Biosynthesis of Pyridoxine in Saccharomyces cerevisiae
-Origin of the Pyridoxine Nitrogen Atom Differs
under Anaerobic and Aerobic Conditions—

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Summary The amide nitrogen atom of glutamine is incorporated into pyridoxine in four
eukaryotes (i.e., Emericella nidulans, Mucor racemosus, Neurospora crassa and Saccharomyces
cerevisiae) and two prokaryotes (i.e., Staphylococcus aureus and Bacillus subtilis). However, in
the prokaryotes Pseudomonas putida, Enterobacter aerogenes and Escherichia coli, it is the ni-
trogen atom of glutamate that is incorporated into pyridoxine (J Nutr Sci Vitaminol (2000)
46, 55–57). As these results were from experiments conducted under aerobic conditions,
we investigated the biosynthesis of pyridoxine on S. cerevisiae under anaerobic conditions.
The results showed that [amide-15N]L-glutamine was not incorporated into pyridoxine, un-
like the results for aerobic conditions. The incorporation of [15N]ammonium salts into pyri-
doxine was not inhibited in the presence of casamino acids and tryptophan. The results
showed that the nitrogen atoms of amino acids are not used for the biosynthesis of pyridox-
ine. The incorporation of 15N into pyridoxine was inhibited in the presence of adenine, but
not in that of hypoxanthine. Thus, the nitrogen atom of pyridoxine may be from the amino
group attached to the C-6 of adenine.

Key Words vitamin B6, pyridoxine, biosynthesis, adenine, anaerobic conditions

The biosynthetic pathway of pyridoxine has been studied by many researchers. In 1990, Lam
and Winkler proposed, based on a gene analysis of Escherichia coli, that pyridoxine is synthesized from
1-deoxy-D-xylulose and 4-hydroxy-L-threonine, and that pdxA and pdxJ are involved in the biosynthesis (1).
Spenser and co-workers showed that the labels of [2,3-13C2]-1-deoxy-D-xylulose and [2,3-13C2]-4-hydroxy-
L-threonine were incorporated into C-2 and C-3 and C-6 and C-5 of pyridoxine, respectively, in E. coli (2, 3). Cane
et al. reported that, in the last stage of pyridoxine biosynthesis in E. coli, pdxA and pdxJ are expressed and
clarified these enzymatic mechanisms (4).

We have studied the origin of the pyridoxine nitrogen atom and reported that there are at least two biosyn-
thetic pathways (5). In E. coli, the amino nitrogen of glutamate is incorporated into pyridoxine, while in S.
cerevisiae, the amide nitrogen atom of glutamine is incorporated. The amide nitrogen atom of glutamine is
also incorporated into pyridoxine in four eukaryotes (i.e., Emericella nidulans, Mucor racemosus, Neurospora
crassa and Saccharomyces cerevisiae) and two prokary-
Ayes (i.e., Staphylococcus aureus and Bacillus subtilis). However, in the following three prokary-
otes, Pseudomonas putida, Enterobacter aerogenes and E. coli, it is the nitrogen atom of glutamate that is incorporated
into pyridoxine (6).

In 1999, Ehrenshaft et al. (7) and Osmani et al. (8)
studied the distribution of SOR1 (pyroA) in eukaryotes
and prokaryotes and compared the results with those
for the genes of pdxA/J found in E. coli. They reported
that plants, fungi and archaeabacteria have SOR1, but
some eubacteria have SOR1 while others have pdxA/J.

In S. cerevisiae, the carbon skeleton of pyridoxine re-
mains to be clarified. When we reported that the pyrim-
idine moiety of thiamin consists of histidine and pyri-
doxine, we proposed that 5-phosphoribosyl-1-amine is
incorporated into N-1, C-6, C-5, and C-5’ of pyridoxine
and the moiety is transferred into N-1, C-6, C-5, and C-
5’ of the pyrimidine (9). We described the unresolved
precursor of the carbon skeleton as being a unit of five
carbons, C-2’, C-2, C-3, C-4, and C-4’. Spenser and his coworkers showed that the precursor
of C-2’ to C-4’ unit of pyridoxine was not 1-deoxyxylu-
lose in S. cerevisiae and proposed that the C-1, C-2, C-3,
C-4, and C-5 of ribose or ribulose are incorporated into
the C-2’, C-2, C-3, C-4, and C-4’ of pyridoxine (10).
This result indicates that the last precursor of pyridox-
ine is 2’-hydroxypyridoxine (11).

These experiments were done under aerobic condi-
tions. As the use of anaerobic conditions may influence
the results, we studied the biosynthesis of pyridoxine on
S. cerevisiae under such conditions. Our results revealed
a different origin of the pyridoxine nitrogen atom in S.
cerevisiae under anaerobic conditions.

MATERIALS AND METHODS

Materials. [15N]Ammonium chloride (99 15N atom

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% and [15N]ammonium sulfate were purchased from Shoko Co., Ltd. (Tokyo, Japan), [amide-15N]-l-glutamine (99 15N atom %) was purchased from Cambridge Isotope Laboratories (MA, USA), and [15N]L-glutamic acid (99 15N atom %) was purchased from ICON Services Inc. (NY, USA), Casamino acids were vitamin-free products from Difco Laboratories (MI, USA). All other chemicals were of analytical grade.

Growth conditions. S. cerevisiae IFO 1234 was grown in synthetic media under the anaerobic conditions described previously (12). The nitrogen sources of the media were substituted with [15N]ammonium chloride (0.5 g/L) and [15N2]ammonium sulfate (0.5 g/L) for tracer experiments. Casamino acids (2.0 g/L) and tryptophan (0.1 g/L) were added as competitors, as were uracil, adenine, and hypoxanthine at 3 mM concentrations. S. cerevisiae was grown at 30°C for 15 h under anaerobic conditions. During this period, S. cerevisiae increased to about three times its original amount, respectively.

Isolation and analysis of pyridoxine from S. cerevisiae. The isolation of pyridoxine from S. cerevisiae and its acetylation and analysis by GC-MS have been described elsewhere (5, 6).

RESULTS AND DISCUSSION

The mass spectrum of authentic acetylpyridoxine has been presented in a previous report (5). The analysis of pyridoxine fragmentation was based on the report by DeJongh and Korytnyk (13). The molecular ion [M+] (i.e., m/z 295), fragment ions (i.e., m/z 253, 235, and 193), and the base peak at m/z 151 have a nitrogen atom from the pyridine ring.

Incorporation of [amide-15N]-l-glutamine and [15N]-glutamate into pyridoxine

As we have reported previously, the label of [amide-15N]-l-glutamine was incorporated into pyridoxine in S. cerevisiae under aerobic conditions (5). However, as can be seen by the comparison in Fig. 1A, under anaerobic conditions, [amide-15N]-l-glutamine was not incorporated into pyridoxine (Fig. 1B), suggesting that the origin of pyridoxine nitrogen is different in yeast under aerobic and anaerobic conditions.

Under aerobic conditions, the origin of the nitrogen atom in E. coli was the nitrogen atom of glutamate (6). Using the same conditions, but in anaerobic state, the nitrogen atom of glutamate was studied using S. cerevisiae. The nitrogen of glutamate was also not incorporated into pyridoxine under anaerobic conditions.

Under aerobic conditions, the origin of the pyridoxine nitrogen atom is the amide-N of glutamine in S. cerevisiae, while under anaerobic conditions, the amide-N of glutamine and the N-atom of glutamate were not incorporated into pyridoxine. Thus, S. cerevisiae seems to have a different biosynthetic pathway for pyridoxine under anaerobic conditions as compared to aerobic conditions. We studied the origin of the pyridoxine nitrogen atom under anaerobic conditions in S. cerevisiae.

Inhibition of 15N incorporation into pyridoxine by nucleobases (adenine and uracil)

As amino acids were not the precursors of pyridoxine, we studied the adenine and uracil nucleobases (Figs. 4 and 5). There was no inhibition of incorporation with uracil. The addition of adenine inhibited the incorporation of 15N into pyridoxine because the peaks of m/z 151, 106, 123, and 193 increased as compared to the MS spectrum of Fig. 2, suggesting that one of the nitrogen atoms of adenine was incorporated into pyridoxine.
Fig. 2. Mass spectrum of the acetylpyridoxine produced by *S. cerevisiae* in the presence of [\(^{15}\text{N}\)]ammonium chloride and [\(^{15}\text{N}_2\)]ammonium sulfate as nitrogen sources under anaerobic conditions. OD values before and after the growth of *S. cerevisiae* were 0.119 and 0.412, respectively.

Fig. 3. Inhibition of [\(^{15}\text{N}\)]incorporation of [\(^{15}\text{N}\)]ammonium salts in the presence of casamino acids and L-tryptophan under anaerobic conditions. OD values before and after the growth of *S. cerevisiae* were 0.133 and 0.315, respectively.

Fig. 4. Inhibition of [\(^{15}\text{N}\)]incorporation from [\(^{15}\text{N}\)]ammonium salts into pyridoxine in the presence of uracil under anaerobic conditions. OD values before and after the growth of *S. cerevisiae* were 0.112 and 0.398, respectively.
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Fig. 5. Inhibition of $^{15}$N incorporation from $^{15}$N ammonium salts into pyridoxine in the presence of adenine under anaerobic conditions. OD values before and after the growth of *S. cerevisiae* were 0.112 and 0.343, respectively.

Fig. 6. Inhibition of $^{15}$N incorporation from $^{15}$N ammonium salts into pyridoxine in the presence of hypoxanthine under anaerobic conditions. OD values before and after the growth of *S. cerevisiae* were 0.108 and 0.407, respectively.

Inhibition of $^{15}$N incorporation by hypoxanthine

As a nitrogen atom of adenine was incorporated into pyridoxine, we studied whether or not the amino group attached to the C-6 of adenine was the origin of the pyridoxine. Hypoxanthine, which does not have an amino group attached to C-6, was used as a competitor for the incorporation of $^{15}$N. As shown in Fig. 6, the incorporation of $^{15}$N was not inhibited by hypoxanthine if the origin of the pyridoxine nitrogen was the amino group attached to the C-6 of adenine.

Our finding that the origin of the pyridoxine nitrogen atom is from the amino group attached to the C-6 of adenine under anaerobic conditions was supported by the results that the incorporation of $[^{15}N]$ ammonium salts into pyridoxine was inhibited by adenine, but not by hypoxanthine.

The biosynthetic pathway is clearly different between some bacteria and eukaryotes as described in the introduction. The question remains about whether the origin of the pyridoxine nitrogen atom differs among species under anaerobic conditions.

REFERENCES


