Nucleotide Pyrophosphatase Activities of Seminal Plasma*

I. THE DEGRADATION OF URIDINE DIPHOSPHATE GLUCOSE BY BOVINE SEMINAL PLASMA ENZYMES

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The occurrence in bovine seminal plasma of 'alkaline' phosphatase (1), 5-nucleotidase (2), adenosine triphosphatase (3), and inorganic pyrophosphatase (4) is well known, and the presence of diprophosphoryl nucleotidase and triphosphoryl nucleotidase in bovine semen has been recently reported by Leone and Bonaduce (5). The present work describes the degradation of uridine diphosphate glucose by what appears to be the combined action of nucleotide pyrophosphatase and 5-nucleotidase present in bovine seminal plasma. A preliminary report of this work has been published (6).

EXPERIMENTAL PROCEDURE

Bovine Seminal Plasma Enzyme Preparation—The short procedure of Heppel and Hilmoe (2) was followed for the preparation of 5-nucleotidase, which also contains the UDP-glucose degradation system reported here. After completion of the described procedure (2), protein was precipitated with ammonium sulfate, pH 8.0, at 67% saturation. The precipitate was collected by centrifugation and was dissolved in a minimal amount of water to yield a solution of 70 mg of protein per ml. Recoveries of 5-nucleotidase and UDP-glucose degrading activity were at least 90%. The enzyme preparation was stored frozen and remained stable for more than 1 year.

Materials—UDP-glucose, UDP, UMP, and uridine were purchased from Sigma Chemical Company. Glucose oxidase, peroxidase, and o-dianisidine (glucostat reagent) were purchased from Worthington Biochemical Corporation. Raw bull semen was obtained through the courtesy of Dr. Sam Tove and Mr. Richard Myers of the Department of Animal Industry, North Carolina State College, Raleigh, North Carolina. Constriction pipettes of the Lang-Levy type in sizes from 5 μl to 50 μl were purchased from Dr. H. E. Pederson, Carlsberg Laboratorium, Valby, Denmark.

Assays and Determinations—Values recorded are the differences between substrate or product determined on suitable aliquots before and after incubation at 38°. Reaction mixtures were routinely run in a volume of 0.20 ml and contained 0.1 M glycine, pH 9.0, 10-4 M MgCl2, 1.05 mg of protein per ml, and 1 × 10-4 M substrate unless otherwise indicated. UDP-glucose was determined spectrophotometrically by transfer of suitable aliquots (5 μl to 50 μl) of reaction mixtures into cuvettes containing the components of the UDP-glucose dehydrogenase assay system (7). The UDP-glucose dehydrogenase (specific activity of 22 μmoles per hour per mg of protein) was prepared by an unpublished purification procedure modified from Strominger et al. (7). Protein was determined by the method of Lowry, et al. (8). Glucose 1-phosphate was estimated with phosphoglucomutase and glucose 6-phosphate dehydrogenase (9). Orthophosphate was determined by the method of Gomori (10). Uridine was determined as non-Dowex 1-chloride-adsorbable 262 μm-absorbing material. Purity of uridine obtained in the Dowex 1-chloride effluent was checked by ultraviolet spectrum and by paper chromatographic comparison with known uridine. The molar absorbancy index of 10.0 × 10³ at 262 μm (11) was used for calculation of amounts of uridine-containing compounds. Measurements were made with a Beckman model DU spectrophotometer. Nucleotides including UDP-glucose, UDP, UMP, and uridine were separated by paper chromatography on Whatman No. 1 paper. Solvent systems used were isobutyric acid-water-concentrated ammonium hydroxide (66:33:1) (11); 1 M pH 3.7 ammonium acetate-ethanol (1:2.5, volume for volume) (12); and 1 M pH 7.5 ammonium acetate-ethanol (1:2.5, volume for volume) (12). Ultraviolet-absorbing materials were visualized with a 2537 A “Mineralight” lamp.

RESULTS

Phosphate Release and Products—With the use of relatively crude bovine seminal plasma 5-nucleotidase preparations for the assay of 5-UMP in the presence of UDP-glucose, it was observed that inorganic phosphate was also liberated from the latter compound. As shown in Fig. 1, the rate of phosphate production from 5-UMP was 10 times greater than from UDP-glucose. Assay for products other than phosphate revealed only uridine and glucose 1-phosphate. Free glucose, UDP, and UMP could not be detected.

Stoichiometry—The data in Table I indicate a 1:1 ratio between UDP-glucose disappearance and the formation of glucose 1-phosphate, uridine, and inorganic phosphate.

Effect of pH—Maximal activity for the reaction in glycine and Tris buffers was found near pH 8.9 as shown in Fig. 2.

Effect of Substrate Concentration—Initial rates of UDP-glucose degradation at several concentration levels were estimated from plots of substrate disappearance against time. From the reciprocal plot (13) shown in Fig. 3, the Michaelis-Menten constant, Ke, was calculated to be 2.1 × 10⁻⁴ M.

Effect of Metals—Activity was enhanced by the presence of

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Table II

Stoichiometry of UDP-glucose degradation

One half of the reaction mixture was removed immediately after the addition of enzyme, neutralized by a predetermined amount of 1 N HCl, and heated 2 minutes in boiling water to stop the reaction. The remainder was incubated at 38° for 60 minutes and then treated similarly. Samples were then removed for assays as indicated in "Experimental Procedure."

Table I

<table>
<thead>
<tr>
<th>Conditions</th>
<th>UDP-glucose destroyed (μmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minus MgCl₂</td>
<td>0.02</td>
</tr>
<tr>
<td>Complete</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Magnesium ions, and inhibited by ethylenediaminetetraacetic acid. Substrate was not degraded in the presence of magnesium ions alone or in the presence of boiled enzyme plus magnesium ions. Table II indicates the dependency for Mg²⁺ observed after treatment of the enzyme preparation with Dowex 50-potassium. Other metals tested with Dowex 50-potassium-treated enzyme were found to be less active in enhancement of the reaction. These included the chlorides of Ni²⁺, Co²⁺, Ca²⁺, and Mn²⁺. The chlorides of Zn²⁺, Cu²⁺, Cd²⁺, Fe²⁺, and Fe³⁺ were not active.

Substrate Specificity—Several other nucleotide pyrophosphate compounds, including DPNH, DPN⁺, FAD, and GDP-choline were also degraded by the enzyme preparation (6). Studies of the degradation of these compounds will be reported elsewhere.

Discussion

The data presented are in agreement with the conclusion that bovine seminal plasma contains one or more nucleotide pyrophosphatase activities which hydrolyze the pyrophosphate bond of UDP-glucose, yielding glucose 1-phosphate and UMP; the latter is immediately hydrolyzed to uridine and inorganic phosphate by excess 5-nucleotidase. These results indicate the necessity for cautious interpretation when seminal plasma 5-nucleotidase is used for the identification of nucleotides in complex mixtures.
SUMMARY

Bovine seminal plasma was found to contain enzyme activities which degrade uridine diphosphate glucose to inorganic phosphate, uridine, and glucose 1-phosphate. Glucose 1-phosphate was not further degraded. The reaction proceeds best near pH 9. Half maximal rates were obtained at a uridine diphosphate glucose concentration of $2.1 \times 10^{-4}$ M. A requirement for a divalent metal ion such as Mg$^{2+}$ was demonstrated.

REFERENCES

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