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SHORT COMMUNICATION

CONVERSION OF OROTATE TO URIDINE BY AN ACETONE POWDER FROM WHEAT SEEDLINGS

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Abstract—Orotic acid-6-^{14}C was incubated with an acetone powder prepared from wheat seedlings and shown to be converted to uridine and uracil. Neither uridine-5′-phosphate nor orotidine was formed under the same conditions.

INTRODUCTION

THE PATHWAY for orotic acid (OA) metabolism has been shown to consist of the condensation of OA with 5-phosphoribosyl-1-pyrophosphate followed by the decarboxylation of the resulting orotidine-5′-phosphate (OMP) to uridine-5′-phosphate (UMP). These reactions are catalysed by OMP pyrophosphorylase and OMP decarboxylase, respectively. Both enzymes, although not separable from each other, have been identified in various organisms including higher plants.[1-3]

In isolated systems the only product of OA metabolism which accumulates is UMP, as OMP decarboxylase activity is usually much higher than that of OMP pyrophosphorylase. However, *in vivo*, in excised wheat seedlings, the first apparent product of OA metabolism was shown to be uridine rather than UMP.[4] Thus, an alternative path of OA utilization, not involving the OMP pyrophosphorylase plus decarboxylase enzyme system, might be operating in wheat seedlings. However, it was difficult to exclude the possibility that uridine was formed by dephosphorylation of the newly synthesized UMP in another part of the cell. To distinguish between these possibilities a cell-free system has been used in the present work.

RESULTS AND DISCUSSION

The data presented in Table 1 indicate that OA can be converted directly to uridine by an acetone powder of wheat seedlings under conditions where no UMP formation can be detected. Thus, UMP cannot be considered as an intermediate in the formation of radioactive uridine, in the system. The possibility that either uracil or orotidine might be an intermediate in the conversion is not supported by the data obtained. The rate of formation of radioactive uracil was much lower than that of uridine, and no radioactive orotidine was found.

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[1] M. KAPOOR and E. R. WAYGOOD, *Can. J. Biochem.* **43**, 143 (1965).

[2] C. ROSS, *Plant Physiol.* **40**, 65 (1965).

[3] J. H. WOLCOTT and C. ROSS, *Plant Physiol.* **42**, 275 (1967).

[4] J. BUCHOWICZ and I. REIFER, *Acta Biochim. Polon.* **9**, 63 (1962).

TABLE 1. RADIOACTIVITY OF ASSUMED PRODUCTS OF OROTATE-6-^{14}C METABOLISM BY ACETONE POWDER FROM WHEAT SEEDLINGS

Incubation time (min)	Total radioactivity of products (cpm)			
	Uridine	UMP	Uracil	Orotidine
0	<6	<6	<6	<6
1	18	<6	<6	<6
3	60	<6	<6	<6
10	488	<6	18	<6
30	900	<6	114	<6
30, inactivated preparation*	<6	<6	<6	<6

* Heated in 0·05 M potassium phosphate, pH 7·5, at 60° for 5 min.

The standard reaction mixtures supplied with uracil, uridine, orotidine and UMP (each 1 μmole) were incubated for the indicated periods.

Since the direct conversion of orotate to uridine is improbable from theoretical considerations, further attempts were made to find out the nature of any intermediary product. Acetone powder and OA-6-^{14}C were incubated with or without addition of an excess of unlabelled possible intermediate (Table 2). Neither uracil, orotidine nor UMP were shown to have a marked dilution effect. The decrease in uridine activity observed in the presence of 100-fold excess of unlabelled orotidine or UMP may be due to some secondary factors. Thus, neither orotidine nor UMP appear to be intermediates in the conversion of OA to uridine.

TABLE 2. INFLUENCE OF PRESUMED INTERMEDIATES ON THE CONVERSION OF OROTATE-6-^{14}C TO URIDINE CATALYSED BY ACETONE POWDER FROM WHEAT SEEDLINGS

Additions, μmoles	Total radioactivity of uridine (cpm)
None	918
Uracil, 1·2	1044
Uracil, 4·0	900
Orotidine, 1·2	942
Orotidine, 4·0	726
UMP, 1·2	1140
UMP, 4·0	696

The standard reaction mixture was incubated with the indicated additions for 30 min.

The results of these preliminary experiments seem to support the idea that in plants orotate may be converted to uridine by a path independent of UMP formation. While the participation of UMP as an intermediate is reasonably well excluded, the actual mechanism of the observed conversion cannot be explained at present.

EXPERIMENTAL

Acetone powder was prepared from green 6-day-old wheat seedlings in the usual manner[5] and stored dry at −20°. OA-6-^{14}C was purchased from The Radiochemical Centre, Amersham, Bucks. The standard

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[5] A. NASON, in *Methods in Enzymology* (edited by S. P. COLOWICK and N. O. KAPLAN), Vol. 1, p. 62, Academic Press, New York (1955).

reaction mixture (5 ml) contained 250 μmoles of K phosphate buffer, pH 7·5, 0·04 μmole of OA (total radioactivity 180,000 cpm) and 200 mg of the acetone powder. The mixture was incubated with gentle shaking at 37°. The reaction was stopped by addition of 5 ml of 0·6 N $HClO_4$. A mixture of non-radioactive OA (5 μmoles), uracil, uridine, orotidine and UMP (each 1 μmole) was then added (carriers were omitted if they had been added to the incubation mixture as additional components).

Acid-soluble pyrimidine derivatives were extracted, preliminarily separated on activated charcoal and applied to a column of Dowex 1 × 1, Cl^- form (1·5 × 90 cm) as described previously.[4] Uracil and uridine were eluted with water and purified using paper chromatography with water-saturated butan-1-ol[6] as the developing solvent. Then the column was eluted with 0·01 N HCl. Fractions containing UMP (1·7–2·2 l. of eluant), orotidine (2·2–2·7 l.) and OA (2·7–3·8 l.) were evaporated to dryness under reduced pressure at room temp. The dried residues were dissolved in suitable volumes of H_2O and the resulting solutions chromatographed on Whatman No. 1 paper using 0·1 N NH_4OH as the solvent. UMP and orotidine had an R_f value of 0·94, OA of 0·87 (descending, at 4°). Eluates from the chromatograms were used for spectrophotometric identification and for determination of radioactivity under conditions described previously.[7] Total losses during the isolation were within the limits of 10–15% in the case of uracil and uridine, and 25–30% in the case of UMP, orotidine and OA.

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[6] J. G. Buchanan, *Nature* **168**, 1091 (1951).

[7] J. Buchowicz, I. Reifer and J. Makowski, *Acta Biochim. Polon.* **8**, 377 (1961).