Regulation and Mechanism of Phosphoribosylpyrophosphate Synthetase

II. EXCHANGE REACTIONS CATALYZED BY THE ENZYME*

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SUMMARY

Phosphoribosylpyrophosphate (PRPP) synthetase from Salmonella typhimurium LT-2 catalyzes an exchange between 14C-AMP and ATP in the absence of added ribose 5-phosphate. The exchange reaction requires Mg++ ions and low concentrations of inorganic phosphate in addition to the substrates. High concentrations of inorganic phosphate (which stimulate over-all PRPP synthesis and inhibit the over-all reverse reaction) inhibit the exchange reaction. Low concentrations (0.2 mM) of ribose 5-phosphate stimulate the exchange reaction from 10- to 55-fold depending on the pH of the reaction mixture, but higher concentrations are strongly inhibitory. The K_m values for the substrates are not substantially altered by ribose 5-phosphate stimulation.

PRPP synthetase also catalyzes an exchange between 14C-ribose 5-phosphate and PRPP in the absence of added adenine nucleotides. The Mg++ and phosphate requirements for this exchange reaction are similar to those of the AMP-ATP exchange reaction. Ribose 5-phosphate-PRPP exchange is stimulated more than 200-fold by low concentrations (0.1 mM) of AMP, but is strongly inhibited by higher concentrations of AMP. AMP stimulation is not a consequence of changes in K_m values for the substrates.

The maximal rates of the unstimulated AMP-ATP exchange reaction and of the unstimulated ribose 5-phosphate-PRPP exchange reaction are slower than the maximal rate of the over-all reverse reaction of PRPP synthetase, but the rates of the stimulated exchanges are at least as fast as the over-all reactions of PRPP synthetase. Evidence is presented that the unstimulated exchange reactions can occur in the complete absence of the stimulating partner substrates.

The results are interpreted as favoring a mechanism in which an enzyme-pyrophosphate intermediate is formed during the PRPP synthesis reaction, but in which this intermediate normally occurs in a ternary complex with AMP or ribose 5-phosphate (or both) noncovalently bound. The stimulation of exchange by AMP and ribose 5-phosphate is viewed as an example of "substrate synergism" (Bridge, W. A., Millen, W. A., and Boyer, P. D., Biochemistry, 7, 3608 (1968)).

The regulation of the biosynthesis of 5-phosphoribosyl a1-pyrophosphate is of interest because of the important roles that this compound plays in metabolism.

A previous paper in this series described the isolation and properties of PRP synthetase from Salmonella typhimurium (1). End product inhibition of the enzyme has been reported (2). Studies of the detailed mechanism of action of PRP synthetase were undertaken both because they would contribute to an understanding of the mechanism of feedback inhibition of the enzyme and because pyrophosphoryl group transfer reactions have not been previously characterized in detail.

The enzymic synthesis of PRPP from ATP and ribose 5-phosphate can be envisioned as proceeding by two general types of mechanisms, each with several possible detailed kinetic pathways. One mechanistic possibility is that an enzyme-pyrophosphate intermediate is formed, which subsequently transfers its pyrophosphoryl group to ribose 5-phosphate:

\[ \text{ATP + enzyme} \rightleftharpoons \text{enzyme-PP + AMP} \] (1)

\[ \text{Enzyme-PP + ribose-5-P} \rightleftharpoons \text{PRPP + enzyme} \] (2)

A second possibility is that the pyrophosphoryl group is transferred directly from ATP to ribose 5-phosphate while the two substrates are bound to the enzyme in a ternary complex:

\[ \text{ATP + ribose-5-P + enzyme} \rightleftharpoons \text{enzyme-ATP-ribose-5-P} \] (3)

The abbreviation used is: PRPP, 5-phosphoribosyl a1-pyrophosphate.
Enzyme-AMP-ribose-5-P = enzyme-AMP-PRPP (4)
Enzyme-AMP-PRPP = enzyme + AMP + PRPP (5)

The experiments in this communication constitute an attempt to distinguish between these possibilities and to obtain any possible additional information about the enzyme mechanism by a study of the properties of AMP-ATP exchange and ribose 5-phosphate-PRPP exchange reactions catalyzed by highly purified preparations of PRPP synthetase.

MATERIALS AND METHODS

Materials—PRPP synthetase was purified from S. typhimurium cells and assayed as described previously (1). Disodium ATP was purchased from Sigma. Ribose 5-phosphate, sodium salt, and dimagnesium PRPP were products of Calbiochem. PRPP solutions were prepared and assayed at the time of use as previously described (1). AMP was obtained from P-L Biochemicals.

Radiochemicals—AMP-8-14C (44 mCi per mmole) and ribose 5-phosphate-14C (30 mCi per mmole) were purchased from Schwarz BioResearch. Both radiochemicals were analyzed by cellulose thin layer chromatography with t-amy1 alcohol-formic acid-water (3:2:1) as the solvent and by high voltage electrophoresis at pH 3.5 and found to be better than 95% radiochemically pure. The 14C-AMP contained about 2% 14C-adenosine. The 14C-ribose 5-phosphate was contaminated with traces (less than 1%) of an unidentified radioactive impurity, possibly ribose. In each case the traces of radioactive impurities were separated from compounds of interest by the techniques used for study of the exchange reactions.

14C-AMP-ATP Exchange Assay—The reaction mixtures contained in a final volume of 50 µl: 50 mM triethanolamine-HCl (pH 7.5), 10 mM MgCl2 and variable amounts of 14C-AMP, ATP, potassium phosphate, and PRPP synthetase. The reactions were initiated by addition of the enzyme and incubated at 37°. The incubation tubes were covered with Parafilm. The reactions were stopped by heating at 100° for 60 sec and immediately cooling on ice, 10 µl of a “carrier” containing 4 mM PRPP and 20 mM ribose 5-phosphate were added to each reaction mixture. The entire reaction mixture was spotted on Whatman No. 3 paper with appropriate standards. After electrophoresis in acetic acid-pyridine-water (3:2:1, by volume), pH 3.5, for 2 hours at 4 kv (Gilson Electrophorator, model D), the paper strips were dried in air or in a warm air oven. The ribose 5-phosphate and FRPP regions were located by staining control strips (5); these regions were cut out and counted in 20 ml of Bray’s solution (8) or in 0.5% 2,5-diphenyloxazole (PPO) in toluene. In some cases the reaction mixtures were analyzed by electrophoresis of 10-µl aliquots in 100 µl solution (5) or in 0.5% 2,5-diphenyloxazole (PPO) in toluene. The radioactivity in the AMP and ATP regions of the thin layer sheets was found to be constant regardless of the extent of exchange and accounted for essentially all of the radioactivity added. Exchange rates were calculated from the formula:

Micromoles exchanged

\[ -2.303 \left( \frac{\text{pmoles of AMP}}{\text{pmoles of ATP}} \right) \frac{\text{pmoles of PRPP}}{} \left( \frac{1}{\text{pmoles of ATP}} \right) \left( \frac{1}{\text{pmoles of AMP}} \right) \left( \frac{\% \text{ exchange}}{100} \right) \]

where R-5-P is d-ribose 5-phosphate. The exchange rate was constant for at least 70 min at 37° when appropriate amounts of enzyme were present. The rate of 14C-ribose 5-phosphate-PRPP exchange was proportional to the amount of PRPP synthetase added, except that, as with the AMP-ATP exchange reaction, the activity was lower than expected when the amount of enzyme per assay was less than 2 µg. This effect was shown to be a consequence of the requirement of the exchange reaction for a low concentration of inorganic phosphate (added with the enzyme solution), since the rate of exchange was shown to be directly proportional to enzyme concentration at all concentrations tested if the potassium phosphate concentration was maintained at 5 to 10 mM. Hence all assays were performed at 5 mM phosphate unless otherwise noted.
Assay of Forward and Reverse Over-all Reactions of PRPP Synthetase—The over-all forward (PRPP synthesis) reaction was assayed with the 32P transfer assay previously described (1). The reaction conditions were as follows: 0.06 M triethanolamine-HCl (pH 7.5), 0.1 M potassium phosphate buffer (pH 8.0), 10 mM MgCl2, 2 mM ATP, and 5 mM ribose 5-phosphate. The reverse of the PRPP synthetase reaction was also assayed as previously described (1). The reaction mixtures contained 2 mM AMP and 0.25 mM PRPP.

RESULTS

14C-AMP-ATP Exchange Reaction

Requirements—Exchange between AMP-S-14C and nonradioactive ATP requires PRPP synthetase, MgCl2, and ATP, in addition to 14C-AMP (Table I). High concentrations of inorganic phosphate (which stimulate PRPP synthesis) inhibit the exchange. This is a true inhibition rather than an activation by the triethanolamine buffer, as is shown by an experiment in which both phosphate and triethanolamine were included. It is especially noteworthy that the addition of ribose 5-phosphate is not required. Details of the effects of ribose 5-phosphate and inorganic phosphate on AMP-ATP exchange are given in later sections.

Substrate Dependence of AMP-ATP Exchange—Dependence of the rate of AMP-ATP exchange at pH 7.5 on the concentration of AMP at two phosphate levels is shown in double reciprocal form in Fig. 1. Phosphate inhibition appears to be of a mixed competitive or noncompetitive rate equations with the computer analysis described by Cleland (6). The values obtained for the apparent Kₐ for AMP were 4.4 ± 1.1 mM (± S.E.) at 5 mM phosphate and 9.3 ± 1.5 mM at 15 mM phosphate. Both values were much higher than the Kₐ for AMP in the over-all reverse reaction (0.15 to 0.4 mM, Reference 1). The maximal velocity of the exchange is approximately twice as great at 5 mM phosphate (1.60 ± 0.24 µmole per min per mg) as at 15 mM phosphate (0.87 ± 0.10 µmole per min per mg). Both the maximal velocity and apparent Kₐ for AMP were significantly (p < 0.01) different at the two phosphate concentrations according to a t test (6).

The effects of varying ATP and Mg++ ion concentration on the rate of AMP-ATP exchange (Fig. 2) show that Mg-ATP inhibits at concentrations above 1 mM. Excess Mg++ increases the apparent maximal velocity about 10-fold, but does not significantly alter the Kₐ for Mg-ATP, which was 1.4 mM under these conditions.

Effects of Ribose 5-Phosphate on AMP-ATP Exchange—Although PRPP synthetase readily catalyzes an AMP-ATP exchange without addition of ribose 5-phosphate, the addition of low concentrations of ribose 5-phosphate stimulates the exchange appreciably. The rates of AMP-ATP exchange at various pH values with and without 0.4 mM ribose 5-phosphate are shown in Fig. 3, which shows that the unassisted exchange reaction has a pH optimum at about 7.0. At the pH optimum for PRPP synthetase (8.0 to 8.5, Reference 1) the exchange reaction is almost absolutely dependent on the presence of ribose 5-phosphate. As the pH is lowered, the exchange reaction becomes much less dependent on ribose 5-phosphate.

The concentration dependence of ribose 5-phosphate stimulation of AMP-ATP exchange is shown in Fig. 4. This dependence was examined at pH 8.3, where the exchange in the absence of ribose 5-phosphate is very slow (about 0.027 µmole per min per mg), and at pH 7.0, where it is about 15 times as fast. In both cases the exchange reaction was stimulated by concentrations of ribose 5-phosphate up to 0.2 mM and was then sharply inhibited by higher levels. The maximal stimulation appears to be higher at pH 8.3 than at pH 7.0 because the unassisted rate at pH 8.3 is very low; the actual maximal rate of exchange is about twice as high at pH 7.0 as at pH 8.3.

The possibility that ribose 5-phosphate exerts its stimulatory

| Requirement | Exchange rate (µmole/15 min) |
|-------------|-------------------------------|
| Complete... | 18.8                          |
| Minus enzyme| 0.1                           |
| Minus MgCl₂| 0.2                           |
| Minus ATP   | 0.1                           |
| Plus 0.1 M potassium phosphate, pH 7.5 (plus triethanolamine) | 0.6 |
| Plus 0.1 M potassium phosphate, pH 7.5 (minus triethanolamine) | 0.8 |

Fig. 1. Effect of AMP concentration on the rate of AMP-ATP exchange. Conditions were as in the legend to Table I, except that the AMP concentration (specific activity 1.42 x 10⁶ cpm per µmole) was varied, the final potassium phosphate concentration was as shown, and the incubation time was 10 min.
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FIG. 2. Effect of ATP concentration on the rate of AMP-ATP exchange. Conditions were as in the legend to Table 1, except that the ATP concentration was varied as shown and incubations were for 10 min. Upper curve, MgCl₂ was added in quantities equimolar to the ATP added; lower curve, 10 mM excess MgCl₂ was added.

FIG. 3. Dependence of the AMP-ATP exchange reaction on pH. Reaction mixtures contained: 10 mM MgCl₂, 1 mM ATP, 2 mM [¹⁴C]-AMP (1.22 × 10⁶ cpm per µmole), 5 mM potassium phosphate, 3.7 µg of PRPP synthetase (30 units per mg), and the following buffers at 0.05 M: □, imidazole-HCl; ○, triethanolamine-HCl. Open symbols, 0.4 mM ribose 5-phosphate (R₅P, R-5-P) was added; solid symbols, no ribose 5-phosphate was added. The dashed line gives the rates which result if the exchange in the absence of ribose 5-phosphate is subtracted from the exchange in the presence of ribose 5-phosphate. The reaction time was 10 min.

FIG. 4. Effects of ribose 5-phosphate on AMP-ATP exchange. The conditions were as in Fig. 3, except that the concentrations shown of ribose 5-phosphate (R₅P) were added and the buffers were 0.05 M imidazole-HCl (pH 7.0) (●) or 0.05 M triethanolamine-HCl (pH 8.3) (○). Rates in the absence of ribose 5-phosphate (Vₒ) are compared with rates in the presence of various levels of ribose 5-phosphate (V). Times of reaction varied from 2 to 10 min.

FIG. 5. Effect of ribose 5-phosphate (R₅-P) on ATP requirement of the AMP-ATP exchange reaction. Conditions were as in Fig. 3, except that ATP and ribose 5-phosphate were added as shown and the buffer was 0.05 M triethanolamine-HCl, pH 8.0.

The substrate inhibition by Mg-ATP appears to be even greater at this pH than at pH 7.5. Ribose 5-phosphate has very little effect on the Kₘ for ATP; from the linear portions of double reciprocal plots of the data of Fig. 5, the Kₘ is changed only slightly (from 0.1 mM to 0.2 mM) by addition of 20 µM ribose 5-phosphate. The maximal velocity, on the other hand, was increased 15-fold. The concentration dependence of AMP for the exchange in the presence and absence of ribose 5-phosphate (Fig. 6) leads to a similar conclusion. The AMP saturation curve is sigmoid at this pH. This appears to be true whether ribose 5-phosphate is present or not, although the phenomenon is
Fig. 6. Effect of ribose 5-phosphate (R-5-P) on AMP requirement of the AMP-ATP exchange reaction. Conditions were as in Fig. 3, except that AMP (1.38 \times 10^6 cpm per pmole) and ribose 5-phosphate were added as shown and the buffer was 0.05 M triethanolamine-HCl, pH 8.0.

less pronounced when ribose 5-phosphate was added (n = 2.1 in a Hill plot of the data obtained in the absence of ribose 5-phosphate; when ribose 5-phosphate was present, n = 1.6). Saturation occurs at about the same concentration of AMP in both cases, but the maximal rate of exchange is clearly increased severalfold by ribose 5-phosphate. The cause of the sigmoid concentration dependence for AMP under these conditions (pH 8.0) is not known.

Inhibition of AMP-ATP exchange by high levels of ribose 5-phosphate (Fig. 4) may be a consequence of depletion of ATP to suboptimal levels by way of PRPP formation, since the equilibrium of the reaction favors PRPP synthesis (1), and the amount of enzyme and substrates present would have permitted extensive reaction. This suggestion receives support from the experiments shown in Table II, in which inhibition of AMP-ATP exchange by increasing ribose 5-phosphate concentrations was examined at three ATP concentrations. The inhibition was abolished by increasing the concentration of ATP from 0.4 mM to 2.0 mM, even though 0.4 mM ATP was optimal at 20 \mu M ribose 5-phosphate (Fig. 5). The inhibition by excess ribose 5-phosphate may also be due in part to a direct effect on the exchange process, as is predicted by the exchange rate expression for a simple ternary mechanism (see "Appendix").

Heat Inactivation of PRPP Synthetase and AMP-ATP Exchange Reactions—Since the PRPP synthetase preparations used in this study were highly purified, but not homogeneous, it is possible that the AMP-ATP exchange reaction—in particular, the unassisted exchange—was catalyzed by an impurity in the preparation rather than by PRPP synthetase itself. This possibility was rendered extremely unlikely by a study of the relative rates of heat inactivation of PRPP synthetase activity and AMP-ATP exchange activity (Fig. 7). At 60°C PRPP synthesis and the unassisted AMP-ATP exchange reactions are rapidly inactivated until a plateau is reached at about 17% of the original level. This residual activity is inactivated only very slowly. The rates of inactivation of PRPP synthetase activity and unassisted AMP-ATP exchange activity are identical throughout the experiment. This result strongly suggests that these two activities reside in the same protein molecule. The shape of the inactivation curve suggests that heating at 50°C yields a modified enzyme which is intrinsically less active, but not completely inactivated. However, an alternative explanation, the existence of two PRPP synthetases with differing sensitivities to heat cannot be excluded.

\[ ^{14}C \text{-Ribose 5-Phosphate-PRPP Exchange Reaction} \]

Requirements—PRPP synthetase catalyzes exchange between \(^{14}C\)-ribose 5-phosphate and nonradioactive PRPP in the presence of PRPP synthetase and MgCl\(_2\) (Table III). In the absence of unlabeled PRPP, no radioactivity was found in the PRPP region. It was not necessary to add AMP to observe this exchange, but it was markedly stimulated by AMP.

Effect of AMP—The relatively slow exchange between \(^{14}C\)-ribose 5-phosphate and PRPP in the absence of added AMP (Table III) is greatly accelerated by AMP (Fig. 8); at as low as 0.1 mM AMP a 200-fold enhancement of the rate was observed. At higher concentrations AMP becomes very inhibitory. The general effects of AMP on this exchange reaction are similar to the effects of ribose 5-phosphate on the AMP-ATP exchange reaction.

The dependence of the rate of the ribose 5-phosphate-PRPP exchange reaction on pH in the presence and absence of a highly stimulatory level of AMP was examined (Fig. 9). The pH optima in both cases were at 7.5, which is somewhat lower than the optimum for the over-all synthesis reaction. The pH rate profile in the presence of AMP is very similar to, although not identical with, that observed in the absence of AMP.
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PRPP synthesis

14C-AMP-ATP exchange (without R5P)

PRPP synthesis

FIG. 7. Heat inactivation of PRPP synthetase and the AMP-ATP exchange reaction. A solution of PRPP synthetase (30 units per mg, 0.7 mg per ml) in 0.05 M potassium phosphate, pH 7.5, was heated at 50° for the times shown. Aliquots were withdrawn and immediately cooled to 0°. PRPP synthesis (○) was assayed as described under "Materials and Methods." The AMP-ATP exchange reaction (■) was assayed at 0.05 mM: imidazole-HCl (pH 7.0), 10 mM MgCl₂, 2 mM 14C-AMP, 1 mM ATP, without ribose 5-phosphate (R5P). Reactions were for 10 min.

TABLE III
Requirements of 14C-ribose 5-phosphate-PRPP exchange reaction

The complete system contained 0.05 M triethanolamine-HCl buffer (pH 7.5), 10 mM MgCl₂, 1 mM 14C-ribose 5-phosphate (3.88 x 10⁶ cpm per µmole), 1 mM PRPP, 5 mM potassium phosphate (added in the enzyme solution), and 2.5 µg of PRPP synthetase (81 units per mg). Rates of exchange were determined as under "Materials and Methods." Incubations were for 10 min at 37°.

| Reaction mixture | Exchange rate (µmoles/min) |
|------------------|----------------------------|
| Complete         | 0.150                      |
| Minus enzyme     | 0.008                      |
| Minus MgCl₂      | 0.010                      |
| Minus PRPP       | 0.008                      |
| Plus 0.1 mM potassium phosphate, pH 7.5 | 0.020 |
| Plus 1 mM AMP    | 1.81                       |

A double reciprocal plot of the rate of ribose 5-phosphate-PRPP exchange as a function of PRPP concentration in the presence and absence of 20 µM AMP is shown in Fig. 10. AMP stimulation is shown to be the consequence of a large increase in the maximal velocity of the exchange rather than an effect on the Kₘ for PRPP, which was 0.1 mM in this experiment. This value is within the range found for the overall reverse reaction, which was 0.05 to 0.13 mM (1).

Similarly, the Kₘ for ribose 5-phosphate in the ribose 5-phosphate-PRPP exchange reaction was not substantially altered by the addition of 20 µM AMP (Fig. 11). The Kₘ for ribose 5-phosphate obtained from the data of Fig. 11 was 0.2 mM. The Kₘ for ribose 5-phosphate in the overall synthetic reaction (2 mM ATP, 5 mM MgCl₂, 0.1 mM potassium phosphate (pH 7.5), Reference 1) was the same within experimental error (0.28 mM). The results indicate that AMP probably does not exert its stimulatory action through changes in the affinity of the system for its substrates.

FIG. 8. Effects of AMP on the ribose 5-phosphate-PRPP exchange reaction. Conditions were as in the legend to Table III, except that the indicated concentrations of AMP were added. Times of incubation ranged from 1 to 10 min. Rates (V) are compared to V₀, the rate observed when no AMP was added. R5P, d-ribose 5-phosphate.

FIG. 9. pH dependence of the ribose 5-phosphate-PRPP exchange reaction. The reaction mixtures contained 10 mM MgCl₂, 1 mM 14C-ribose 5-phosphate (1.73 x 10⁴ cpm per µmole), 1 mM PRPP, 5 mM potassium phosphate, and 3.7 µg of PRPP synthetase (30 units per mg). Buffers were: ○, imidazole-HCl; ●, triethanolamine-HCl. AMP was added as shown. Note the change in scale of the ordinates in the two figures. Times of incubation ranged from 1 to 20 min. R5P, d-ribose 5-phosphate.
The inhibition of ribose 5-phosphate-PRPP exchange by high levels of AMP deserves further attention. In the case of the AMP-ATP exchange, inhibition by high levels of ribose 5-phosphate could be explained as resulting from depletion of ATP by way of PRPP formation (see Table II and the accompanying discussion), although other causes were not excluded. It is possible that high levels of AMP might inhibit the ribose 5-phosphate-PRPP exchange through depletion of the PRPP pool via ATP formation, rather than through some direct effect of AMP on the enzyme. No experiments were performed to test this possibility, but it appears to be ruled out by the unfavorable equilibrium of the reaction in the direction of ATP formation (1). In order for the highest level of AMP tested (4 mM) to bring about the observed inhibition through PRPP depletion alone, the concentration of PRPP would have to be reduced to less than the $K_m$ for PRPP for the exchange reaction (0.1 mM, see Fig. 10). This requires a greater than 90% conversion of PRPP to ATP and ribose 5-phosphate, which is not permitted by the initial concentrations of the substrates and the measured equilibrium constant for the reaction. Thus, the inhibition of ribose 5-phosphate-PRPP exchange by high levels of AMP is not simply a consequence of PRPP depletion, and results from an effect of AMP itself (or possibly ATP) on the exchange reaction (see "Appendix").

**Heat Inactivation of Ribose 5-Phosphate-PRPP Exchange Reaction**—The possibility that the ribose 5-phosphate-PRPP exchange studied here was catalyzed by an impurity in the enzyme preparation, rather than by PRPP synthetase itself, was tested by heat inactivation experiments. In Fig. 12 it may be seen that heating the enzyme solution at 50°C very rapidly inactivates slightly over 80% of the PRPP synthetase activity and the ribose 5-phosphate-PRPP exchange activity. The remaining activity is then only very slowly inactivated by further heating. These results reproduce very well the results of an earlier experiment on the heat lability of the AMP-ATP exchange reaction (Fig. 7). The lability to heat of the PRPP synthesis and unassisted ribose 5-phosphate-PRPP exchange reactions are clearly identical, but the AMP-stimulated exchange appears to be inactivated to a slightly greater extent. This difference may not be significant; after 30 min of heating, 10% of the PRPP synthetase activity...
remained, while 15% of the original assisted exchange activity was found. These rather small differences are exaggerated by the semilogarithmic plot. In any case, the striking similarity between the heat inactivation curves provides strong evidence that the ribose 5-phosphate-PRPP exchange reaction—like the AMP-ATP exchange reaction—is catalyzed by PRPP synthetase rather than an impurity in the preparation.

**Effects of Phosphate on Exchange and Over-all Reactions of PRPP Synthetase**

The effects of inorganic phosphate on the reactions catalyzed by PRPP synthetase are complex. In a previous paper (1) it was shown that the enzyme has a specific and apparently absolute requirement for high levels of phosphate when the synthesis of PRPP was measured. Double reciprocal plots of the effects of phosphate on PRPP synthesis are bimodal and are described by two linear portions corresponding to apparent \( K_m \) values of 2.3 mM and 40 mM at pH 7.5. Removal of phosphate from the enzyme by dialysis or by simply diluting the enzyme into cold-distilled water inactivates PRPP synthetase; under some conditions activity can be restored by adding phosphate to enzyme solutions that contain very low concentrations of phosphate.

**Effects of Phosphate on AMP-ATP Exchange**—The AMP-ATP exchange reaction is strongly inhibited by inorganic phosphate (Table I and Fig. 13). Even though this inhibition was observed at all concentrations above 5 mM, removal of phosphate from the enzyme solution by overnight dialysis against Tris-Cl, pH 7.5, or triethanolamine-HCl abolished the ability of the enzyme to catalyze the exchange reaction. Simultaneously the enzyme lost over 90% of its ability to catalyze PRPP synthesis (\(^{32}\)P transfer assay). Phosphate is an effective inhibitor at quite low concentrations (Fig. 13); for example, a 76% decrease in activity was observed on increasing the phosphate concentration from 5 mM to 15 mM. Concentrations of phosphate which are optimal for PRPP synthesis (0.1 M or higher) virtually abolish the exchange reaction.

**Effects of Phosphate on Ribose 5-phosphate-PRPP Exchange Reaction**—As with the AMP-ATP exchange reaction, ribose

The exchange between AMP and ATP which is catalyzed by PRPP synthetase is sensitive to inhibition by phosphate whether the exchange is stimulated by ribose 5-phosphate or not (Fig. 14); however, the concentration dependences of phosphate inhibition are altered by ribose 5-phosphate. In Fig. 14 it is seen that the presence of 0.1 mM ribose 5-phosphate lowers the sensitivity of the exchange to phosphate inhibition. It is significant that the relative sensitivity of AMP-ATP exchange to phosphate is shifted by ribose 5-phosphate to a curve which is identical with the phosphate sensitivity curves of the reverse over-all reaction and the ribose 5-phosphate-PRPP exchange reaction (see below). This finding suggests that ribose 5-phosphate may act by altering the rate-determining step of the exchange so that it becomes the same step that determines the rate of these other reactions.

**Fig. 13. Effects of phosphate on reactions catalyzed by PRPP synthetase. All rates are expressed as percentage of the rate under the same conditions at 5 mM potassium phosphate, pH 7.5.**

- \( \Delta \), reverse reaction, measured as under "Materials and Methods";
- \( \nabla \), ribose 5-phosphate-PRPP exchange reaction without AMP, determined as in Table III or Fig. 15, respectively;
- \( \square \), ribose 5-phosphate-PRPP exchange reaction in the presence of 4 X 10\(^{-4}\) M AMP (data of Fig. 15);
- \( \bigcirc \), AMP-ATP exchange reaction, measured as in Table 1. RuP, \( \nu \)-ribose 5-phosphate; CONC, concentration.

**Fig. 14. Effect of ribose 5-phosphate (RuP, R-5-P) inhibition of AMP-ATP exchange.** The conditions were as in Fig. 3, except that potassium phosphate, pH 7.0, and ribose 5-phosphate were added as shown and the buffer was imidazole-HCl, pH 7.0. The effect of phosphate on the \(^{32}\)P-ribose 5-phosphate-PRPP exchange reaction (from Fig. 13) is shown for comparison (\( \times \)).

**Fig. 15. Relation between phosphate inhibition and AMP stimulation of ribose 5-phosphate-PRPP exchange.** Conditions were as in Table III, except that the concentration of potassium phosphate, pH 7.5, was as shown and 2.5 \( \mu \)g of PRPP synthetase with a specific activity of 22.5 units per mg were used. Incubations varied from 2 to 30 min. \( \bigcirc \), no AMP was added; \( \bullet \), 4 X 10\(^{-5}\) M AMP was added. In 5 mM phosphate the exchange rate in the presence of 4 X 10\(^{-5}\) M AMP was 120 times as fast as without AMP. RuP, \( \nu \)-ribose 5-phosphate; CONC, concentration.
5-phosphate-PRPP exchange is inhibited by potassium phosphate (Table III and Fig. 13). As a function of phosphate concentration, inhibition of this exchange reaction is more gradual than phosphate inhibition of AMP-ATP exchange. Enzyme solutions which have had phosphate removed by dialysis have less than 3% of the activity of an undialyzed control; such a dialyzed preparation has also lost its ability to catalyze PRPP synthesis and the AMP-ATP exchange reaction.

A possible interrelation between phosphate inhibition and AMP stimulation of the ribose 5-phosphate-PRPP exchange reaction was probed by examining the concentration dependence of phosphate inhibition of the exchange in the presence and absence of 40 mM AMP (Fig. 15). The results show that the sensitivity of the exchange to phosphate inhibition was nearly the same, if not identical, whether AMP was present or not, even though the rate of exchange was more than 100 times as fast when AMP was added.

Effects of Phosphate on Reverse Over-all Reaction—It has been shown that the removal of inorganic phosphate from PRPP synthetase by dialysis inactivates the enzyme, both with respect to its ability to catalyze PRPP synthesis and the exchange reactions. Since the requirement for phosphate is a very unusual one, this observation may be taken as additional evidence that the exchange reactions and the over-all PRPP synthesis reaction are catalyzed by the same protein, and, further, that the exchange reactions are kinetically significant components of the over-all reaction. On the other hand, the fact that concentrations of phosphate above 5 mM inhibit the exchange reactions while stimulating the synthetic reaction seems to contradict this conclusion.

The probable resolution of this apparent contradiction has been obtained from studies on the effects of phosphate on the reverse reaction of PRPP synthetase, i.e. on the formation of ATP from PRPP and AMP. Fig. 13 shows that the reverse reaction requires low levels of phosphate but that, when the concentration of phosphate exceeds 5 mM, the reaction is inhibited. For comparative purposes the effects of phosphate on the exchange reactions are superimposed in the same figure. It is clear that the effect of increasing phosphate concentration on the ribose 5-phosphate-PRPP exchange reaction is essentially the same as on the reverse reaction. The AMP-ATP exchange reaction is also inhibited by phosphate, but it is somewhat more sensitive to increasing phosphate concentration than the reverse reaction. However, it will be recalled that, when the AMP-ATP exchange reaction is inhibited by ribose 5-phosphate, the phosphate inhibition curve also shifts to one which is the same as that of the reverse reaction (Fig. 14). Data about the effects of phosphate levels below 5 mM on the exchange reactions have not been determined, but it is probable that the exchange reactions also require low concentrations of phosphate since removal of phosphate by dialysis inactivates them and since 5 mM phosphate must be included to yield a linear response of the exchange rates to enzyme level (see "Materials and Methods").

DISCUSSION

Maximal Rates of Exchange Reactions—The maximal rates of the unassisted exchange reactions appear to be appreciably slower than the maximal rate of either the reverse or the forward over-all reactions of PRPP synthetase. Comparison of these rates is somewhat uncertain because of the complex effects of phosphate on the system. Since 5 mM phosphate is optimal for the reverse reaction and for both of the exchange reactions, I have chosen the assay condition containing 5 mM potassium phosphate, pH 7.5, as a "standard state" for comparing these rates. There is evidence that PRPP synthetase is not stable at this phosphate concentration, however. Dilution of the enzyme into 2.5 mM phosphate brings about a very rapid decline in synthetic activity to a value that is about 30% of the initial value and does not decline further (1). It is possible that a similar partial inactivation occurs during dilution of the enzyme into the 5 mM phosphate which was present in the exchange assays. Furthermore, the forward reaction requires much higher concentrations of phosphate and proceeds at only about 20% of the maximal rate at 5 mM phosphate. These complexities should be kept in mind in assessing the following comparisons.

In a previous paper (1) it was shown that the reverse of the PRPP synthetase reaction is only about 15% as fast as the synthesis of PRPP. This comparison was made from reaction rates obtained with 50 mM phosphate, a concentration at which the enzyme is known to be stable. The reverse reaction is about 1.85 times as fast at 5 mM phosphate (Fig. 13); this yields an estimate for the maximal velocity of the reverse reaction of 10.9 μmoles per min per mg of enzyme. The forward reaction can be estimated in a similar fashion to be about 20% of its maximal rate at 0.1 mM phosphate, or approximately 13 μmoles per min per mg.

The maximal velocity of AMP-ATP exchange at saturating AMP concentrations, 1 mM ATP (the optimum concentration), and 5 mM phosphate was 1.6 μmoles per min per mg (Fig. 1). Thus, even if appreciable inactivation results from diluting the enzyme into 5 mM phosphate, the maximal rate of AMP-ATP exchange is probably less than half of that of the reverse reaction. The presence of low levels of ribose 5-phosphate stimulates the AMP-ATP exchange from 10-fold at pH 7.0 to 55-fold at pH 8.3, bringing about rates of exchange that are clearly faster than the reverse reaction and are probably as fast as the forward reaction. The phosphate inhibition curve and the pH activity profile of the ribose 5-phosphate-stimulated AMP-ATP exchange—rather than the unstimulated exchange—most closely resemble those of the reverse reaction. These observations suggest that the stimulated exchange proceeds by steps that are involved in the over-all reaction, while the unstimulated exchange may not.

The maximal rate of the ribose 5-phosphate-PRPP exchange is much slower than the AMP-ATP exchange unless AMP is added. From the data of Fig. 10 a maximal velocity of 0.006 μmoles per min per mg was obtained at saturating PRPP concentrations and 1 mM ribose 5-phosphate. Therefore, even after extrapolation to saturating ribose 5-phosphate concentrations and allowing for a 3-fold inactivation on dilution into 5 mM phosphate, the maximal rate of the unstimulated ribose 5-phosphate-PRPP exchange is not above 0.4 μmoles per min per mg. On the other hand, low concentrations of AMP have been shown to stimulate the exchange by more than 200-fold—yielding rates which are easily as rapid as the reverse reaction.

It might be expected that the maximal rates of the exchange reactions would be equal to or greater than the maximal rate of the reverse or forward reaction, whichever is the slower, if the exchange reactions proceed by the same reaction path as the
over-all reaction. Actually, it can be shown for some of the mechanisms applicable to this system that this condition need not be met (see "Appendix"). Nonetheless, the stimulation of the exchange reactions by partner substrates provides valuable information about the mechanism of the PRPP synthetase reaction even if no assumptions about the maximal rate of exchange and over-all reactions are made.

It should be understood that the exchange rates determined in this work were not obtained at reaction equilibrium. In the cases of the unassisted exchange reactions, this presents no problem since there is no net reaction during the exchange assay. However, when the stimulating substrates were added, net reaction as well as exchange takes place. This leads to errors in the determination of the exchange rate, especially in those cases in which the level of stimulating substrate is high. The data are adequate to support the qualitative conclusions drawn in this paper, but the use of exchange rates in conjunction with a detailed steady state kinetic analysis will require determination of true equilibrium exchange rates.

Do Exchange Reactions Absolutely Require AMP and Ribose 5-Phosphate?—It is useful to consider the results of this study in relation to the two general mechanisms described in the introductory section, namely, a mechanism involving formation of an enzyme-pyrophosphate intermediate and a mechanism in which direct pyrophosphate transfer occurs on a ternary complex. The latter mechanism requires that neither AMP-ATP exchange nor ribose 5-phosphate PRPP exchange can occur in the absence of the other substrate, i.e. ribose 5-phosphate or AMP, respectively (see "Appendix"). Both of the exchange reactions were readily observed without adding these compounds. However, since both AMP and ribose 5-phosphate bring about marked stimulation, is it possible that these compounds were present as impurities? In the case of AMP-ATP exchange, the fact that the assisted and unassisted exchange reactions have different pH dependencies (Fig. 3) makes it unlikely that ribose 5-phosphate-independent exchange is an artifact caused by ribose 5-phosphate contamination of the enzyme or substrates. If the exchange reaction had an absolute requirement for ribose 5-phosphate and was observed in the absence of added ribose 5-phosphate only because of contamination, the assisted and unassisted exchange reactions should have the same pH rate profile, since they would be determined by the same enzymic events. Furthermore, no ribose 5-phosphate has been detected in the reactants with the very sensitive 3P transfer assay (1). With respect to the ribose 5-phosphate-PRPP exchange, the pH dependencies of the exchange with and without AMP (Fig. 9) are very similar, so no argument can be made that an AMP impurity is absent. In the case with AMP-ATP exchange, however, if either substrate contained AMP as an impurity, one would not expect the exchange rate to reach a maximum as substrate was added until the system was saturated with both substrate and AMP. Yet the rate enhancement by added AMP is observed when the system is saturated with either ribose 5-phosphate or PRPP (Figs. 10 and 11). Therefore, it is unlikely that either substrate contained AMP as an impurity. Similarly, if the enzyme contained AMP as an impurity, a curve of rate plotted against enzyme concentration might be expected to curve upward; it does not. The enzyme which was used in these studies has been purified some 450-fold by a procedure which includes repeated ammonium sulfate and acid precipitations. Thus, while the presence of very small quantities of very tightly bound AMP cannot be excluded, it is unlikely that the enzyme contains significant amounts of AMP. The results justify the tentative conclusion that at least one and possibly both exchange reactions can occur without the involvement of the stimulating partner substrate.

Mechanism of PRPP Synthetase Reaction—If one accepts the conclusion that the stimulation of the exchange reactions catalyzed by PRPP synthetase is the consequence of an acceleration of previously existing exchange processes, what can be deduced from these findings about the reaction mechanism? It is likely that the unassisted exchange reactions proceed by way of an enzyme-pyrophosphate intermediate:

\[
\text{ATP} + \text{enzyme} \rightleftharpoons \text{enzyme-PP} + \text{AMP} \quad (6)
\]

and

\[
\text{PRPP} + \text{enzyme} \rightleftharpoons \text{enzyme-PP} + \text{ribose-5-P} \quad (7)
\]

The occurrence of these reactions tends to implicate the two-step mechanism of Equations 1 and 2 (a ping-pong bi bi mechanism in the terminology of Cleland, Reference 7). This simple mechanism must be excluded, however, because it predicts that the only effect of adding the second substrate will be to inhibit the exchange reaction (see "Appendix"). A mechanism involving an enzyme-pyrophosphate intermediate can be accommodated only if binding of the second substrate in some way increases the rate of the exchange process.

A ternary mechanism involving direct transfer of pyrophosphate from ATP to ribose 5-phosphate predicts that the non-exchanging substrate will first stimulate and then inhibit the exchange reaction as the concentration is increased (see the "Appendix" for a discussion of a simple case). Such a model accounts qualitatively for the observed effects of AMP and ribose 5-phosphate on the exchange reactions, but also requires that the exchange cannot occur in the absence of the stimulating substrate.

From the above considerations it appears that only a mechanism involving ternary complexes can account for the observed properties of the enzyme reactions. There are two general possibilities. The first, which I favor, envisions a scheme in which an enzyme-pyrophosphate intermediate is formed as in the unassisted exchange reactions, but in which the partner substrates remain attached to the active site and alter the rates of individual catalytic steps. Thus, the normal reaction sequence might be as follows (without necessarily requiring the order of binding given):

\[
\text{ATP} + \text{enzyme} \rightleftharpoons \text{enzyme-PP} \quad (8)
\]

\[
\text{Enzyme-PP} + \text{AMP} + R-5-P \rightleftharpoons \text{enzyme-PP} + \text{AMP-R-5-P} \quad (9)
\]

\[
\text{Enzyme-PP} + \text{AMP} + R-5-P \rightleftharpoons \text{enzyme} + \text{AMP-PRPP} \quad (10)
\]

\[
\text{Enzyme-AMP-PRPP} \rightleftharpoons \text{enzyme} + \text{AMP} + \text{PRPP} \quad (11)
\]

It will be seen that this scheme differs only from the mechanism of Equations 3, 4, and 5 by the identification of enzyme-bound ATP as an enzyme-pyrophosphate intermediate with AMP non-covalently attached. The postulate must also include reactions involving the free enzyme-pyrophosphate intermediate, which participates in the unassisted exchange reactions (Equations 6 and 7), but which is not a participant in the over-all reaction or the assisted exchange reactions. The hypothesis is shown in schematic form in Fig. 16. In such a scheme the stimulatory effects of AMP and ribose 5-phosphate on the exchange reactions may be thought of as examples of "substrate synergism," as
Enzyme-pyrophosphate intermediates have been proposed for expressions for two enzymes on the basis of exchange studies. In these PRPP synthetase reactions catalyzed by PRPP synthetase, which tend to implicate an enzyme-pyrophosphate intermediate, are mechanistically unrelated to the over-all catalysis. In this case the stimulation of the AMP-ATP exchange reaction by ribose 5-phosphate, for example, would be the result of a shift in mechanism from one involving an enzyme-pyrophosphate intermediate to a reaction path in which ribose 5-phosphate serves as the intermediate pyrophosphate carrier. A similar shift in mechanism would presumably apply to AMP stimulation of the ribose 5-phosphate-PRPP exchange reaction. The over-all reaction mechanism of PRPP synthesis in this formulation would proceed by direct pyrophosphate transfer in ternary complexes as shown in Equations 3, 4, and 5. The data in this paper do not permit a conclusive choice between these alternatives, but the a priori assumption of a shift in reaction mechanism on the addition of a second substrate seems unwarranted. If the enzyme is capable of forming a covalent pyrophosphoryl intermediate in both unassisted exchange reactions, it seems unlikely that this reaction pathway is mechanistically unrelated to the over-all reaction pathway which involves the same substrates and the same general chemistry, i.e., pyrophosphoryl group transfer. For this reason, I conclude that the results favor the reaction scheme summarized in Fig. 16. It should be possible to test the consistency of the proposed mechanism with kinetic observations, although steady state kinetics will not distinguish between the two possibilities described above.

The suggested mechanism predicts that it should be possible to isolate an enzyme-pyrophosphate intermediate with substrate amounts of purified PRPP synthetase and 32P-labeled ATP. In fact, it has been possible to isolate a phosphorylated derivative of PRPP synthetase (9). A detailed study of this derivative may provide a definitive examination of the mechanistic possibilities suggested in this paper.

If the PRPP synthetase reaction does indeed proceed by way of an enzyme pyrophosphate intermediate, it will be of great interest to compare this enzyme with the phosphoenolpyruvate synthetases from Escherichia coli (10, 11) and propionibacteria (12). Enzyme-pyrophosphate intermediates have been proposed for both of these enzymes on the basis of exchange studies. In these cases, however, the pyrophosphoryl group does not appear to be transferred directly, but rather it is cleaved to yield an enzyme-phosphate derivative. It seems likely that these enzymes and PRPP synthetase will be found to share chemical and mechanistic properties. It will also be of interest to learn whether the PRPP synthetase reaction is mechanistically related to other pyrophosphate-transferring enzymes such as thiamine pyrophosphokinase (13) and 7,8-dihydro-2-amino-4-hydroxy-6-hydroxyethyldine pyrophosphokinase (14).

Acknowledgments—The early part of this work was performed by L. N. Ornston and B. V. Plapp for critical readings of the manuscript. It is a pleasure to acknowledge Professor Paul Eoyer for valuable assistance with the ideas developed in the “Appendix” and Drs. L. N. Ornston and B. V. Plapp for critical readings of the manuscript.

APPENDIX

Derivation and Properties of Equilibrium Exchange Rate Expressions for Two Simple Mechanisms for PRPP Synthetase

Mechanism Involving Free Enzyme-Pyrophosphate Intermediate—

\[ E + ATP \xrightarrow{k_1} E \cdot ATP \]
\[ E \cdot ATP \xrightarrow{k_2} E \cdot PP + AMP \]
\[ E \cdot PP + R-5-P \xrightarrow{k_3} E \cdot PRPP \]
\[ E \cdot PRPP \xrightarrow{k_4} E + PRPP \]

The general approach is that of Boyer (15, 16). For AMP-ATP exchange, as an example, let \( R_1 \) equal the rate of appearance of label from AMP into ATP at equilibrium. \( R_1 = k_{-1} \)
\[(E\cdot ATP)_{a}\] where \((E\cdot ATP)_{a}\) is the concentration of that fraction of \(E\cdot ATP\) which is labeled by AMP.

The rate of \(E\cdot ATP\) formation from \(E\) and ATP = \(k_{1} (E) (ATP) = k_{-1} (E\cdot ATP)\) at equilibrium. The rate of \(E\cdot ATP\) formation from AMP = \(k_{-2} (E\cdot PP) (AMP) = k_{2} (E\cdot ATP)\) at equilibrium.

Then the fraction of \(E\cdot ATP\) from AMP \(E + ATP \rightarrow \) 
\[\frac{\text{rate of } E\cdot ATP \text{ formation from AMP}}{\text{total rate of } E\cdot ATP \text{ formation}} = \frac{k_{2}}{k_{-1} + k_{2}}\]

Therefore,
\[R_1 = \frac{k_{1}k_{2}}{k_{-1} + k_{2}} (E\cdot ATP)\]

From the enzyme conservation equation,
\[E_{t} = E + E\cdot ATP + E\cdot PP + E\cdot PRPP\]
and the equilibrium involved, one can obtain
\[E_{t} = E\cdot ATP \left[1 + \frac{k_{-1}}{k_{2}(ATP)} + \frac{k_{2}}{k_{-2}(AMP)} + \frac{k_{2}k_{3}(R-5-P)}{k_{-2}k_{3}(AMP)}\right]\]

so that,
\[R_1 = \frac{k_{1}k_{2}(E_{t})}{[k_{-1} + k_{2}]\left[1 + \frac{k_{-1}}{k_{2}(ATP)} + \frac{k_{2}}{k_{-2}(AMP)} + \frac{k_{2}k_{3}(R-5-P)}{k_{-2}k_{3}(AMP)}\right]}\]

The rate expression has the following properties.
1. \(R_1\) is finite when \((R-5-P) = 0\); therefore, AMP-ATP exchange does not require R-5-P.
2. The only effect of increasing \((R-5-P)\) is to decrease \(R_1\); i.e. the mechanism predicts inhibition of AMP-ATP exchange.
3. At very high \((ATP)\) and \((AMP)\) and \((R-5-P) = 0\) or \((R-5-P) \ll (AMP)\)

\[R_{t(\text{max})} = \frac{k_{1}k_{2}(E_{t})}{k_{-1} + k_{2}}\]

For this mechanism, it can be shown by the usual steady state treatment that
\[V_{\text{max}} \text{ (forward)} = \frac{k_{1}k_{2}(E_{t})}{k_{-1} + k_{2}}\]
and
\[V_{\text{max}} \text{ (reverse)} = \frac{k_{-1}k_{2}(E_{t})}{k_{-1} + k_{2}}\]

This leads to the conclusion that the relative magnitude of \(R_{t(\text{max})}\) and \(V_{\text{max}} \text{ (forward)}\) or \(V_{\text{max}} \text{ (reverse)}\) depends on the relative magnitude of the kinetic constants only. Thus \(R_{t(\text{max})}\) may be larger than, equal to, or smaller than either \(V_{\text{max}} \text{ (forward)}\) or \(V_{\text{max}} \text{ (reverse)}\).

Bridger et al. (8) have presented an equilibrium exchange expression for a similar but slightly more complex mechanism, which includes steps for the \(E\cdot ATP \Rightarrow E\cdot PP\cdot AMP\) and \(E\cdot PP\cdot R-5-P \Rightarrow E\cdot PRPP\) reactions. This expression predicts the same general properties of the exchange as the equation derived above.

Mechanism Involving Single Ternary Complex with Compulsory Binding Order—

\[E + ATP \xrightarrow{k_{1}} E\cdot ATP\]
\[E\cdot ATP + R-5-P \xrightarrow{k_{2}} E - X\]
\[E - X \xrightarrow{k_{3}} E\cdot PRPP + AMP\]
\[E\cdot PRPP \xrightarrow{k_{4}} E + PRPP\]

\((E - X = E\cdot ATP - R-5-P = E\cdot AMP - PRPP)\)

For AMP-ATP exchange, let \(R_1\) equal the rate of appearance of label from ATP into AMP. \(R_1 = k_{4} (E - X)_{a}\) where \((E - X)_{a}\) is the concentration of that fraction of E - X which was labeled by ATP.

The rate of \(E\cdot ATP\) formation from \(E\) and ATP = \(k_{1} (E) (ATP) = k_{-1} (E\cdot ATP)\) at equilibrium. The rate of \(E\cdot ATP\) formation from AMP = \(k_{2} (E\cdot PP) (AMP) = k_{2} (E\cdot ATP)\) at equilibrium.

Then the fraction of \(E\cdot ATP\) from AMP \(E + ATP \rightarrow \) 
\[\frac{\text{rate of } E\cdot ATP \text{ formation from AMP}}{\text{total rate of } E\cdot ATP \text{ formation}} = \frac{k_{2}}{k_{-1} + k_{2}}\]

Therefore,
\[R_1 = \frac{k_{1}k_{2}}{k_{-1} + k_{2}} (E\cdot ATP)\]

From the enzyme conservation equation,
\[E_{t} = E + E\cdot ATP + E\cdot PP + E\cdot PRPP\]
and the equilibrium involved, one can obtain
\[E_{t} = E\cdot ATP \left[1 + \frac{k_{-1}}{k_{2}(ATP)} + \frac{k_{2}}{k_{-2}(AMP)} + \frac{k_{2}k_{3}(R-5-P)}{k_{-2}k_{3}(AMP)}\right]\]

so that,
\[R_1 = \frac{k_{1}k_{2}(E_{t})}{[k_{-1} + k_{2}]\left[1 + \frac{k_{-1}}{k_{2}(ATP)} + \frac{k_{2}}{k_{-2}(AMP)} + \frac{k_{2}k_{3}(R-5-P)}{k_{-2}k_{3}(AMP)}\right]}\]

The rate of \(E - X\) formation from \(E\) and ATP = \(k_{3} (E\cdot ATP)\) \(f\). At equilibrium \(k_{3} (E\cdot ATP) = k_{-2} (E - X)\), so that the rate of \(E - X\) formation from \(E\) and ATP = \(k_{1}k_{2}(E - X)\) \(f\). The rate of \(E - X\) formation from AMP = \(k_{4} (E\cdot AMP)\) \(f\). The rate expression has the following properties:
1. \(R_1\) is finite when \((R-5-P) = 0\); therefore, AMP-ATP exchange does not require R-5-P.
2. The only effect of increasing \((R-5-P)\) is to decrease \(R_1\); i.e. the mechanism predicts inhibition of AMP-ATP exchange.
3. At very high \((ATP)\) and \((AMP)\) and \((R-5-P) = 0\) or \((R-5-P) \ll (AMP)\)

\[R_{1(\text{max})} = \frac{k_{1}k_{2}(E_{t})}{k_{-1} + k_{2}}\]

For this mechanism, it can be shown by the usual steady state treatment that
\[V_{\text{max}} \text{ (forward)} = \frac{k_{1}k_{2}(E_{t})}{k_{-1} + k_{2}}\]
and
\[V_{\text{max}} \text{ (reverse)} = \frac{k_{-1}k_{2}(E_{t})}{k_{-1} + k_{2}}\]

This leads to the conclusion that the relative magnitude of \(R_{1(\text{max})}\) and \(V_{\text{max}} \text{ (forward)}\) or \(V_{\text{max}} \text{ (reverse)}\) depends on the relative magnitude of the kinetic constants only. Thus \(R_{1(\text{max})}\) may be larger than, equal to, or smaller than either \(V_{\text{max}} \text{ (forward)}\) or \(V_{\text{max}} \text{ (reverse)}\).

Bridger et al. (8) have presented an equilibrium exchange expression for a similar but slightly more complex mechanism, which includes steps for the \(E\cdot ATP \Rightarrow E\cdot PP\cdot AMP\) and \(E\cdot PP\cdot R-5-P \Rightarrow E\cdot PRPP\) reactions. This expression predicts the same general properties of the exchange as the equation derived above.
and then decreases to zero because $R_{1(\text{max})}$ becomes proportional to $1/(R-5-P)$.

For this mechanism, a steady state treatment yields

$$V_{\text{max}} (\text{forward}) = \frac{k_b E_i}{k_3 + k_4}$$

and

$$V_{\text{max}} (\text{reverse}) = \frac{k_{-1} k_{-2} E_i}{k_{-1} + k_{-2}}$$

The relation between $R_{1(\text{max})}$ and the maximal velocities clearly depends on $(R-5-P)$.

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J. Biol. Chem. 1970, 245:483-495.

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