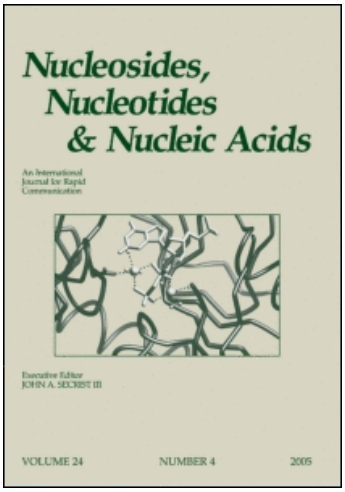

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RIBOSYLUREA ACCUMULATES IN YEAST *urc4* MUTANTS

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□ *Yeast* Saccharomyces *(*Lachancea*)* kluyveri urc4 *mutants, unable to grow on uracil, biotransformed* $^{14}C_2$*-uracil into two labeled compounds, as detected by high performance liquid chromatography (HPLC). These two compounds could also be obtained following organic synthesis of ribosylurea. This finding demonstrates that in the URC pyrimidine degradation pathway, the opening of the uracil ring takes place when uracil is attached to the ribose moiety. Ribosylurea has not been reported in the cell metabolism before and the two observed compounds likely represent an equilibrium mixture of the pyranosyl and furanosyl forms.*

KEYWORDS Uracil degradation; ribosylurea; URC pathway; *Saccharomyces* (*Lachancea*) *kluyveri*

INTRODUCTION

The reductive pathway of uracil degradation is well understood and in humans involved also in degradation of anticancer nucleoside compounds. We have recently reported a novel and thereby second pathway of uracil degradation in eukaryotes, called URC (uracil catabolism).[1] It is a highly efficient system enabling our yeast model *Saccharomyces* (*Lachancea*) *kluyveri* to grow on uracil as sole nitrogen source. Five URC genes have been reported, encoding a putative gene expression regulator (*URC2*), urea amidolyase (*URC3,5*), uracil phosphoribosyl transferase (UPRTase) (*URC6*), and two conserved, putative enzymes involved in the opening of the pyrimidine ring (*URC1* and *URC4*). This article deals with *URC4* whose gene product has no homolog among the known proteins and protein domains. However, *URC4* is widespread in fungi and bacteria.[1] In this article, we report the fate of uracil in the *urc4* mutants.

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MATERIALS AND METHODS

Labeling of Cells

Two *S. kluyveri urc4* mutants, the EMS induced Y814 and the knock-out Y1167 were labeled according to Andersen et al.[1] Briefly, cells grown in 50 ml of the medium containing uracil were harvested (at OD_{600} ~1.0), washed and concentrated to 0.5 ml for labeling during one hour with 0.7 mM $^{14}C_2$-uracil (1.8 Ci/mol). The low molecular weight fraction from the cell lysate was obtained by the addition of PCA followed by neutralization with KOH and centrifugation. The supernatants were used in high performance liquid chromatography (HPLC) analysis.

Synthesis of Ribosylurea

Solution of 0.6 M ribose and 0.6 M urea ([^{14}C]-urea with specific radioactivity 3 mCi/mol was used for synthesis of labeled ribosylurea) in 90% ethanol with 0.1 M HCl was incubated for 24 hours at 50°C.[2,3] The mixture was neutralized with NaOH, a yellow insoluble by-product was removed by centrifugation, and the supernatant used for analysis.

Separation on HPLC

Samples were analyzed on a 100 mm × 4.6 mm 200 Å 5μ ZIC-pHILIC column with a mobile phase of 85% acetonitrile (ACN) and 2 mM ammonium carbonate pH 9.6. 100 μL of sample was injected and peaks were detected by an in-line liquid scintillation counter and by UV at 260 nm (uracil, uridine, and UMP).

RESULTS AND DISCUSSION

We have previously used the approach of labeling, extraction and separation to identify the accumulation of urea in the *urc3,5* mutants.[1] When *urc4* cells from Y814 or Y1167 were fed with $^{14}C_2$-uracil, almost 100% of the label appeared in two peaks, A (at 8 ml) and B (at 14.5 ml), which did not correspond to any of our reference compounds (Figure 1). These references were uracil, uridine, UMP, UDP, UTP, urea, and various other metabolites from the known uracil catabolic pathways. The essential role of UPRTase encoded by *URC6* for the growth on uracil, and our previous observation that *urk1* mutants (defective in uridine kinase) display growth retardation on uridine, have clearly shown that UMP is an intermediate in the URC pathway.[1] The finding of urea as an intermediate[1] has suggested that ribosylurea or phosphoribosylurea might be an intermediate in the URC pathway. In our experiments, alkaline phosphatase treatment of the *urc4* extracts did not

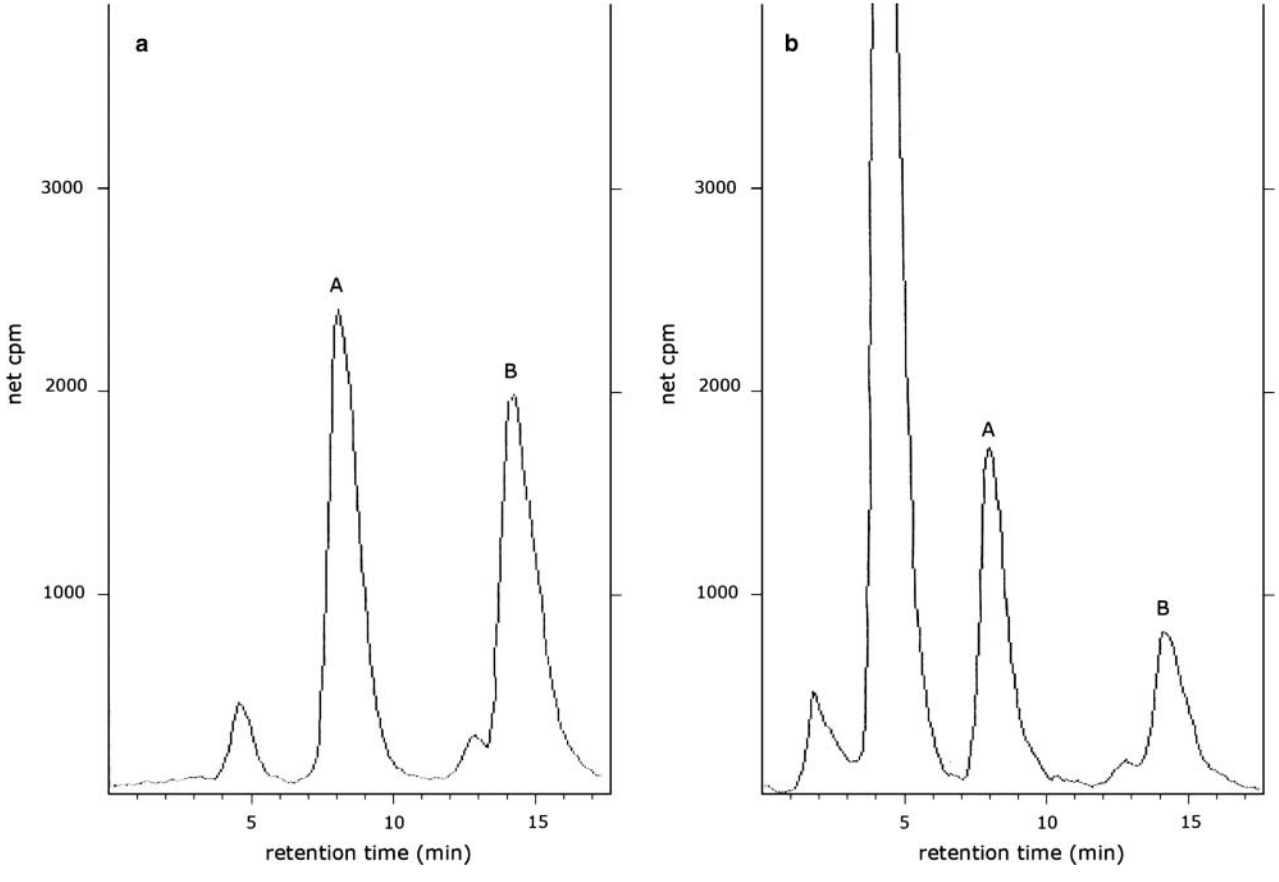

FIGURE 1 Radiolabeled HPLC peaks from (a) the extract from the *urc4* cells labeled with ^{14}C-2 uracil, and (b) the organic synthesis of ribosylurea from ribose and ^{14}C- urea. The first peak (4.7 ml) in the cell extract (a) elutes at the same position as the uridine standard, whereas the large peak (3.6–6 ml) in the case of organic synthesis (b) is unreacted ^{14}C-urea. We propose that compounds A and B, eluting at 8 and 14.5 ml, are ribopyranosylurea and ribofuranosylurea, respectively.

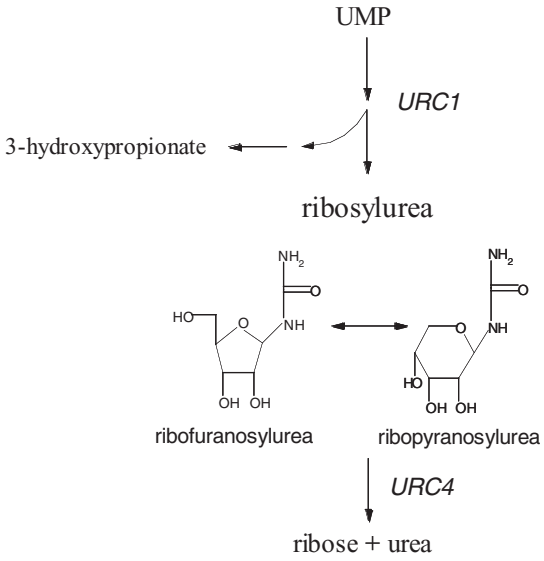

FIGURE 2 The first step in metabolism of uracil is a conversion to the monophosphate level (UMP) by uracil phosphoribosyltransferase encoded by the *URC6* gene.[1] Further phosphorylation of UMP to the di- and/or triphosphate level is so far not clear. The activity of Urc1p is unknown but one can speculate about it on the basis of homology to GTP cyclohydrolase II, a Zn^{2+}-containing enzyme activating hydrolytic water molecules in the first step of flavin biosynthesis. Stoichiometric amounts of 3-hydroxypropionate, originating from uracil, are secreted from cells growing on uracil as sole nitrogen source.[1] The reaction product of the Urc1p action is phosphoribosylurea or its dephosphorylated form ribosylurea. In this study, we showed that in *urc4* mutants the label from $^{14}C_2$ uracil ended up in two compounds, ribopyranosylurea and ribofuranosylurea. One of them may be an "off product" while the other is the "real" substrate of Urc4p. In wild type, the resulting urea is targeted by the two-headed urea amidolysase (encoded by *URC3,5)* generating ammonia and carbon dioxide.

change the appearance of the two peaks indicating that none of the compounds was phosphorylated. In addition, UMP run as a standard was eluted late (at 30 ml).

The ribosylurea moiety is present in every pyrimidine nucleoside or nucleotide, and as an independent compound it exists as a mixture of ribopyranosylurea and ribofuranosylurea.[2,3] To confirm the presence of these compounds we subjected our synthesis reaction to HPLC analysis. Unreacted urea eluted as a large peak at the beginning (4–6 ml) but about 10% of the label eluted as peaks A (8 ml) and B (14.5 ml), presumably corresponding to the pyranosyl and furanosyl forms of ribosylurea. Moreover, the *urc4* cells and the organic synthesis gave similar proportions of the two peaks. We made a separate extraction from the labeled *urc4* cells with the detergent digitonin (0.1%) for 1 hour, thus avoiding the strongly acidic PCA treatment. In this case about four times larger quantity of B than of A was obtained. Compound B seems therefore likely to be the main intermediate in the cell and it is most likely that this form resembles the one originating from uridine and UMP, with a five-member furanosyl ring.

CONCLUSIONS

Hereby we identified ribosylurea as a physiologically important compound in the yeast cell. The identification of ribosylurea suggests that the ring opening of uracil takes place when uracil is attached to ribose, in contrast to the classical reductive pathway[4] in which the uracil base itself is targeted by the catabolic enzymes. Thus, ribosylurea might be a true intermediate of the URC pathway (Figure 2) or a result of further transformations into a dead end product.

REFERENCES

1. Andersen, G.; Björnberg, O.; Polakova, S.; Pynyaha, Y.; Rasmussen, A.; Møller, K.; Hofer, A.; Moritz, T.; Sandrini, M.P.B.; Merico, A.-M.; Compagno, C.; Åkerlund, H.-E.; Gojkovic, Z.; Piškur, J. The second pathway to degrade pyrimidine nucleic acid precursors in eukaryotes. *J. Mol. Biol.* **2008**, 380, 656–666.
2. Benn, M.H.; Jones, A.S. Glycosylureas. part I. preparation and some reactions of D-glucosylureas and D-ribosylureas. *J. Chem. Soc.* **1960**, 3837–3841.
3. Ukita T.; Hamada, A.; Yoshida, M. Synthesis of 1-(β-D-ribofuranosyl) urea and related compounds. *Chem. Pharm. Bull. (Tokyo)* **1964**, 12, 454–459.
4. Piškur, J.; Schnackerz K.D.; Andersen, G.; Björnberg, O. (**2007**) Comparative genomics reveals novel biochemical pathways. *Trends Genet.* **2007**, 8, 369–72.