QUINOLINIC ACID: A PRECURSOR TO NICOTINAMIDE ADENINE DINUCLEOTIDE IN *Escherichia coli**

Anthony J. Andreoli+, Masayuki Ikeda
Yasutomi Nishizuka and Osamu Hayaishi

Department of Medical Chemistry
Kyoto University Faculty of Medicine
Kyoto, Japan

Received May 22, 1963

Nicotinamide adenine dinucleotide (NAD) biosynthesis from niacin *via* niacin ribonucleotide and niacin adenine dinucleotide (deamido-NAD) has been demonstrated in mammalian tissue, yeast and *Escherichia coli* (Preiss and Handler 1958; Imsande, 1961). Several studies have clearly established tryptophan and its metabolites as precursors of niacin in mammals and *Neurospora* (Heidelberger, Abraham and Lepkovsky, 1949; Partridge, Bonner and Yanofsky, 1952). In addition, evidence was provided recently that quinolinic acid is a key intermediate in the conversion of tryptophan to niacin ribonucleotide in rat liver (Nishizuka and Hayaishi, 1963). However, *Xanthomonas pruni* appears to be the only bacterial species capable of forming niacin from tryptophan (Davis, Henderson and Powell, 1951). In contrast, it has been concluded that in *E. coli*, tryptophan, quinolinic acid and other

* This investigation was supported in part by research grants from the National Institutes of Health (C-4222), the Rockefeller Foundation, the Jane Coffin Childs Memorial Fund for Medical Research, the Squibb Institute for Medical Research, and the Scientific Research Fund of the Ministry of Education of Japan.

+ Special Fellow, National Institutes of Health, U. S. Public Health Service. Permanent address: Los Angeles State College, Los Angeles, California.

tryptophan-metabolites do not serve as precursors to niacin (Yanofsky, 1954; Ortega and Brown, 1960). Radioisotope studies indicate that a high rate of niacin biosynthesis is achieved when adenine, ribose, a 4-carbon dicarboxylic acid, e.g. succinic acid, and glycerol are offered to resting cells of E. coli (Ortega and Brown, 1960). Their results suggest that in E. coli quinolinic acid biosynthesis occurs and as demonstrated in rat liver, may also serve as a key intermediate in NAD synthesis.

This communication reports the 5-phosphoribosyl-1-pyrophosphate (PRPP)-dependent conversion of quinolinic acid to niacin ribonucleotide by an enzyme preparation from E. coli, K-12.

Cells were grown in a salts-acetate medium, supplemented with mono-sodium glutamate (0.2 %) and yeast extract (0.02 %) (Smith and Gunsalus, 1957), for 16 hours at 25° with constant aeration. The frozen cells (5 g) were suspended in 20 ml of 0.05 M phosphate buffer, pH 6.8, disrupted by sonic oscillation and the preparation centrifuged at 20,000 x g for 30 minutes at 2°. The cell-free supernatant recovered was used after treatment either with charcoal (5 - 10 % w/v) or with protamine sulfate and ammonium sulfate fractionation.

A reaction mixture (1.0 ml) containing 1.1 μmoles of quinolinic acid-C^{14} 1/ (2.45×10^5 c.p.m.), 20 μmoles of PRPP, 20 μmoles of $MgCl_2$, 200 μmoles of phosphate buffer, pH 6.8 and 0.5 ml of the charcoal-treated extract was incubated for 3 hours at 30°. In the presence of PRPP a total of 11,050 c.p.m. of $C^{14}O_2$ were evolved and with PRPP omitted (control), no $C^{14}O_2$ was detected. The reaction mixture was resolved by Dowex 1-formate column chromatography and radioactive products were located in the niacin (50,220 c.p.m.), niacin ribonucleotide (19,700 c.p.m.), deamido-NAD

1/ Quinolinic acid, uniformly labeled with C^{14} except for the β-carboxyl carbon, was prepared as previously described (Nishizuka and Hayaishi, 1963).

(1,330 c.p.m.), and NAD (5,580 c.p.m.) fractions. The products were further identified by paper chromatography, in the presence of authentic samples, with three solvent systems and high-voltage paper electrophoresis[2]. The niacin recovered was also repeatedly recrystallized to constant specific activity[3].

In marked contrast to the results obtained with the rat liver system (Nishizuka and Hayaishi, 1963), significant levels of free niacin were consistently detected among the products derived from quinolinic acid by the E. coli preparation. A time-course experiment was conducted in which the formation of niacin and niacin nucleotides from quinolinic acid was demonstrated (Fig. 1) by microbiological assay (Leuconostoc mesenteroides ATCC 9135)[4].

As shown in Fig. 1, enzymic $C^{14}O_2$ evolution parallels the formation of niacin nucleotides, while the accumulation of free niacin occurs at a somewhat lower rate[5].

In order to establish the role of niacin in the conversion of quinolinic acid to NAD, i.e. whether or not niacin is a free intermediate, experiments were conducted utilizing niacin and niacin ribonucleotide as cosubstrates of quinolinic acid (metabolic traps) and a partially purified enzyme preparation.

The data in Table I show that in the presence of niacin ribonucleotide (reaction 3) a significant increase in the accumulation of radioactive niacin ribonucleotide and concomitant decrease in

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2/ Ascending paper chromatography and high-voltage electrophoresis were carried out as previously described (Nishizuka and Hayaishi, 1963).

3/ Niacin was recrystallized five times from water and ethanol to constant specific activity, 175, 173, 170, 171, 171 c.p.m./mg and melting point, 233°.

4/ We are indebted to Drs. F. Tanaka and M. Nakamura, Research Laboratories, Takeda Chemical Industries, Ltd. for conducting the assay.

5/ Leuconostoc mesenteroides was unable to utilize niacin ribonucleotide and deamido-NAD as a growth factor.

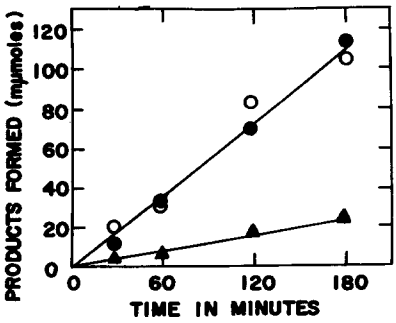

Fig. 1. Each reaction mixture (2.0 ml) contained: quinolinic acid-C^{14} (1.6 x 104 c.p.m.), 0.4 μmole; PRPP, 4 μmoles; $MgCl_2$, 10 μmoles; phosphate buffer, pH 6.8, 200 μmoles; charcoal-treated extract, 0.5 ml. The reactions were incubated for the intervals indicated at 30° and terminated by the addition of perchloric acid. One-half of each deproteinized reaction mixture was then hydrolyzed (in 0.1 N NaOH, 100°, 30 minutes), the second-half remained untreated. The niacin present in each sample was determined by microbiological assay with Leuconostoc mesenteroides ATCC 9135. (●——●), $C^{14}O_2$; (○——○) niacin, after alkaline hydrolysis; (▲——▲), niacin, before alkaline hydrolysis. In a control reaction mixture (PRPP omitted) no $C^{14}O_2$ was evolved nor were niacin nor niacin nucleotides formed. The amount of $C^{14}O_2$ evolved was calculated from the specific activity of the quinolinic acid. The values were not corrected for self-absorption.

niacin was observed. In contrast, the presence of niacin as cosubstrate did not decrease the level of niacin ribonucleotide-C^{14} or $C^{14}O_2$. It is also evident that niacin ribonucleotide-C^{14} was not formed to a significant extent from niacin-C^{14}, either by exchange (reaction 4) or in the presence of PRPP (reaction 5). No radioactive deamido-NAD or NAD was formed. These results clearly indicate that niacin is not an intermediate in the PRPP-dependent conversion of quinolinic acid to niacin ribonucleotide. It should also be noted, that the conversion of niacin to the ribonucleotide by E. coli is both PRPP and ATP dependent (Imsande, 1961) while niacin ribonucleotide formation from quinolinic acid is ATP independent.

Since niacin is not an intermediate in the conversion of quinolinic acid to NAD in E. coli, it is quite possible that formation of a nucleotide derivative, e.g. quinolinic acid ribonucleotide, occurs prior to decarboxylation.

Table I.

Enzymic Products from Quinolinic Acid

The complete reaction mixture (1.0 ml) contained: either quinolinic acid-C^{14} (2.45×10^5 c.p.m.), 1 µmole (series A) or niacin-C^{14} (2.56×10^5 c.p.m.), 1 µmole (series B); PRPP, 4 µmoles; phosphate buffer, pH 6.8, 200 µmoles; $MgCl_2$, 10 µmoles; $(NH_4)_2SO_4$ fraction (40 - 60 % saturation) of the charcoal and protamine sulfate (0.3 %)-treated extract, 0.4 ml (10.8 mg protein), and where indicated, 4 µmoles of niacin and 3 µmoles of niacin ribonucleotide.

	Additions	C^{14}-Labeled products recovered (c.p.m.)			
		CO_2*	niacin	niacin ribonucleotide	quinolinic acid
Series A					
1	none	10,050	9,770	36,700	169,500
2	niacin	9,760	10,260	39,750	162,900
3	niacin ribonucleotide	9,510	385	50,790	158,400
Series B					
4	niacin ribonucleotide, PRPP omitted	—	246,500	300	—
5	none	—	249,200	50	—

* Radioactive CO_2 was trapped in alkali and counted as thin samples. The values were not corrected for self-absorption.

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