Purification and Properties of Pyridine Nucleosidase (Glycosidase) from Bull Semen*  
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(Received for publication, June 30, 1961)

A soluble pyridine nucleosidase was first described in bull semen by Leone and Bonaduce (1). During studies on the specificity of this enzyme, we observed that passage of dilute semen through a column composed of calcium phosphate gel and diethylaminoethyl cellulose anion exchanger (Whatman) resulted in a preparation of high specific activity. In this single purification step the specific activity of the enzyme achieved was two to three times that of the best reported preparations (1). This material could be further purified to a high degree through relatively simple steps involving various forms of cellulose columns.

The purified enzyme catalyzed the hydrolysis and imidazolysis of both di- and triphosphopyridine nucleotides. Most interestingly, it also catalyzed the hydrolysis and imidazolysis of nicotinamide mononucleotide. N-(ring)-Ribosyl nicotinamide was also attacked. In the present paper we report the procedure for the purification of this enzyme and certain observations on its specificity and physicochemical properties.

**Experimental Procedure**

**Materials and methods—** Diphosphopyridine nucleotide, TPN, NMN, and other nucleotides mentioned in this communication were products of the Pabst Laboratories. Histamine (free base) and histamine dihydrochloride were Fisher reagents. Alcohol and isocitric dehydrogenases were purchased from Sigma Chemical Company. Soluble, partially purified beef spleen DPNase was prepared as previously reported (2, 3).

Pyridine nucleosidase activity was expressed in terms of DPNase activity. Incubation conditions for the determination of the latter were as follows: DPN (0.7 mM) was incubated at 38° for 15 minutes in the presence of Tris (0.1 M, pH 8.0) with approximately 1 unit (see below) of the enzyme. The volume of the mixture was 1 ml. At the end of the incubation period 6 ml of 2 M sodium cyanide solution were added. Appropriate controls (DPN or DPNase omitted) accompanied the experimental vessel. Readings at 327 mμ with quartz cuvettes of 1 cm light path were taken against blanks prepared by mixing 1 ml of 0.1 M Tris, pH 8.0, with 6 ml of 2 M sodium cyanide. A unit of enzyme was defined according to Zatman, Kaplan, and Colowick (4) as the amount of enzyme that would cause a splitting of 1 μmole of DPN in 1 hour. Due to differences in the initial concentration of the substrate and the period of incubation, the presently used unit is slightly larger than that utilized previously (4). Thus, activity values reported in this paper are, as a rule, a little smaller than values reported previously (2, 4).

Wherever possible, protein concentrations were estimated from the absorbancy values of appropriate solutions at 260 and 280 mμ (5). In opaque preparations (homogenates, etc.) proteins were determined by the biuret method (6) with use of bovine albumin (Armour and Company) as a standard. Ribose (7), total phosphorus (8), and inorganic phosphorus (9) determinations, as well as studies on periodate consumption (10), were performed as previously described (3).

Methods applied for the determination in solutions or detection on paper of nicotinamide, various purines, and histamine were the same as those utilized previously (3, 11). Ionophoretic (3) and ascending paper chromatographic techniques (11), as well as separations of mixtures by means of ion exchange resins (12) used in this study, have been described previously in detail (3).

**Preparation of Columns for Protein Purification**

**Calcium Phosphate Gel-DEAE-cellulose** To a slurry of 25 g of DEAE-cellulose in 0.5 liter of deionized water, 415 ml of calcium phosphate gel (13) (dry weight 9.04 mg per ml) were added with constant stirring. The mixture was then poured into a column container plugged with glass wool. The column was packed with suction to dimensions 5 cm in height and 6 cm in diameter. It was first washed with 0.1 M potassium phosphate, pH 7.1, until the effluent acquired the same pH value (about 1 liter). Washing (about 400 ml) was then continued with deionized water until absorbancy readings in the effluent at 260 and 280 mμ were smaller than 0.010. At this point, the pH of the effluent had dropped to 7.0. During operations, the rate of flow was regulated to approximately 2 ml per minute.

**Alumina Gel Cy-DEAE-cellulose**—To a slurry of 0.5 g of DEAE-cellulose in a few milliliters of deionized water, were added with
constant stirring 5 ml of alumina gel Cg (11) (aged for 3 months before use; dry weight 14.6 mg per ml). The mixture was then poured into a small column container plugged with glass wool. The column was packed with suction to dimensions 1.6 cm in diameter and 2 cm in height. It was washed with 0.1 m potassium phosphate, pH 7.0, until the pH of the effluent reached this value (approximately 500 ml). At this point, the washing liquid was changed to water and the flow was continued until absorbancy readings in the effluent at 260 and 280 m\(\mu\) were below 0.010. When this was achieved (approximately 1 liter of water), the pH of the effluent was 6.4. During operation the rate of flow was regulated to approximately 3 ml per minute.

Carboxymethyl Cellulose A slurry of 0.3 g Cellex-CM cation exchange cellulose (CM-W)2 in a few milliliters of deionized water was poured into a column container. The column was washed first with 0.1 m potassium phosphate, pH 7.0, and then with deionized water (approximately 1 liter) until absorbancy readings in the effluent at 260 and 280 m\(\mu\) were below 0.010. At this point, the pH of the effluent was 7.7. During the operation the rate of flow was regulated to approximately 3 ml per minute. All of the above columns were operated at 4°.

**Results**

Purification of Enzyme—Raw bull semen3 was received frozen and was either processed immediately or stored in the frozen state for several weeks before use. In a typical experiment (Table I), 50 ml of semen (specimen from Viking and Emperor of the American Breeders Service) were diluted with cold deionized water to 400 ml. The mixture was stirred at 4° for 10 minutes and then centrifuged at 2-4° and at 16 \(\times 10^6\) \(\times g\) for 60 minutes. The clear supernatant solution (390 ml, pH 7.7; No. I, Table I) was filtered through a calcium phosphate gel-DEAE-cellulose column (see "Materials and Methods") and the column was further washed with deionized water. There was no activity in the first 225 ml of the effluent. The next 385 ml contained a large portion of the activity originally present in the dilute semen. It was also rich in nucleic acids (No. II, Table I).

Further fractions obtained by washing with water (160 ml) were devoid of activity. Most of the activity still remaining on the column was eluted with 0.1 m potassium phosphate, pH 7.0. The first 290 ml of this eluent contained 7,900 units. The latter fraction contained 672 units of specific activity 650. Further elution with 0.2 m potassium phosphate, pH 7.0, yielded two fractions, one (11 ml) containing 461 units of specific activity 1,430 (No. VI, Table I) and a second containing 160 units in 8 ml. The latter fraction did not show measurable absorbancies at 260 and 280 m\(\mu\). The over-all purification in the case of Fraction VI, followed by deionized water. The first effluents (84 ml) did not contain enzyme activity. Elution was continued with 0.1 m potassium phosphate, pH 7.0. Although no activity was present in the first 10.5 ml of this eluent, 7,050 units were collected in the next 17.5 ml (No. IV, Table I). This was followed by an additional fraction containing 730 units of specific activity 454 in 23 ml of the same buffer. The most active fraction (No. IV, Table I), was concentrated by precipitation with ammonium sulfate (0°, pH 7.0) and the precipitate was redissolved in a buffer (pH 7.3) containing 0.1 m NaCl, 0.0037 m NaH2PO4, and 0.0115 m NaH2P04 (No. V, Table I).

After repeated dialysis against the same buffer, this sample (No. V, Table I, 3 mg of protein per ml) was utilized in ultra centrifugal studies (Spinco model E ultracentrifuge; analytical rotor AN-A; 59,789 r.p.m.; temperature 6.3° (corrected (15)). It showed a well defined peak with \(s_{20, w} = 3.25\). After prolonged (2 hours) centrifugation, a slight anomaly could be detected in the branch of the boundary closer to the axis of rotation. This indicated that a slower sedimenting material, not exceeding \(\frac{1}{2}\) of the total, could be present in the preparation.

The degree of purification achieved by the above mentioned steps varied, in different experiments, from 250-fold to 330-fold. As a rule specimens with initially lower specific activity (see footnote 2) could be purified successfully, but the specific activity of the end product was in those instances lower, varying from 700 to 960. In one experiment, several such preparations with specific activity below 1,000 were combined (27 ml, 3,000 units, specific activity 860; Fraction Va, Table I) and passed through a carboxymethyl cellulose column (see "Materials and Methods") and the column was then washed with 13 ml of water, followed by 9.5 ml of 0.1 m potassium phosphate, pH 7.0. The latter fraction contained 672 units of specific activity 650. Further elution with 0.2 m potassium phosphate, pH 7.0, yielded two fractions, one (11 ml) containing 461 units of specific activity 1,430 (No. VI, Table I) and a second containing 160 units in 8 ml. The latter fraction did not show measurable absorbancies at 260 and 280 m\(\mu\).

### Table I

**Purification of pyridine nucleosidase from bull semen**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Specific activity</th>
<th>Nucleic acids, % of protein</th>
<th>Activity, % of previous step</th>
<th>Total units</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Raw bull semen(^b)</td>
<td>5</td>
<td>0.39</td>
<td>100</td>
<td>22,400</td>
</tr>
<tr>
<td>II. Ca-phosphate gel-DEAE-cellulose step</td>
<td>237</td>
<td>21.50</td>
<td>45</td>
<td>10,050</td>
</tr>
<tr>
<td>IIa. — (c)</td>
<td>307</td>
<td>0.37</td>
<td>78</td>
<td>9,250</td>
</tr>
<tr>
<td>III. 46-60% saturation ammonium sulfate</td>
<td>1,070</td>
<td>0.37</td>
<td>76</td>
<td>7,050</td>
</tr>
<tr>
<td>IV. Alumina gel Cg-DEAE-cellulose step</td>
<td>1,210</td>
<td>0.38</td>
<td>75</td>
<td>5,300</td>
</tr>
<tr>
<td>V. 0-60% saturation ammonium sulfate</td>
<td>880</td>
<td>0.38</td>
<td>75</td>
<td>3,000</td>
</tr>
<tr>
<td>Va. — (c)</td>
<td>1,430</td>
<td>15</td>
<td>461</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Reference (5).

\(^b\) Diluted 1:8 with water; insoluble material removed by centrifugation before test.

\(^c\) Pooled fractions of the same step from various preparations; actually used in the next step.

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2 Fresh bull semen of different origin exhibits great differences in pyridine nucleosidase content (200 to 500 units per ml) and the specific activity of the enzyme (1.9 to 5.5 units per mg of protein).

3 Purification studies attempted up to now to correlate such differences to the breed of bull. Human sperm is also rich in the enzyme.

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\(^2\) CM-W, California Corporation for Biochemical Research.

\(^3\) Footnote 2) could be purified successfully, but the specific activity of the end product was in those instances lower, varying from 700 to 960. In one experiment, several such preparations with specific activity below 1,000 were combined (27 ml, 3,000 units, specific activity 860; Fraction Va, Table I) and passed through a carboxymethyl cellulose column (see "Materials and Methods") and the column was then washed with 13 ml of water, followed by 9.5 ml of 0.1 m potassium phosphate, pH 7.0. The latter fraction contained 672 units of specific activity 650. Further elution with 0.2 m potassium phosphate, pH 7.0, yielded two fractions, one (11 ml) containing 461 units of specific activity 1,430 (No. VI, Table I) and a second containing 160 units in 8 ml. The latter fraction did not show measurable absorbancies at 260 and 280 m\(\mu\). The over-all purification in the case of Fraction VI,
The incubation mixtures (1 ml) were buffered with 0.2 M Tris-HCl at pH 8.1. The initial substrate concentration was 0.7 mM. The enzyme preparation was identical with No. Vc of Table II. In the controls, either substrate or enzyme was omitted. At the end of the incubation period (15 minutes at 38°C), 6 ml of 2 M sodium cyanide were added to each sample and readings were taken at 327 nm (see text).

Table I, was approximately 500-fold (starting from an average initial specific activity of 3). Scarcity of raw material prevented a duplication and standardization of this step.

Other Studies with Purified Pyridine Nucleosidase—No liberation of inorganic phosphate was observed during a 2-hour incubation of 1.8 μmoles AMP with 100 μmoles MgCl₂ and 6.6 units of a preparation of specific DPNase activity of 670 (1 ml; 38°C; pH 8.1 with Tris). DPN was completely protected from hydrolysis during a 2-hour incubation at 38°C, when nicotinamide (final concentration, 0.10 M) was included in mixtures containing 6.6 units of pyridine nucleosidase of specific activity 670 (alcohol dehydrogenase) in a volume of 1 ml. These experiments demonstrated that the purified preparation was free from phosphatases and pyrophosphatases and that protection against hydrolysis of the nicotinamide-ribose bond could be achieved by adding free nicotinamide (see also (16)).

Hydrolytic Properties of Purified Enzyme—A purified preparation (specific activity, 1,210) catalyzed the hydrolysis of DPN, TPN and NMN (Fig. 1). Michaelis constants for the hydrolysis of these substrates are given in Table II. It is noteworthy that at higher stages of purification the values of these constants were found to be slightly elevated (Table II). Ratios of the hydrolytic activities for DPN and TPN at various stages of the purification are also given in Table II. The hydrolytic activities of the enzyme with DPN and TPN as substrates as a function of pH are shown in Fig. 2. Relatively concentrated enzyme preparations stored in the frozen state at −20°C retained most of their activity for long periods of time. Thus, a preparation

### Table II

**Determination of Michaelis constants of bull semen pyridine-nucleosidase at various stages of purification**

For a definition of DPNase units see “Materials and Methods”; TPNase and NMNase units are defined in an identical manner but with the corresponding substrates. Protein concentration was determined from absorbancy values at 200 and 280 nm (5).

<table>
<thead>
<tr>
<th>Stage of purification</th>
<th>DPN</th>
<th>TPN</th>
<th>NMN</th>
<th>Ratio of DPNase to TPNase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Specific activity</td>
<td>$K_m \times 10^b$</td>
<td>Specific activity</td>
<td>$K_m \times 10^b$</td>
</tr>
<tr>
<td>I</td>
<td>2.0</td>
<td>1.7</td>
<td>9.8</td>
<td>5.8</td>
</tr>
<tr>
<td>IIB&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11.8</td>
<td>3.9; (0.013)</td>
<td>9.3; (0.018)</td>
<td>3.8; (0.013)</td>
</tr>
<tr>
<td>III</td>
<td>266.0</td>
<td>3.2; (0.021)</td>
<td>220.0</td>
<td>3.8; (0.018)</td>
</tr>
<tr>
<td>IV</td>
<td>670.0</td>
<td>405.0</td>
<td>405.0</td>
<td>331.0</td>
</tr>
<tr>
<td>V</td>
<td>1210.0</td>
<td>263.0</td>
<td>263.0</td>
<td>9.9</td>
</tr>
<tr>
<td>V&lt;sup&gt;5&lt;/sup&gt;</td>
<td>900.0</td>
<td>331.0</td>
<td>331.0</td>
<td>9.9</td>
</tr>
<tr>
<td>V&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1800.0</td>
<td>1260.0</td>
<td>1260.0</td>
<td>9.9</td>
</tr>
<tr>
<td>VI</td>
<td>1450.0</td>
<td>8.7; (0.019)</td>
<td>1260.0</td>
<td>9.9</td>
</tr>
</tbody>
</table>

<sup>a</sup> Latin numerals correspond to stages of purification indicated in Table I. However, individual preparations employed in the present table do not necessarily correspond to the preparations described in Table I.

<sup>b</sup> Michaelis constants were calculated from the regression lines of 1/S against 1/V plots (17) by the method of least squares. Of the two figures in each group, the first represents the actual constant multiplied by 10<sup>b</sup> and the second is the mean square deviation from the regression line. Each constant was determined from 6 experimental points. All incubation mixtures (1 ml) were buffered with 0.2 M Tris at pH 8.15. The initial substrate concentration varied from 0.2 mM to 1.2 mM for all three substrates. At the end of the incubation (15 minutes at 38°C) 6 ml of 2 M sodium cyanide were added and readings were taken at 327 nm.

<sup>c</sup> This preparation was an eluate from a calcium phosphate gel-DEAE-cellulose column obtained with 0.1 M potassium phosphate (second peak of activity; see “Results”).

<sup>d</sup> The preparation No. V after standing for 6 days at 0°C in a medium 20% saturated with ammonium sulfate (pH was 7.3). Before testing, this preparation was dialyzed 3 times against 0.05 M Tris, pH 7.6 and insoluble material formed during this treatment was removed by centrifugation.

<sup>e</sup> The preparation No. V diluted 1:10 (about 0.3 mg of protein per ml) and stored in the frozen state for 5 weeks before testing its activity.
of specific activity 940 (158 units per ml) lost 9% of its initial activity in 13 days and 27% in 50 days. Lyophilized preparations retained full activity for the limited period of 2 weeks during which they were assayed.

In another experiment, 38.3 μmoles of NMN, 300 μmoles of MgCl₂, 900 μmoles of Tris (pH 8.5), and 600 units of 5'-nucleotidase (phosphatase (18)) were incubated in a volume of 1.9 ml at 38° for 150 minutes. The mixture was then immersed in boiling water for 90 seconds. Cyanide tests and phosphate determinations indicated that during this preliminary period there was no destruction of the nicotinamide-ribose bond, while all esterified phosphorus was set free. The mixture was then diluted to approximately 15 ml with water and passed through a Dowex 1 chloride form column (1 x 2 cm) followed by water. This treatment removed the liberated inorganic phosphate. N-(ring)-Ribosynicotinamide was obtained almost quantitatively in the first 25 ml of effluent. This material was lyophilized and redissolved in a small volume of water. The solution contained approximately 10 μmoles of N-(ring)-ribosynicotinamide per ml (cyanide test). It was free from esterified phosphorus and it contained less than 0.014 μmole of inorganic phosphate per μmole of the ribonucleoside. This material was utilized in the experiment described in Fig. 3. It was demonstrated (Fig. 3) that the enzyme is capable of effecting the breakdown of the nicotinamide-ribosy bond in the ribonucleoside even when inorganic phosphate is virtually absent. It is not yet established whether the traces of phosphates present in the preparation were a necessary component of the mixture (see "Discussion").

Imidazolytic Properties of Enzyme—Imidazolysis of "onium" structures was studied with both TPN and NMN. Thus, in one experiment 60 μmoles of TPN, 2,500 μmoles of histamine, 150 μmoles of sodium pyrophosphate, and 100 μmoles of Tris (pH 8.0), were incubated with 270 units of enzyme (specific DPNase activity 427) in a volume of 2.55 ml for 8 hours at 38°. At the end of the incubation the mixture was immersed in boiling water for 90 seconds. Ribose determinations (7) at zero time and at intervals indicated in the abscissa, 0.1-ml aliquots were withdrawn and utilized in the cyanide test (7 ml total volume).

Dependence of pyridine nucleosidase activity on the pH. Incubation mixtures (1 ml) were buffered with either 0.2 M Tris-HCl or 0.2 M potassium phosphate, at pH 8.0. The initial N-(ring)-ribosynicotinamide concentration was 0.0015 M. The enzyme preparation (15 units per sample) was identical to No. Vc of Table II. The total phosphate content of samples designated as free from phosphate ions was less than 0.1 μmole. In the control vessels enzyme was omitted. Incubation was carried out at 38°. At zero time and at intervals indicated on the abscissa, 0.1-ml aliquots were withdrawn and utilized in the cyanide test (7 ml total volume).

FIG. 3. Hydrolysis of N-(ring)-ribosynicotinamide. Incubation mixtures (1 ml) were buffered with either 0.2 M Tris-HCl or 0.2 M potassium phosphate, at pH 8.0. The initial N-(ring)-ribosynicotinamide concentration was 0.0015 M. The enzyme preparation (15 units per sample) was identical to No. Vc of Table II. The total phosphate content of samples designated as free from phosphate ions was less than 0.1 μmole. In the control vessels enzyme was omitted. Incubation was carried out at 38°. At zero time and at intervals indicated in the abscissa, 0.1-ml aliquots were withdrawn and utilized in the cyanide test (7 ml total volume).

The degree of purification of pyridine nucleosidase achieved in this study is, to our knowledge, the highest reported for a mammalian enzyme of similar nature (1, 2, 20, 21). It is hoped that with forthcoming supplies of semen the homogeneity of our preparations may further be checked. However, on the basis of pH. Incubation mixtures (1 ml) were buffered with either 0.2 M Tris-HCl or 0.2 M potassium phosphate, at pH 8.0. The initial N-(ring)-ribosynicotinamide concentration was 0.0015 M. The enzyme preparation (15 units per sample) was identical to No. Vc of Table II. The total phosphate content of samples designated as free from phosphate ions was less than 0.1 μmole. In the control vessels enzyme was omitted. Incubation was carried out at 38°. At zero time and at intervals indicated in the abscissa, 0.1-ml aliquots were withdrawn and utilized in the cyanide test (7 ml total volume).

TABLE III

Histaminolysis of NMN

Two-hour incubation in a total volume of 0.51 ml at 38°. Each sample contained NMN (0.001 m) and Tris-HCl at pH 8.3 (0.35 m with respect to Tris). A 2 μ histamine solution was prepared from histamine-free base and from its dihydrochloride so as to achieve a pH value of 8.5 (3). When histamine was present, 0.25 ml of this solution was included in the incubation mixture at a final concentration of 1 m. Appropriate quantities of the enzyme (3 DPNase units of preparation No. Vc of Table II; 300 units per ml) and sodium-pyrophosphate-phosphoric acid (final concentration in vessel 0.05 m with respect to pyrophosphate; pH 8.4) were also included as indicated. The pyrophosphate-phosphate ions were included to exclude the possibility of a phosphorolytic or, alternatively, pyrophosphorolytic mechanism. No inorganic phosphorus (9) could be detected in 0.25-ml aliquots taken from sample Nos. 1, 2, and 3 at the end of the incubation period. For ribose determinations (7) 0.05-ml aliquots were utilized. Readings were taken in a Coleman Junior spectrophotometer with cylindrical cuvettes of 1 cm inside diameter against a blank containing water instead of incubation mixture. Our thanks are due to Mr. Julius Bulyovszky for this experiment.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Additions</th>
<th>Optical density at 670 mμ</th>
<th>Enzymea</th>
<th>Porphosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Histamine</td>
<td>Enzyme†</td>
<td>Zero time</td>
<td>2 hours</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>0.179</td>
<td>0.078</td>
</tr>
<tr>
<td>2</td>
<td>--</td>
<td>+</td>
<td>0.185</td>
<td>0.190</td>
</tr>
<tr>
<td>3</td>
<td>--</td>
<td>+</td>
<td>0.184</td>
<td>0.174</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>--</td>
<td>0.178</td>
<td>0.067</td>
</tr>
<tr>
<td>5</td>
<td>--</td>
<td>+</td>
<td>0.172</td>
<td>0.175</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>+</td>
<td>0.179</td>
<td>0.179</td>
</tr>
<tr>
<td>7</td>
<td>--</td>
<td>+</td>
<td>0.170</td>
<td>0.162</td>
</tr>
</tbody>
</table>

* This preparation was dialyzed three times against 0.005 M Tris, pH 7.5.
† Ribose determinations (7).

In addition to the hydrolysis of pyridine coenzymes, the bull semen enzyme could also catalyze their imidazolysis. Leone and Santoianii (16) reported that the bull semen enzyme is not capable of promoting exchange reactions with nicotinamide. These observations further emphasize differences in the mechanism of exchanges with pyridine bases on the one hand and imidazolytic processes on the other (22).

Of particular interest is the hydrolysis and histaminolysis of NMN as catalyzed by this preparation. On several occasions crude diphosphopyridine nucleotidases were reported capable of catalyzing the hydrolysis of NMN. Thus, rabbit erythrocyte preparations, which are rich in DPNase (23, 24), were reported (25) to attack NMN but at a rate 200 times less than that of DPNase. Human erythrocytes also catalyzed the hydrolysis of NMN (26). However, the ability of the latter cells to hydrolyze the nicotinamide-ribose bond in either DPN or NMN is negligible as compared to the DPNase activity of rabbit erythrocytes. In contrast to the above, bull semen preparations were quite active toward NMN (Table II).

Bull semen preparations of specific DPNase activity of 1,210 were also capable of splitting the nicotinamide-ribose bond of N-(ring)-ribosynicotinamide. Grossman and Kaplan (26) found that crude human erythrocyte preparations could catalyze the phosphorolytic breakdown of this bond. In our studies, the quantities of inorganic phosphate present in reaction mixtures could conservatively be estimated as below 7% of the quantities of the ribonucleoside (Table IV and “Results”). The extent of splitting of the nicotinamide-ribose bond during incubation of such mixtures was approximately 84% of the amount of the ribonucleoside originally present. This leaves little room for a consideration of a mole per mole interaction of inorganic phosphate with N-(ring)-ribosynicotinamide. However, the possibility that presence of traces of inorganic phosphates is a prerequisite for the action of preparation Vc (Table II) upon N-(ring)-ribosynicotinamide cannot be excluded at present.

Similar considerations and reservations may be raised when examining the mechanism of the imidazolytic breakdown of N-(ring)-ribosynicotinamide (see Table IV). In any case, the

* A Table IV

Histaminolysis of N-(ring)-ribosynicotinamide

Incubation was carried out in a total volume of 0.51 ml at 38°. The mixtures were buffered with 0.35 m Tris, pH 8.4. The initial N-(ring)-ribosynicotinamide concentration was 0.001 m. The enzyme preparation (18 units per sample) was identical with No. Vc of Table II. The solution of histamine was identical with that described in Table III. When present, 0.25 ml of this histamine solution was included in the samples. Sample Nos. 4 to 6 were 0.1 m with respect to potassium phosphate (pH 8.0). At zero time and at intervals indicated in the table, 0.05-ml aliquots were withdrawn and utilized for ribose (7) determinations. Readings were made as shown in Table III.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Additions</th>
<th>Optical density at 670 mμ</th>
<th>Total phosphorousa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Histamine</td>
<td>Enzyme†</td>
<td>Zero time</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>0.195</td>
</tr>
<tr>
<td>2</td>
<td>--</td>
<td>+</td>
<td>0.190</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>--</td>
<td>0.190</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td>0.190</td>
</tr>
<tr>
<td>5</td>
<td>--</td>
<td>+</td>
<td>0.190</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>+</td>
<td>0.190</td>
</tr>
</tbody>
</table>

a Ribose determination (7).
† Reference (8).
observed histaminolysis of the ribonucleotide and the ribonucleoside are of particular interest. Thus, histaminolysis of NMN revealed a new pathway for the biosynthesis of histamine mononucleotide (3, 19, 27, 28) according to the following equation:

$$\text{NMN}^+ + \text{histamine} \rightarrow \text{5'-ribosylhistamine phosphate} + \text{nicotinamide} + \text{H}^+$$


Preliminary observations on the histaminolysis of N-(ring)-ribosynicotinamide also reported in this communication established the possibility of biosynthesis of N-(ring)-ribosylhistamine at the ribonucleoside level:

$$\text{N-(ring)-Ribosynicotinamide} + \text{histamine} \rightarrow \text{N-(ring)-Ribosylhistamine} + \text{nicotinamide} + \text{H}^+$$

A more complete account of the imidazolytic capabilities of this enzyme and of the subsequent fate of the various imidazolytic products is now in progress.

The role of the pyridine nucleosidase in the semen is not known. It is of interest, however, that this soluble enzyme is extracellular, and in this respect it resembles the enzyme of rabbit erythrocytes (24) that were shown capable of hydrolyzing DPN present in the medium in which the cells were suspended.

**SUMMARY**

Pyridine nucleosidase from bull semen was purified mainly through chromatography. Best preparations obtained in this manner had approximately 1,430 diphosphopyridine nucleotidase units per mg of protein. Ultra centrifugal pictures of a purified preparation consisted, almost entirely, of a single well defined peak with $s_{20, w} = 3.25$. A slower sedimenting material, not exceeding one-twentieth of the total, could be detected as an anomaly in the corresponding branch of the boundary pattern. Purified preparations of specific diphosphopyridine nucleotidase activity of 1,210 or more catalyzed the hydrolysis and imidazolysis of diphosphopyridine nucleotide, triphosphopyridine nucleotide, and nicotinamide mononucleotide. They were also capable of splitting the nicotinamide-ribose bond of N-(ring)-ribosynicotinamide, both by hydrolysis and by imidazolysis. The nature of this breakdown at the ribonucleoside level is under study.

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Purification and Properties of Pyridine Nucleosidase (Glycosidase) from Bull Semen
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