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INHIBITION OF UDP-*N*-ACETYLGLUCOSAMINE PYROPHOSPHORYLASE BY URIDINE

KENJI YAMAMOTO [a], MITSUAKI MORIGUCHI [a], HIROYASU KAWAI [b] and TATSUROKURO TOCHIKURA [a]

[a] *Department of Food Science and Technology, Kyoto University, Sakyo-ku, Kyoto 606 and* [b] *Department of Food Science and Nutrition, Nara Women's University, Kitauoya, Nara 630 (Japan)*

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Summary

UDP-*N*-acetylglucosamine pyrophosphorylases (UTP: 2-acetamido-2-deoxy-α-D-glucose-1-phosphate uridylyltransferase, EC 2.7.7.23) from baker's yeast and *Neurospora crassa* IFO 6178 were inhibited by uridine which is the nucleoside moiety of UDP-GlcNAc. The inhibition was shown in both directions of pyrophosphorolysis and of synthesis of UDP-GlcNAc. Kinetic analysis revealed that uridine demonstrated a noncompetitive type of inhibition with UDP-GlcNAc and competitive inhibition with PP_i. The K_i values for the baker's yeast enzyme were 1.8 mM for UDP-GlcNAc and 0.16 mM for PP_i, and the values for the *Neurospora* enzyme were 1.1 mM for UDP-GlcNAc and 0.15 mM for PP_i, respectively. Uridine did not bind irreversibly to the enzyme, as the activity was restored with dialysis. No other nucleosides caused inhibition of the enzyme activity except uridine.

Some uridine derivatives, such as 5-hydroxyuridine, 5,6-dihydrouridine and pseudouridine, also inhibited the enzyme activity. But deoxyuridine showed only slight inhibition, and 5'-UMP and orotidine caused no inhibition of the enzyme activity.

Introduction

UDP-*N*-acetylglucosamine pyrophosphorylase (UTP: 2-acetamido-2-deoxy-α-D-glucose-1-phosphate uridylyltransferase, EC 2.7.7.23) catalyzes the formation of UDP-GlcNAc and PP_i from UTP and *N*-acetylglucosamine 1-phosphate (GlcNAc-1-*P*). The enzyme has been found in various sources and some properties of the partially purified enzymes from calf liver [1], sheep brain [2] and

Staphylococcus aureus [1] have been previously reported. We have recently purified the enzyme to a homogeneous state from baker's yeast [3,4] and *Neurospora crassa* IFO 6178 [5] and described some properties of the enzymes [3—6]. In microorganisms, the enzyme is presumed to play a physiological role in the biosynthesis of cell walls, that is, in the biosynthesis of peptidoglycan and teichoic acid [7].

Concerning the biosynthesis of aminosugar nucleotides, there is some evidence suggesting the presence of various inhibition mechanisms. One of the enzymes responsible for the de novo synthesis of aminosugars is L-glutamine D-fructose-6-phosphate aminotransferase (2-amino-2-deoxy-D-glucose-6-phosphate Ketol-isomerase (amino-transferring), EC 5.3.1.19) which catalyzes the formation of glucosamine 6-phosphate from fructose 6-phosphate and glutamine. It is well known that the aminotransferase of rat liver is subject to feedback control by UDP-GlcNAc [8]. Feedback inhibition of the amidotransferase was also seen in baker's yeast [9] and *N. crassa* [10]. Therefore, the control of the synthesis of UDP-GlcNAc may play a physiological role in the biosynthesis of cell walls of microorganisms.

Many studies on the biosynthesis of UDP-GlcNAc have been carried out with respect to aminosugar metabolism. However, no study has been done to show the effect of a pyrimidine nucleoside precursor on the biosynthesis of UDP-GlcNAc.

In the present study, evidence is presented showing that UDP-*N*-acetylglucosamine pyrophosphorylase activities of baker's yeast and *N. crassa* are inhibited by uridine which is the pyrimidine nucleoside moiety of UDP-GlcNAc.

Materials and Methods

Materials. UDP-GlcNAc was prepared according to the fermentative method described previously [11]. All other chemicals were available commercially.

Enzyme preparations. UDP-*N*-acetylglucosamine pyrophosphorylases were prepared from baker's yeast [4] and *N. crassa* [5] as described previously.

Assay of enzyme activity. The enzyme activity was measured by estimation of UTP formed in the UDP-GlcNAc pyrophosphorolysis reaction as described previously [5].

Protein determination. Protein was determined by the method of Lowry et al. [12].

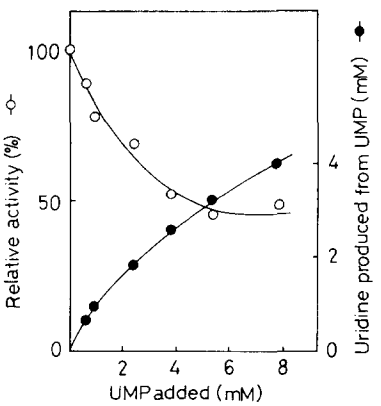
Results

Effect of a degradation product of 5'-UMP on enzyme activity. When a crude enzyme preparation of *N. crassa*, which was prepared by disruption in a mill followed by treatment with protamine sulfate, was incubated with 5'-UMP the inhibition of UDP-*N*-acetylglucosamine pyrophosphorylase activity was observed. The enzyme activity of the crude preparation decreased with increasing concentrations of 5'-UMP. However the purified enzyme itself was not inhibited by 5'-UMP. This result shows that the inhibition may be due to a degradation product of 5'-UMP, which was identified as uridine by means of

paper chromatography with a solvent system of 95% ethanol/1 M ammonium acetate (7.5 : 3) [13], because the crude enzyme preparation contained the phosphatase activity. As shown in Fig. 1, the degree of inhibition definitely increased with an increase in the amount of uridine produced from 5'-UMP.

Inhibition by uridine. To examine the effect of uridine on the activity of UDP-*N*-acetylglucosamine pyrophosphorylase, the purified enzymes of baker's yeast and *N. crassa* were incubated in the presence of uridine under the enzyme assay conditions. It was shown that the inhibition of the enzyme activities was dependent on the concentration of uridine (Fig. 2). Half-maximal inhibitions of both enzyme activities were attained at about 1 mM uridine, as shown in Fig. 2. When the enzyme was preincubated with uridine at 30°C inactivation of the enzyme occurred, but the enzyme activity was recovered by dialyzing the preincubation mixture against 0.01 M potassium phosphate buffer (pH 7.5). A similar inhibition by uridine was obtained in the direction of UDP-GlcNAc synthesis (Fig. 3). From these results, both reactions of the synthesis and the pyrophosphorolysis of UDP-GlcNAc catalyzed by UDP-*N*-acetylglucosamine pyrophosphorylase were significantly inhibited by uridine.

Kinetic analysis of inhibition. The effect of substrate concentration on the enzyme activity at different concentrations of uridine was investigated. In the case of both enzymes, the reciprocal plots showed that the inhibition by uridine of UDP-GlcNAc was of a noncompetitive type, whilst the inhibition was competitive toward PP_i (Figs. 4 and 5). The baker's yeast enzyme had inhibition constants of $1.8 \cdot 10^{-3}$ M for UDP-GlcNAc and $1.6 \cdot 10^{-4}$ M for PP_i. On the other hand, the inhibition constants for the *Neurospora* enzyme were $1.1 \cdot 10^{-3}$ M for UDP-GlcNAc and $1.5 \cdot 10^{-4}$ M for PP_i. The inhibitory behavior of uridine toward the two substrates suggests that the binding site of the enzyme molecule for uridine is the same as that for PP_i, but distinct from that for UDP-GlcNAc.



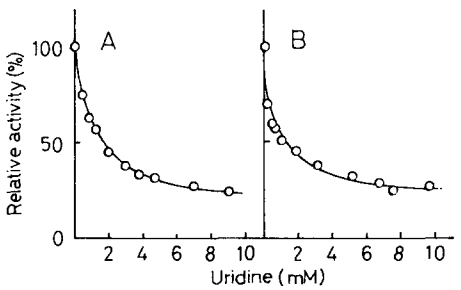


Fig. 1. Inhibitory effect of uridine produced with different concentrations of 5'-UMP added on the UDP-*N*-acetylglucosamine pyrophosphorylase activity of *N. crassa*. Assays were performed as described in Materials and Methods except for the addition of varying amounts of 5'-UMP. The enzyme preparation was found to possess a high phosphatase activity.

Fig. 2. Inhibition of enzyme activity by varying concentrations of uridine. The percentages of inhibition by uridine were calculated against the control tube without uridine. A, baker's yeast enzyme. B, *N. crassa* enzyme.

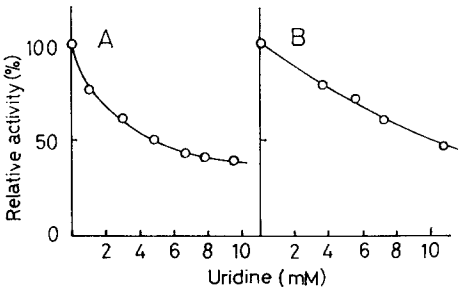


Fig. 3. Inhibitory effects of uridine on UDP-GlcNAc synthetic activities of the enzyme. Enzyme assays were carried out at 30°C with 10 mM UTP and about 3 mM GlcNAc-1-*P* as substrates in 100 mM Tris-HCl buffer, pH 7.5, containing 5 mM $MgCl_2$ and $1 \cdot 10^{-4}$ M dithiothreitol. The percentages of inhibition by uridine were calculated against the control tube without uridine. A, baker's yeast enzyme. B, *N. crassa* enzyme.

Effect of other nucleosides on enzyme activity. UDP-*N*-acetylglucosamine pyrophosphorylase activities of baker's yeast and *N. crassa* were not affected by cytidine, thymidine, adenosine, guanosine or inosine. No inhibition was observed when uracil and ribose were added together or separately.

Effects of various uridine derivatives on enzyme activity. The effects of various uridine derivatives on the enzyme activity were investigated, and the inhibitory effects are shown in Table I. 5-Hydroxyuridine, 5,6-dihydrouridine and pseudouridine (5-D-ribosyluracil) significantly inhibited the activity of both enzymes. The degree of inhibition of both enzymes by these compounds was nearly equal at various concentrations, and as is the case for uridine, were competitive with respect to PP_i. However, deoxyuridine showed only slight inhibition. This is possibly due in the lack of a hydroxyl group at the C2′ position of the ribose moiety. Although 5-bromouridine and 5-iodouridine were less effective inhibitors, 5-bromodeoxyuridine and 5-iododeoxyuridine did not inhibit enzyme activity. From these results, the hydroxyl group at the C2′ position of the ribose moiety of uridine derivatives appeared to be necessary at least for the inhibition of the enzyme.

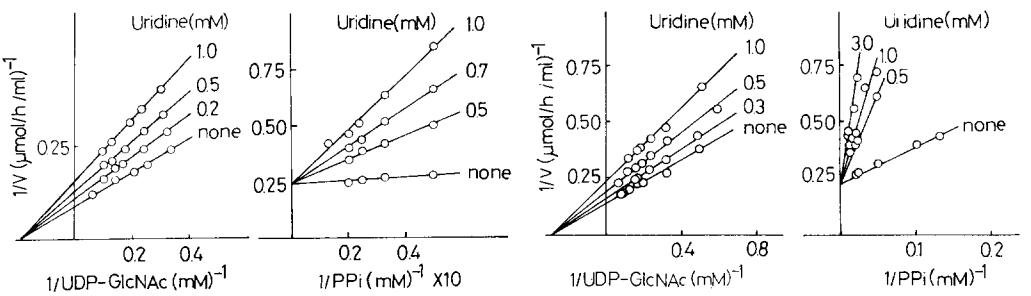


Fig. 4. Double-reciprocal plots of velocity against substrate concentration with different concentrations of uridine for the enzyme of baker's yeast.

Fig. 5. Double-reciprocal plots of velocity against substrate concentration with different concentrations of uridine for the enzyme of *N. crassa*.

TABLE I

INHIBITION OF UDP-*N*-ACETYLGLUCOSAMINE PYROPHOSPHORYLASE ACTIVITIES OF BAKER'S YEAST AND *N. CRASSA* BY VARIOUS URIDINE DERIVATIVES

Assays were carried out as described in Materials and Methods in the presence of the uridine derivatives indicated. The percentage of enzyme activity was taken as 100 in the absence of a uridine derivative.

Compound	mM	Relative activity (%)	
		Baker's yeast	*N. crassa*
None		100	100
Uridine	2	45	46
5-Hydroxyuridine	2	68	37
5,6-Dihydrouridine	2	65	73
Pseudouridine	2	83	75
5-Bromouridine	2	87	94
5-Iodouridine	2	85	88
Orotidine	10	100	100
Deoxyuridine	10	88	85
5-Bromodeoxyuridine	10	95	97
5-Iododeoxyuridine	10	100	100
5'-UMP	10	95	95

Discussion

Although a number of studies on the regulation of UDP-GlcNAc synthesis by L-glutamine D-fructose-6-phosphate aminotransferase have been made [8—10, 14—16], little is known about the control of the intracellular concentration of UDP-GlcNAc by enzymes responsible for the UDP-GlcNAc formation. This study showed that the UDP-*N*-acetylglucosamine pyrophosphorylase activity of both baker's yeast and *N. crassa* was inhibited by uridine, the nucleoside moiety of the substrate. This inhibition was observed specifically in the presence of uridine in both the reactions of pyrophosphorolysis and the synthesis of UDP-GlcNAc. These results indicate that uridine may have some role in controlling the biosynthesis of UDP-GlcNAc which serves as a precursor in cell wall synthesis in microorganisms. Therefore, the specific inhibition of UDP-*N*-acetylglucosamine pyrophosphorylase by uridine may suggest that the metabolic pathway of the pyrimidine nucleotide has some relation to the control of the biosynthesis of UDP-GlcNAc.

In the direction of pyrophosphorolysis of GlcNAc, uridine was a noncompetitive inhibitor toward UDP-GlcNAc and competitive with PP_i. This suggests that uridine binds to the site of the enzyme for PP_i but not for UDP-GlcNAc. Moreover, studies on the effects of uridine derivatives showed that the basic structure for the inhibitor to bind the enzyme seems to require the presence of a hydroxy group at the C2' position of the ribose. These results may give insight into the structure of the active site of the enzyme.

Hori et al. [17] reported that the chitin synthetase of the phytopathogenic fungus *Piricularia oryzae* was inhibited by uridine which was found to be a competitor for UDP-GlcNAc. Therefore, it is possible that uridine is concerned in the synthesis of cell wall chitin as a regulatory compound. Further studies of

the inhibition by uridine are needed to clarify the significance of this compond.

References

1 Strominger, J.L. and Smith, M.S. (1959) J. Biol. Chem. 234, 1822—1827
2 Pattabiraman, T.N. and Bachhawat, B.K. (1961) Biochim. Biophys. Acta 50, 129—134
3 Kawai, H., Yamamoto, K., Moriguchi, M. and Tochikura, T. (1976) J. Ferment. Technol. 53, 463—465
4 Yamamoto, K., Kawai, H., Moriguchi, M. and Tochikura, T. (1976) Agric. Biol. Chem. 40, 2275—2281
5 Yamamoto, K., Kawai, H., Moriguchi, M. and Tochikura, T. (1979) Can. J. Microbiol. 25, 1381—1386
6 Yamamoto, K., Kawai, H., Moriguchi, M. and Tochikura, T. (1978) J. Ferment. Technol. 56, 57—58
7 Anderson, R.G., Douglas, L.J., Hussey, H. and Baddiley, J. (1973) Biochem. J. 136, 871—876
8 Kornfeld, S., Kornfeld, R., Neufeld, E.F. and O'Brien, P.J. (1964) Proc. Natl. Acad. Sci. U.S.A. 52, 371—379
9 Moriguchi, M., Yamamoto, K., Kawai, H. and Tochikura, T. (1976) Agric. Biol. Chem. 40, 1655—1656
10 Endo, A., Kakiki, K. and Misato, T. (1970) J. Bacteriol. 103, 588—594
11 Tochikura, T., Kawai, H. and Gotan, T. (1971) Agric. Biol. Chem. 35, 163—176
12 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 263—275
13 Caputto, R., Leloir, L.F., Cardini, C.E. and Paladini, A.C. (1950) J. Biol. Chem. 184, 333—350
14 Trujillo, J.L. and Gan, J.C. (1973) Biochim. Biophys. Acta 304, 32—41
15 Kikuchi, H. and Tsuiki, S. (1976) Biochim. Biophys. Acta 422, 241—246
16 Tsuiki, S. and Miyagi, T. (1977) Adv. Enzyme Regul. 15, 35—52
17 Hori, N., Kakiki, K., Suzuki, S. and Misato, T. (1971) Agric. Biol. Chem. 35, 1280—1291