JOURNAL OF BACTERIOLOGY, Jan., 1965 Vol. 89, No. 1
Copyright © 1965 American Society for Microbiology Printed in U.S.A.

Metabolism of Pentoses and Pentitols by *Aerobacter aerogenes*

III. Physical and Immunological Properties of Pentitol Dehydrogenases and Pentulokinases[1]

R. P. MORTLOCK,[2] D. D. FOSSITT, D. H. PETERING,[3] AND W. A. WOOD

Department of Biochemistry, Michigan State University, East Lansing, Michigan

Received for publication 26 August 1964

ABSTRACT

MORTLOCK, R. P. (Michigan State University, East Lansing), D. D. FOSSITT, D. H. PETERING, AND W. A. WOOD. Metabolism of pentoses and pentitols by *Aerobacter aerogenes*. III. Physical and immunological properties of pentitol dehydrogenases and pentulokinases. J. Bacteriol. **89**:129–135. 1965.—Four pentulokinases and three pentitol dehydrogenases were purified from *Aerobacter aerogenes* PRL-R3, and the properties of the enzymes within each family were compared. D-Ribulokinase was purified from cells grown on ribitol, D-arabitol, and xylitol, and from a mutant constitutive for ribitol dehydrogenase; D-xylulokinase, from cells grown on D-xylose and xylitol; L-ribulokinase, from cells grown on L-arabinose; and L-xylulokinase, after growth on L-xylose. Similarly, ribitol dehydrogenase was purified after growth on ribitol, D-arabinose, and xylitol, and from the ribitol dehydrogenase-constitutive mutant. D-Arabitol dehydrogenase was obtained after growth on D-arabitol or D-xylose, and xylitol dehydrogenase was obtained after growth on xylitol. Except for L-xylulokinase, which also had a different S_{20} value, the pentulokinases had identical *p*H optima, K_m for the ketopentose, and sedimentation constants, and fractionated identically by a number of procedures. These kinases could be distinguished only by their substrate and immunological specificity. Ribitol dehydrogenase and D-arabitol dehydrogenase could be distinguished by several properties, but the properties of xylitol dehydrogenase were always similar to ribitol dehydrogenase. In all cases, individual kinases or dehydrogenases produced by different inducers had identical properties. These data constitute evidence against multiple forms of the same enzyme being produced by different inducers and against the dehydrogenase family containing essentially identical proteins differing only at the active site. For the kinases, three of the four appear to differ only at the active site.

Previous publications have established the existence in *Aerobacter aerogenes* PRL-R3 of kinase activities for phosphorylation of the four ketopentoses, and nicotinamide adenine dinucleotide-linked dehydrogenase activities for oxidation of the four pentitols (Mortlock and Wood, 1964*a*, *b*). D-Xylulokinase, L-ribulokinase, and L-xylulokinase, as well as ribitol (→ D-ribulose) dehydrogenase and D-arabitol (→ D-xylulose) dehydrogenase, have been purified and partially characterized from this strain (Simpson, Wolin, and Wood, 1958; Anderson and Wood, 1962*b*; Bhuyan and Simpson, 1962; Wood, McDonough, and Jacobs, 1960). In many instances, enzyme activities were induced which were not required for growth of the organism on a given substrate (Mortlock and Wood, 1964*a*). For instance, D-ribulokinase activity was induced by growth on D-arabinose, ribitol, xylitol, D-ribose, L-xylose, and L-arabinose, but this kinase is known to be involved in the utilization of only the first two carbohydrates.

It is possible that all inductions of one kind of activity (i.e., ribitol-oxidizing activity) may not be due to production of the same protein. Thus, different proteins possessing the same or similar activities may be synthesized in response to different inducers. This possibility was recognized by Stadtman (1963) for aspartokinase, because

[1] Contribution No. 3401 of the Michigan Agricultural Experiment Station. A preliminary report was presented at the Annual Meeting of the American Society for Microbiology, Cleveland, Ohio, May, 1963.

[2] Postdoctoral Fellow, National Institutes of Health. Present address: Department of Microbiology, University of Massachusetts, Amherst.

[3] National Science Foundation Undergraduate Research Participant.

more than one type of aspartokinase was necessary to obtain independent control of L-lysine and L-threonine synthesis from the common precursor, aspartate. In a somewhat similar situation with this organism, D-ribulokinase activity as found in extracts may be attributed either to a true D-ribulokinase, or to the ability of L-ribulokinase to phosphorylate D-ribulose (Simpson et al., 1958).

Since all of the pentulokinases or pentitol dehydrogenases constitute enzyme families whose members catalyze the same reaction but differ in specificity, the experiments to be reported were performed to analyze the following characteristics: (i) the similarity among the various members of the pentulokinase and the pentitol dehydrogenase families, and (ii) the number of different proteins catalyzing the same pentitol dehydrogenation or pentulose phosphorylation. For this purpose, several characteristics, including sedimentation constants and immunological responses, of several members of each family were compared.

Materials and Methods

Bacteriological. A. aerogenes PRL-R3 was grown on 0.5% of the different aldopentoses and pentitols in a minimal medium and under conditions described previously (Mortlock and Wood, 1964*a*). A mutant constitutive for ribitol dehydrogenase, obtained by growth on xylitol and isolated by the method of Lin, Lerner, and Jorgensen (1962), was grown on a 2% peptone-salts medium as above. Extracts were prepared by treating cell suspensions in a 10-kc sonic oscillator or a French pressure cell.

Immunological. For preparation of antisera, partially purified enzyme (1 mg of protein) was mixed with an equal volume of Freund's complete adjuvant (Difco) and injected into the intraperitoneal cavity of the rabbit. A second injection was given after 2 weeks, and the rabbit was bled 3 weeks after the second injection. A new purification of all kinases was required for the second injection, because the kinases lost appreciable activity before the time of the second injection. Ouchterlony (1949) plates were prepared by standard techniques with Oxoid Ionagar No. 2 (Consolidated Laboratories, Inc., Chicago Heights, Ill.)

Chemical. Protamine sulfate and diethylaminoethyl (DEAE)-cellulose were obtained from Calbiochem. The DEAE cellulose was treated with 0.005 M potassium phosphate buffer (*p*H 7.5). Salt concentrations were determined with a precalibrated Barnstead purity meter. Carbohydrates used as growth substrates were prepared or obtained as described previously (Mortlock and Wood, 1964*a*).

Determinations. Protein content was obtained from the ratio of absorbancies at 280 and 260 mμ (Warburg and Christian, 1941), and by the method of Lowry et al. (1951). Sedimentation coefficients were determined by the sucrose density centrifugation method of Martin and Ames (1961). Michaelis-Menten constants (K_m) were obtained by plotting kinetic data by the method of Lineweaver and Burk (1934).

Enzymatic. The kinase and dehydrogenase activities were determined spectrophotometrically as described previously (Mortlock and Wood, 1964*a*). A unit of activity for each was defined as an absorbance change of 1.0 per minute at 340 mμ. The reduction of pentulose by reduced nicotinamide adenine dinucleotide was used as the reaction for determining pentitol dehydrogenase activity.

Results

Purification of pentulokinases and pentitol dehydrogenases. To make comparisons among immunological and physical properties of the several kinases and dehydrogenases formed in the presence of various inducers, a generalized scheme was developed for partial purification of all of these enzymes. In this procedure, the temperature was maintained at 0 to 5 C unless otherwise stated, and the dilutions, except for assay, were made in 0.001 M ethylenediaminetetraacetic acid (EDTA), *p*H 7.5.

Pentulokinases. The crude extract was diluted to 10 mg of protein per ml, and solid ammonium sulfate was added to make a 0.1 M solution. One-tenth volume of protamine sulfate solution (40 mg/ml) was added dropwise with stirring; the mixture was then centrifuged at 12,000 × *g* for 10 min, and the pellet was discarded. The supernatant liquid was adjusted to 1.9 M with solid ammonium sulfate and centrifuged for 5 min, and the pellet was dissolved in 0.001 M EDTA. This fraction was then heated to 50 C for 5 min and then cooled. After centrifugation, the supernatant solution was dialyzed overnight against 0.01 M tris(hydroxymethyl)aminomethane (Tris) buffer (*p*H 7.5), containing 0.001 M EDTA. After dialysis, the ammonium sulfate concentration was adjusted to 0.01 M, the fraction was passed through a DEAE-cellulose phosphate column, and the proteins were eluted either with one volume each of 0.03, 0.05, and 0.08 M potassium phosphate buffer (*p*H 7.5), or with a linear gradient from 0.005 to 0.2 M potassium phosphate (*p*H 7.5). Generally, the kinases were found in the eluate obtained with 0.08 M phosphate. If the protein concentration of the eluate was greater than 1 mg/ml, the next ammonium sulfate fractionation was carried out directly on the eluate. If the protein concentration was less than 1 mg/ml, the fraction was further diluted to give a salt concentration of 0.02 M. The fraction was

then passed through a small DEAE-cellulose column. The cellulose was removed from the column, the kinase was eluted with several small portions of 0.1 M phosphate buffer, and the eluates were combined. The eluate obtained by either method was then fractionated with saturated ammonium sulfate (0 C, *p*H 7.0). The fraction precipitating between 0.45 and 0.54 saturation was collected by centrifugation and dissolved in water. Treatment of the final ammonium sulfate fraction with alumina-C-γ gel usually resulted in an additional purification of 2- to 3-fold, but the details of the procedure varied with different gel preparations. Additional purification could then be obtained by further ammonium sulfate fractionation.

A typical purification of D-ribulokinase is shown in Table 1. For this fractionation, the ribitol-constitutive mutant was grown with aeration in 20 liters of salts containing 2% peptone. The ribulokinase was purified 216-fold with a 28% yield of activity. The ribitol dehydrogenase-ribulokinase ratio changed from 10.4 to 0.077, representing a relative decrease of ribitol dehydrogenase of 132-fold.

In this manner, pentulokinases were purified for 7 of the 10 observed instances of their induction. These included four inducers of D-ribulokinase, one of D-xylulokinase, and one each of L-ribulokinase and L-xylulokinase (Table 2). Presumably, the L-xylulokinase could also be obtained after growth on L-arabitol (Fossitt et al., 1964), and D-xylulokinase could be obtained after growth on D-lyxose or xylitol; but extensive purification of these was not attempted. All of these kinases fractionated identically in this procedure, and all were quite labile during storage at −10 C. As observed by Bhuyan and Simpson (1962), EDTA and glutathione were partially effective in maintaining kinase activity.

The pentitol dehydrogenases were fractionated by a similar procedure with the following exceptions. The heat step was omitted, and a different segment of the eluate from DEAE cellulose (approximately 0.05 M) was used in additional purification steps. Separation of ribitol dehydrogenase was most efficient when fractions were eluted from DEAE cellulose by a phosphate buffer gradient (Fig. 1). The dehydrogenase was eluted at a phosphate concentration of approximately 0.04 M, and ribulokinase (not shown) was eluted at 0.065 M phosphate. Among the pentitol dehydrogenases, xylitol dehydrogenase accompanied ribitol dehydrogenase, whereas D-arabitol dehydrogenase eluted intermediate between ribitol dehydrogenase and D-ribulokinase.

Pentitol dehydrogenases were purified for seven instances of their induction, four of ribitol dehydrogenase, two of D-arabitol dehydrogenase, and one of xylitol dehydrogenase (Table 2).

Michaelis constants and sedimentation coefficients of the pentitol dehydrogenases. Table 3 gives the Michaelis constants (K_m) and the sedimentation coefficients (S_{20}) obtained from sucrose gradient centrifugations for the three pentitol

TABLE 1. *Purification of D-ribulokinase by generalized procedure for kinases*

Step	Total activity	Yield	Specific activity (units/mg of protein)	Purification (fold)	Dehydrogenase-kinase ratio
	units	%			
Crude extract	30,000	100	0.945	1	10.4
Protamine supernatant fraction	33,000	100	1.10	1	9.7
Ammonium sulfate fraction	28,000	93	2.23	1.2	9.3
Heat treatment	25,200	84	5.98	6.34	5.6
Dialysis	24,080	80	6.52	6.90	4.6
DEAE cellulose	13,200	44	31.3	33.2	0.18
Ammonium sulfate	8,510	28	47.9	50.6	0.19
Alumina Cγ gel	8,450	28	205	216	0.077

TABLE 2. *Purification (characteristics) of pentulokinases and pentitol dehydrogenases*

Enzyme	Inducer	Specific activity (units/mg of protein)	Purification (fold)	Yield
				%
D-Ribulokinase	Ribitol	1,040	68	—
	D-Arabinose	735	100	10
	Xylitol	114	13	35
	None*	205	217	28
D-Xylulokinase	D-Xylose	2,010	270	27
L-Ribulokinase	L-Arabinose	278	105	12.8
L-Xylulokinase	L-Xylose	221	24	11.0
Ribitol dehydrogenase	Ribitol	3,520	126	49
	D-Arabinose	12,500	186	18.1
	Xylitol	7,400	9	22
	None*	38,000†	59	10
D-Arabitol dehydrogenase	D-Arabitol	3,000	60	—
	D-Xylose	1,100	15	45
Xylitol dehydrogenase	Xylitol	740	9	22

* Constitutive mutant.

† Same specific activity as obtained from crystalline dehydrogenase (Fossitt, Mortlock, and Wood, *unpublished data*).

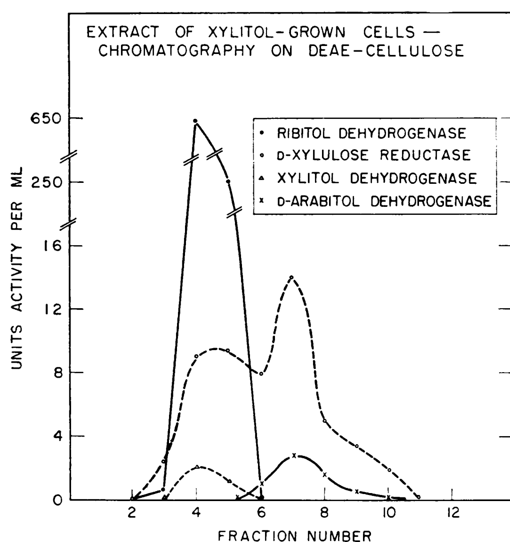

FIG. 1. *Chromatography of ribitol, D-arabitol, and xylitol dehydrogenase activities on DEAE cellulose. Nucleic acids were first removed from a crude extract with protamine sulfate. After dialysis, a portion equivalent to 50 mg of protein was applied to the column (0.8 cm² × 12 cm) and eluted with a linear gradient of 0.005 to 0.2 M potassium phosphate (pH 7.5).*

TABLE 3. *Physical properties of pentitol dehydrogenases*

Dehydrogenase	Inducer	K_m*	$S_{20,w}$	Molecular wt†
D-Arabitol dehydrogenase	D-Arabitol	20	4.6	70
	D-Xylose		4.8	75
	Xylitol	20	4.5	68
Ribitol dehydrogenase	Ribitol	4.5	6.2	110
	D-Arabitol	4.5	6.2	110
	Xylitol	2.8	6.0	102
Xylitol dehydrogenase	Xylitol	420	6.0	102

* Michaelis constant for reduction of ketopentose. Values have been multiplied by 10^4.

† Estimated molecular weight (±12,000). Values have been multiplied by 10^{-3}.

dehydrogenases induced by growth on several substrates. It is apparent that the Michaelis constants differed greatly among ribitol (→ D-ribulose) dehydrogenase, D-arabitol (→ D-xylulose) dehydrogenase, and xylitol (→ D-xylulose) dehydrogenase. However, the values were identical, or nearly so, for the three D-arabitol dehydrogenases and the three ribitol dehydrogenases produced in response to different inducers.

Similarly, D-arabitol dehydrogenase and ribitol dehydrogenase could be readily separated by centrifugation in a sucrose gradient between 5 and 20%. D-Arabitol dehydrogenase had $S_{20,w}$ values of 4.6*S* to 4.8*S*, whereas ribitol dehydrogenase gave values of 6.0*S* to 6.2*S*. Xylitol dehydrogenase, however, had an $S_{20,w}$ value identical to that of ribitol dehydrogenase. Within experimental error, the various ribitol dehydrogenases had the same $S_{20,w}$ values as did the three D-arabitol dehydrogenases. On the assumption of sphericity of the proteins, and of a partial specific volume of 0.73 cm³/g (Martin and Ames, 1961), the values of 4.8*S* and 6.2*S* correspond to molecular weights of 75,000 and 110,000 for D-arabitol dehydrogenase and ribitol dehydrogenase, respectively.

Chromatography of pentitol dehydrogenases. When ribitol dehydrogenases, isolated from cells grown on several inducers, were mixed and chromatographed on DEAE cellulose, only a single, symmetrical peak of ribitol dehydrogenase activity was eluted.

Immunospecificity of pentitol dehydrogenases. It had been observed that antibodies, produced by injection of purified enzyme preparations into rabbits, were strong inhibitors of the same enzyme when added to the assay system. This observation yielded a valuable method for determining antigenic specificity of the various kinases and dehydrogenases. Antisera were prepared against D-arabitol dehydrogenase isolated from D-arabitol-grown cells, and against ribitol dehydrogenase isolated from D-arabinose-grown cells. As shown in Table 4, antiserum against ribitol dehydrogenase inhibited ribitol dehydrogenase from all four sources almost completely and, surprisingly, also inhibited xylitol dehydrogenase. However, these antibodies had only a small effect upon D-arabitol dehydrogenase and no effect upon the pentulokinases. The common immunological identity of the four ribitol dehydrogenases was confirmed by diffusion in an Ouchterlony plate. The four dehydrogenase samples and an extract from glucose-grown cells (control) were arranged pentagonally around a well containing ribitol dehydrogenase antibodies. A single, thin, continuous pentagonal zone of precipitation, open opposite the glucose control, was obtained. Antiserum against D-arabitol dehydrogenase inhibited only D-arabitol dehydrogenase. These antibodies were less effective in inhibiting the D-arabitol dehydrogenase activity than were the antibodies which inhibited

ribitol activity. Whether this was due to low titer or only partial binding in the region of the active site is not known.

Properties of the pentulokinases. There are no significant differences in the *p*H optima or Michaelis constants of the four pentulokinases (Table 5). The *p*H optima are in the region of 7.4 to 7.5, and the K_m values for the primary substrate range from 4.0×10^{-4} to 6.5×10^{-4} M. The sedimentation coefficients derived from sucrose density gradient centrifugation were essentially identical ($S_{20,w}$ = 5.7*S* to 6.3*S*) for two D-xylulokinases, four D-ribulokinases, and L-ribulokinase. These correspond to a minimal molecular weight of 65,000.

Inhibitions of the various pentulokinases by antibodies prepared against D-xylulokinase from cells grown on D-xylose, L-ribulokinase from cells grown on L-arabinose, and D-ribulokinases obtained from the ribitol dehydrogenase-constitutive mutant are shown in Table 6. A high degree of specificity was observed in that there was no cross reactivity among the three dehydrogenases and the three antisera. Also of interest is the fact that the kinase possessing dual substrate specificity, L-ribulokinase, was inhibited by antibodies against L-ribulokinase when either L-ribulose or the secondary substrate, D-ribulose (Simpson et al., 1958), was used as substrate. In contrast, the antibodies against D-ribulokinase did not inhibit L-ribulokinase when D-ribulose was the substrate.

TABLE 4. *Inhibition by antisera against pentitol dehydrogenases**

Enzyme	Inducer	Inhibition by antibodies against	
		D-Arabitol dehydrogenase†	Ribitol dehydrogenase‡
		%	%
Ribitol dehydrogenase	D-Aribinose		96
	Ribitol	0	93
	Xylitol		93
	Constitutive		91
Xylitol dehydrogenase	Xylitol	0	100
D-Arabitol dehydrogenase	D-Arabitol	25	14
	D-Xylose	32	
D-Ribulokinase	Ribitol	0	0
	D-Arabinose		6
D-Xylulokinase	D-Xylose	0	0
L-Ribulokinase	L-Arabinose	0	0

* Antiserum (0.02 ml) was mixed with 0.02 ml of an appropriate dilution of enzyme. Incubation was carried out at 25 C in microcuvettes. After 5 min, the additional assay reagents were added, and enzymatic activity was determined.

† Prepared from dehydrogenase purified after growth on D-arabitol.

‡ Prepared from dehydrogenase purified after growth on D-arabinose.

TABLE 5. *Physical properties of the pentulokinases*

Kinases	Inducer	*p*H Optimal	K_m[a]	$S_{20,w}$	Molecular wt[b]
D-Xylulokinase	D-Xylose	7.5	6.5	6.1	107
	Xylitol	—	—	6.0	102
D-Ribulokinase	D-Arabinose	7.5	5.0	6.2	110
	Ribitol	7.5	5.0	6.2	110
	Xylitol	7.5	—	6.3	120
	L-Xylose	—	—	5.7	98
L-Ribulokinase	L-Arabinose	7.5	5.7	6.1	107
		7.5[c]	14[c]	6.1[c]	107[c]
L-Xylulokinase	L-Xylose	7.4[d]	4.0	4.4	65

[a] Values have been multiplied by 10^4.

[b] Estimated molecular weight ± 12,000. Values have been multiplied by 10^{-3}.

[c] D-Ribulose as substrate. (Simpson and Wood, 1958).

[d] Anderson and Wood, 1962*b*.

DISCUSSION

Previous studies have established the existence of a family of pentulokinases which can be defined by their specificity for induction and by their substrate specificity. Three of the four, D-ribulokinase, D-xylulokinase, and L-xylulokinase, are produced by growth on more than one of the pentitols or aldopentoses (Mortlock and Wood, 1964*a*; Anderson and Wood 1962*a*). Similarly, there is evidence for four pentitol dehydrogenase activities (one for each of the pentitols) and, in this case also, each is produced by growth on more than one pentitol or aldopentose. Because of specificity differences, each member must differ from the others with respect to the structure of a part of the active site. At the same time, the finding of identical catalytic ability raises questions as to (i) the possible structural similarities among members of the same family and (ii) possible multiple forms of the same enzyme produced in response to the different inducers.

The data presented for several members of the dehydrogenase family seem to give clear answers to each question. There are marked differences in K_m for three of the four members; the fourth

TABLE 6. *Inhibition by antisera against pentulokinases*[a]

Enzyme	Inducer	Inhibition by antibodies against		
		D-Xylulokinase[b]	D-Ribulokinase[c]	L-Ribulokinase[d]
		%	%	%
D-Xylulokinase	D-Xylose	95	0	7
D-Ribulokinase	D-Arabinose	0	90	0
	Ribitol	0	81	0
	Constitutive	0	66	—
L-Ribulokinase	L-Arabinose	0	0	88
		—	0[d]	85[e]
L-Xylulokinase	L-Xylose	0	0	0
D-Arabitol dehydrogenase	D-Arabitol	0	0	0
Ribitol dehydrogenase	Ribitol	0	5	0

[a] Antiserum (0.02 ml) was mixed with 0.02 ml of an appropriate dilution of enzyme. Incubation was carried out at 25 C in a microcuvette. After 5 min, the additional assay reagents were added and enzymatic activity was determined.
[b] From cells grown on D-xylose.
[c] From constitutive mutant.
[d] From cells grown on L-arabinose.
[e] D-Ribulose as substrate.

was not studied. Also, there is a substantial difference in S_{20} value for xylitol and ribitol dehydrogenase as compared with D-arabitol dehydrogenase, and there is complete immunological distinction between D-arabitol dehydrogenase and ribitol dehydrogenase. Unfortunately, the K_m values and the immunological differences, based upon inhibition of activity, only reflect differences in the active site, as is already implicit from the specificity differences. In spite of this, it is quite obvious that ribitol dehydrogenase and D-arabitol dehydrogenase have substantial differences in structure.

For the kinases, three of the four are very similar as to *p*H optimum, K_m and S_{20} values. In addition, these fractionate identically and have the same elution position from DEAE cellulose. The sedimentation coefficient of L-xylulokinase, 4.4*S*, is considerably lower than that of the other kinase, 6.1*S*. Thus, only the substrate specificity and interference of the immunological reaction with the site of catalysis serve to distinguish three of the four members of the kinase family. The possibility, therefore, remains that these three may have common elements of structure. However, other methods more discerning of structural differences will be necessary to determine this.

For four of these enzymes, D-arabitol dehydrogenase, ribitol dehydrogenase, D-xylulokinase, and D-ribulokinase, the same catalytic activities produced by growth on two to four inducers were purified and compared. For example, the ribulokinases formed during growth on D-arabinose, ribitol, xylitol, and L-xylose were compared. In all instances (four enzymes, 14 examples), the same enzyme, e.g., D-ribulokinase, exhibited identical properties. It is thus concluded that only one kind of each kinase and dehydrogenase exists, rather than multiple forms as found by Stadtman (1963) for aspartokinase.

The similarity of the dehydrogenases for xylitol and ribitol with respect to all properties, including immunological cross reactivity, suggests that the same protein catalyzes both activities.

ACKNOWLEDGMENT

This investigation was supported by a grant-in-aid from the National Science Foundation.

LITERATURE CITED

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

ANDERSON, R. L., AND W. A. WOOD. 1962*b*. Purification and properties of L-xylulokinase. J. Biol. Chem. **237**:1029–1033.

BHUYAN, B. K., AND F. J. SIMPSON. 1962. Some properties of the D-xylulokinase of *Aerobacter aerogenes*. Can. J. Microbiol. **8**:737–745.

FOSSITT, D., R. P. MORTLOCK, R. L. ANDERSON, AND W. A. WOOD. 1964. Pathways of L-arabitol and xylitol metabolism in *Aerobacter aerogenes*. J. Biol. Chem. **239**:2110–2115.

LIN, E. C. C., S. A. LERNER, AND S. E. JORGENSEN. 1962. A method for isolating constitutive mutants for carbohydrate-catabolizing enzymes. Biochim. Biophys. Acta **60**:422–424.

LINEWEAVER, H., AND D. BURK. 1934. The determination of enzyme dissociation constants. J. Amer. Chem. Soc. **56**:658–666.

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.

MARTIN, R. G., AND B. N. AMES. 1961. A method for determining the sedimentation behavior of enzymes: application to protein mixtures. J. Biol. Chem. **236**:1372–1379.

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

MORTLOCK, R. P., AND W. A. WOOD. 1964b. Metabolism of pentoses and pentitols by *Aerobacter aerogenes*. II. Mechanism of acquisition of kinase, isomerase, and dehydrogenase activity. J. Bacteriol. **88**:845-849.

OUCHTERLONY, O. 1949. Antigen-antibody reactions in gels. Acta Pathol. Microbiol. Scand. **26**:507-515.

SIMPSON, F. J., M. J. WOLIN, AND W. A. WOOD. 1958. Degradation of L-arabinose by *Aerobacter aerogenes*. I. A pathway involving phosphorylated intermediates. J. Biol. Chem. **230**:457-472.

SIMPSON, F. J., AND W. A. WOOD. 1958. Degradation of L-arabinose by *Aerobacter aerogenes*. II. Purification and properties of L-ribulokinase. J. Biol. Chem. **230**:473-486.

STADTMAN, E. R. 1963. Symposium on multiple forms of enzymes and control mechanisms. II. Enzyme multiplicity and function in the regulation of divergent metabolic pathways. Bacteriol. Rev. **27**:170-181.

WARBURG, O., AND W. CHRISTIAN. 1941. Isolierung und Krystallization des Garungsferments. Enolase. Biochem. Z. **310**:384-421.

WOOD, W. A., M. J. MCDONOUGH, AND L. B. JACOBS. 1961. Ribitol and D-arabitol utilization by *Aerobacter aerogenes*. J. Biol. Chem. **236**:2190-2195.