ON THE FORMATION OF AN INTERMEDIATE IN THE ADENINE PHOSPHORIBOSYLTRANSFERASE REACTION*

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Summary. Highly purified rat liver adenine phosphoribosyltransferase (EC 2.4.2.7) exhibits an "initial burst" reaction in the presence of either PP-ribose-P or Mg-ADP indicating that the purified enzyme contains a bound phosphoribosyl group. The initial burst reaction was studied separately by inhibiting the overall reaction between PP-ribose-P and adenine with low incubation temperatures or EDTA. The results are consistent with a mechanism involving the intramolecular transfer of a phosphoribosyl group as the rate determining step.

The purine (Pu) phosphoribosyltransferases catalyze the synthesis of purine mononucleotide according to the following general reaction:

$$Pu + PP\text{-ribose-}P \underset{}{\overset{Mg^{++}}{\rightleftharpoons}} Pu\text{-ribose-}P + PP$$

Recent extensive kinetic studies (1-4) on the purine phosphoribosyltransferases have indicated that at least two possible mechanisms are each partially consistent with the results obtained. Utilizing a preparation from Ehrlich ascites cells Henderson and coworkers originally proposed a ping-pong mechanism involving a phosphoribosyl-enzyme intermediate but later favored an ordered reaction involving a ternary complex of enzyme and both substrates (3). Krenitsky and Papaioannou (4) proposed that both of the above mechanisms were possible, and that the quantitative participation of each mechanism was controlled by the Mg++ concentration. These authors observed an "initial burst" of IMP synthesis with human hypoxanthine (guanine) phosphoribosyltransferase that had been previously incubated with Mg-PP-ribose-P. Initial burst kinetics have also been observed with an apparently homogeneous preparation of rat liver adenine phosphoribosyltransferase recently obtained in our laboratory (5). The present studies on an initial burst of AMP synthesis obtained when the rat liver enzyme is employed indicates that the isolated enzyme contains

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a bound phosphoribosyl group which is retained during the purification procedure.

Methods. Radioactive AMP synthesis from adenine-8 ^{14}C (specific activity 51.5 milli c./millimole, Nuclear-Chicago) and PP-ribose-P (Na^+ salt) was measured essentially as described by Hori and Henderson (6). In each type of experiment performed selected samples were cochromatographed with carrier AMP using the solvent system of Krebs and Hems (7). Greater than 95% of the ^{14}C applied to the papers was recovered as ^{14}C-AMP in every experiment. The enzyme used was purified from the soluble fraction of rat liver homogenates (5). Polyacrylamide disc-gel electrophoresis of the enzyme preparation at pH 7.2 or pH 8.3 on 7.5% gels or with an ampholyte gradient (pH 5-8) on 3% gels indicated enzyme homogeneity as only a single sharp band of protein was seen in all of these analytical gels. Further chemical evidence of homogeneity is described below utilizing the size of the initial burst of AMP synthesis in the presence of EDTA.

Results and Discussion. An initial burst of ^{14}C-AMP synthesis by the purified

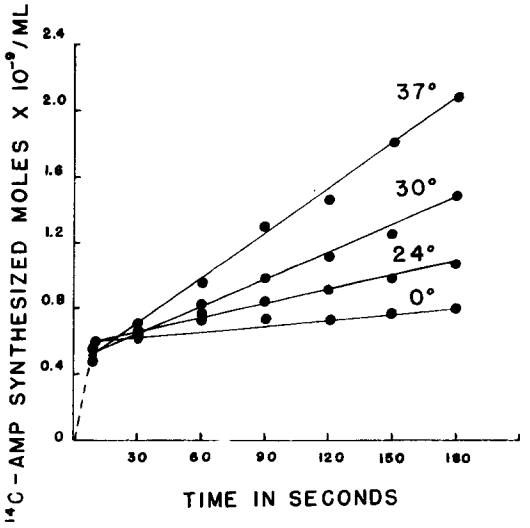

Fig. 1 Effect of temperature upon the initial burst and overall reaction phases of rat liver adenine phosphoribosyltransferase. In a volume of 1 ml were combined 50 mM TRIS-HCl, pH 7.5, 2 mM $MgCl_2$, 0.2 mM PP-ribose-P, 0.05 mM adenine-8 ^{14}C and 10.7 µg enzyme. Incubations were carried out at 0°, 24°, 30°, and 37°. A 0.1 ml aliquot was removed at the indicated times from 10 to 180 seconds and assayed for ^{14}C-AMP.

enzyme can be easily demonstrated and delineated from the overall ^{14}C-AMP synthesis from PP-ribose-P and adenine-8 ^{14}C either by lowering the incubation temperature from 37° to 0° or by chelating endogeneous Mg++ with EDTA. The initial burst synthesis, which was complete in 10 seconds, was essentially the same at 0°, 24°, 30°, and 37°. The overall synthesis from PP-ribose-P and adenine was markedly temperature dependent, however (Fig. 1).

Although the overall synthesis of AMP from PP-ribose-P and adenine is Mg++ dependent, the initial burst synthesis has no apparent Mg++ requirement. If sufficient EDTA (0.2 mM) was added to increasing concentrations of enzyme, PP-ribose-P (Na^+ salt), and adenine, an initial burst of AMP synthesis was observed. Continued synthesis of AMP via the overall reaction, however, was completely blocked (Fig. 2, top). The molecular weight of the rat liver

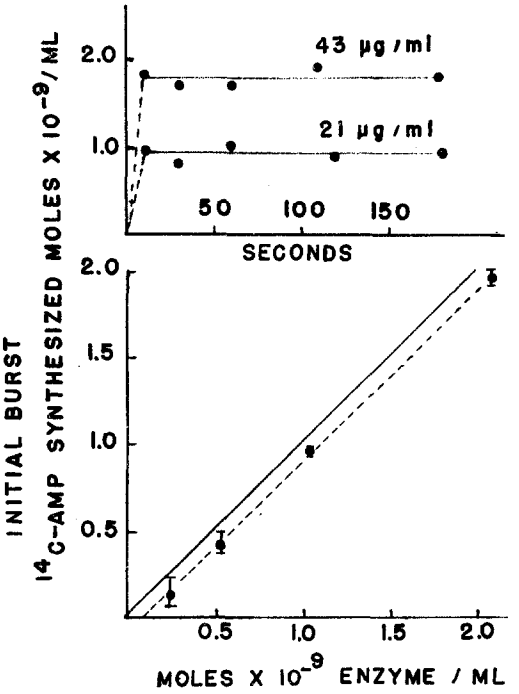

Fig. 2 Total initial burst of ^{14}C-AMP synthesis at different enzyme concentrations. In a volume of 0.9 ml were combined 50 mM TRIS-HCl, pH 7.5, 0.2 mM PP-ribose-P and the indicated enzyme concentrations. The reaction was initiated by the addition of 0.1 ml of a mixture of 0.5 mM adenine-8 ^{14}C and 2 mM EDTA. Incubations were carried out at 30°. A 0.1 ml aliquot was removed at the indicated times from 10 to 180 seconds (Fig. 2, top) and assayed for ^{14}C-AMP. The plateau values ± S.E. were then replotted for all enzyme concentrations including these given at the top. (Fig. 2, bottom: solid line-theoretical, dotted line-experimental.)

adenine phosphoribosyltransferase was estimated to be 20000 daltons by sucrose density gradient technics (8). If it is assumed that one mole of phosphoribosyl group is bound per mole of enzyme, a straight line of unit slope should be obtained when moles of ^{14}C-AMP synthesized in the initial burst are plotted against moles of enzyme added. It is apparent that the experimentally determined initial burst reactions at four different enzyme concentrations closely follow a line of unit slope (Fig. 2, bottom). These results also indicate that our preparations of rat liver enzyme possess considerable chemical homogeneity (if the assumption of one mole of bound phosphoribosyl group per mole of enzyme is valid) as approximately one mole of AMP was synthesized per mole of enzyme during the initial burst reaction in the presence of 0.2 mM EDTA.

The initial burst synthesis of ^{14}C-AMP from adenine-8 ^{14}C at 0° or at 30° in the presence of 0.2 mM EDTA requires the addition of PP-ribose-P (Na^+ salt). The PP-ribose-P cannot be replaced by AMP or pyrophosphate, either in

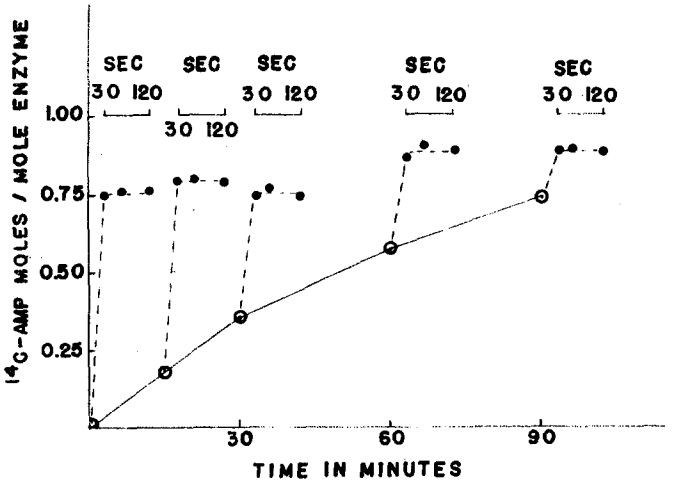

Fig. 3 Effect of preincubation of rat liver adenine phosphoribosyltransferase with Mg-ADP upon the initial burst reaction. In separate incubation mixtures of 0.4 ml were combined 50 mM TRIS-HCl, pH 7.5, 0.5 mM adenine-8 ^{14}C, 10 mM Mg-ADP and 10 µg of enzyme. The mixtures were incubated at 30° for the indicated times in minutes. 3.6 ml of H_2O were added, the mixture was chilled to 0°, and an aliquot was taken and assayed for ^{14}C-AMP. (Solid line in figure). The diluted Mg-ADP preincubated mixtures were kept in an ice bath, and 0.2 mM PP-ribose-P was added to a final concentration of 0.2 mM. The residual initial burst of ^{14}C-AMP synthesis elicited by PP-ribose-P at 0° was then measured at 30, 60, and 120 seconds. (Dotted lines in figure).

the presence or absence of 2 mM Mg++. When ADP (10 mM) and Mg++ (2 mM) are added, however, the bound "phosphoribosyl" group was slowly made available for condensation with adenine-8 ^{14}C to form ^{14}C-AMP. If PP-ribose-P was added at various periods of time following preincubation of the enzyme with Mg-ADP and adenine, a progressively smaller residual initial burst was obtained at 0°. Mg-ATP, Mg-GDP, in addition to Mg-AMP and Mg-PP, did not substitute for Mg-ADP in this modified "burst" reaction (Fig. 3).

Rat liver adenine phosphoribosyltransferase can therefore be subjected to a purification procedure which gives high resolution of an enzyme species containing a bound "phosphoribosyl" group. The addition of either PP-ribose-P (Na^+) or Mg-ADP to the purified enzyme probably induces a conformational change in a portion of the enzyme molecules in the preparation, the portion undergoing the conformational change being much larger with PP-ribose-P than with Mg-ADP. These conformationally altered molecules can enter into a rapid (initial burst) reaction in which the bound phosphoribosyl group reacts with adenine. From the studies with EDTA inhibition it would appear that the rate determining step in the overall reaction between PP-ribose-P and adenine is a Mg++ requiring intramolecular transition of PP-ribose-P from its initial binding site (conformational) to a second stable site (phosphoribosyl (~RP))

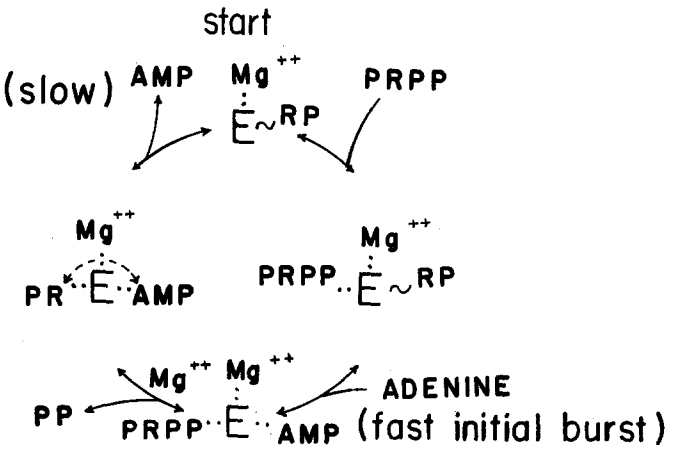

Fig. 4 Proposed mechanism of rat liver adenine phosphoribosyltransferase.

(Fig. 4). Studies now in progress indicate that the initial burst reaction is unaffected by borohydride reduction but is markedly inhibited by periodate oxidation. PP-ribose-P labeled with ^{14}C and ^{32}P is also being used to more fully characterize the "phosphoribosyl" group.

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