JOURNAL OF BACTERIOLOGY, Oct. 1983, p. 198–204
0021-9193/83/100198-07$02.00/0

Vol. 156, No. 1

# Uridine Phosphorylase from *Acholeplasma laidlawii*: Purification and Kinetic Properties

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Received 8 March 1983/Accepted 3 August 1983

Uridine phosphorylase was purified 1,370-fold from sonicated extracts of *Acholeplasma laidlawii* by ammonium sulfate precipitation, DEAE-Sephadex column chromatography, hydroxylapatite chromatography, and Sephadex G-200 fractionation. The molecular weight of the enzyme as determined by gel filtration was approximately 65,000. [*U*-$^{14}$C]ribose-1-phosphate (Rib-1-P), prepared enzymatically from [*U*-$^{14}$C]inosine, was utilized in initial velocity studies of uridine synthesis, which indicated a sequential reaction with a $K_{m_{Ura}}$ of 110 μM and a $K_{m_{Rib\text{-}1\text{-}P}}$ of 17 μM. The kinetics of uridine cleavage were assessed at a saturating cosubstrate concentration, resulting in a $K_{m_{Urd}}$ of 170 μM and a $K_{m_{P_i}}$ of 120 μM. These results indicate that an intracellular flux from uracil to uridine is kinetically feasible. However, such flux would be metabolically unproductive, since the low affinity of uridine kinase ($K_{m_{Urd}}$ = 3.2 mM) precludes the operation of uridine phosphorylase and uridine kinase in tandem to convert uracil to UMP. We conclude that uridine phosphorylase performs only a catabolic function in *A. laidlawii*.

Uridine phosphorylase (uridine:orthosphosphate ribosyltransferase, EC 2.4.2.3) catalyzes the reversible, phosphate-dependent cleavage of uridine to uracil and ribose-1-phosphate. This enzyme, first observed by Peage and Schlenk (20) in bacterial extracts, has been purified from *Escherichia coli* (11, 12) and from rat liver (10). In addition, its presence has been reported in many different microorganisms (19), including mycoplasmas (8, 13), and also in a number of mammalian tissues and cell lines (24, 26; R. M. Wohlhueter, R. S. McIvor, and P. G. W. Plagemann, Abstr. Int. Congr. Biochem. 11th, Toronto, abstr. no. 06-8-R52, p. 467, 1979).

Although the synthesis of uridine is thermodynamically favored in this reaction ($K_{eq}$ = 0.031 at pH 7.4 [2]), uridine phosphorolysis has been the primary physiological function associated with this enzyme (1), and in cultured mammalian cells, the uptake of uracil through this enzyme (with subsequent phosphorylation by uridine kinase) is slow (6, 9).

The cleavage of pyrimidine nucleosides by mycoplasmas, although originally noted in 1957 (R. J. Lynn, Bacteriol. Proc. Soc. Am. Bacteriol. 1957, P56, p. 126), did not receive much attention until it became of practical importance in the contamination of mammalian cell cultures (7). Uridine phosphorylase activity was demonstrated in a variety of mycoplasma species, and its presence in mammalian cell cultures has been construed as a convenient diagnostic for contamination (13). Uridine cleavage is rapid in mycoplasma cultures (17), and measurements of the specific activity of uridine phosphorylase have given consistently high values from a variety of these organisms (8, 18).

Within the context of pyrimidine metabolism in mycoplasmas (see accompanying paper [15], we address here the possibility of an anabolic role for uridine phosphorylase acting in tandem with uridine kinase [EC 2.7.1.48). The phosphorylase was purified from sonicated extracts, and its kinetics were studied in both the synthetic and phosphorolytic directions. The kinetic characteristics of uridine kinase have also been determined, albeit in crude extracts. The data are interpreted in terms of the feasibility of intracellular conversion of uracil to UMP via uridine.

## MATERIALS AND METHODS

**Culture conditions.** *A. laidlawii* ATCC 14192 was grown in tryptic soy broth, and cytosol was prepared as described in the accompanying paper (15).

**Enzyme assays.** During the course of this study, it became apparent that a single strategy for assaying uridine phosphorylase activity was not sufficient to analyze initial velocity kinetics with respect to each of its four substrates: Rib-1-P + Ura ⇆ $P_i$ + Urd. Therefore, different assay systems were devised, each tailored to optimize sensitivity with respect to the variable substrate. All assays were conducted at 37°C.

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For the purposes of enzyme purification, uridine phosphorylase activity was conveniently monitored by using a continuous spectrophotometric assay. Enzyme test samples were brought to 270 μl with PEM buffer (20 mM potassium phosphate–5 mM 2-mercaptoethanol–1 mM EDTA [pH 7.5]) and warmed to 37°C, and the reaction was initiated by the addition of 30 μl of 10 mM uridine in 0.4 M potassium phosphate (pH 7.5). The decrease in absorbance at 282 nm and 37°C was followed on a Beckman model 35 spectrophotometer equipped with a temperature-controlled cuvette holder and a chart recorder, from which initial velocities were taken ($\Delta\epsilon_{282}$ = 1,450 $M^{-1}$ $cm^{-1}$ at pH 7.5). For the kinetic analysis of uridine cleavage with respect to uridine, an intermittent spectrophotometric assay was employed whereby the product was measured in alkali. Reaction mixtures contained 120 μmol of triethanolamine (pH 7.5), 48 μmol of potassium phosphate (pH 7.5), uridine as indicated, and enzyme in a total volume of 1.2 ml. At various times after the addition of enzyme, 270-μl samples were removed and added to 30 μl of 10 N NaOH. Absorbance was read for all samples, and initial velocities were computed ($\Delta\epsilon_{285}$ = 4,470 $M^{-1}$ $cm^{-1}$).

The kinetics of uridine cleavage with respect to $P_i$ were measured by using $^{32}P_i$ in a method similar to that of Friedkin and Roberts (5). Reaction mixtures (37°C) contained 25 μmol of triethanolamine (pH 7.5), 2 mM uridine, 1 μCi of $^{32}P_i$ (at the indicated concentration) per ml, and enzyme in a final volume of 500 μl. At various times after the addition of enzyme, 100-μl samples were taken and heated for 3 min in a boiling water bath. Ribose-1-$^{32}$P formed during the reaction was separated from unreacted $^{32}P_i$ by adding to the heat-quenched samples 50 μl of 10 mM potassium phosphate (pH 7.5) and then 0.35 ml of a solution containing 0.7% $CaCl_2$ and 71.4 mM glycine (pH 9.6). The $Ca_3(PO_4)_2$ precipitate was removed by centrifugation, the supernatant (containing the ribose-1-$^{32}$P) was collected, and the pellet was resolubilized in 0.05 N HCl. The radioactivity of both fractions was assessed by liquid scintillation counting.

For determination of the kinetics of uridine synthesis with respect to uracil and ribose-1-phosphate, [*U*-$^{14}$C]ribose-1-phosphate was employed. It was synthesized enzymatically from [*U*-$^{14}$C]inosine (23) in a reaction mixture containing 18 μCi of [*U*-$^{14}$C]inosine (34.3 nmol), 50 nmol of potassium phosphate (pH 7.5), 10 μmol of triethanolamine (pH 7.5), 0.1 μmol of NAD, and 0.002 U of xanthine oxidase (final volume, 1.0 ml). The reaction was initiated by the addition of 0.048 U of purine nucleoside phosphorylase and followed by continuous measurement of the increase in absorbance at 290 nm (as described above for the continuous uridine phosphorylase assay). The coupling of xanthine oxidase to the phosphorolysis of inosine is necessary to drive the thermodynamically unfavorable ($K_{eq}$ = 0.018 [16]) phosphorolytic reaction to completion. When the reaction was 90% complete (about 2 h), ribose-1-phosphate was separated from the other labeled reaction components by high-pressure liquid chromatography (Altex model 420) (Fig. 1). When a sample of the purified [*U*-$^{14}$C]ribose-1-phosphate was incubated with 2 mM uracil and uridine phosphorylase purified from *A. laidlawii*, 95% of the radioactivity was converted to uridine, indicating ≥95% isotopic purity.

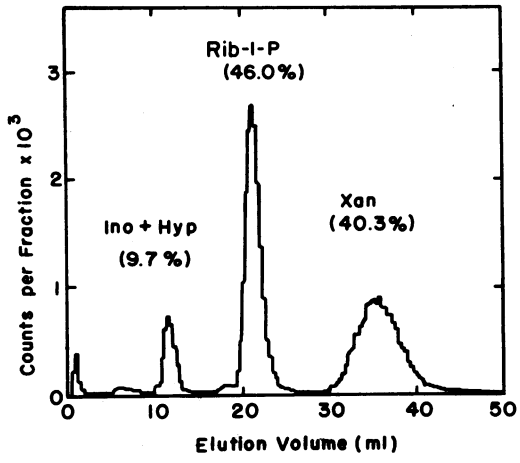


FIG. 1. Purification of [*U*-$^{14}$C]ribose-1-phosphate (Rib-1-P). The reaction is described in the text. Separation of ribose-1-phosphate from other labeled compounds in the reaction mixture by high-pressure liquid chromatography on Bio-Rad MP-1 (see text) was monitored by an in-line scintillation flow cell mounted in a Beckman LS-230 liquid scintillation spectrometer. The column eluant was mixed 1:3 with triton-xylene-based scintillant. Ino, Inosine; Hyp, hypoxanthine; Xan, xanthine.

For kinetic studies in the synthetic direction, reaction mixtures (37°C) contained 25 μmol of triethanolamine (pH 7.5), 0.5 μCi of [*U*-$^{14}$C]ribose-1-phosphate (concentration as indicated) per ml, uracil (concentration as indicated), and enzyme in a final volume of 500 μl. At various time points after adding enzyme, 100-μl samples were removed and heated in a boiling water bath for 3 min. [*ribosyl*-$^{14}$C]uridine formed during the reaction was then separated from unreacted [*U*-$^{14}$C]ribose-1-phosphate by adding the samples to small columns of DEAE-Sephadex A-25 equilibrated with 10 mM triethanolamine (pH 7.5). Labeled uridine was washed through the columns with the equilibration buffer and collected. [*U*-$^{14}$C]ribose-1-phosphate was eluted with 0.05 N HCl, and the radioactivity of both fractions was assessed by liquid scintillation counting.

Uridine kinase was assayed by measuring the appearance of anionic product described by Wohlhueter (27) for hypoxanthine phosphoribosyltransferase. Reaction mixtures (37°C) contained 5 μmol of triethanolamine (pH 7.5), 0.2 mol of ATP, 0.5 mol of $MgCl_2$, 5 μCi of [5-$^3$H]uridine per ml (2 mM unless otherwise indicated), and enzyme in a final volume of 100 μl. At various time points after the addition of enzyme, 20-μl samples were removed, spotted onto thin layers of an anion exchanger, polyethyleneimine-cellulose, dried under a stream of hot air, and processed as previously described for the assay of uracil phosphoribosyltransferase (15).

**Other methods.** Protein assay and the analyses of kinetic data were carried out as described in the accompanying paper (15). Liquid scintillation counting was conducted with modified Bray solution (without ethylene glycol [3]) or, for monitoring high pressure liquid chromatography effluents, with xylene–Triton-

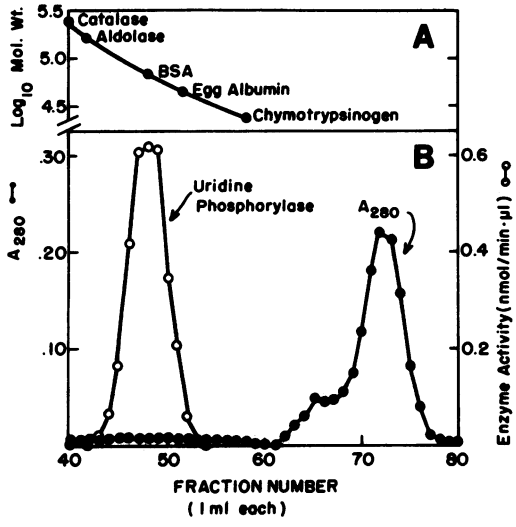


FIG. 2. Purification and determination of molecular weight of uridine phosphorylase on Sephadex G-200. (A) The column was prepared, equilibrated in PEM buffer, and calibrated as described in the legend to Fig. 1 of the accompanying paper (15). A 3.5-ml amount of enzyme purified through the hydroxylapatite step was loaded, and 1-ml fractions were collected at a flow rate of 2.0 ml/h. (B) Fractions were assayed for protein by measurement of absorbance at 280 nm (●) and enzyme activity by continuous spectrophotometric assay (○). Calibration of the column against known molecular weight standards is shown in (A).

X114 (3:1, vol/vol) containing 4 g of Omnifluor (New England Nuclear Corp., Boston, Mass.) per liter (21).

**Chemicals.** All pyrimidine and purine compounds, as well as ribose-1-phosphate and the enzymes purine nucleoside phosphorylase (25 to 40 U/mg) and xanthine oxidase (0.76 U/mg), were obtained from Sigma Chemical Co., St. Louis, Mo. [5-$^3$H]uridine (specific activity, 20 Ci/mmol) was obtained from Moravek Biochemicals, Brea, Calif., [*U*-$^{14}$C]inosine (575 mCi/mmol) was obtained from Amersham Corp., Arlington Heights, Ill., and carrier-free $^{32}P_i$ was obtained from ICN, Irvine, Calif.

## RESULTS

**Purification of uridine phosphorylase.** The fraction of *A. laidlawii* cytosol precipitating between 55 and 80% ammonium sulfate (15) served as starting material for purification of the enzyme. All manipulations were carried out at approximately 4°C.

The ammonium sulfate precipitate was dissolved in 8.5 ml of PEM buffer containing 0.06 M KCl and further purified by column chromatography on DEAE-Sephadex, first by elution with a negative pH gradient and then with a salt gradient.

In the pH gradient elution, the column (Sephadex A-50, 0.9 cm [diameter] by 4.3 cm [length]) was eluted first with 5 ml of a solution (pH 6.4) containing 0.01 M sodium succinate, 5 mM 2-mercaptoethanol, 1 mM EDTA, and 0.06 M KCl and then with a linear mixture of this solution and increasing proportions of one of similar components, but at pH 4.2. Since the enzyme was somewhat unstable at a lower pH, fractions from the pH-eluted column were checked immediately for pH and then adjusted to approximately pH 7.5 by the addition of 0.1 ml of 1 M triethanolamine. The fractions containing the most enzyme activity were pooled, dialyzed against PEM buffer, and loaded onto a second DEAE-Sephadex column (A-50; 0.9 cm [diameter] by 15.5 cm [length]), equilibrated with PEM buffer, and eluted with a linear gradient to 0.4 M KCl in PEM buffer. Uridine phosphorylase eluted at about 0.2 M KCl. Phosphorylase-containing fractions were pooled and dialyzed against PEM buffer.

A portion of the DEAE-salt eluant was chromatographed on hydroxylapatite, which effected a significant concentration of enzyme activity as well as a 7.3-fold purification. Uridine phosphorylase was eluted from this column (0.9 cm [diameter] by 13.3 cm [length]) with a linear gradient of $P_i$ (5 to 100 mM) in PEM buffer. The phosphorylase activity emerged in a sharp band at about 60 mM $P_i$. The fractions containing the most enzyme activity were pooled and fractionated on a column of Sephadex G-200 calibrated against molecular weight standards. Uridine phosphorylase eluted with a molecular weight of approximately 65,000 (Fig. 2).

The purification of uridine phosphorylase

TABLE 1. Purification of uridine phosphorylase from *A. laidlawii*

| Purification step | Total protein (mg) | Total activity (µmol · min) | Sp act (µmol/min · mg) | Purification (fold) |
|---|---|---|---|---|
| Sonication | 132 | 12.5 | 0.0948 | 1.0 |
| Cytosol | 57.8 | 9.40 | 0.163 | 1.72 |
| $(NH_4)_2SO_4$ precipitate | 10.3 | 18.6 | 1.811 | 19.1 |
| DEAE-Sephadex, pH elution | 1.26 | 5.70 | 4.54 | 47.9 |
| DEAE-Sephadex, KCl elution | 0.478 | 4.43 | 9.27 | 97.8 |
| Hydroxylapatite | 0.0455 | 3.09 | 67.9 | 716 |
| Sephadex G-200 | 0.0136 | 1.76 | 129 | 1,370 |

from *A. laidlawii* is summarized in Table 1. A purification of 1,370-fold was achieved; this material contained no detectable phosphatase activity.

**Kinetic studies on uridine phosphorylase.** Preliminary experiments in which [$^3$H]uracil was used as the labeled substrate to assay the kinetics of uridine phosphorylase with respect to ribose-1-phosphate indicated that this method lacks the necessary sensitivity to obtain accurate initial velocities at low ribose-1-phosphate concentrations. At a saturating uracil concentration and a concentration below the $K_m$ ribose-1-phosphate concentration, only a small amount (<1%) of the [$^3$H]uracil substrate was converted to [$^3$H]uridine by the time that ribose-1-phosphate was exhausted. An assay in which [*U*-$^{14}$C]ribose-1-phosphate is used was designed to circumvent this problem (see Materials and Methods).

Initial velocities were measured at a range of uracil concentrations, each at several fixed concentrations of ribose-1-phosphate. Initial velocity equations for various bimolecular mechanisms were fitted to the data by nonlinear least-squares analysis (15) (Table 2). A finite $K_{ia}$ was determined from the ordered sequential fit, so the reaction is deemed to be of the sequential rather than of the "ping-pong" type. These data do not distinguish between ordered and random sequences of substrate addition. The parameters shown are those obtained for an ordered sequential mechanism. The $K_m$s with respect to ribose-1-phosphate and uracil were 17 and 110 μM, respectively. Since no information is available concerning the order of addition, assignment of substrate A (the first substrate to add to the enzyme) is arbitrary. Assigning uracil as A gave a $K_{ia}$ of 392 μM, whereas assigning ribose-1-phosphate as A gave a $K_{ia}$ of 61 μM.

Graphic analysis of these data, using the Hanes-Woolf linear transform, is shown in Fig. 3, with uracil (Fig. 3A) and ribose-1-phosphate (Fig. 3B) as the variable substrate. The kinetic parameters thus obtained ($K_m$ from the $x$ intercept of the slope replot and $K_{ia}$ from the $x$ intercept of the intercept replot) were not substantially different from those obtained by nonlinear least-squares analysis (Table 2), implying that neither method mis-weighted the data in a biased way. The family of lines generated in each plot intersect far to the left of the $y$ axis, as expected for a sequential reaction (for a contrasting example, see the plots for uracil phosphoribosyltransferase in the accompanying paper [15]).

The kinetics of uridine phosphorolysis were analyzed with respect to both uridine and phosphate at a single, saturating cosubstrate concentration. A spectrophotometric assay was utilized for the kinetics with respect to uridine. Sensitivity problems analogous to those explained above were observed when we attempted to utilize [$^3$H]uridine to follow the reaction rate at low phosphate concentrations. Therefore, an assay in which $^{32}P_i$ is used was designed to resolve this problem (see Materials and Methods). The Michaelis-Menten equation ($v = V_{max}(S)/[K_m + (S)]$) was fitted to the data by nonlinear regression, yielding Michaelis-Menten constants of 170 and 95 μM with respect to uridine and phosphate, respectively (Fig. 4).

**Uridine kinase.** The ability of *A. laidlawii* to anabolize uracil to UMP via uridine depends on the activity of uridine kinase as well as that of uridine phosphorylase, so it was desirable to analyze the kinetics of uridine kinase also. We made little headway with the purification of the kinase, which proved very labile in our hands. Furthermore, kinetic studies of this enzyme in crude cytosol is problematic, since cytosol contains relatively high levels of uridine phosphorylase activity; the [$^3$H]uridine substrate in the uridine kinase assay is rapidly cleaved to [$^3$H]uracil, making it unavailable for the uridine kinase reaction. This problem was observed also by Mitchell and Finch (18) in working with *Mycoplasma mycoides*. Theoretically, this problem can be eliminated by using $P_i$-free reaction mixtures, but we have found that commercial ATP preparations are sufficiently contaminated with $P_i$ as to support a hefty phosphorolysis. We found, however, that uridine phosphorolysis could be minimized by including 1 mM ribose-1-phosphate in the reaction mixture. Under these conditions, phosphorolysis is held in abeyance by mass action, and paper chromatography (22) indicated that, in fact, no more than 10% of the uridine had been cleaved to uracil by the end of any of the uridine kinase assays shown in Fig. 5.

TABLE 2. Kinetic parameters for uridine phosphorylase from *A. laidlawii*[a]

| Method of parameter evaluation | $K_m$ (μM) | | $K_{ia}$ (μM) | | $v_{max}$ (nmol/min · μg) |
|---|---|---|---|---|---|
| | Ura | Rib-1-P | Ura | Rib-1-P | |
| Rate equation fit | 110 ± 19 | 17 ± 1.9 | 392 ± 75 | 61 ± 16 | 72 ± 2.4 |
| Hanes-Woolf plot | 102 | 14 | 333 | 108 | 68 |

[a] Rate equation for an ordered sequential mechanism was fitted to the data by least-squares regression. The ranges specified are the standard errors of parameter estimate. Ura, Uracil;' Rib-1-P, ribose-1-phosphate.

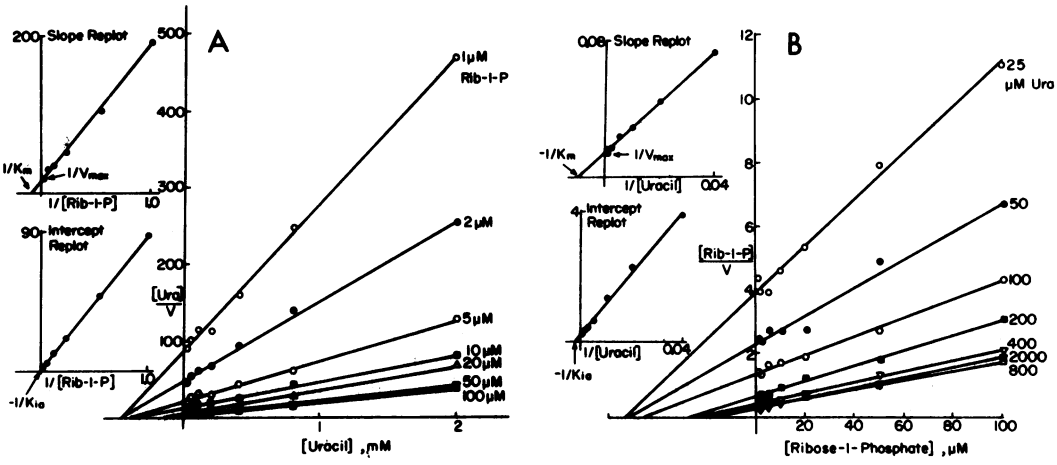


FIG. 3. Hanes-Woolf plot of uridine phosphorylase kinetics with respect to uracil and ribose-1-phosphate. (A) Uracil was plotted as the variable substrate (abscissa; micromolar) at several fixed concentrations of ribose-1-phosphate as indicated. The uracil concentration divided by the initial velocity (in nanomoles per minute per microgram) was plotted on the ordinate. $V_{max}$ (67 nmol/min · μg) and $K_m$ with respect to ribose-1-phosphate (14 μM) are obtained from the $y$ and $x$ intercepts of the slope replot, respectively. A hypothetical $K_{i_{Rib\text{-}1\text{-}P}}$ (108 μM) was obtained from the $x$ intercept of the intercept replot. (B) The same data shown in (A) were plotted with ribose-1-phosphate as the variable substrate (abscissa; micromolar) at several fixed concentrations of uracil as indicated. The ribose-1-phosphate concentration divided by the initial velocity (in nanomoles per minute per microgram) was plotted on the ordinate. $V_{max}$ (69 nmol/min · μg) and $K_m$ with respect to uracil (102 μM) are obtained from the $y$ and $x$ intercepts of the slope replot, respectively. A hypothetical $K_{i_{Ura}}$ (333 μM) was obtained from the $x$ intercept of the intercept replot. Ura, Uridine; Rib-1-P, ribose-1-phosphate.

Ribose-1-phosphate at concentrations of up to 10 mM did not inhibit the uridine kinase raction. The approach yielded well-behaved velocities which, measured at a range of uridine concentrations, generated a Michaelis-Menten curve (Fig. 5) with a $K_{m_{Urd}}$ of 3.2 mM.

## DISCUSSION

The observation that the kinetics of uridine phosphorylase from *A. laidlawii* conform to a sequential reaction comes as no surprise, since similar results have been obtained with this enzyme from *E. coli* (11) and rat liver (10). It is interesting, however, that the $K_m$s obtained for a number of substrates in this study are far below those reported for the enzyme from other sources. Thus, the $K_m$ with respect to ribose-1-phosphate observed here (17 μM) is 100-fold lower than that reported for the *E. coli* enzyme (12). The Michaelis-Menten constants with respect to uridine and phosphate reported for the phosphorylase in crude extracts of *M. mycoides* ($K_{m_{Urd}}$ = 2.2 mM; $K_{m_{Pi}}$ = 2.0 mM [18]) are 10- and 20-fold higher than those reported here for the enzyme from *A. laidlawii*. These kinetic differences might reflect structural differences in the phosphorylase from various sources, but, particularly for the apparent Michaelis-Menten constants with respect to ribose-1-phosphate and $P_i$, they might reflect methodological differences. All of the studies cited used the conversion of base to nucleoside (or vice versa), followed photometrically or isotopically, to gauge reaction velocities even when ribose-1-phosphate or $P_i$ was the varied substrate. We found it difficult to obtain accurate initial velocities at low concentrations of phosphate and ribose-1-phosphate when the measured signal was a saturating cosubstrate, and this approach yielded estimates of $K_m$ with respect to phosphate and ribose-1-phosphate consistently higher than those reported here.

In any case, it is clear that the ability of uridine phosphorylase to function anabolically in *A. laidlawii* depends on the concentration of ribose-1-phosphate available intracellularly. We have measured ribose-1-phosphate in heat-quenched extracts of this organism by an enzymatic procedure (14). Intracellular concentrations of ribose-1-phosphate in these cells were high, ranging from 0.5 to 1.0 mM. The phosphate concentration in such extracts of *A. laidlawii* assayed essentially by the method of Fiske and Subbarow (4) corresponded to an intracellular concentration of 9 mM. Taking these concentrations for ribose-1-phosphate and $P_i$, the kinetic constants calculated for uridine phosphorylase from *A. laidlawii*, and an equilibrium constant of 0.031 (at pH 7.4 [2]) and imposing several simpli-

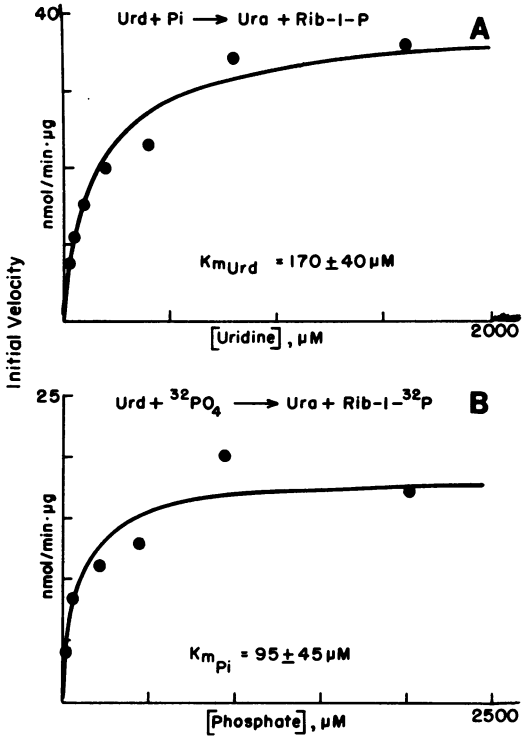


FIG. 4. Kinetic analysis of uridine phosphorylase with respect to uridine and phosphate. Initial velocities were measured at various uridine concentrations (A) assayed spectrophotometrically at alkaline pH as described in Materials and Methods ($P_i$ concentration, 40 mM) and at various phosphate concentrations (B) assayed with $^{32}P_i$ as described in Materials and Methods (uridine concentration, 10 mM). The curves shown were obtained by fitting the Michaelis-Menten equation to the data as described in the accompanying paper (15). The best-fitting Michaelis-Menten constants were 170 µM with respect to uridine (A) and 95 µM with respect to phosphate (B). Urd, Uridine; Ura, uracil; Rib-1-P, ribose-1-phosphate.

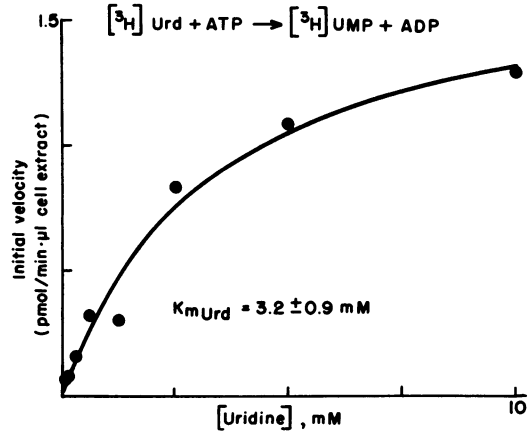


FIG. 5. Kinetic analysis of uridine kinase with respect to uridine. Initial velocities were measured at various uridine concentrations (in the presence of ribose-1-phosphate to suppress phosphorolysis of uridine) as described in the text. The source of the enzyme was dialyzed crude cytosol from *A. laidlawii*. The Michaelis-Menten equation was then fitted to the data (see accompanying paper [15]). The best-fitting $K_m$ was 3.2 mM.

fying assumptions on the net velocity equation for an ordered bi-bi mechanism (namely, that the inhibition constants and Michaelis-Menten constants with respect to any given substrate are equal; that forward and reverse maximal velocities are equal; and that *A*, *B*, *P*, and *Q* correspond to ribose-1-phosphate, uracil, uridine, and phosphate, respectively [25]), one can estimate an intracellular flux from uracil to uridine on the order of 4 nmol/min · mg of cytosol protein for 10 µM concentrations of uracil and uridine. The point of this mathematical exercise is to suggest that the conversion of uracil to uridine in *A. laidlawii* is kinetically feasible and potentially substantial at physiological concentrations of substrate.

However, the properties of uridine kinase mitigate the further conversion of uridine to UMP. With a $K_m$ of 3.2 mM and a specific activity of 0.37 nmol/min · mg of protein, uridine kinase would clearly limit the flux through this anabolic sequence. The disparity between kinase and phosphorylase activities suggests that the phosphorylase would maintain near equilibrium between uracil and uridine (i.e., 36 µM uridine for the concentrations of coreactants noted above), whereas the kinase would support the production of 0.004 nmol of UMP per min · mg of cytosol protein (assuming saturation with ATP). The estimated flux from uracil (at 10 µM) to UMP via uracil phosphoribosyltransferase operating under physiological conditions (see accompanying paper [15]) is 1 nmol/min · mg of cytosol protein.

We conclude that, in *A. laidlawii*, uridine phosphorylase functions only in the direction of phosphorolysis. Taken with the results of the accompanying paper (15), we can conclude further that the only productive route of uracil anabolism is that mediated by uracil phosphoribosyltransferase.

ACKNOWLEDGMENTS

The work described in this paper is included in the doctoral dissertation submitted by R.S.M. to the University of Minnesota and was supported by Public Health Service grant GM24468 (to P.G.W.P.) and training grant CA 09138 (to R.S.M.) from the National Institutes of Health.

Timothy Leonard contributed the graphic arts and Linda Livermore did the clerical work.

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