material remaining after dialysis was lyophilized and then hydrolyzed with 10\% platinum on Darco. After hydrolysis and analysis for amino acids, the yields of alanine and of \( \alpha \)-aminobutyric acid were found to be at least 20 and 35\% of the theoretical values, respectively. Keratosulfate from shark cartilage was allowed to react with alkali in the presence of Adams catalyst (5) and hydrogen. At least 50\% of the theoretically expected yields of alanine and \( \alpha \)-aminobutyric acid were obtained.

The formation of alanine and of \( \alpha \)-aminobutyric acid confirms the postulated mode of linkage and the mechanism of its cleavage in alkali. The similarity of the action of alkali on cartilage keratosulfate and some glycoproteins further underlines the previously indicated similarity of this type of keratosulfate with the blood group substances (6).

**REFERENCES**


**Regulation of Nicotinamide Adenine Dinucleotide Biosynthesis by Adenosine Triphosphate**

Shigenobu Nakamura, Yasutomi Nishizuka, and Osamu Hayashi

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

(Received for publication, May 18, 1964)

In 1958, Preiss and Handler (1, 2) reported the formation of niacin ribonucleotide from niacin and 5-phosphoribosyl 1-pyrophosphate by extracts of human erythrocytes (Scheme 1, Reaction I). The enzyme was subsequently purified from beef liver and referred to as niacin ribonucleotide pyrophosphorylase by Imsande and Handler (3). Recent reports from this laboratory indicated that niacin ribonucleotide is also produced from quinolinate by a 5-phosphoribosyl 1-pyrophosphate-dependent reaction catalyzed by quinolinate transphosphoribosylase (Reaction II) (4-6). In the presence of ATP, niacin ribonucleotide thus formed is converted to deamido-NAD (Reaction III), which is further reduced to niacin by a specific phosphatase (Reaction IV, the enzyme will be referred to as niacin ribonucleotidase), followed by phosphorolytic cleavage of the niacin ribonucleoside produced (Reaction VII).

**Scheme 1. Biosynthesis of NAD from tryptophan and niacin, and the regulation of these pathways by ATP.** PP-ribose-P, 5-phosphoribosyl-1-pyrophosphate.

![Scheme 1](http://www.jbc.org/)

**Fig. 1. Effects of ATP on the activity of niacin ribonucleotidase (A) and niacin ribonucleotide pyrophosphorylase (B).**

- **A,** the reaction mixture (0.5 ml) contained 7 mmoles of niacin ribonucleotide-\(^{14}\)C (8 X 10\(^6\) c.p.m. per pmole), 3 mmoles of MgCl\(_2\), 50 mmoles of phosphate buffer, pH 7.3, 20 \(\mu\)g of the purified enzyme preparation, and ATP as indicated.
- **B,** the reaction mixture (0.5 ml) contained the same ingredients as A, except that 7 mmoles of niacin-\(^{14}\)C (8 X 10\(^6\) c.p.m. per pmole) and 0.4 pmole of 5-phosphoribosyl 1-pyrophosphate were added in the place of niacin ribonucleotide-\(^{14}\)C. Incubation was carried out for 30 minutes at 37\°C.

Another report indicated that niacin ribonucleotidase to niacin ribonucleotide pyrophosphorylase, thus controlling the biosynthesis of NAD from both niacin and tryptophan by regulating the interconversion of the two enzyme activities.

The activity of niacin ribonucleotidase was assayed by measuring the formation of niacin ribonucleoside-\(^{14}\)C from niacin ribonucleotide-\(^{14}\)C in the absence of ATP. Niacin ribonucleoside-\(^{14}\)C was separated by paper chromatography and the radioactivity was determined by the method described previously (3). The activity of niacin ribonucleotide pyrophosphorylase was assayed in the presence of ATP according to the method of Imsande and Handler (3). The enzyme protein was purified about 1000-fold from beef liver acetone powder extracts by ammonium sulfate fractionation, acid treatment, adsorption on and elution from calcium phosphate gel, DEAE-cellulose chromatography, and by acetone fractionation. The relative ratio of the two enzyme activities remained constant, and the activities could not be...
At 1 X 10^{-5} M ATP, K_m and V_{max} values were 1.4 x 10^{-4} M and 5.2 mmoles per mg of protein per hour, respectively. At 1 x 10^{-5} M ATP, these were 1.0 x 10^{-5} M and 0.9 mmole per mg of protein per hour, respectively.

The inhibition was not competitive in nature and was accompanied by a concomitant appearance of the inactivated ribonucleotide pyrophosphorylase activity (Fig. 1B). In the latter reaction, the higher concentrations of ATP increased the affinity of niacin to the enzyme and also the maximal velocity of the reaction. These effects of ATP on both Reactions I and V were specific, and all other ribo- and deoxyribonucleosides and the respective nucleotides so far tested did not affect the activity at 10^{-4} M.

When the enzyme preparation was subjected to sedimentation analyses in a sucrose density gradient without addition of ATP, both activities of niacin ribonucleotidase and niacin ribonucleoside pyrophosphorylase were detected. The reaction was specific for niacin ribonucleotide, and other ribo- and deoxyribonucleotides were inert as substrate. The reaction was stoichiometric conversion of niacin ribonucleotide to niacin and P_i, and practically no activity of niacin ribonucleotide pyrophosphorylase was detected. The reaction was inhibited by ATP at low concentrations as shown in Fig. 1A. The inhibition was not competitive in nature and was accompanied by a concomitant appearance of the niacin ribonucleotide pyrophosphorylase activity (Fig. 1B). In the latter reaction, the higher concentrations of ATP increased the affinity of niacin to the enzyme and also the maximal velocity of the reaction. These effects of ATP on both Reactions I and V were specific, and all other ribo- and deoxyribonucleosides and the respective nucleotides so far tested did not affect the activity at 10^{-4} M.

When the enzyme preparation was subjected to sedimentation analyses in a sucrose density gradient without addition of ATP, both activities of niacin ribonucleotidase and niacin ribonucleotide pyrophosphorylase (assayed in the presence of ATP) appeared in Fractions 12 to 16 (Fig. 2A), and the ratio of the two activities was constant in each fraction. When the preparation was centrifuged after preincubation with ATP (2 X 10^{-4} M) for 10 minutes at 25^\circ, only niacin ribonucleotide pyrophosphorylase was found in Fractions 7 to 10 and niacin ribonucleotidase was separated into more than one component throughout the entire purification procedure.

In the absence of ATP, the purified preparation catalyzed a stoichiometric conversion of niacin ribonucleotide to niacin ribonucleoside and P_i, and practically no activity of niacin ribonucleotide pyrophosphorylase was detected. The reaction was specific for niacin ribonucleotide, and other ribo- and deoxyribonucleotides were inert as substrate. The reaction was inhibited by ATP at low concentrations as shown in Fig. 1A. The inhibition was not competitive in nature and was accompanied by a concomitant appearance of the niacin ribonucleotide pyrophosphorylase activity (Fig. 1B). In the latter reaction, the higher concentrations of ATP increased the affinity of niacin to the enzyme and also the maximal velocity of the reaction. These effects of ATP on both Reactions I and V were specific, and all other ribo- and deoxyribonucleosides and the respective nucleotides so far tested did not affect the activity at 10^{-4} M.

When the enzyme preparation was subjected to sedimentation analyses in a sucrose density gradient without addition of ATP, both activities of niacin ribonucleotidase and niacin ribonucleotide pyrophosphorylase were detected. The reaction was specific for niacin ribonucleotide, and other ribo- and deoxyribonucleotides were inert as substrate. The reaction was inhibited by ATP at low concentrations as shown in Fig. 1A. The inhibition was not competitive in nature and was accompanied by a concomitant appearance of the niacin ribonucleotide pyrophosphorylase activity (Fig. 1B). In the latter reaction, the higher concentrations of ATP increased the affinity of niacin to the enzyme and also the maximal velocity of the reaction. These effects of ATP on both Reactions I and V were specific, and all other ribo- and deoxyribonucleosides and the respective nucleotides so far tested did not affect the activity at 10^{-4} M.
enzyme activities and also to see if the enzyme is phosphorylated in the presence of ATP or if ATP acts as a modifier of protein conformation.

REFERENCES
Regulation of Nicotinamide Adenine Dinucleotide Biosynthesis by Adenosine Triphosphate
Shigenobu Nakamura, Yasutomi Nishizuka and Osamu Hayaishi


Access the most updated version of this article at http://www.jbc.org/content/239/8/PC2717.citation

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/239/8/PC2717.citation.full.html#ref-list-1