DEAMIDATION OF NICOTINAMIDE AND NMN*

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Deamidation of nicotinamide was first demonstrated in lactic acid bacteria by Hughes and Williamson (1953). Rajagopalan et al. (1958) were the first to report the presence of this enzyme nicotinamide deamidase in vertebrates. Among the vertebrates studied, the enzyme activity was exhibited only by the avian species. The present communication deals with, 1) a detailed survey of distribution of nicotinamide deamidases in avain species, and 2) for the first time, deamidation of nicotinamide at nucleotide level by mouse liver. Further, a possible biological role for deamidases was suggested.

Experimental details were similar to that reported by Rajagopalan et al. (1959).

Results and Discussion:

The results presented in Table I indicated that the deamidase activity was found to be present in the tissues of all avian species studied. There was, however, a specific distribution of deamidase in the tissues of various avian species.

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* Abbreviations: NMN: Nicotinamide mononucleotide; NAMN: Nicotinic acid mononucleotide; DPN: Diphosphopyridine nucleotide; Desamido DPN: Desamido diphosphopyridine nucleotide; TCA: Trichloroacetic acid.

Table I

Distribution of nicotinamide deamidase in avian and mammalian species

(Incubation mixture consisted of 800 µg of nicotinamide in 0.3 ml. 1 ml. of 10% enzyme extract in 0.1 M phosphate buffer pH 7.5 (corresponding to 100 mg. fresh tissue) and 0.1 M phosphate buffer of pH 7.5 in a final volume of 2 ml. After 1 hr. incubation at 37° the reaction was arrested by keeping the tubes in a boiling water bath for 5 min. Nicotinic acid released was assayed by the method described by Rajagopalan et al. (1959)).

Species	Nicotinic acid released (µgm.)	
	Liver	Kidney
Dove	400	200
Pigeon*	252	760
Parrot	30	400
Duck	Nil	400
Turkey	Nil	400
Chick*	0.7	219
Bat	Nil	Nil
Mouse	Nil	Nil
Monkey	Nil	Nil

* Literature values (Rajagopalan et al., 1958).

Thus the enzyme was found to be present both in the kidney as well as the liver of dove, pigeon and parrot. In the case of duck, turkey and chick only the kidney possessed the enzyme activity, while the liver exhibited either feeble or negligible activity. Mammalian species studied including bat, a flying mammal, did not exhibit any deamidase activity either in the liver or kidney.

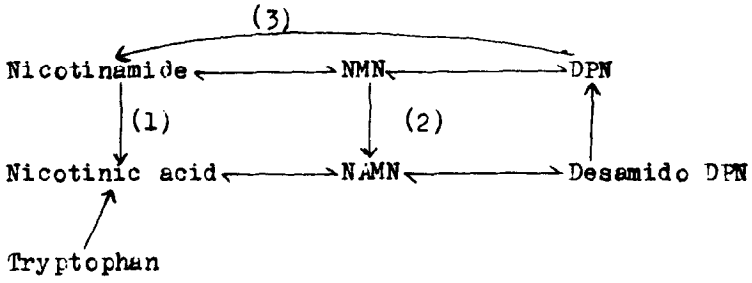
Langan et al. (1959) observed enhanced levels of desamido DPN in the liver of mouse, when administered intraperitoneally extraphysiological doses of nicotinamide. Further, they reported that this increase in desamido DPN

content was not due to the degradation of DPN. In the course of our survey of distribution of deamidases, it was observed that mouse liver did not exhibit any nicotinamide deamidase activity. These observations strongly suggested that the deamidation might occur at nucleoside or nucleotide level. Therefore, experiments were carried out to demonstrate the NMN-deamidase activity in mouse liver. A 10% homogenate of mouse liver in 0.1 M phosphate buffer of pH 7.5 was prepared using Potter-Elvehjem type Teflon homogenizer and the homogenate was centrifuged to remove cell debris and unbroken cells. The turbid supernatant was used as the enzyme source. The incubation mixture consisted of 200 µgm. of NMN (Sigma product) in 0.2 ml., 0.5 ml. of enzyme and 0.1 M phosphate buffer in a final volume of 1 ml. The reaction mixture was incubated for 30 min. at 37°C. The reaction was arrested by the addition of 0.1 ml. of 20% TCA and was centrifuged to sediment the protein. The reaction was followed by 1) measuring the optical density of the cyanide complex at 325 mµ or 2) by estimating the residual NMN by the fluorimetric method (Levitas et al., 1947). Further a peak at 315 mµ characteristic of NAMN was observed. From the results obtained it was observed that as much as 50% of NMN was destroyed in 30 minutes by 100 mg. of fresh tissue. In a duplicate experiment the TCA was removed from the incubation mixture by ether extraction and the mixture was concentrated and tested for free nicotinamide by paper chromatography using n-butanol saturated with water as the solvent system. No spot corresponding to nicotinamide could be detected, when the chromatogram was exposed to cyanogen bromide and sprayed with benzidine reagent. The fall in optical density at 325 mµ; the appearance of peak at 315 mµ and the absence of nicotinamide as the reaction product proved conclusively the

enzymatic deamidation of NMN by mouse liver homogenates. Such enzymatic deamidation of nicotinamide at nucleotide level, has not been reported earlier.

Although the presence of nicotinamide deamidase was reported by many investigators, the exact biological role for deamidases has yet to be suggested. According to Sundaram et al. (1960) and Imsande (1961) nicotinamide deamidase plays a prominent role in the synthesis of DPN from nicotinamide. A potent biological source for nicotinamide would thus be obligatory for the operation of such a biosynthetic pathway in the cell. So far no pathway has been suggested for the in vivo biogenesis of nicotinamide. Apart from the controversial observations by Porcellati (1954) and Ellinger (1948), the only biological source for nicotinamide appears to be the action of DPN-ase on DPN. Microorganisms such as A. niger, N. crassa, larvae of the insect Corcyra cephalonica St., pigeon liver and kidney and chicken kidney were found to contain both DPN-ase and nicotinamide deamidase. In these organisms, therefore, the breakdown of DPN by DPN-ase to nicotinamide would provide the substrate for the action of deamidase, resulting in the formation of nicotinic acid. The nicotinic acid thus produced could be reutilized for the synthesis of DPN. A cyclic pathway for the biosynthesis of DPN could be visualized as represented in the accompanying diagram.

However, in the case of mouse liver, deamidation takes place at nucleotide level. The NAMN thus formed could be utilized for DPN biosynthesis via desamido DPN. Thus deamidation appears to play an important role in the maintenance of DPN level in the cell.



(1) Nicotinamide deamidase; (2) NMN deamidase;
(3) DPN-ase.

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