Specific Basal Bioconversion (Biochemical Conversion) related to Nicotinamide Methylation (Metabolic Process) found in the Hepatocytes of Rat and Mouse regarding Ehrlich Ascites Tumor Host

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Specific Basal Bioconversion (Biochemical Conversion) related to Nicotinamide Methylation (Metabolic Process) found in the Hepatocytes of Rat and Mouse regarding Ehrlich Ascites Tumor Host

by

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Introduction

The studies related to nicotinamide methyltransferase in rats and mice bearing ascites tumor that Ehrlich was transplanted intraperitoneally were carried on and published obtaining various results[1)~11)]. This experiment which could no avoid to detect the metabolic bio-conversion in the liver of tumor host was thought as so much important basal one with regard to the above described with regard to the above-described study.

The activities of N1-methylnicotinamide(MNA) oxidizing enzymes which have catalyzed the conversion of MNA to N1-methyl-2-pyridone-5-carboxamide(2-PY) and N1-methyl-4-pyridone-5-carboxamide(4-PY),have been found exclusively in the livers of mammals[12)~17)].

The fractions included MNA oxidizing activities (MNAOAs) were revealed in the livers of male Wistar strain rats using DEAE-cellulose column chromatography[18), 19)]. Fraction I was responsible for the conversion of MNA to 2-PY, and identified as a xanthine oxidase(XO) because of the inhibition of the activity by allopurinol. Fraction II catalyzed the simultaneous conversion of MNA to the two pyridones, 2-PY and 4-PY, with preference for 4-PY to 2-PY. Fraction III catalyzed the conversion to 4-PY only. Fraction II and III were considered as MNA oxidase I (MNAOI) and MNA oxidase II (MNAO II) respectively. For the assay of aldehyde oxidase (AO), MNA was used as the substrate like other aldehydes, acetaldehyde and benzaldehyde, to detect the broad spectrum of the substrate specificity of the enzyme.

The livers of many animals contained MNA oxidizing activities(MNAOAs) with a variety of ratios of the formation of 2-PY and 4-PY[15)]. The variation in the level of MNAOAs of these livers from three strains of rats and the existence of MNAO II in connection with aldehyde-oxidizing activities were determined as one of the objects of this experiment.

This experiment was performed at Chiba University School of Medicine and Osaka University School of Medicine parially.

Experimental and results

Chemicals: 1-[Me-^{14}C] methylnicotinamide (M-^{14}C MNA, Cl form, 5.74 mCi/n mol), MNA Cl, and 2-hydroxypyrimidine HCl were purchased from New England Nuclear, Tokyo Kasei Kogyo Co., and Nakarai Chemicals Co. respectively. Propion aldehyde purchased from Wako Pure Chemicals Co. was redistilled, and its 1 M solution was stored at -20℃ until use. 2-PY was synthesized from MNA by alkaline ferricyanide oxidation. 4-PY was isolated from urine of male CD strain rats by the modified method[21), 22)] of Chang et al.

Animals: Male Wistar rats (18-23 week-old),

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male Donryu strain rats(8 week-old), and male CD(Spraque-Dawley) strain rats(30 week-old) were obtained from Nihon Rat Co. and Charles River Japan Inc. Inbred male A/J strain mice (19 week-old) and male ICR strain mice (23 week-old) wee purchased from Shizuoka Ag. Coop. Asso. and CLEA Japan Inc.

Enzyme preparations: Animals wee sacrificed (at 9:30, each case) under ether-anesthesia. The livers were removed, weighed and homogenized in 3 volumes of either 50 mM Tris-HCl buffer(pH 8.0) or 3 mM sodium pyrophosphate-HCl buffer (pH 8.0), containing 1 mM PMSF, 0.1 mM EDTA, and 1% isopropanol with a teflon-glass homogenizer. After the homogenates were centrifuged at 10500G for 60 min at 4℃, the supernatants were used as the enzyme preparation.

DEAE-cellulose column chromatography: 10 ml of the enzyme preparation was applied to a column(30 x 1 cm) of DEAE-cellulose(Brown, 0.9 meq/g) which had been equilibrated in advance with either of the buffers used in enzyme preparation. After washing with a sufficient amount of the buffer, namely 10 volumes of the bed-volume of the column, the elution was performed with a concave gradient of NaCl concentration from 0 to 0.3 M in 400 ml of the buffer, with fractionation of each 5 ml of the eluate every 12 min.

Determination of MNA oxidizing activity (MNAOA): The assay mixture, according to the slight modified method described before[18], contained 1.4 mM M-^{14}C MNA(0.4 μ Ci), 0.001% EDTA, 1.25 mg/ml BSA, and 75.4 mM potassium phosphate buffer(pH 7.8), and was added to the enzyme preparation in a final volume of 50 μl. After incubation at 25℃ for an appropriate time, the mixture was boiled for 2 min and centrifuged. 20 μl of the mixture was applied to Whatman No.1 filter paper, developed in H_2O saturated n-butanol and again 1 N HCl saturated n-butanol for 24 h. The radioactivities of the areas on the paper corresponding to 2-PY and 4-PY were determined in toluene based scintillater cocktail with a liquid scintillation counter.

Determination of aldehyde oxidizing activity (AOA): The assay mixture was prepared with the slight modified method[22] of Krenitsaky et al., containing 1 mM potassium ferricyanide as an electron acceptor, 0.13 mM EDTA, one of various substances such as benzaldehyde, propionaldehyde and 2-hydroxypyrimidine as the substrate at concentration of 20 mM, the enzyme fraction and 25 mM sodium pyrophosphate-HCl buffer(pH 8.0) in a total volume of 1 ml. After incubation at 37℃ for 30 min, 0.1 ml of 1 N HCl was added to the mixture. The absorbance of the supernatant was determined at 420 nm. Activity was represented as m mol of reduced ferricyanide/h/ml of the enzyme fraction.

Determination of xanthine oxidase activity (XOA): XOA was determined by uric acid formation at 25℃ for 25 min from 60 μM xanthine in 0.1 M Tris-HCl buffer(pH 8.1) in a total volume of 1 ml, with determing the absorbance at 292 nm by the method[23] of Stripe et al. Protein concentrations were determined by the method[24] of Lowry et al.

MNAOAs in the hepatocytosol homogenate;

1) Wistar, Donryu and CD strain rats: as shown in Table 1, all three strain rats showed the lower pyridone forming activities with ratios between 0.3-1.1 of 2-PY and 4-PY at fixed ages. Wistar and Donryu strain rats showed the larger variation in the level of both pyridone forming activities(S.D.: 50% of average in each activity) than CD strain rats(S. D. 5%). About Wistar strain rats, both pyridone forming activities which were at low frequently showed one reasonable correction. Among the three enzyme fractions of hepatocytosol included MNAOA, allopurinol at the concentration of

0.5mM inhibited completely XOA, but a little the MNAO I and not at all the MNAO II. MNAO I catalyzed 2-PY/4-PY formation at a ratio of (71.0+2.3)/(29.0+3.4) in almost all of the rats, but MNAOAs were different. Thus, the activities of XO(represented by 2-PY formation), MNAO I(represented by 2-PY formation) and MNAO II(represented by 4-PY formation) were calculated by both pyridone forming activities determined with or without allopurinol in 16 cases of rats. XOA and MNAO IA were calculated against MNAO IIA about each rat. Although M NAO II As showed 0-3.56 nmol/h/mg protein, XOAs were almost constant(0.40-0.05 nmol/h/mg protein). The correlation between 2-PY and 4-PY forming activities in Donryu strain rats showed the almost same range of the activities seen in Wistar strain rats. In CD rats of 8 weeks, the lower activities were shown than in Wistar strain rats.

2) Mice: as shown in Table 1, A/J strain mice were higher (20 times) than Wistar strain rats about both pyridone forming activities without the large variations seen in Wistar strain rats. Both activities of ICR strain mice were about 3 times higher than those of A/J strain mice with the prominent variations. The ratio of 2-PY to 4-PY formation in both strain rats was as low as that of Wistar strain rats.

Table 1. Nl-methylnicotinamide oxidizing activities in the hepatocytosol of rats and mice

Species	strain	number of animals	oxidizing activities (nmole/h/mg protein)		2-PY/4-PY
			2−PY	4−PY	
Rat	Wistar	30	1.1+0.5#	1.0+0.4	1.1
	Donryu	5	1.9+1.0	5.5+4.4	0.4
	CD	5	0.4+0.02	1.2+0.06	0.6
Mouse	A/J	4	24.3+1.7	18.7+1.4	1.4
	ICR	4	68.3+16.1	49.2+12.1	1.4

#: means ± S.D.

MNAOAs in DEAE-cellulose column chromatography of the hepatocytosol;

1) CD strain rats: as shown in Fig. 1, three MNA oxidizing enzyme fractions contained XO, MNAO I, and MNAO II were eluted from DEAE-cellulose column by 50mM Tris-HCl buffer(pH 8.0) at the concentration of NaCl of 0.03, 0.075, and 0.15M respectively[19], as the previous reported case of Wistar strain rats. XOA was defined by using xanthine.

Fig. 1: MNA oxidizing activities of DEAE-cellulose column chromatography of the hepatocytosol from a male CD strain rat. 10ml of enzyme solution prepared in 50 ml Tris-HCl buffer(pH 8.0) was applied to the column. 2-PY(○-○), 4-PY(●-●) forming activities were determined. −•−•− : NaCl concn., ---: absorbance at 280 nm

2) Mice: MNAOAs of A/J strain mice in DEAE-cellulose chromatography performed by 3 mM sodium pyrophosphate-HCl buffer(pH 8.0) was shown in Fig. 2.

Fig. 2: MNA oxidizing activities of DEAE-cellulose column chromatography of the hepatocytosol from a male A/J strain mouse. 12ml of enzyme solution prepared in 3 mM sodium pyrophosphate-HCl buffer(pH 8.0) was precipitated by adding ammonium sulfate up to 50% saturation level, desalted by filtration through Sephadex G-25 gel, and applied to the column. 2-PY(○-○) and 4-PY(●-●) forming, and aldehyde(benzaldehyde and propionaldehyde) oxidizing activities were determined. Among aldehyde, propionaldehyde(▲-▲) oxidizing activities were determined. −•−•−: NaCl concn., ---: absorbance at 280 nm

In contrast to the case of rats, XO which catalyzed only 2-PY formation was confirmed by XO assay.

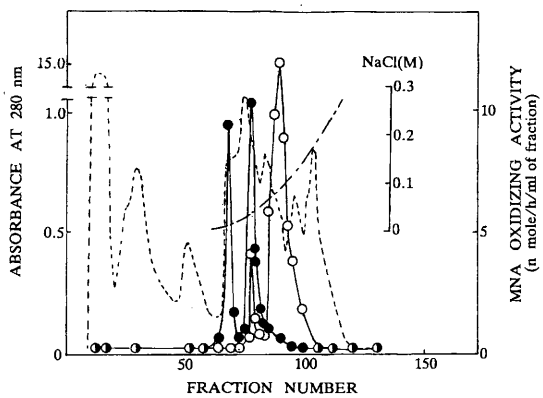

Fig.1. N_1-methylnicotinsmide Oxidizing activity of CD strain rat

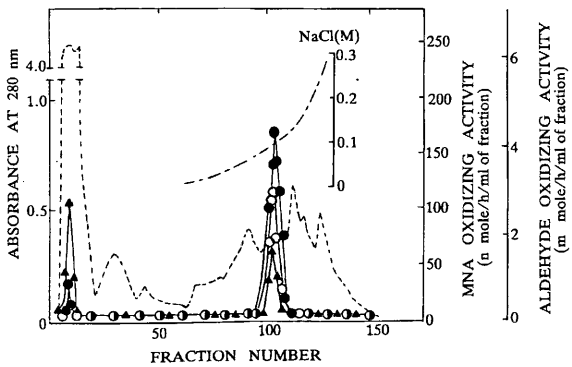

Fig.2. N_1-methylnicotinsmide·aldehyde oxidizing of male A/J strain mouse

The second MNAOA was shown in the fraction eluted by 0.1 M NaCl under the condition that the formation ratio of 2-PY to 4-PY was about 1.3 to 1. And this fraction showed aldehyde oxidase activity(AOA) for oxidizing propionaldehyde and benzaldehyde, but no XOA. Then, the enzyme of the second fraction was defined as MNAOI. On the other hand, XO fraction catalyzed the oxidation of these aldehydes.

Also, the third MNAOA which corresponded to MNAO II of the hepatocytosol was not found at all, although the ratio of both pyridones formation by the hepatocytosol was as low as Wistar strain rats(Table 1). In this column chromatography, sodium pyrophosphate-HCl buffer was used for elution in order to detect the AOA instead of the aldehyde trapping Tris-HCl buffer[25]. However, the enzyme activities eluted with the two buffers were the same profile.

The ICR strain mice showed a similar profile of MNAOAs in DEAE-cellulose column chromatography to A/J strain mice, and also did not show the third MNAOA.

Discussion

It was revealed in this study that the rat livers showed three enzyme fractions catalyzing the oxidation of Nl-methylnicotinamide(MNA); xanthine oxidase(XO), MNA oxidase I (MNAO I), and MNA oxidase II (MNAO II). Among the rats of three strains examined, Wistar and Donryu strains showed the large variation between the levels of MNAO I and MNAO II activities, while the level of XO activity(XOA) was rather homogenous. It was reported that the activities of 2-PY and 4-PY formation from MNA in the livers of inbred mice were homogenous and genetically controlled[26), 27)]. The genetical polymorphism in rat liver has been suggested[28)]. Wistar and Donryu strain rats examined in the present study were maintained in a closed colony, and were able o assume the demonstration of genetical polymorphism in their activities. Also, CD strain rat was kept in a closed colony but showed a restrained activity, suggesting its genetic homogeneity. The result that the levels of activities of MNAO I and MNAO II varied in the compared experiment might point the genes of these two enzymes belonged to the same gene family. After these alkylating agents such as methymethane sulfonate or methylnitrosourea were administered, the increased excretion of 2-PY in urine was observed

in rats.

This increase might reflect the increase of MNA oxidizing activity of the liver, since the oxidizing activities were found exclusively in the liver. So, for the investigation of changes of the activities, CD strain rats were more suitable than Wistar strain rats in spite of the fact that their activities were relatively low. In mice, these activities seemed to increase the maturation, and could be partially explained by the effect of androgenic hormone. In fact, female or castrated mice showed lower activities than male mice. In CD strain rats, the activities were observed to increase steadily after birth. In Wistar strain rats, the activities were not detectable just before and after birth. Thus, it could be considered in Wistar strain rats that the variation of activities might be less.

The ratio of 2-PY to 4-PY formation by the hepatocytosol was observed in mice(C57BL/6 and DBA/2 strains) at 1.5 and 2.2[15] respectively. This result was quite compatible with the data in Table 1. Mice having low ratio of pyridone formation were expected to contain the MNAO II fraction which catalyzed the formation of 4-PY only.

In order to detect aldehyde oxidase activity (AOA) in the fractions obtained using DEAE-cellulose column chromatography, sodium pyrophosphate-HCl butter was used rather than Tris-HCl buffer owing o its effective aldehyde bonding property. AOA was detected in XO and MNAO fractions but not other fractions in DEAE-cellulose column chromatography of the hepatocytosol from mouse. Among the various substances so far examined, the slight oxidation of benzaldehyde catalyzed by MNAO II of the CD strain rats was detected using ferricyanide as an electron acceptor. The detection of oxidation of aldehyde might depend on the affinity of the enzyme for electron acceptors. In polyacrylamide disc gel electrophoresis, MNAO II A as well as MNAO I A were detected by incubation of the gel with MNA and nitro blue tetrazolium[18]. MNAO II might be equal molecular weight but be immunologically different from MNAO I [18]. Moreover, 4-PY formating activity(mostly derived from MNAO II) was more strongly inhibited by the alkylating agent, N-methyl-N'-nitro-N-nitrosoguanidine, than 2-PY forming activity in the liver of Wistar strain rat. During the dietary administration of hepatocarcinogen 2-acetylaminofluorene, the compensating stimulation of MNAO I A with the decrease of MNAO II A was revealed[19]. It is thus of particular interest to study on the characteristic substrate of MNAO II.

It is proving to investigate the human liver for the possible existence of MNAO II A having a great interest.

Summary

This study was performed for the basal experiment with regard to nicotinamide methylation in the livers of Ehrlich ascites tumor hosts, rats and mice, under administration of nicotinamide at the first step.

Male Donryu and CD(Spraque-Dawley) strain rats showed three enzyme fractions catalyzing the oxidation of N1-methylnicotinamide(MNA); xanthine oxidase(XO), MNA oxidase I (MNAO I) and MNAO II, as Wistar strain rats in DEAE-cellulose chromatography of the hepatocytosol. The aldehyde oxidase activity determined by the usage of benzaldehyde, propionaldehyde or 2-hydroxypyrimidine as the substrate was detected in XO and MNAO fractions only, but its activity could not be detected in any other fractions. Wistar and Donryu strain rats showed larger variations than CD strain rats in the levels of MNAO I and MNAO II activities.

The ratios of 2-PY to 4-PY formation in mice was low(1.4) like rats(0.3-1.1).

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By the limitation of page, the descriptions of Experimental and others should be cut or abbreviated. But this publication could not be cut by the obligation of scientific funds.

エールリッヒ腹水腫瘍宿主に関する鼠と二十日鼠の肝細胞におけるニコチンアミドメチル化（代謝過程）についての特定な基礎的生変換（生化学的変換）　（英文）

堀津　圭佑

（平成９年10月２日受理）

本実験は第１段階において，ニコチンアミド（ＮＡ）投与下のエールリッヒ腹水腫瘍宿主，ラツテとマウス，の肝におけるニコチンアミドメチル化に関する基礎的実験のためになされた．

肝細胞液のDEAE—セルローズクロマトにおけるWistar系統ラッテと同様に雄性 Donryu とCD系統のラッテはキサンチンオキシダーゼ〔酸化酵素（XO)〕，メチル（M）ＮＡオキシダーゼＩ〔酸化酵素（MNAOＩ)〕，アルデヒドオキシダーゼ〔酸化酵素〕，MNAオキシダーゼⅡ〔酸化酵素（MNAOⅡ)〕，すなわち，MNAの酸化を触媒する３酵素区分を示した．基質としてベンツアルデヒド，プロピオンアルデヒドまたは２-ハイドロキシピリミジンの使用により測定されたアルデヒドオキシダーゼ活性はXOとMNAO区分だけで検出され，他の３区分には検出されなかった．WistarとDonryu系統のラッテはMNAOＩとMNAOⅡ活性の準位について，ＣＤ系統ラッテより大きい変動が示された．マウスにおける２-ＰＹの４-ＰＹ生成比はラッテ（0.3〜1.1）のように低く（1.4）かった．