

SUPPORTING INFORMATION

Figure 1. Time course for the full reaction of wild-type PPDK under steady state conditions. For each time point a 40 μ l solution containing 2 μ M enzyme active sites, 20 units inorganic pyrophosphatase, 10 mM $MgCl_2$, 20 mM NH_4Cl , and 50 mM K^+ HEPES (pH 7.0) was mixed with an equal volume solution containing 4 mM $[^{14}C]$ ATP, 10 mM pyruvate, 10 mM P_i , and 50 mM K^+ HEPES (pH 7.0). Final concentrations of each species after mixing were as follows: 1 μ M enzyme active sites, 2 mM $[^{14}C]$ ATP, 5 mM pyruvate, 5 mM P_i , 20 units inorganic pyrophosphatase, 5 mM $MgCl_2$, and 10 mM NH_4Cl . The inset shows the computer fit of the initial time points to a linear equation. The initial velocity is given by the slope of inset plot ($V_0 = 0.32$ mM/min; $R^2 = 0.990$).

Figure 2. Time course for the full reaction of R337A PPDK under steady state conditions. For each time point a 40 μ l solution containing 40 μ M enzyme active sites, 20 units inorganic pyrophosphatase, 10 mM $MgCl_2$, 20 mM NH_4Cl , and 50 mM K^+ HEPES (pH 7.0) was mixed with an equal volume solution containing 4 mM $[^{14}C]$ ATP, 10 mM pyruvate, 10 mM P_i , and 50 mM K^+ HEPES (pH 7.0). Final concentrations of each species after mixing were as follows: 20 μ M enzyme active sites, 2 mM $[^{14}C]$ ATP, 5 mM pyruvate, 5 mM P_i , 20 units inorganic pyrophosphatase, 5 mM $MgCl_2$, and 10 mM NH_4Cl . The inset shows the computer fit of the initial time points to a linear equation. The initial velocity is given by the slope of inset plot ($V_0 = 0.019$ mM/min; $R^2 = 0.990$).

Figure 3. (A) Time course for the full reaction of R337K PPDK under steady state conditions. For each time point a 40 μ l solution containing 4 μ M enzyme active sites, 20 units inorganic pyrophosphatase, 10 mM $MgCl_2$, 20 mM NH_4Cl , and 50 mM K^+ HEPES (pH 7.0) was mixed with an equal volume solution containing 4 mM $[^{14}C]ATP$, 10 mM pyruvate, 10 mM P_i , and 50 mM K^+ HEPES (pH 7.0). Final concentrations of each species after mixing were as follows: 2 μ M enzyme active sites, 2 mM $[^{14}C]ATP$, 5 mM pyruvate, 5 mM P_i , 20 units inorganic pyrophosphatase, 5 mM $MgCl_2$, and 10 mM NH_4Cl . (B) graph of the initial AMP time points from the full reaction of R337K under steady state conditions. The initial velocity is given by the slope of the line obtained by the computer fit of the data to a linear equation ($V_0 = 0.20$ mM/min; $R^2 = 0.990$).

Figure 1- Sup. mat

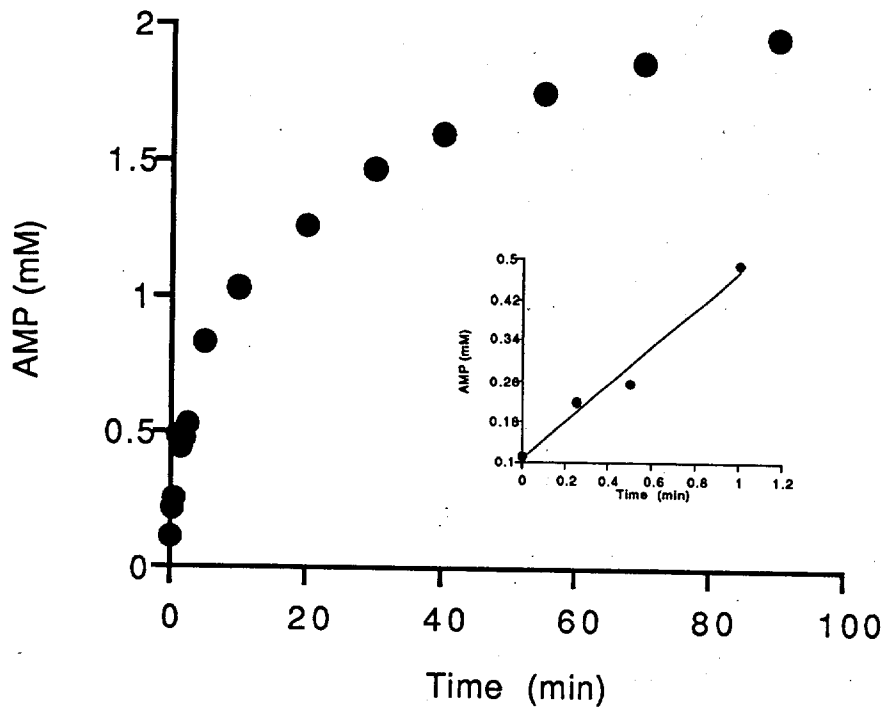


Figure 2- Sup. mat

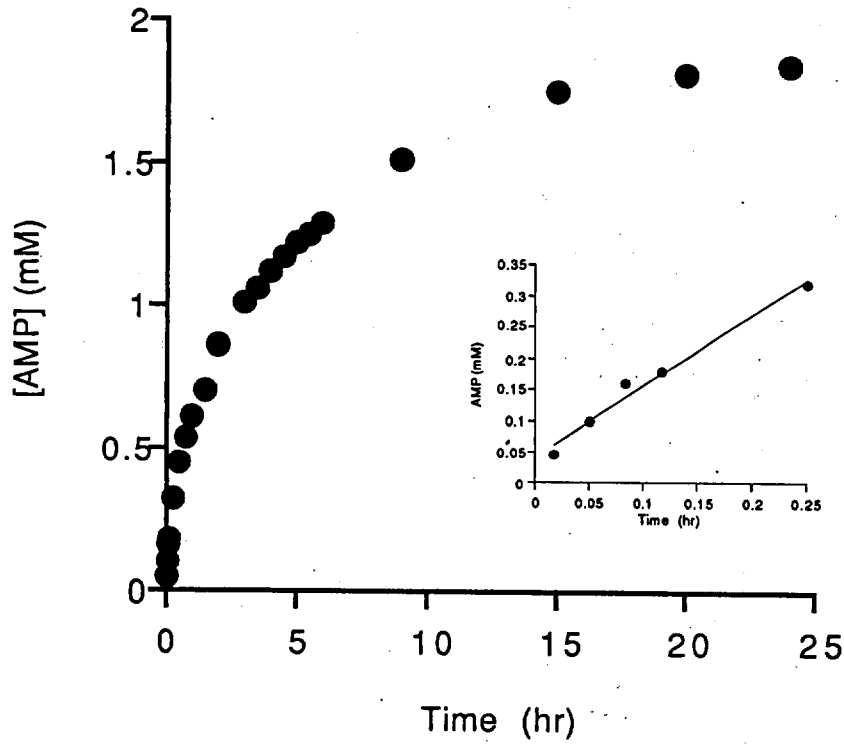
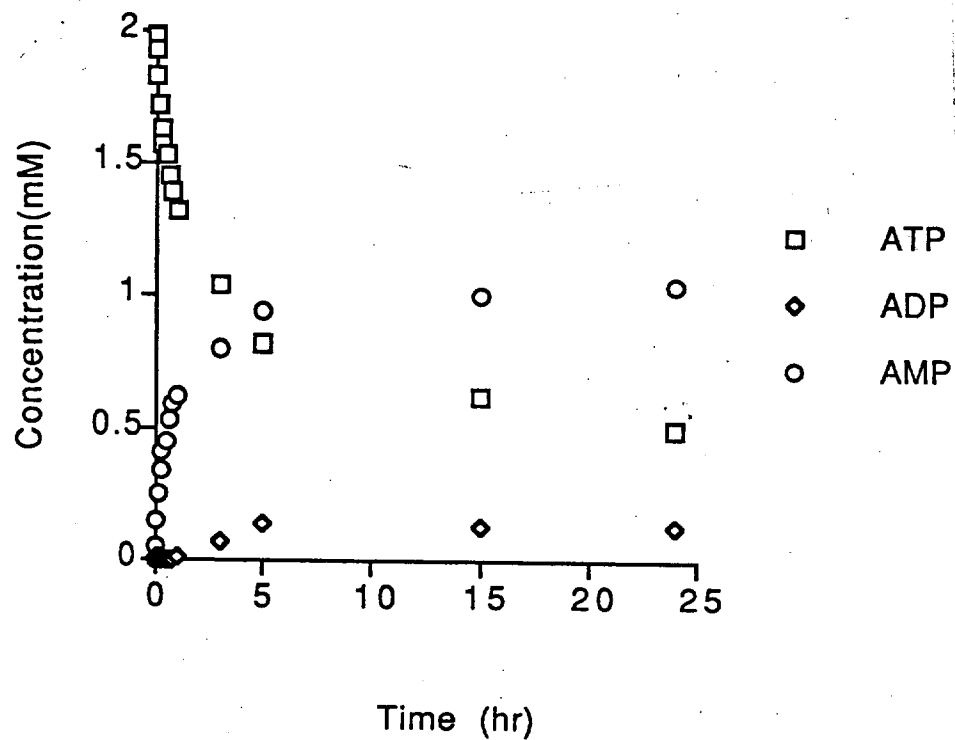


Figure 3- Sup. mat

A



B

