Metabolism of Tryptophan to Niacin in *Saccharomyces uvarum*

Mariko SHIN, Keiji SANO, and Chisae UMEZAWA*

School of Pharmacy, Kobe-Gakuin University, Nishi-ku, Kobe 651-21, Japan
(Received December 19, 1990)

Summary In *Saccharomyces uvarum*, the effect of metabolic intermediates of the tryptophan-NAD pathway on the niacin-production was investigated. Exogenously added kynurenine and 3-hydroxyanthranilic acid raised the content of total niacin of the cells 2-fold as compared to the control cells, although anthranilic acid and tryptophan were less effective. Tryptophan was taken up into the cells faster than kynurenine, and the intracellular pool of tryptophan was larger than that of kynurenine. Of kynurenine (0.05 mM) added to the medium, 55% went through the transaminase flux (2-H liberation), 20% through the kynureninase flux, but none through the acetyl-CoA flux. As for tryptophan, only 2% went through the kynureninase flux. The products through the transaminase flux were identified as kynurenic acid (85%) and xanthurenic acid. 3-Hydroxykynurenine, 3-hydroxyanthranilic acid, quinolinic acid and niacin were also detected. The metabolism of tryptophan via the kynureninase flux reached a plateau above 0.05 mM. The production of kynurenine and kynurenic acid gradually increased above 0.05 mM. Tryptophol was formed in parallel with the amount of tryptophan consumed, while the rate of niacin production increased after glucose and tryptophan were exhausted. Based on the data obtained, a possible regulatory mechanism of the tryptophan-NAD pathway was discussed.

Key Words Saccharomyces uvarum (S. carlsbergensis), tryptophan-niacin flux, kynurenine-niacin flux, catabolite derepression, tryptophan pyrrolase, kynurenine 3-hydroxylase

---

* To whom all correspondence should be addressed.

Abbreviations: Trp, L-tryptophan; TOH, tryptophol; KN, L-kynurenine; OHKN, L-3-hydroxykynurenine; KA, kynurenic acid; XA, xanthurenic acid; OHAA, 3-hydroxyanthranilic acid; AA, anthranilic acid; QA, quinolinic acid; Amino acids and their derivatives were all L-isomers, except for DL-[benzene-U-14C]tryptophan. TPase, tryptophan 2,3-dioxygenase (tryptophan pyrrolase); KNOHase, L-kynurenine 3-hydroxylase; TTase, tryptophan transaminase; KTase, kynurenine transaminase; KNase, kynureninase.
It is well known that niacin mainly acts as NAD(H) or NADP(H), important coenzymes for oxidoreductases. Niacin is unique in respect of its being biosynthesized from an amino acid, tryptophan (Trp) (1). The Trp-NAD pathway was detected in mammalian liver (1), Neurospora (2) and aerobically grown yeasts (3). Most bacteria (4) do not appear to utilize Trp for the niacin biosynthesis, except for Xanthomonas pruni (5). Several papers on the regulation of the Trp-NAD pathway have been reported. The first reaction in this pathway, catalyzed by tryptophan 2,3-dioxygenase (tryptophan pyrrolase; Tpase) [EC 1.13.11.11], has been reported to be regulated differently in different organisms. In Neurospora, it appears to be subjected to repression control (6) and feedback inhibition by NADPH (7). In Xanthomonas pruni, the first three enzymes are induced coordinately by Trp (8), Tpase being under feedback control and inhibited by reduced pyridine nucleotides (9). In rat liver, Tpase is stabilized and/or activated by Trp (10, 11). In yeast, NAD is biosynthesized through 2 routes depending on aeration condition (3), that is, oxygen is one of the regulatory factors of L-kynurenine 3-hydroxylase (KNOHase) [EC 1.14.13.9], a supposed rate-limiting enzyme in the Trp-NAD pathway (12). The activity of Tpase, another molecular oxygen requiring enzyme, however, has never been reported in yeast (3). Tpase is mainly metabolized to tryptophol (TOH) (13), the flux from Trp to niacin being far smaller in yeast (14). The smallness of this flux has made studies on the Trp-NAD pathway more difficult.

Smith et al. (15) developed a method to study Trp metabolism in rat hepatocytes by measuring released radioisotope from several radioisomers of Trp. Studies on the accumulation of metabolic intermediates were the keys for understanding metabolic disturbances in the B<sub>6</sub>-deficient status in pregnancy and pellagra. In this study, we strove for better understanding of regulation of niacin biosynthesis from Trp in yeast by combining the method developed by Smith et al. (15) with metabolites assays.

MATERIALS AND METHODS

Chemicals. L-Trp, L-kynurenine (KN) sulfate, kynurenic acid (KA), xanthurenic acid (XA), quinolinic acid (QA) and charcoal Norit A were obtained from Nacalai tesque. Anthranilic acid (AA), L-3-hydroxykynurenine (OHKN) and niacin were purchased from Wako Pure Chemicals, DL-kynurenine (KN) from Sigma and tryptophol (TOH) from Tokyo Kasei. Scintillation cocktail ACS II and Soluene-350 were obtained from Amersham and Packard, respectively. L-[Methylene-14C]Trp (specific radioactivity; 54 mCi/mmoll) and DL-[benzene ring-U-14C]-Trp (specific radioactivity; 84 mCi/mmoll) were purchased from Radiochemical Centre, Amersham. L-[Side chain 2,3-3H]Trp (specific radioactivity 62 Ci/mmoll) was from Commissariat à l'Énergie Atomique. Each radiolabelled Trp was purified before use by high-performance liquid chromatography (HPLC) (column, Fine pak SIL C<sub>18</sub>) with 10% methanol as the mobile phase.

Organism and growth conditions. Saccharomyces uvarum (Saccharomyces

J. Nutr. Sci. Vitaminol.
carlsbergensis) ATCC 9080 was used throughout the experiments. Stock culture was incubated at 30°C for 20 h on yeast extract-malt extract agar slants. Cells were grown in the synthetic liquid medium (16) aerobically at 30°C. Cells, in mid-logarithmic growth phase, were centrifuged, washed and resuspended with the same fresh medium to a final density of 2.5 mg dry wt. cells/ml. In each experiment, prepared cell suspensions were mixed with one fifth volume of additional substrate and incubated at 30°C for 3 h, aerobically at 110 oscillations min⁻¹.

Preparation of radiolabelled kynurenines. A male Sprague-Dawley rat, weighing 400 g, was injected intraperitoneally with 0.5 mmol of Trp. After 5.5 h, the liver was removed and homogenized with 3 volumes of 0.14 M KCl. The homogenate was centrifuged at 20,000 × g for 20 min. The supernatant was used to convert several kinds of radiolabelled Trp to the corresponding KNs. Radiolabelled Trp (about 140 nmol) previously purified by HPLC was incubated with 150 μl of the enzyme solution in the presence of phosphate buffer, pH 7.0 at 37°C for 3.5 h shaking. At the end of incubation, 30 μl of 60% HClO₄ were added. The precipitate was washed with 3% HClO₄. Combined supernatant and washings (7 ml) were applied to the column of Dowex 50W × 8 (H⁺ form, 7 × 50 mm). After successive washing with 50 ml of water, 20 ml of 1 N HCl and 30 ml of 2.5 N HCl, labelled KN was eluated with 20 ml of 5 N HCl (17). The eluates were evaporated to dryness, the residue was dissolved in a small volume of distilled water and subjected to HPLC (Fine-pack SIL C₁₈, 10% methanol). A peak corresponding to KN was subjected to paper chromatography [Toyo No. 51A filter paper, ascending, solvent system: pyridine–methanol–water, 4:1:1]. In the case of DL-[benzene ring-U-¹⁴C]-Trp, a small amount of D-KN (Rf value, 0.36) was contaminated in L-KN (Rf value, 0.45) sample. Therefore, L-[benzene ring-U-¹⁴C]KN was further purified by paper chromatography. Prepared radiolabelled KN was shown to be free of radioactive impurities. Specific radioactivity of [methylene-¹⁴C]-, [benzene ring-U-¹⁴C]-, and [2,3-³H]KN were, 1,500, 800 and 1,400 dpm/nmol, respectively.

Analyses of tryptophan, total niacin, quinolinic acid and glucose. Trp and its metabolites were extracted from broth with cold 60% ethanol twice. Combined supernatants were evaporated to dryness and dissolved in adequate volume of distilled water. When cellular distribution of metabolites was investigated, broth was separated by centrifugation. Both neutralized supernatant and the extract of cells with cold 60% ethanol were subjected to metabolite analysis. Trp was assayed by the method of Denckla and Dewey (18). The total niacin (including nicotinamide nucleotides, nicotinamide and niacin) was determined microbiologically using Lactobacillus plantarum ATCC 8014 as a test organism (19). QA was determined as niacin after decarboxylation with glacial acetic acid (20). Glucose was extracted with 0.45 N HClO₄ and assayed by the method of Bergmeyer and Bernt (21). Simultaneously, absorbance at 610 nm of broth was determined and converted into dry cell weight/ml.

Analyses of other tryptophan metabolites. Metabolites, except for TOH, were extracted from broth with 0.45 N HClO₄ and the extract was subjected to HPLC
after neutralization for analysis of KN, OHKN, OHAA, KA, and XA [column, Finepack SIL C18-5; mobile phase, 10mM phosphate buffer (pH 4.0)-methanol (19:1); 0.8 ml/min; absorbance, 360 nm]. When AA was not detected on the chromatograms, extraction with acidic ethyl acetate (85.7±1.1% efficiency) was attempted prior to HPLC to concentrate the sample. KN (17) and KA (22) were applied to the columns of Dowex 50W×8 (200-400 mesh, H+, 7×50 mm) and Dowex 1×8 (200-400 mesh, formate, 7×50 mm), respectively, prior to determination by HPLC. TOH was extracted with acidic ethyl acetate and then subjected to HPLC [column, Fine pack SIL C18-5; mobile phase, 10 mM phosphate buffer (pH 4.0)-methanol-acetonitrile (9:1:1); absorbance, 280 nm].

Determination of metabolic fates of tryptophan and kynurenine. The metabolic fluxes of Trp were estimated by the method of Smith et al. (15), developed for the study on Trp metabolism in rat hepatocytes. Similarly radiolabelled KN was shown useful for studying the metabolic fluxes of KN in yeast in our preliminary experiment. Therefore, purified radiolabelled [methylene-14C]Trp and prepared several kinds of radiolabelled KNs ([methylene-14C], [benzene ring-U-14C], and [side chain-2,3-3H]) were used to estimate metabolic fates of Trp in this investigation. Metabolic fates of Trp were estimated by assaying charcoal non-adsorbed radioactivity, 14CO2 and radioactivity incorporated into acid-insoluble fraction. Trp metabolized via the KN flux (alanine liberation) was estimated as the sum of nmoles of Trp metabolized to charcoal non-adsorbed compounds (aliphatic compound) plus nmoles of Trp metabolized to 14CO2 using L-[methylene-14C]Trp. KN metabolized via the KNase flux was estimated as the sum of nmoles of KN metabolized to charcoal non-adsorbed metabolites, nmoles of KN metabolized to 14CO2 and nmoles of alanine liberated from KN incorporated into acid-insoluble fraction. The transaminase flux (2-H liberation) was calculated from the following expression: (3H radioactivity in the acid insoluble fraction + charcoal non-adsorbed radioactivity –nmole of alanine liberated from KNx specific radioactivity of [3H]-KN)/(specific radioactivity of [3H]KN/3) (nmol). Nmoles of alanine liberated from KN were obtained from the KNase flux. The flux through the acetyl-CoA flux was estimated by using [benzene ring-U-14C]KN. 14CO2 may derive from oxidative degradation through the acetyl-CoA flux and also from one carbon liberation reaction via the QA-NAD pathway. The flux was calculated from the expression: (14CO2 dpm×6/specific radioactivity of KN) – (n mole of total niacin produced) + (charcoal non-adsorbed radioactivity + radioactivity in the acid insoluble) ÷ specific radioactivity of KN.

Extraction of enzymes and determination of their activities. After 3 h incubation, broth was centrifuged and the cells were washed with 10mM potassium phosphate buffer, pH 7.0. The cells were resuspended in the same buffer and sonicated by ultrasonic disruptor (Model UR-200P, Tomy Seiko) with glass beads (MK-2GX, 0.25–0.5 mm φ, Shinmaru Enterprises). After centrifugation at 7,000×g for 10 min, the supernatant was used to assay tryptophan transaminase (TTase) and kynurenine transaminase (KTase) activities. For KNase, 10mM potassium
phosphate buffer (pH 7.2) containing 10 μM PLP and 0.01% 2-mercaptoethanol was used as buffer for sonication and the supernatant after centrifugation for 20 min at 20,000 × g was used as crude enzyme.

TTase and KTase activities of the cell-free preparations were determined by the spectrophotometric methods described by Nakamura et al. (23). KNase activity was determined by the fluorometric method (24). One unit of the enzyme is defined as the amount of enzyme that catalyzes the conversion of 1 nmol of substrate per h. Specific activity is expressed as units per mg of protein. Protein concentrations were determined by the method of Lowry et al. (25) with bovine serum albumin as a standard.

**Results**

1. **Effects of various precursors on niacin levels**

The effects of metabolic intermediates in the Trp-NAD pathway on niacin production were shown in Fig. 1. When no intermediate was added, niacin content was about 8 nmol/ml (2.8 nmol/mg dry cells), which possibly derived from endogenous Trp synthesized from glucose. Most niacin remained in the cells, and only 2% was extracellular. The cell growth after 3 h-incubation was similar regardless of added precursors. Addition of 0.5 mM AA or Trp had no effect on niacin production. On the other hand, KN or OHAA increased niacin content two times of the control value.

2. **Cellular uptake of tryptophan or kynurenine and their intracellular pools**

To elucidate the cause of the difference in the effects of Trp and KN on niacin production, their incorporation into the cells was examined. As shown in Fig. 2, both Trp and KN were incorporated linearly into the cells, the rate of Trp incorporation being faster than that of KN. The intracellular pool of Trp was larger than that of KN. KN incorporated into the cells was quickly and linearly metabolized with time. On the other hand, incorporated Trp was sigmoidally.

Vol. 37, No. 3, 1991
Fig. 1. Effects of various precursors on niacin level. Cells were incubated aerobically with 0.5 mM each of substrate at 30°C. After 3 h-incubation, total niacin in the broth was determined and expressed as nmol/ml (■) or nmol/mg dry cells (□).

Fig. 2. Incorporation and intracellular pool of tryptophan. Cells were incubated with 0.05 mM Trp (A) or KN (B) at 30°C. At the indicated time, Trp or KN incorporated into cells was determined as shown in MATERIALS AND METHODS. □, metabolized; ■, intracellular; ●, incorporated.

metabolized with a lag of 15 min.

3. Metabolic fates of tryptophan and kynurenine

Scheme 1 outlines metabolic fates of Trp. As shown in Table 1, 54.5% of added KN was metabolized through the flux of 2-H liberation reaction. On the other hand, 20.4% of KN was metabolized via the KNase flux, which is in contrast to 1.68% in Trp. The acetyl-CoA flux was not detected with this amount of Trp or KN.

J. Nutr. Sci. Vitaminol.
Scheme 1. Metabolic fate of radioisotope from specifically radiolabelled L-kynurenine or L-tryptophan. Outlines of Trp- or KN-metabolism expected in the yeast was shown with radiolabelled substrates (Trp: \( \nabla \), L-[methylene-\(^{14}\)C]; KN: \( \circ \), L-[benzene ring-\(U\)-\(^{14}\)C]; \( \nabla \), L-[methylene-\(^{14}\)C]; \( \star \), L-[side chain-2,3-\(^{3}\)H]).

(a), the KNase flux (alanine liberation); (b), the transaminase flux (2-H liberation); (c) the acetyl-CoA flux; \( \odot \) TTase; \( \odot \) KTase; \( \odot \) KNase; \( \odot \) TPase; \( \odot \) KNOHase.

Vol. 37, No. 3, 1991
Table 1. Metabolic fates of kynurenine or tryptophan.

| Kynurenine (KN) | Fluxes (%) | Transaminase flux (2-H liberation) | Kynureninase flux (Ala liberation) | Acetyl-CoA flux |
|-----------------|------------|----------------------------------|----------------------------------|----------------|
| Metabolites     | KN         | KA                               | XA                               | OHKN           | OHAA | QA   | NA |
| 18.1            | 46.5       | 9.38                             | 1.64                             | 19.9           | 0.41 | 4.93 |
| Tryptophan (Trp)| Fluxes (%) | Kynureninase flux                | 1.68                             |                |      |      |

Cells were incubated with 0.05 mM each of radiolabelled substrate ([methylene-14C]-Trp, [methylene-14C]-KN, [benzene ring-U-14C]-KN and [2,3-3H]-KN) at 30°C under O₂. After 3h-incubation, Trp metabolized via each flux and amounts of metabolites were determined as shown in MATERIALS AND METHODS. Values were expressed as percentage to an amount of substrate added.

To investigate these fluxes in detail, metabolites were analyzed by HPLC and microbioassay (Table 1). As metabolites in the broth extract were well separated as authentic samples, pretreatment of the extract was not needed. AA was not detected, and the sum of KA and XA accounted for the amount of Trp metabolized via the 2-H liberation reaction. The sum of OHAA, QA and total niacin accounted for the amount metabolized via the KNase flux. The sum of KN metabolized via the 2-H liberation flux, the KNase flux, the amounts of residual KN and accumulated OHKN coincided well with the amount of KN added at the beginning.

4. Cellular distribution of kynurenine metabolites

Figure 3 shows amounts of metabolites present and their cellular distribution with 0.05 mM or 0.5 mM Trp and KN added. In the basal medium, only niacin and QA were detected. With 0.5 mM Trp, small amounts of KA and OHAA were found in addition to niacin and QA.

When 0.05 mM KN was added, niacin was increased to 174% of the control value and OHKN became detectable. When an amount of KN was increased 10 times, niacin level was further raised to 240% of the control and OHKN was increased 3 times as high as that with 0.05 mM KN.

KA, mostly distributed outside of the cells, increased 15 and 100 times with 0.05 mM and 0.5 mM KN added, respectively. KA added to the medium was not metabolized further and seemed to be an end product of KN. About 50% of metabolized KN was recovered as KA.

XA was found at the 3.4 and 22.1 nmol/ml level, respectively, with 0.05 and 0.5 mM KN. AA was not detected.

5. Dose responses of the kynureninase flux and kynurenine metabolites formation from tryptophan

When KN added to the medium was increased 10 times, the amount of KA
increased 6 times, and niacin or the intermediates in the Trp-NAD pathway, such as OHKN, OHAA, and QA increased only 2 to 3 fold (Fig. 3). When dose response of the KNase flux from Trp was examined, alanine liberation from Trp reached a plateau above 0.05 mM (Fig. 4). On the other hand, amounts of KN and KA continued to increase above 0.05 mM Trp. Most of the produced KN or KA was released into the culture medium.

6. Activities of enzymes related to tryptophan metabolism

To support the data shown in Figs. 3 and 4, activities of several enzymes related to Trp metabolism were measured in crude extracts of the cells. Among the three enzymes listed in Table 2, KTase was the least saturated with PLP. The maximum velocities of TTase and KTase in the presence of PLP were larger than that of KNase by one order, which favors formation of TOH and KA. When 0.5 mM Trp was added to the medium, TTase activity slightly decreased, while KTase activity increased especially in the presence of PLP. KNase activity had a tendency to decrease with Trp added, but no change with KN added. The apparent $K_m$ values for TTase (Trp) ($3.30 \times 10^{-3}$ M) and KTase (KN) ($1.11 \times 10^{-2}$ M) were much higher than the values previously reported with purified enzymes (13, 26, 27). $V_{max}$
Fig. 4. Dose response of the kynureninase flux and accumulated metabolites from tryptophan. Cells were incubated for 1 h with various concentrations of L-[methylene-14C]Trp and the KNase flux and metabolites (KN and KA) were determined as shown in MATERIALS AND METHODS. ⅋, KA; ■, KN; □, KNase flux (alanine liberation).

for KNase estimated with OHKN as a substrate was 10-fold higher than the value with KN, and the $K_m$ value for OHKN ($1.47 \times 10^{-6}$ M) was far less than that for KN ($1.84 \times 10^{-4}$ M).

7. Niacin production related to glucose in the medium

In the preceding experiments, incubation lasted for 3 h. Niacin production from tryptophan was much smaller than from other intermediates. Therefore, niacin- or tryptophol-production was investigated in a longer incubation period (Fig. 5). No difference in the cell growth was observed between the mediums with or without added tryptophan. Glucose and tryptophan were consumed linearly with time by 8 h. TOH, a main metabolite, increased in the first 3 h and reached a plateau at 5.5 h. On the other hand, total niacin in the tryptophan-added medium

| Incubation (PLP in assay) | None | 0.5 mM Trp | 0.5 mM KN | $K_m$ value (M) |
|--------------------------|------|------------|----------|----------------|
| Tryptophan transaminase  | 198  | 456        | 114      | 3.30 $\times 10^{-3}$ |
| Kynurenine transaminase  | 20.9 | 230        | 23.7     | 1.11 $\times 10^{-2}$ |
| Kynureninase (OHKN) (KN) | 32.9 | 36.4       | 27.0     | 1.47 $\times 10^{-6}$ |
|                          | 2.75 | 2.81       | 2.63     | 1.84 $\times 10^{-4}$ |

Details of assay methods were shown in MATERIALS AND METHODS. Values were expressed as specific activity (units/mg protein) in the absence or presence of PLP. KNase activity was assayed with OHKN or KN as a substrate. nd; not determined.
Fig. 5. Time course of niacin- or tryptophol-production and glucose- or tryptophan-consumption. Cells were incubated in the absence (○) or in the presence of 0.5 mM Trp (●). Details were described in MATERIALS AND METHODS. A: ——, ——, growth (OD 610 nm); ———, TOH; ——, ——, glucose; —,—; Trp. B: ——, ——, niacin level.

increased very slowly at the beginning and then at a faster rate after most of the added tryptophan and glucose were consumed. An increase in niacin was observed after 5.5 h, therefore, it must have been brought about by utilizing previously formed intermediates in the tryptophan-NAD pathway.

DISCUSSION

The existence of the Trp-NAD pathway (3, 14) and properties of a few enzymes related with this route (12, 26, 28) have been reported in aerobically grown yeast. However, the metabolic flux of Trp via this pathway and its regulation in yeast are not fully known yet. This is probably because the flux is so small that it is difficult to handle. Our results obtained using intermediates in the Trp-NAD pathway (Fig. 1) suggest that niacin synthesis from Trp might be regulated somewhere between Trp and KN.

In yeast, all amino acids, including Trp, are transported by the general amino acid transport (GAP) system (29). KN is a kind of amino acid which resembles Trp. Shetty and Gaertner (26) reported that exogenous Trp was used only slowly as a nitrogen source, indicating a low rate of Trp degradation is *Saccharomyces*
It was reported in *Neurospora crassa* (30) that externally supplied Trp and endogenously synthesized Trp had different effects. In *Saccharomyces*, the vacuole was reported to be the organelle functioning to maintain cellular homeostasis (31). The Trp pool of the yeast grown in the minimal medium is very small, a thousandth of that of alanine (32, 33). Taking these into consideration, Trp incorporated from the medium may mostly exist in the vacuole, although no such evidence has been presented.

To elucidate the cause of the difference in the effects of Trp and KN on niacin production, their incorporation into the cells was examined. The data shown in Fig. 2 clearly indicate that the difference in the effects of Trp and KN on niacin synthesis is not due to the difference in the rates of incorporation into the cells. The lag phase in Trp metabolism may be due to high $K_m$ of TOH formation (13), the main metabolic flux of Tip in the yeast.

To investigate the difference in the effects of Trp and KN on niacin production, metabolic fluxes and metabolites were studied compared to that of Tip (Table 1). The data indicated that the metabolic flow of KN via the KNase flux was much bigger than that of Trp, which explained the different effect of KN and Trp on niacin production. In rat hepatocytes, large proportion of Trp metabolized through KN flux was further metabolized through the acetyl-CoA flux to be degraded oxidatively as an energy source (15). Trp was not degraded oxidatively at all in yeast, on the contrary. Soda *et al.* (34) reported on broad substrate specificity of KTase in yeast (*Hansenulla schneggi*). Formation of KA and XA in the culture of *Saccharomyces uvarum* indicates the existence of the same enzyme in this yeast.

Metabolites of KN or Trp accumulated in the cells are considered to be direct precursors of niacin. KN and OHKN in the intracellular pool increased as KN concentration in the medium increased, which favors niacin production from KN (Figs. 1 and 3). The results indicate that the KNase flux from KN downward has the capacity of increasing niacin synthesis as long as enough KN was provided. In other words, the primary regulatory step(s) is between Trp and KN. KN or KA was mostly excreted in the medium (Fig. 3). The yeast probably may have a small intracellular pool for these metabolites. AA was not detected, which agreed with the report that in *Saccharomyces cerevisiae* Trp was converted to OHAA rather than AA by constitutive 3-hydroxy-kynureninase (26–28, 30). As shown in Figs. 3 and 4, the degree of increase in OHKN, OHAA or QA was less than that of KN or KA with Trp or KN as a precursor. However, intracellular Trp concentration of the yeast cultivated in S medium was 60 $\mu$M, at which concentration the KN flux was predominant compared to KN or KA formation (Fig. 4). Data on enzymes related to Trp metabolism explain why Trp and KN added exogenously in large amounts had tendencies to be converted to TOH and KA, rather than to be metabolized via the KNase flux.

Activity of KNOHase (catalyzes step 5 in Scheme 1) could not be detected in the crude extract, though its activity was found in the isolated mitochondria (35). As for TPase (catalyzes step 4 in Scheme 1), we also had difficulty in estimating its
TRYPTOPHAN-NIACIN METABOLISM IN YEAST

activity, as has been reported by others (3, 6).

In the previous paper (36), we reported that the niacin level of the yeast was raised by exogenously added hemin, which is known to derepress catabolite repression. Catabolite repression is known to be due to inhibition of enzymes for heme biosynthesis and biogenesis of mitochondria by glucose in yeast (37). Though both TPase and KNOHase are expected to be under the influence of catabolite repression, present results (Figs. 1–5, Table 1) point out TPase as the primary regulatory site. Further study on TPase activity in the yeast is now under way.

REFERENCES

1) Nishizuka, Y., and Hayaishi, O. (1963): Studies on the biosynthesis of nicotinamide adenine dinucleotide. I. Enzymic synthesis of niacin ribonucleotides from 3-hydroxyanthranilic acid in mammalian tissues. J. Biol. Chem., 238, 3369–3377.
2) Partridge, C. W. H., Bonner, D. M., and Yanofsky, C. (1952): A quantitative study of the relationship between tryptophan and niacin in Neurospora. J. Biol. Chem., 194, 269–278.
3) Ahmad, F., and Moat, A. G. (1966): Nicotinic acid biosynthesis in prototrophs and tryptophan auxotrophs of Saccharomyces cerevisiae. J. Biol. Chem., 241, 775–780.
4) Ortega, M. V., and Brown, G. M. (1960): Precursors of nicotinic acid in Escherichia coli. J. Biol. Chem., 235, 2939–2945.
5) Wilson, R. G., and Henderson, L. M. (1963): Tryptophan-niacin relationship in Xanthomonas pruni. J. Bacteriol., 85, 221–229.
6) Lester, G. (1971): End-product regulation of the tryptophan-nicotinic acid pathway in Neurospora crassa. J. Bacteriol., 107, 448–455.
7) Brody, S. (1972): Regulation of pyridine nucleotide levels and ratios in Neurospora crassa. J. Biol. Chem., 247, 6013–6017.
8) Brown, A. T., and Wagner, C. (1970): Regulation of enzymes involved in the conversion of tryptophan to nicotinamide adenine dinucleotide in a colorless strain of Xanthomonas pruni. J. Bacteriol., 101, 456–463.
9) Wagner, C., and Brown, A. T. (1970): Regulation of tryptophan pyrrolase activity in Xanthomonas pruni. J. Bacteriol., 104, 90–97.
10) Shimke, R. T., Sweeney, E. W., and Berlin, C. M. (1965): The roles of synthesis and degradation in the control of rat liver tryptophan pyrrolase. J. Biol. Chem., 240, 322–331.
11) Knox, W. E. (1966): The regulation of tryptophan pyrrolase activity by tryptophan. Adv. Enzyme Regul., 4, 287–297.
12) Schott, H.-H., and Staudinger, H. (1971): The regulatory function of L-kynurenine 3-hydroxylase (EC 1.14.1.2) for the biosynthesis of pyridine nucleotides in anaerobically and aerobically grown Saccharomyces cerevisiae. Hoppe-Seyler's Z. Physiol. Chem., 352, 1654–1658.
13) Kradolfer, P., Niderberger, O., and Hütter, R. (1982): Tryptophan degradation in Saccharomyces cerevisiae: Characterization of two aromatic aminotransferases. Arch. Microbiol., 133, 242–248.
14) Heilmann, H.-D., and Lingens, F. (1968): Zur Regulation der Nicotinsäure-Bio-
synthese in *Saccharomyces cerevisiae*. *Hoppe-Seyler’s Z. Physiol. Chem.*, 349, 231–236.

15) Smith, S. A., Carr, F. P. A., and Pogson, C. I. (1980): The metabolism of L-tryptophan by isolated rat liver cells. Quantification of the relative importance of, and the effect of nutritional status on, the individual pathways of tryptophan metabolism. *Biochem. J.*, 192, 673–686.

16) Rose, A. H., and Nickerson, W. J. (1956): Secretion of nicotinic acid by biotin-dependent yeasts. *J. Bacteriol.*, 72, 324–328.

17) Brown, R. R. (1957): The isolation and determination of urinary hydroxykynureninase. *J. Biol. Chem.*, 227, 649–652.

18) Denckla, W. D., and Dewey, H. K. (1967): The determination of tryptophan in plasma, liver and urine. *J. Lab. Clin. Med.*, 69, 160–168.

19) Snell, E. E., and Wright, L. D. (1941): A microbiological method for the determination of nicotinic acid. *J. Biol. Chem.*, 139, 675–686.

20) Henderson, L. M., and Hirsch, H. M. (1949): Quinolinic acid metabolism. I. Urinary excretion by the rat following tryptophan and 3-hydroxyanthranilic acid administration. *J. Biol. Chem.*, 181, 667–675.

21) Bergmeyer, H. U., and Bernt, E. (1974): Determination with glucose oxidase and peroxidase, in *Method of Enzymatic Analysis*, 2nd ed., ed. by Bergmeyer, H. U., Academic Press, New York pp. 1205–1212.

22) Price, J. M., and Dodge, L. W. (1956): Occurrence of the 8-methyl ether of xanthurenic acid in normal human urine. *J. Biol. Chem.*, 223, 699–704.

23) Nakamura, J., Noguchi, T., and Kido, R. (1973): Aromatic amino acid transaminase in rat intestine. *Biochem. J.*, 135, 815–818.

24) Tanizawa, K., and Soda, K. (1979): Comparison of inducible and constitutive kynureninases of *Neurospora crassa*. *J. Biochem.*, 85, 1367–1375.

25) Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951): Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, 193, 265–275.

26) Shetty, A. S., and Gaertner, F. H. (1973): Distinct kynureninase and hydroxykynureninase activities in microorganisms: Occurrence and properties of a single physiologically discrete enzyme in yeast. *J. Bacteriol.*, 113, 1127–1133.

27) Gaertner, F. H., and Shetty, A. S. (1975): Hydroxykynureninase and the excretion of 3-hydroxyanthranilate by yeast. *Acta Vitaminol. Enzymol.*, 29, 332–334.

28) Schott, H.-H., and Krause, U. (1979): Purification and characterization of 3-hydroxykynureninase from yeast. *Hoppe-Seyler’s Z. Physiol. Chem.*, 360, 481–488.

29) Grenson, M., Hou, M., and Crabeel, M. (1970): Multiplicity of the amino acid permeases in *Saccharomyces cerevisiae*. IV. Evidence for a general amino acid permease. *J. Bacteriol.*, 103, 770–777.

30) Matchett, W. H., and Demoss, J. A. (1964): Physiological channeling of tryptophan in *Neurospora crassa*. *Biochim. Biophys. Acta*, 86, 91–99.

31) Wiemken, A., and Duerr, M. (1974): Characterization of amino acid pools in the vacuolar compartment of *Saccharomyces cerevisiae*. *Arch. Microbiol.*, 101, 45–57.

32) Fantes, P. A., Roberts, L. M., and Huetter, R. (1976): Free tryptophan pool and tryptophan biosynthetic enzymes in *Saccharomyces cerevisiae*. *Arch. Microbiol.*, 107, 207–214.

33) Messenguy, F., Colin, D., and Have, T. J. (1980): Regulation of compartmentation of amino acid pools in *Saccharomyces cerevisiae* and its effects on metabolic control. *Eur. J. Nutr. Sci. Vitaminol.*
TRYPTOPHAN-NIACIN METABOLISM IN YEAST

34) Soda, K., Sawa, Y., and Tanizawa, K. (1980): Kynurenine aminotransferase with low substrate specificity of Hansenula schneggi, in Current Developments in Yeast Research [Proceedings of the International Yeast Symposium], 5th, ed. by Stewart et al., Russell, Inge, Pergamon Press, London, pp. 441–446.

35) Shin, M., Sano, K., and Umezawa, C. (1982): Inhibition of L-kynurenine 3-hydroxylase from Saccharomyces carlsbergensis by α-keto acid derivatives of branched chain amino acids. J. Nutr. Sci. Vitaminol., 28, 191–201.

36) Shin, M., Sano, K., and Umezawa, C. (1990): Effect of exogenous hemin on the niacin content of aerobically grown Saccharomyces uvarum. FEMS Lett., 78, 17–20.

37) Jayaraman, J., Padmanabai, G., Malathi, K., and Sharma, P. S. (1971): Haem synthesis during mitochondrogenesis in yeast Biochemical J., 121, 531–535.