at 30 C and samples were removed at intervals for analyses.

RESULTS

Cells grown in D-glucose were harvested, washed, and incubated with shaking for 2 days prior to use in induction studies. During this "starvation" period the D-glucokinase activity observed in cells grown in D-glucose rapidly disappeared, and after several hours endogenous oxygen utilization was greatly reduced (Table 1). The direct cell count increased slightly, and the dry weight of cell material gradually decreased only slightly over long periods of time. Thus, such cells may be comparable to the starvation-resistant mutants of *A. aerogenes* described by Harrison and Lawrence (1963). After this starvation period, the cells were still capable of induced enzyme formation, depending upon the inducer added.

Short-term induction. As described previously (Mortlock and Wood, 1964), *A. aerogenes* PRL-R3, grown on D-glucose as the source of carbon and energy, upon transfer to medium containing either D-ribose, D-xylose, L-arabinose, ribitol, or D-arabitol, is capable of growth upon any of these new substrates within 24 hr. The time required for the induction of relevant enzyme activities by these carbohydrates in suspensions of starved cells could be reduced considerably by the addition of an amino acid source such as casein hydrolysate. Table 2 shows the rates of induction of D-xylulokinase, L-ribulokinase, D-arabitol dehydrogenase, and ribitol dehydrogenase in induction system A containing added amino acids; and with D-xylose, L-arabinose, D-arabitol, and ribitol, respectively, as inducers. With a single inducer, simultaneous induction of more than one kinase and dehydrogenase was detectable in 2 hr or less, and the rate of formation was exponential for a short time, presumably until the inducer concentration became limiting. Upon transfer to the same medium containing D-glucose, kinase and dehydrogenase activity immediately decreased in direct proportion to the increase in new cell material.

Long-term induction. With D-arabinose and L-arabitol where there is a lag of about 24 hr before the initiation of growth, induction system B was utilized. Thus, in addition to enzyme activity, growth could be measured. Table 3 illustrates enzyme induction and growth with D-arabinose as the substrate. D-Ribulokinase and ribitol dehydrogenase activities were first de-

tected after 12 hr of incubation. The time required for the appearance of these activities correlated with the beginning of growth and substrate utilization. D-Arabinose (→ D-ribulose) isomerase, measured in a separate experiment, was first detectable after 13 hr. The appearance of D-glucokinase activity after exhaustion of the substrate was typical.

TABLE 2. *Induction of kinases and dehydrogenases involved in utilization of D-xylose, L-arabinose, D-arabitol, and ribitol**

Time	Inducer					
	D-Xylose	L-Arabinose	D-Arabitol		Ribitol	
	D-Xylulokinase	L-Ribulokinase	D-Xylulokinase	D-Arabitol dehydrogenase	D-Ribulokinase	Ribitol dehydrogenase
hr						
0	0†	0	0	0	0	0
1	0	0	1	2.1	0	0
2	6.4	1.4	7.4	15	3.2	12
3	6.6	2.6	6.0	44	6.5	25
4	15	3.9	9.5	50	9.3	118
5	36	2.6	13	53	14	84

* Induction system A (with casein hydrolysate) was used for these experiments. Samples were removed for analysis at intervals indicated. Pentitol dehydrogenases were assayed by measuring pentulose reduction. A value of 0 indicates activity was less than 1 unit per milligram of protein.

† Figures indicate kinase activity expressed as units per milligram of protein.

With D-lyxose and xylitol as substrates, a lag of approximately 3 days was observed before growth was initiated (Mortlock and Wood, 1964). Table 4 shows the growth response and induction of enzyme activity with D-lyxose as the substrate. For these experiments, induction system B was used and cells grown in D-glucose, freshly harvested and washed, were added as the inoculum. The appearance of the first enzyme activity again correlated with the first disappearance of substrate and the beginning of growth. D-Xylulokinase activity was first detectable 83 hr after addition of the inducer. Activity of those enzymes cross-induced by D-lyxose (D-ribulokinase, D-xylulose reductase, and ribitol dehydrogenase) appeared from 83 to 89 hr after addition of the inducer. During the first day of incubation, the inoculum behaved similarly to that described in Table 1.

Measurement of induction with L-xylose proved to be difficult, owing to the long incubation period prior to growth (Anderson and Wood, 1962). Upon incubation of cells grown in D-glucose with this substrate, L-xylulokinase

TABLE 3. *Adaptation to D-arabinose utilization**

Time	Cell count × 10^{-9}	Dry wt	D-Arabinose	D-Arabinose oxidation rate†	Activity‡		
					D-Ribulokinase	D-Glucokinase	Ribitol dehydrogenase
hr		*mg/ml*	*mg/ml*				
0	2.2	0.56	5.2	0	0	0	0
2	2.2	0.57	5.2	0	0	0	0
4	2.2	0.56	5.1	0	0	0	0
6	2.2	0.60	—	32	0	0	0
12	2.2	0.73	5.0	91	2.3	0	3.4
17	2.2	1.01	—	73	—	0	—
24	2.2	1.16	3.5	—	3.2	0	5.1
35	6.9	1.70	3.2	89	—	—	—
41	6.4	2.00	0.7	169	5.2	7.0	11.3
48	7.6	2.12	—	—	12	14.8	52.3

* A starved inoculum of glucose-grown cells was added to induction system B containing D-arabinose. Samples were removed at intervals for analysis. The reduction of D-ribulose was utilized as a measure of ribitol dehydrogenase.

† Expressed as microliters of O_2 per milligram of cells per hour.

‡ Expressed as units per milligram of protein.

TABLE 4. *Adaptation to D-lyxose utilization**

Time	Cell count $\times 10^{-9}$	Cell dry wt	D-Lyxose	D-Lyxose oxidation rate†	Activity‡				
					D-Xylulo-kinase	D-Ribulo-kinase	D-Gluco-kinase	D-Arabitol dehydro-genase	Ribitol dehydro-genase
hr		*mg/ml*	*mg/ml*						
0	2.5	0.74	4.6	0	0	0	5.7	0	0
12	3.8	0.60	4.7	0	0	0	0	0	0
17	3.0	0.58	4.8	0	0	0	0	0	0
35	2.6	0.58	4.5	0	0	0	0	0	0
41	2.5	0.50	4.7	0	0	0	0	0	0
60	3.4	0.51	4.6	0	0	0	0	0	0
66	3.6	0.50	4.0	0	0	0	0	0	0
83	3.6	0.80	2.5	55	2.6	0	0	4.4	0
89	7.0	1.08	0	122	3.9	0.8	6.0	4.3	1.2

* Induction system B was utilized with washed cells grown in D-glucose as inoculum. Samples were removed at time intervals for analysis. Pentitol dehydrogenases were measured by assaying pentulose reduction.

† Expressed as microliters per milligram of cells per hour.

‡ Expressed as units per milligram of protein.

TABLE 5. *Minimal time required for appearance of activity**

System	Substrate (inducer)	Kinases					Dehydrogenases		
		D-Xylulo-	L-Xylulo-	D-Ribulo-	L-Ribulo-	D-Ribo	D-Arabitol	L-Arabitol	Ribitol
A									
	D-Xylose	2†	—	—	—	—	4	—	—
	L-Arabinose	—	—	—	2	—	—	—	—
	D-Ribose	—	—	4	—	4	—	—	—
	D-Arabitol	1	—	—	—	—	1	—	—
	Ribitol	—	—	2	—	—	—	—	2
B									
	L-Xylose	—	400	—	—	—	—	—	—
	D-Arabinose	—	—	12	—	—	—	—	12
	D-Lyxose	83	—	89	—	—	83	—	89
	L-Arabitol	—	23	—	—	—	—	23	23
	Xylitol	45	—	45	—	—	45	—	45

* Induction system A was utilized for the first set of carbohydrates and induction system B for the second. Pentulose reduction was utilized for detection of pentitol dehydrogenase activities.

† Indicates time in hours.

activity was first detectable 17 days after the addition of the L-xylose.

Table 5 lists the minimal time required for the induction of detectable activity for some of the kinases and pentitol dehydrogenases that are induced by the six aldopentoses and the four pentitols. The organism clearly possesses the necessary genetic information to synthesize, in the presence of an appropriate inducer, kinases which phosphorylate D-xylulose, D-ribulose, L-ribulose, and D-ribose, as well as dehydrogenases which reduce D-xylulose and D-ribulose (or oxidize D-arabitol and ribitol). L-Arabinose (→ L-ribulose) isomerase could be detected 1 hr after the addition of L-arabinose, and D-xylose (→ D-xylulose) isomerase was detected 1 hr after the addition of D-xylose (induction system A). D-Arabinose (→ D-ribulose) isomerase required

13 hr for synthesis in detectable amounts with D-arabinose as inducer (induction system B).

DISCUSSION

In view of the observed short-term induction under nonproliferating conditions, *A. aerogenes* PRL-R3 contains the genetic information for the synthesis of pentulokinases, and pentitol dehydrogenases or pentose isomerases required for the metabolism of D-xylose, L-arabinose, D-ribose, ribitol, and D-arabitol. However, with D-arabinose, L-arabitol, xylitol, D-lyxose, or L-xylose, growth and appearance of enzyme activity was delayed. Thus, a requirement for mutation prior to growth upon these latter carbohydrates cannot be eliminated. Because the kinases involved in the degradation of D-arabinose, D-lyxose, and xylitol (of the second group) are rapidly synthesized in response to other inducers such as ribitol and D-xylose, acquisition of these activities presumably does not require prior mutation and selection of a new population. Thus, such a mutation, if required for growth upon D-lyxose, D-arabinose, and xylitol, would be expected to involve the acquisition of a pentitol dehydrogenase, a pentose isomerase, or possibly a specific permease. The delay in induction of D-xylulokinase by xylitol and by D-lyxose, even in the presence of added amino acid and energy sources, implies that these carbohydrates themselves do not function as inducers for D-xylulokinase, and that the appearance of this kinase results from the subsequent accumulation of D-xylulose. Evidence that ketopentoses may induce low levels of pentitol dehydrogenase in *A. aerogenes* was reported by Hulley, Jorgenson, and Lin (1963).

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LITERATURE CITED

ANDERSON, R. L., AND W. A. WOOD. 1962. Pathway of L-xylose and L-lyxose degradation in *Aerobacter aerogenes*. J. Biol. Chem. **237**:296–303.

HARRISON, A. P., JR., AND F. R. LAWRENCE. 1963. Phenotypic, genotypic, and chemical changes in starving populations of *Aerobacter aerogenes*. J. Bacteriol. **85**:742–750.

HULLEY, S. B., S. B. JORGENSON, AND E. C. C. LIN. 1963. Ribitol dehydrogenase in *Aerobacter aerogenes* 1033. Biochim. Biophys. Acta **67**:219–225.

LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, AND R. J. RANDALL. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.

MORTLOCK, R. P., AND W. A. WOOD. 1964. Metabolism of pentoses and pentitols by *Aerobacter aerogenes*. I. Demonstration of pentose isomerase, pentulokinase, and pentitol dehydrogenase enzyme families. J. Bacteriol. **88**:838–844.