Metabolism of Inorganic Pyrophosphate

I. MICROSONAL INORGANIC PYROPHOSPHATE PHOSPHOTRANSFERASE OF RAT LIVER*

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The formation of large amounts of inorganic pyrophosphate as a by-product of numerous synthetic reactions in nearly all living tissues makes the metabolic reactions of this compound of interest. Rapid hydrolysis to orthophosphate is presumed, and extensively studied (3, 4), knowledge of mammalian tissues makes the metabolic reactions of this compound a subject of speculation (1, 2).

Whereas a yeast pyrophosphatase has been isolated, crystallized, and extensively studied (3, 4), knowledge of mammalian enzymes acting upon PPi is in a much less satisfactory state. At least four inorganic pyrophosphatases, differing in their pH optima, have been reported to occur in rat liver (5, 6). The properties of a pyrophosphatase occurring in mouse liver particles have been studied (7) and the wide distribution of activity in subcellular fractions of rat liver homogenates has been noted (8). Two distinct pyrophosphatase activities differing in pH optima and Mg++ requirements have been reported to exist in rat liver mitochondria (9).

Of particular interest is the finding of Rafter (10) that mouse liver mitochondrial preparations at pH 5.2 are capable of utilizing PPi for the formation of glucose 6-phosphate. Under his optimal conditions the synthetic function represented only a small fraction of the total PPi which was cleaved, but the two activities were parallel.

The present paper reports the results of studies of the occurrence, distribution, and some of the properties of the rat inorganic pyrophosphate phosphotransferase enzyme which catalyzes the synthesis of glucose-6-P. The results indicate that the two reactions,

\[
\text{PPi} + \text{H}_2\text{O} \rightarrow 2 \text{Pi} \tag{1}
\]

\[
\text{PPi} + \text{glucose} \rightarrow \text{Pi} + \text{glucose-6-P} \tag{2}
\]

are catalyzed by a single enzyme.

EXPERIMENTAL PROCEDURE

Enzyme Preparations—All enzyme preparations were made from tissues of white rats of the Sprague-Dawley strain, killed by decapitation and worked up in the cold as rapidly as possible. In most cases homogenization and cell fractionation were carried out by the method of Hogeboom (11), with 0.25 M sucrose containing 0.001 M ethylenediaminetetraacetate (12).

Studies were done with washed “microsomal” fractions which sedimented between either 8,500 or 12,500 x g and 105,000 x g. Cell fractions were frozen and stored in small batches before being assayed.

For localizing the enzymatic activity within the cell, fractionation followed closely the method of de Duve et al. (12, 13). Fraction N, consisting of nuclei and cellular debris, was that crude fraction which precipitated in 10 minutes at 1,000 x g and was resuspended in 0.25 M sucrose and recentrifuged at 700 x g. The heavy mitochondrial fraction (M) was obtained by centrifugation at 3,300 x g for 10 minutes, and was resuspended and recentrifuged twice in 0.25 M sucrose. The light mitochondrial fraction (L) was that precipitating in 10 minutes between 3,300 and 25,000 x g; it was twice resuspended and recentrifuged in 0.25 M sucrose. The microsomal fraction (P), in this case, was that portion of the cell particulate precipitating from the first supernatant of Fraction L in 30 minutes at 105,000 x g; it was resuspended in 0.25 M sucrose and recentrifuged once. The soluble fraction (S) was the 105,000 x g supernatant.

Assay Procedures—Conditions used for the pyrophosphatase enzymatic reactions are given with each table or graph. Inorganic pyrophosphate was measured on protein-free supernatant solutions obtained by stopping the enzyme reactions by the addition of equal volumes of cold 10% trichloroacetic acid. Modifications of the Fiske-SubbaRow method (14) or the Lowry-Oxidase (Worthington Biochemical Corporation).

Glucose 6-phosphate dehydrogenase was measured at 340 nm. Glucose 6-phosphate dehydrogenase obtained from Sigma Chemical Company was found to reduce only 1 mole of triphosphopyridine nucleotide per mole of glucose-6-P.

Glucose was measured by means of oxidation with glucose oxidase.

Protein was determined by the method of Lowry et al. (17).

Acid phosphatase was estimated by the method of Appelmans, Wattiaux, and de Duve (13) with slight modifications. Enzyme fractions were incubated for 10 minutes at 30° with 0.05 M
\(\beta\)-glycerophosphate and 0.04% Triton X-100 (18) in 0.1 M sodium acetate buffer at pH 5.0. The reaction was stopped by the addition of an equal volume of cold 10% trichloroacetic acid, and Pi was measured.

Glucose 6-phosphatase activity was measured by incubation of the enzyme fractions for 10 minutes at 30° with 0.05 M glucose-6-P and 0.04% Triton X-100, at optimal PPi concentration (0.08 M) with and without 0.4 M glucose. The amounts of Pi and glucose formed were the same when suitably corrected for preexisting glucose and Pi in the enzyme preparations and reagents.

RESULTS

Subcellular Distribution of Enzyme Activity in Rat Liver

Liver homogenates fractionated by the method of de Duve et al. (12), including lysosomal and heavy mitochondrial fractions, were assayed for several enzymes. The results, summarized in Table I, show that the pyrophosphatase activity, when measured at pH 5.4 in the absence of added Mg\(^{++}\), is found predominantly in the microsomal fraction. The very closely parallel distribution between this pyrophosphatase activity and glucose 6-phosphatase activity, which is well known to be found exclusively in the microsomal fraction (19), was striking. The pyrophosphatase could clearly be distinguished from the acid phosphatase which occurs in lysosomes (13). The activity was found distributed to some extent in all the other cell fractions as well, but this can readily be explained by the very incomplete separation of fractions which is obtained by the relatively crude methods employed. The approximately constant ratio of 1.2 between the pyrophosphatase and the glucose 6-phosphatase activity in the whole homogenate and in the various fractions obtained from it, indicates that the activity is probably exclusively microsomal and that the activity occurring in other fractions, including the mitochondria, is due to microsomal contamination.

Since the intent of these experiments was to study the properties of that inorganic pyrophosphatase which was also responsible for the glucose-6-P synthesizing activity, it was necessary to use conditions which as much as possible eliminated measurement of the various other widely distributed enzymes which hydrolyze PPi. Measurements of the hydrolytic and the phosphotransferase activities were carried out at pH 5.4 and at pH 7.5 with high and low PPi concentrations, with and without added Mg\(^{++}\) and with and without added glucose. Typical results obtained with the 105,000 \(\times g\) supernatant fraction are reported in Table II and those with the washed liver microsome fraction in Table III. Since the enzyme preparations were made in sucrose solutions containing EDTA it may be assumed that there was no significant concentration of free Mg\(^{++}\) ions present except when added.

In the absence of added Mg\(^{++}\) the soluble fraction exhibited no appreciable amount of activity of either type under any of the conditions used (Table II). When the concentration of PPi greatly exceeded the Mg\(^{++}\) concentration, relatively small amounts of Pi were hydrolyzed. The hydrolytic activity was greatest with the soluble fraction when the Mg\(^{++}\) concentration exceeded the PPi concentration, in agreement with the theory that magnesium pyrophosphatase is the substrate for similar enzymes (20, 21). Whereas there was appreciable hydrolytic activity at pH 5.4, the activity was very much greater at pH 7.4. These results are in accord with the observations of Swanson (22) and of Nordlie and Lardy (8) that the bulk of the "neutral pyrophosphatase," requiring Mg\(^{++}\), occurs in the soluble fraction. The reaction was not greatly influenced by the presence of large amounts of glucose and no glucose-6-P was formed. The soluble fraction was thus free of that pyrophosphatase with which we are concerned.

In contrast, the pyrophosphatase activity of the microsomal fraction (Table III) was much greater at pH 5.4 than at 7.5. At high glucose concentrations relatively large amounts of glucose-6-P accumulated and the hydrolytic reactions were depressed. Magnesium ion was not only not required, but was

### Table I

<table>
<thead>
<tr>
<th>Subcellular fraction</th>
<th>Protein per g of liver (wt. wt.)</th>
<th>Pyrophosphatase</th>
<th>Acid phosphatase</th>
<th>Glucose-6-phosphatase</th>
<th>Ratio of pyrophosphatase to glucose-6-phosphatase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Without glucose</td>
<td>With 0.4 M glucose</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PPi cleaved</td>
<td>PPi cleaved</td>
<td>Glucose-6-P formed</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mg</td>
<td>(\mu)moles</td>
<td>(\mu)moles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unfractionated cytoplasmic extract (homogenate - Fraction N)</td>
<td>140</td>
<td>24.8</td>
<td>32.7</td>
<td>26.3</td>
<td>80</td>
</tr>
<tr>
<td>Fraction N (nuclei + cellular debris)</td>
<td>64</td>
<td>5.0</td>
<td>7.0</td>
<td>5.3</td>
<td>78</td>
</tr>
<tr>
<td>Fraction M (heavy mitochondrial)</td>
<td>5.3</td>
<td>0.89</td>
<td>0.70</td>
<td>0.32</td>
<td>74</td>
</tr>
<tr>
<td>Fraction L (light mitochondrial)</td>
<td>6.9</td>
<td>2.6</td>
<td>1.67</td>
<td>1.32</td>
<td>79</td>
</tr>
<tr>
<td>Fraction P (microsomal)</td>
<td>45</td>
<td>20.1</td>
<td>26.1</td>
<td>22.5</td>
<td>85</td>
</tr>
<tr>
<td>Fraction S (soluble)</td>
<td>66</td>
<td>Trace</td>
<td>Trace</td>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>

* Per cent transfer = (glucose-6-P formed)/(total PPi reacting) \(\times 100\).

† Determined in the absence of glucose.
**TABLE II**

Effect of pH, Mg++, and glucose on pyrophosphatase activity of soluble fraction of liver homogenate

Reaction mixtures contained 0.08 M or 0.0016 M PPi, 0.4 M or no glucose, 0.02 M or no Mg++, 0.04% Triton X-100, and a portion of the 105,000 x g supernatant fraction of rat liver homogenate, containing 0.33 mg of protein, in a final volume of 1 ml of either 0.1 M acetate buffer, pH 5.4, or 0.1 M Tris buffer, pH 7.5. Incubation was for 10 minutes at 30°C. Reactions were stopped by the addition of 1 ml of cold 10% trichloroacetic acid for Pi measurement and by heating 3 minutes at 100°C before glucose-6-P measurement. Each value was corrected for the results obtained when trichloroacetic acid was added to the reaction mixture before addition of the enzyme.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPi</td>
<td>Glucose-6-P</td>
</tr>
<tr>
<td>Mg++</td>
<td>Pi</td>
</tr>
<tr>
<td>pH 5.4</td>
<td></td>
</tr>
<tr>
<td>pH 7.5</td>
<td></td>
</tr>
<tr>
<td>μmoles/ml</td>
<td>μmoles/min/mg protein</td>
</tr>
</tbody>
</table>

80 0 0 0 Trace 0.076 Trace 0.079 1.6 0 Trace Trace 0 Trace 1.6 0 Trace 0 1.6 0 Trace 0 1.6 0 Trace 0 1.6 0 Trace 0

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPi</td>
<td>Glucose-6-P</td>
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<tr>
<td>Mg++</td>
<td>Pi</td>
</tr>
<tr>
<td>pH 5.4</td>
<td></td>
</tr>
<tr>
<td>pH 7.5</td>
<td></td>
</tr>
<tr>
<td>μmoles/ml</td>
<td>μmoles/min/mg protein</td>
</tr>
</tbody>
</table>

80 0 0 0 Trace 0.076 Trace 0.079 1.6 0 Trace Trace 0 Trace 1.6 0 Trace 0 1.6 0 Trace 0 1.6 0 Trace 0 1.6 0 Trace 0

*Table III*

**Effect of pH, Mg++, and glucose on pyrophosphatase activity of liver microsomes**

Conditions were identical with those of Table II except that the enzyme was a suspension of rat liver microsomes. At the higher PPi concentration the reaction mixtures contained 1.13 mg of protein; at the lower PPi, 0.28 mg of protein per ml.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPi</td>
<td>Glucose-6-P</td>
</tr>
<tr>
<td>Mg++</td>
<td>Pi</td>
</tr>
<tr>
<td>pH 5.4</td>
<td></td>
</tr>
<tr>
<td>pH 7.5</td>
<td></td>
</tr>
<tr>
<td>μmoles/ml</td>
<td>μmoles/min/mg protein</td>
</tr>
</tbody>
</table>

80 0 0 0 Trace 0.08 0.08 0.76 0.22 0.60 0.42 0.05 0.019 0.56 0.39 0.12 0.057 0.52 0.08 0.29 0.061 0.24 0.10 0.003 0 0.26 0.02 0.51 0.001 0.16 0.08 0.005 0 0.26 0.02

The enzyme was found to occur in about equal concentrations in liver and in kidney. It was found also in the livers and kidneys of fetal rats 3 days before term. All other organs and tissues studied exhibited no pyrophosphate phosphotransferase activity when measured at low pH in the absence of added Mg++. Negative results were obtained with spleen, intestinal mucosa, brain, lung, blood, skeletal muscle, and heart muscle. These same tissues contained, of course, appreciable amounts of various pyrophosphatases having other properties.

**Properties of Microsomal Enzyme**

**Dependence upon Enzyme Concentration—Formation of glucose-6-P is linear with respect to enzyme concentration (Fig. 1).** In the absence of enzyme and with boiled enzyme no glucose-6-P is formed.

**Effect of Triton X-100—Without added Triton X-100 the rate of formation of glucose-6-P, catalyzed by microsomes at 30°C, is approximately linear for 30 minutes at several different pH values (Fig. 2).** In the presence of Triton X-100, a nonionic detergent which is known to liberate a number of enzymes by causing the rupture of subcellular particles (18), the initial rate of reaction was increased and the rate fell off more rapidly with time. When Triton X-100 was added after 10 minutes, the rate of reaction was abruptly accelerated. It is possible that a rate-limiting step in the measurement in the absence of Triton is the rupture of the microsomal particles which takes place more rapidly at pH 5 than at higher pH values. The enzyme, once inhibitory at the lower pH, at both high and low PPi concentration, and with and without added glucose. With the microsomal fraction the small amounts of hydrolytic and transfer activity occurring at the higher pH were increased by the inclusion of Mg++, indicating the action of a different particulate enzyme, possibly that studied in mitochondria by others (9). By the use of washed microsomes, a high PPi concentration, low pH, and no added Mg++, the action of other inorganic pyrophosphatases is effectively excluded. The enzyme here studied is similar to that studied by Rafter (7, 10) in mouse mitochondria.

**Distribution of Pyrophosphate Phosphotransferase Activity in Rat Tissue**

The enzyme was found to occur in about equal concentrations in liver and in kidney. It was found also in the livers and kidneys of fetal rats 3 days before term. All other organs and tissues studied exhibited no pyrophosphate phosphotransferase activity when measured at low pH in the absence of added Mg++. Negative results were obtained with spleen, intestinal mucosa, brain, lung, blood, skeletal muscle, and heart muscle. These same tissues contained, of course, appreciable amounts of various pyrophosphatases having other properties.
Effect of Temperature - At optimal substrate concentration and pH the rate of reaction at 37° falls off rapidly with time, nearly all of the enzyme being inactivated in an hour (Fig. 4). At 30° the initial rate of reaction as well as the rate of inactivation is lower. The apparent activity measured at 30° and at

liberated from the particulate by rupture due to either low pH or the presence of Triton X-100, is relatively unstable in its optimum pH range.

The optimal concentration of Triton X-100 was found to be 0.04%, so this concentration was used in the kinetic studies. Activation was found to be immediate and was optimal when Triton X-100 was included in the reaction mixture before the addition of the enzyme.

To see if the enzyme was solubilized or was retained in the particulate, a portion of a microsomal suspension which had been treated with 0.1% Triton X-100 at 0° for 30 minutes was centrifuged for 1 hour at 105,000 x g. The clear supernatant was free of enzyme while only about 50% of the initial activity was recovered in the precipitate. It thus appears that the enzyme is either not solubilized by the Triton X-100 at the concentration used, or that the part made soluble is very unstable. In contrast, extraction of the microsomes with 1% digitonin resulted in transferring an appreciable amount of the activity into the fraction not precipitated by centrifugation at 105,000 x g.

Effect of pH - Optimal pH determined in the presence of 0.04% Triton X-100 was about pH 5.3 to 5.4 (Fig. 3A). In the absence of the detergent, where the rupture of the microsomes by the acidity of the medium may be rate-limiting, the apparent optimal pH was a little lower, pH 5.0 to 5.1 (Fig. 3B). Although the true optimum may be a little lower, kinetic studies were done at pH 5.4 in the presence of Triton X-100 as a compromise to minimize the effect of the instability of the enzyme in acidic solutions.

Fig. 2. Effect of Triton X-100 upon pyrophosphate phosphotransferase measurement. Each milliliter of reaction mixture contained 100µmoles of PPi, 400µmoles of glucose, and microsomes containing 1.16 mg of protein. In the upper curves, 0.1 m acetate buffers were used with and without 0.1% Triton X-100. In the lower curve, where the Triton X-100 concentration was varied, the buffer was 0.1 m acetate at pH 5.4 and incubation was for 10 minutes at 30°. , no Triton X-100; , with Triton X-100.

Fig. 3. Effect of pH. , P; formed; , glucose-6-P formed. A, the curves were obtained with 100 µmoles of PPi, 400 µmoles of glucose, 0.04% Triton X-100, and 1.16 mg of microsomal protein per ml of 0.1 M acetate buffer of the indicated pH. B, the curves represent the same concentrations of substrates used with 1.0 mg of protein and no Triton X-100. Incubation in all cases was at 30° for 30 minutes.

Fig. 4. Effect of temperature upon rate of production of glucose-6-P. Each milliliter of reaction mixture contained 100 µmoles of PPi, 400 µmoles of glucose, 0.04% Triton X-100, and 1.16 mg of microsomal protein in 0.1 m acetate buffer, pH 5.4. , 37°; , 30°; , 0°.
37° is about the same at 30 minutes. The pyrophosphate phosphotransferase is remarkably active at 0°, the amount of glucose-6-P produced at that temperature in 8 hours approximately equaling that found in the same time at 30°. At 0° enzyme activity continues for days.

The percentage of transfer, i.e. the percentage of the total PPi used which appears as glucose-6-P, remains approximately constant for a considerable length of time until the concentration of glucose-6-P builds up to the point where the enzymatic hydrolysis of glucose-6-P becomes significant under the assay conditions used. The interrelationships between the pyrophosphate phosphotransferase activity and the glucose 6-phosphatase of microsomes will be the subject of a separate communication.

Effect of Adenine Nucleotides on Transferase Reaction

Under conditions favorable for the formation of a glucose-6-P from glucose and PPi the microsomal enzyme preparation is unable to make glucose-6-P from ATP (Table IV). Thus traditional hexokinase action is absent. The addition of equimolar amounts of ATP, ADP, or AMP to a system containing PPi had no effect, either inhibitory or stimulatory, on the synthesis of glucose-6-P. This is in contrast to the inhibitory action of these compounds on the magnesium-requiring mitochondrial pyrophosphatase studied at pH 7.25 by Nordlie and Lardy (9).

Under the conditions studied, fluoride inhibited the transferase reaction about 90% and a parallel inhibition of Pi production was observed. This inhibition was not reversed by equimolar Mg++. Mg++ itself caused about 25% inhibition, while EDTA had no effect.

Effect of Concentration of Glucose

A comparison of the relative proportions of the two reactions of inorganic pyrophosphate occurring with the microsomal enzyme at pH 5.4 (see Reactions 1 and 2) was obtained by measuring the production of Pi and glucose-6-P at constant optimal PPi concentration and varying glucose concentration (Table V). As the glucose concentration was increased, the quantity of glucose-6-P formed increased and the Pi yield decreased. Optimal glucose concentration was found to be about 0.4 M, higher concentrations of glucose causing inhibition of both reactions.

From the data of Table V the quantities of PPi reacting with glucose and with water were calculated (PPi reacting with water = (total Pi formed - glucose-6-P formed)/2). In the insert, the reciprocal of the glucose-6-P formation is plotted against the reciprocal of the glucose concentration and the apparent Michaelis constant for glucose obtained by the method of Lineweaver and Burk (23).
Fig. 6. Effect of PP$_i$ concentration on pyrophosphate phosphotransferase activity at various glucose levels. Reaction mixtures contained glucose and PP$_i$, as indicated, 0.04% Triton X-100, and a suspension of washed rat liver microsomes containing 1.09 mg of protein in 1.0 ml of 0.1 M acetate buffer, pH 5.4. Incubation was at 30° for 10 minutes. The quantities of P$_i$ and glucose 6-P formed were measured.

Fig. 7. Inhibition of inorganic pyrophosphatase by glucose. From the data plotted in Fig. 6, the quantities of PP$_i$ reacting with water at different levels of glucose were calculated. $K_p = K_n$ in presence of inhibitor.
is to very greatly inhibit the reaction between PPi and water. An increase of the glucose concentration to 1 m, with a resulting decrease of only about 2% in the H2O concentration, reduced the hydrolytic action of the pyrophosphatase to about 10%, whereas the percentage of reacting PPi involved in the transfer reaction rose to about 90%. This inhibition of the pyrophosphatase by glucose is taken as evidence that Reactions 1 and 2 may be catalyzed by the same enzyme.

From the data of Table V the apparent $K_m$ for glucose has been calculated as 0.06 m by the method of Lineweaver and Burk (23) (Fig. 5, insert). Approximately the same value for the apparent Michaelis constant was obtained for glucose with microsomes in the absence of Triton X-100.

**Effect of PPi Concentration**

The formation of glucose-6-P was followed as a function of PPi concentration at several different concentrations of glucose (Fig. 6). The optimum PPi concentration was found to be about 0.08 m. From a double reciprocal plot of this data, the apparent Michaelis constant for PPi in the pyrophosphate phosphotransferase reaction was determined as about $6.8 \times 10^{-3}$ and was independent of the concentration of glucose over a wide range.

**Nature of Glucose Inhibition of PPi Hydrolysis**

From the quantities of orthophosphate and of glucose-6-P formed, the amount of PPi reacting with water in the absence of glucose and in the presence of several different concentrations of glucose was calculated (Fig. 7). The $K_m$ for PPi in the hydrolytic reaction is $1.2 \times 10^{-2}$. The effect of increasing glucose concentrations is simply to diminish hydrolysis progressively without appreciably changing the apparent Michaelis constant, indicating the noncompetitive nature of the inhibition by glucose.

**Table VI**

**Effect of PPi, glucose-6-P, and P1 on inactivation of enzyme**

A suspension of microsomes equivalent to 1 mg of protein per ml, in 0.08 m acetate buffer at pH 5.0, was preincubated at 30° for 30 minutes with the additions indicated. The solutions were chilled, aliquots were taken for preliminary analysis and enzyme assays were carried out, after the addition of glucose (0.4 m) and PPi (0.1 m) at 30° for 30 minutes.

<table>
<thead>
<tr>
<th>Preincubation additions</th>
<th>Products of enzymatic assay</th>
<th>Transfer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose-6-P</td>
<td>P1</td>
</tr>
<tr>
<td>None</td>
<td>0</td>
<td>0.12</td>
</tr>
<tr>
<td>Glucose, 0.4</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Pyrophosphate</td>
<td>4.16</td>
<td></td>
</tr>
<tr>
<td>0.02</td>
<td>4.10</td>
<td>6.42</td>
</tr>
<tr>
<td>0.01</td>
<td>2.03</td>
<td>4.98</td>
</tr>
<tr>
<td>0.005</td>
<td>1.93</td>
<td>2.95</td>
</tr>
<tr>
<td>Glucose-6-P</td>
<td>3.88</td>
<td></td>
</tr>
<tr>
<td>0.02</td>
<td>2.20</td>
<td>3.85</td>
</tr>
<tr>
<td>P1, 0.02</td>
<td>0.84</td>
<td></td>
</tr>
<tr>
<td>No preincubation</td>
<td>6.65</td>
<td>11.34</td>
</tr>
</tbody>
</table>

* Micromoles present at end of assay minus micromoles present after preincubation.

Although the initial rate of reaction of PPi is more rapid with H2O than with glucose, glucose readily displaces H2O as the preferred acceptor. At high glucose concentration the transfer reaction predominates over the hydrolytic reaction to the extent of 80 to 85% transfer over a wide range of PPi concentration.

**Effect of Substrates and Products on Stability of Enzyme**

In the absence of substrate the microsomal enzyme is very unstable in the range of its optimal pH. Portions of microsomal suspensions, without added Triton X-100, were preincubated at 30° and pH 5.0 with several concentrations of substrates or products for 30 minutes. Pyrophosphatase assays were then carried out with 0.4 m glucose and 0.1 m PPi at 30° for an additional 30 minutes. The quantities of P1 and glucose-6-P formed, corrected for the amounts formed during preincubation, are given in Table VI.

The enzyme was completely inactivated in 30 minutes at 30° and pH 5 in the absence of substrate. Whereas glucose was entirely ineffective, PPi provided considerable protection to the enzyme. Glucose-6-P was nearly as effective as PPi in inhibiting the inactivation of the enzyme and the protecting effect of both of these substances was less as their concentration was decreased. Orthophosphate was appreciably less effective. The percentage of transfer activity remained approximately constant despite the different conditions of inactivation.

**DISCUSSION**

The inorganic pyrophosphate phosphotransferase activity of rat liver microsomes with which we are here concerned resembles in some regards the phosphotransferase activity observed in recent years for many acid and alkaline phosphomonoesterases obtained from a variety of plant and animal sources (24-27). Many different arylphosphates as well as creatine phosphate have been studied as donors and a variety of aliphatic hydroxy compounds used as acceptors. Rafter (10) was the first to use pyrophosphate as donor with glucose as acceptor.

Evidence in support of the assumption that the hydrolytic and the phosphotransferase activities are properties of the same enzyme is found in the parallel distribution of the two activities (Table I), in the nature of the competition between glucose and water as acceptors (Fig. 5), and in the parallel loss of the two kinds of enzymatic activities under a variety of conditions (Table VI). In view of the fact that whole microsomes rather than a purified protein were used, the possibility of the presence in the system of an additional enzyme with pyrophosphate hydrolytic activity cannot be entirely eliminated. However, the action of any such enzyme must be slight, since under optimal conditions the pyrophosphate phosphotransferase activity accounts for about 80 to 90% of the inorganic pyrophosphate used.

The existence of an enzymatic pathway leading to the formation of glucose-6-P from PPi independent of ATP and hexokinase or glucokinase action is of considerable interest. This reaction would conserve the otherwise wasted energy of inorganic PPi which is abundantly available as one of the products of many synthetic reactions in the animal (28). However, the possible importance of this method of glucose-6-P synthesis in the total body economy is difficult to assess.
SUMMARY

The occurrence, distribution, and some of the properties of an inorganic pyrophosphate phosphotransferase enzyme in rats have been studied.

The enzyme was found to occur only in liver and kidney and exclusively in the microsomal fraction, its distribution in subcellular fractions exactly paralleling that of glucose 6-phosphatase.

Hydrolysis of inorganic pyrophosphate by this enzyme is very effectively inhibited in a noncompetitive fashion by glucose. At high glucose concentrations the synthesis of glucose 6-phosphate from inorganic pyrophosphate predominates over the hydrolytic reaction to the extent of about 80 to 90% transfer.

Glucose 6-phosphate synthesis by the action of this pyrophosphate phosphotransferase is independent of the action of hexokinase and adenosine triphosphate.

Inhibition studies, anatomical distribution, and parallel inactivation conditions indicate that the transfer and hydrolytic activities are functions of the same enzyme.

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Metabolism of Inorganic Pyrophosphate: I. MICROSONAL INORGANIC PYROPHOSPHATE PHOSPHOTRANSFERASE OF RAT LIVER
Marjorie R. Stetten


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