Review

Organ Co-Relationship in Tryptophan Metabolism and Factors That Govern the Biosynthesis of Nicotinamide from Tryptophan

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Summary The pathway of tryptophan (Trp)-nicotinamide is very important nutritionally because a vitamin nicotinamide is biosynthesized from an amino acid Trp. Until we started studying the factors that affect the Trp-nicotinamide conversion rate, little data existed. Data obtained from TDO (Trp 2,3-dioxygenase)-KO (knock-out) mice have revealed that mice can biosynthesize a necessary amount of nicotinamide from Trp by indoleamine 2,3-dioxygenase (IDO) even when TDO is lacking. It has also been shown that 3-hydroxyanthranilic acid is a key intermediate. Urine upper metabolites such as kynurenic acid and xanthurenic acid originate from non-hepatic tissues but not from the liver. Data obtained from quinolinic acid phosphoribosyltransferase (QPRT)-KO mice indicated that the Trp→quinolinic acid conversion ratio was 6%. Urine quinolinic acid levels and the conversion ratio of Trp to nicotinamide were the same between hetero and wild mice. These findings indicate that QPRT is not the rate-limiting enzyme in the conversion. Thus, the limiting factors in the conversion of Trp to nicotinamide are the amounts of 3-hydroxyanthranilic acid and quinolinic acid in the liver and the activity of liver 3-hydroxyanthranilic acid 3,4-dioxygenase. Studies on factors have shown that conversion of Trp to nicotinamide is increased by adequate intake of good quality protein, and adequate intake of unsaturated fatty acids and starch. However, conversion was decreased by deficient niacin, vitamin B2, or vitamin B6, excessive intake of protein, saturated fatty acids, or glucose and fructose, or intake of protein with low Trp content, and insufficient mineral intake.

Key Words tryptophan, nicotinamide, amino acid, vitamin, metabolism

The tryptophan (Trp) degradation pathways can be divided into the serotonin pathway, the kynurenine (Kyn) pathway, the glutaric acid pathway, and the nicotinamide (Nam) pathway. Many biologically important compounds, such as serotonin, melatonin, Kyn, kynurenic acid (KA), quinolinic acid (QA), NAD1, and Nam are synthesized from Trp. Of these pathways, we are interested in the conversion pathway of Trp to Nam (Fig. 1). Nam is synthesized from Trp via QA and NAD+.

This review summarizes 40 y of the author’s studies into the biosynthesis of Nam from Trp. To do these studies, we had to develop many methods to measure the intermediates of the Trp-Nam pathway. Our group has developed methods to measure the following compounds in urine and blood: Trp (1), Kyn (2), anthranilic acid (AnA) (3), KA (4), 3-hydroxykynurenine (3-HK) (5), xanthurenic acid (XA) (6), 3-hydroxyanthranilic acid (3-HA) (6), Nam (7), N1-methylnicotinamide (MNA) (8), N1-methyl-2-pyridone-5-carboxamide (2-Py) (9), and N1-methyl-4-pyridone-3-carboxamide (4-Py) (9). In addition, methods have been developed for detecting: NAD+ and NADH (10) in blood; and NADP+ and NADPH (11) in blood, and serotonin (12) and 5-hydroxyindole-3-acetic acid (12) in blood and urine. Although our group did not develop the original method for detecting QA, we have measured QA using a reported method (13).

We have previously reported that the molar conversion ratio of Nam from Trp is approximately 2%, and the rate-limiting enzyme is QA phosphoribosyltransferase (EC 2.4.2.19; QPRT). It was generally accepted that this pathway mainly takes place in the liver, and not in non-hepatic tissues (14–16). However, our recent findings have revealed that non-hepatic tissues do contribute to the conversion of Nam from Trp. Here, we review the body of work revealing the whole process of Trp-Nam metabolism.

Trp 2,3-Dioxygenase (TDO)-KO Mice

TDO-KO mice do not have TDO (EC 1.13.11.11) but do have indoleamine 2,3-dioxygenase (IDO, EC 1.13.11.52) (17). We investigated how Trp metabolism is disrupted when TDO is lacking. We investigated whether or not TDO-KO mice can sustain normal body weight gain if TDO-KO mice are fed a niacin-free diet (18). Retardation of body weight gain was not observed, even in TDO-KO mice. We found that the necessary amount of Nam was synthesized from Trp by IDO in non-hepatic tissues. From our experiments with TDO-KO mice, we also
found that urinary excretion amounts of upper intermediates of the Trp-Nam pathway, such as Kyn, KA, XA, and 3-HA, were greater in TDO-KO mice than in wild mice. However, such excretions of lower intermediates, including QA and Nam, MNA, 2-Py, and 4-Py, were lower in the TDO-KO mice than in the wild mice. These results indicate that the urinary excretion of upper metabolites reflects the ability of 3-HA to form from Trp in non-hepatic tissues; however, these results do not reflect this transformation in the liver. The ability to form Nam from Trp was very low in TDO-KO mice, owing to the lack of TDO.

The relative niacin activities of exogenous Trp, Kyn, 3-HK, and 3-HA have been reported (19–21). Each compound was fed orally to niacin-deficient animals. Trp and 3-HA showed a niacin activity of 1/10 and 1/12 (molar ratio) against Nam, respectively; however, Kyn and 3-HK did not show niacin activity. These findings indicate that liver cells can incorporate Trp and 3-HA, but cannot incorporate Kyn or 3-HK. 3-HA 3,4-dioxygenase (3-HADO, EC 1.13.11.6) does not exist in non-hepatic tissues (18); hence, 3-HA synthesized from Trp by IDO in non-hepatic tissues is sent to the bloodstream where it is then taken up by the liver. The 3-HA is then metabolized to Nam in the liver. The Nam is distributed among non-hepatic tissues via the bloodstream and Nam is synthesized to NAD+ in non-hepatic tissues. The kidneys contain 3-HADO and aminocarboxymuconate-semialdehyde decarboxylase (EC 4.1.1.45; ACMSD), and the kidneys have a role in Trp degradation in the elimination of extra 3-HA formed in non-hepatic tissues; however, this is not the case for Nam biosynthesis (see Fig. 2).

**QPRT-KO Mice**

In QPRT-KO mice (22), QA is not metabolized. Therefore, QA is completely excreted into urine in QPRT-KO mice. The molar ratio of urine QA was approximately 6% of the dietary intake of Trp. This value was higher than expected and almost the same between the groups of qprt+/+ and qprt+/-, which was an unexpected result. We anticipated that urine QA would be higher in hetero mice than in wild mice, because we thought that QPRT was the rate-limiting enzyme in the conversion pathway of Trp to Nam. These results indicate that the QPRT is not the rate-limiting enzyme. The Trp→Nam conversion percentages in qprt+/+ and qprt+/- mice were 1.4% and 1.2%, respectively. These findings also...
indicate that QPRT was not the rate-limiting enzyme in the Trp→Nam conversion.

Organ Correlation of Trp-Nam Metabolism in Wild Mice

Figure 2 shows the organ correlation of Trp-Nam metabolism in wild mice (23). The first enzyme in Trp degradation by the liver is TDO. A small amount of Trp is also degraded in non-hepatic tissues. In this case, the first enzyme is IDO. We investigated the distribution of enzymes involved in the Trp-Nam metabolism. As a result, 3-HADO was found only in the liver and in small amounts in the kidneys, but was not detected in non-hepatic tissues (23). Therefore, 3-HA is an end product of the Trp degradation pathway in non-hepatic tissues. Thus, non-hepatic tissues cannot biosynthesize Nam from Trp. Notably, some 3-HA is taken up by the liver and is used as a precursor to Nam. In non-hepatic tissues, NAD is synthesized only from Nam. Upper metabolites in the Trp-Nam pathway, such as 3-HA, KA and XA are detected in urine and their origin is non-hepatic tissues.

All of the Trp in liver is catabolized to Kyn→3-HK→3-HA→ACMS→acetyl-CoA; however, approximately 6% of ACMS is spontaneously cyclized to form QA (see Fig. 2). Lower metabolites in the Trp-Nam pathway such as QA, MNA, 2-Py, and 4-Py, originate from the liver and are detectable in urine.

Enzyme That Governs the Biosynthesis of Nam from Trp

It is unclear which enzyme governs the biosynthesis of Nam from Trp. First, we investigated the relationships between Nam in urine and its catabolites and other Trp catabolites in urine (24). A significant correlation was observed between Nam in urine and its catabolites and QA in urine. Namely, the formation of Nam was found to depend on the formation of QA. Next, we examined which enzyme governs the formation of Nam from Trp in the liver. The enzyme that governs the biosynthesis of Nam from Trp was found to be 3-HADO.

Nam Formation Does Not Increase with Dietary Protein Levels

It was widely accepted that Nam formation depends on the intake of Trp levels until we reported that the con-
version ratio of Trp to Nam did not show a dependence on dietary casein levels (25). In addition, we found that only when the total nitrogen intake was constant Nam formation increase with increasing intake of Trp (26).

High Protein Food Demands Niacin itself

A high-protein diet requires niacin for normal growth of animals (27). As expected, the average body weight gain of weaning rats fed a 20%-casein diet without niacin was the same as that of rats fed niacin-containing food: however, a very high-protein diet without niacin, or a niacin-free 70% casein diet, retarded the body weight gain of weaning rats. This retardation could be recovered by feeding food including niacin itself. This result reflects a strange phenomenon, where increased dietary protein means an increased intake of Trp. Namely, increased Trp intake means increased formation of Nam. It is unclear why this behavior was observed.

To resolve this mystery, we measured all the enzymes and all of the intermediates involved in the Trp-Nam pathway. As shown in Fig. 3A (14), the sum of Nam and Nam catabolites for the 20% casein diet and 40% casein diet groups was found to be almost the same. But, the sum in the 70% casein diet group was lower when compared with the 20% and 40% casein diet groups. The reason for this was attributed to an abnormal increase of ACMSD activity in the group of 70% casein diet (Fig. 3B).

As shown in Fig. 3C, 3D, and 3E, the enzyme ACMSD exists at the branching point between the Trp-ACMS-QA pathway (leading to the formation of Nam) and the Trp-ACMS-aminomuconate-semialdehyde (AMS) pathway (leading to the formation of acetyl-CoA). The transformation of ACMS to QA is a non-enzymic reaction, and the transformation of ACMS to AMS is enzymatic, based on ACMSD. Hence, the formation of QA from ACMS is controlled by the activity of ACMSD. As shown in Fig. 3C, 3D, and 3E, increased activity of ACMSD decreases the formation of QA. Therefore, the abnormally increased ACMSD activity considerably reduces the formation of QA. As a result, the amount of Nam required cannot be supplied from Trp in the body. These results indicate that the Trp-Nam pathway has a critical role in Nam supply in rats.

Mechanism for Maintaining NAD+ Concentration

NAM phosphoribosyltransferase (NamPRT, EC 2.4.2.12) plays a critical role in maintaining normal NAD+ concentration (Fig. 4) (28–30). This enzyme is inhibited when a certain amount of NAD+ exists in the cell. Certain NAD+ concentrations inhibit the reaction from Nam to Nam mononucleotide (NMN). Under these...
conditions, surplus Nam is catabolized into MNA, 2-Py, and 4-Py, which are excreted into urine. However, as the NAD$^+$ concentration decreases, the enzyme activity of MNA oxidase also decreases; then MNA, the substrate of MNA oxidase, accumulates, and the increased MNA concentration inhibits the reaction from Nam to MNA (Fig. 5). As a result, the catabolism of Nam is suppressed, and Nam is synthesized to NMN by NamPRT. As a result, the NAD$^+$ concentration is restored.

**Actual Concentration of Nam in the Human Body**

We examined the concentration of Nam in the human body from the urinary excretion of Nam and its catabolites (31). In this human study, the subjects were young male Japanese adults, who received 75 mg of Nam or 150 mg of Nam daily over 44 wk. They ate food freely during the experimental period. Twenty-four-hour urine samples were collected periodically. Nam and its catabolites MNA, 2-Py, and 4-Py were measured. The urinary excretion levels of respective MNA, 2-Py, and 4-Py from the administration of 150 mg Nam were almost twice as high as the levels of those administered 75 mg Nam. Notably, the excretion of Nam was below the detection limit. This result indicates that humans can catabolize up to 150 mg of Nam daily.

**Diurnal Variation of Nam Catabolism**

We examined the diurnal variation of Nam catabolism from the urinary excretion of Nam and its catabolites (32). Figure 6 shows the diurnal variation in the urinary excretion of the sum of Nam catabolites, MNA+2-Py+4-Py. The points are plotted at 07:30, 10:45, 15:45, 20:00, and 26:00. The sum of the urinary output was significantly higher in samples collected from 06:30 to 08:30, from 08:30 to 13:00, and from 13:00 to 18:30 than in the samples collected from 18:30 to 21:30, and from 21:30 to 06:30 the next day. The urinary excretions reflected the surplus amount.
stable during sleep (the reaction Nam→NAD⁺ is high) and transcription might be less active. However, chromosomes are in an unstable state during the period of activity in the daytime (the reaction Nam→Nam catabolites is high) when transcription is activated. Thus, sleep might suppress aging processes, reflecting an inherent anti-aging mechanism. However, these implications are speculative.

**Increased NAD⁺ Concentration through Negation of the Mechanism for Maintaining NAD⁺ Concentration**

As mentioned previously, the cellular NAD⁺ concentration is controlled at the point of the NamPRT reaction by NAD⁺ itself. Even if a large amount of Nam is administered, most of it is treated by the catabolic pathway (see Fig. 4) and the intracellular Nam concentration does not increase. If we want to increase the NAD⁺ concentration, we should consider negating the pathway that maintains the NAD⁺ concentration. A candidate compound for negating the pathway maintaining NAD⁺ concentration is NMN.

We intraperitoneally injected 45 μmol of Nam or NMN per kg body weight into rats at 0 h (34). Urine samples were collected over defined time ranges (0–3, 3–6, 6–9, 9–12, 12–24 h) after the injection. Notably, the urinary excretion percentage was lower in the NMN group than in the Nam group at 3–6 h.

Nam is mainly catabolized to form inactive niacin compounds including MNA, 2-Py, and 4-Py (see Fig. 4), which are eliminated in the urine (34). This is because the reaction, Nam+5-phosphoribosyl-1-pyrophosphate (PRPP)+ADP→NMN+ADP+pyrophosphate+phosphate, which is catalyzed by NamPRT, is inhibited by the physiological concentration of NAD⁺. Therefore, administration of Nam does not contribute to increased turnover of NAD⁺ biosynthesis.

A proposed NAD⁺ biosynthesis pathway from NMN has been reported in Ref. 34. NMN moves into the bloodstream, where it is dephosphorylated to form Nam riboside (NR). NR is then transported to liver cells and is re-phosphorylated to form NMN. This step (NR+ADP→NMN+ATP) should be the rate-limiting step for the conversion of NR to NAD⁺. Thus, accumulation of NR should be observed in liver cells. This increased concentration of NR might inhibit the reaction of Nam→MNA. This effect should result in accelerated turnover of the salvage biosynthesis of NAD⁺, which activates the SIRT1 reaction, because SIRT1 (histone deacetylase) requires NAD⁺. Deacetylated histone molecules induce DNA silencing, contributing to anti-aging and longevity.

**Amino Acid Compositions Significantly Affect the Biosynthetic Pathway from Trp to Nam**

The conversion percentages of Trp-Nam were compared for rats fed a zein diet and a casein diet (35). The conversation percentage of the casein diet was approximately 2%. The addition of zein reduced the conversion percentage to approximately 0.5%, and further addition
The effects of amino acid addition on the conversion ratio of Trp-Nam in rats were investigated (36). We found that when Trp was the first limiting amino acid, the Trp-Nam conversion ratio showed the greatest decrease (to approximately 0.2%). However, the reduced conversion ratio was completely recovered by the addition of Trp.

Factors That Influence the Conversion Rate of Trp-Nam in Rats

Table 1 is a summary of factors that influence the conversion rate of Trp-Nam in rats. These reviews have been published (14–16, 37).

Conclusion

The formation of QA is catalyzed by 3-HADO but this enzyme activity is higher than that of other enzymes involved in the Trp degradation pathway (25). Hence, it had been thought that 3-HADO is not the rate-limiting enzyme in the Trp-Nam conversion pathway. Nevertheless, our finding suggests that the rate-limiting step was the transformation of 3-HA→QA (24). The supply of 3-HA from non-hepatic tissues to the liver might have a key role. We previously thought that the supply of 3-HA was lower in non-hepatic tissues than in the liver. However, the amount was much higher than expected. Thus, IDO in non-hepatic tissues plays a key role in the supply of 3-HA. An uptake transporter of 3-HA in the liver membrane might also have a critical role. The transformation of QA from 3-HA is catalyzed by 3-HADO. Hence, a better understanding of organ-organ relationships might elucidate the whole metabolism of Trp. In addition, the effects of 3-HADO should be studied in greater detail.

The key point to increase the NAD$^+$ concentration is...
the formation of QA. Its formation is subject to the two enzymes, 3-HADO and ACMSD in the liver. Thus, if we can find some compounds that control the two reactions, we would be able to freely change the cellular NAD\(^+\) concentration.

**Future Direction**

In the conversion pathway of Trp-Nam, the reactions of 3-HA→ACMS, ACMS→QA, ACMS→AMS, AMS→ciocinic acid (PiA), and AMS→2-aminoenomic acid are very interesting and important ones. The reactions of 3-HA→ACMS, ACMS→AMS, and AMS→2-aminoenomic acid are catalyzed by 3-HADO, ACMSD, and AMS reductase, respectively. But, we have not yet succeeded in the in vitro synthesis of 2-aminoenomic acid from 3-HA. It is because ACMS and AMS are very unstable compounds. ACMS spontaneously cyclizes to be QA, and AMS also to be PiA. If we can develop a method for measuring QA, PiA, and 2-aminoenomic acid, we can speculate the fate of 3-HA by in vitro experiments. To perform such experiments, we must develop the respective measurement methods for QA, PiA, 2-aminoenomic acid, and AMS reductase (EC 1.2.1.32) (Fig. 7). If someone can succeed in developing these methods, it will be possible to infer the fate of 3-HA in the body by in vitro experiments.

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