HHS Public Access

Author manuscript

Biochemistry. Author manuscript; available in PMC 2022 September 07.

Published in final edited form as:

Biochemistry. 2021 September 07; 60(35): 2672–2676. doi:10.1021/acs.biochem.1c00535.

Adenylate Kinase-Catalyzed Reaction of AMP in Pieces: Enzyme Activation for Phosphoryl Transfer to Phosphite Dianion

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Abstract

The binding of adenosine 5'-triphosphate (ATP) and adenosine 5'-monophosphate (AMP) to adenylate kinase (AdK) drives closure of lids over the substrate adenosyl groups. We test the hypothesis that this conformational change activates AdK for catalysis. The rate constants for *Homo sapiens* adenylate kinase 1 (*Hs*AdK1)-catalyzed phosphoryl group transfer to AMP, k_{cat}/K_m = 7.0 x 10^6 M^{-1} s^{-1}, and phosphite dianion, $(k_{HPi})_{obs} \leq 1 \times 10^{-4}$ M^{-1} s^{-1}, show that the binding energy of the adenosyl group effects a ≥7.0 x 10^{10}-fold rate acceleration of phosphoryl transfer from ATP. The third-order rate constant of $k_{cat}/K_{HPi}K_{EA}$ = 260 M^{-2} s^{-1} for 1-(β-D-erythrofuranosyl)adenine (EA)-activated phosphoryl transfer to phosphite dianion was determined, and the isohypophosphate reaction product characterized by ^{31}P NMR. The results demonstrate: (i) a ≥14.7 kcal/mol stabilization of the transition state for phosphoryl transfer by the adenosyl group of AMP and a ≥2.6 x 10^6-fold rate acceleration from the EA-driven conformational change, and (ii) the recovery of ≥8.7 kcal/mol of this transition state stabilization for EA-activated phosphoryl transfer from ATP to phosphite.

Graphical Abstract

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Author Contributions

All authors have given approval to the final version of the manuscript.

Supporting Information

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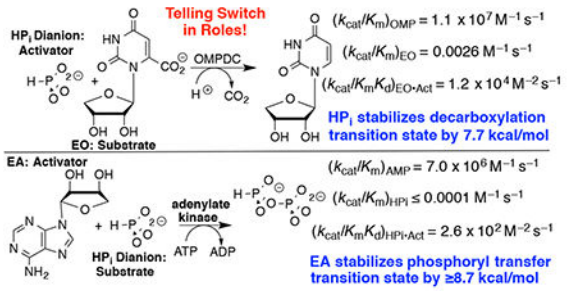
Experimental Section with (i) the sources of the enzymes and chemicals used in this work, (ii) procedures for the preparation and purification of TEV protease and *Hs*AdK1, and (iii) protocol for the determination of kinetic parameters for *Hs*AdK1-catalyzed reactions of the whole substrate AMP and the substrate pieces phosphite and EA. Results Section with (i) SDS-PAGE gel for purified TEV protease and *Hs*AdK1 (Figure S1), (ii) kinetic data for *Hs*AdK1-catalyzed reactions of ATP with AMP (Figure S2), and (iii) kinetic data for *Hs*AdK1-catalyzed hydrolysis of the ATP (Figure S3A) and for the effect of increasing $[HP_i]$ on the velocity *Hs*AdK1-catalyzed conversion of ATP to ADP (Figure S3B). (PDF)

The authors declare no competing financial interests.



Keywords

AK1; P00568; p1 protease; Q0GDU8

The phosphodianion of phosphate monoester substrates for metabolic reactions provides *ca.* 12 kcal/mol of binding energy for stabilization of transition states for enzyme-catalyzed proton transfer, hydride transfer,[1, 2] decarboxylation,[2, 3] and phosphoryl transfer reactions.[4] From 33–66% of this binding energy is utilized in phosphite dianion activation of these enzymes for catalysis of the reactions of phosphodianion truncated substrates.[1–5] Our model to rationalize the reactions of substrate pieces can be generalized to any enzyme that undergoes a substrate-driven conformational change,[6] but phosphite dianion is the only identified activating substrate piece.

Adenylate kinase (AdK) catalyzes the transfer of a phosphoryl group from ATP to AMP to form two molecules of ADP (Scheme 1). The binding of nucleotide substrates to AdK drives the closure of ATP- and AMP-lids over the substrate adenosine groups.[7] We predict that these protein-adenosine interactions activate AdK for catalysis of phosphoryl transfer, and that AdK is an effective catalyst of the reaction of the AMP pieces 1-(β-D-erythrofuranosyl)adenine (EA) and phosphite dianion. This is a reversal of the roles of phosphite dianion (substrate) and truncated nucleoside EA (activator) from that for phosphite dianion activation of orotidine monophosphate decarboxylase (OMPDC, Scheme 2).[8]

The commercial sources for all materials, and the methods for the preparation of *Homo sapiens* adenylate kinase 1 (*Hs*AdK1) and tobacco etch virus (TEV) protease are given in the Supporting Information (SI). The following protocols are described in the SI: (i) assays for AdK-catalyzed phosphoryl transfer from ATP to AMP, for AdK-catalyzed hydrolysis of ATP, and for unactivated and EA-activated AdK-catalyzed phosphoryl transfer from ATP to phosphite dianion and (ii) protocol for product determination using ^{31}P NMR.

His$_6$-tag labelled *Hs*AdK1 was expressed in *E. coli,* purified over a Ni^{2+}-column, and the His$_6$-tag removed by tobacco etch virus (TEV)-protease. The purified *Hs*AdK1 showed a single band by SDS-PAGE (Figure S1). The initial velocity, v_o, at 25 °C for *Hs*AdK1-catalyzed phosphoryl transfer from ATP to AMP was determined by a standard enzyme assay (SI).[9] Figure S2 shows a plot of v_o/[E] against AMP for *Hs*AdK1-catalyzed phosphoryl transfer from 1 mM ATP (saturating)[10] to AMP to form 2 ADPs at 25 °C

and pH 7.5, which gave the kinetic parameters k_{cat}/K_m = (7.0 ± 0.4) x 10^6 M^{-1} s^{-1}, $k_{cat}t$ = 475 ± 8 s^{-1} and K_m = 68 ± 4 μM.

*Hs*AdK1-catalyzed hydrolysis of ATP to ADP was monitored by coupling the formation of ADP to the oxidation of NADH by pyruvate, catalyzed by lactate dehydrogenase. Figure S3A shows the dependence of v_o on [*Hs*AdK1] for catalysis of hydrolysis of 1 mM ATP (saturating)[10] to form ADP. The slope of this correlation, k_{hyd} = 5 x 10^{-6} s^{-1}, is similar to k_{hyd} = 2 x 10^{-6} s^{-1} reported for adenylate kinase from *E. coli.*[7]

Figure S3B shows the increase in k_{hyd} = 5 x 10^{-6} s^{-1} for *Hs*AdK1-catalyzed (72 μM) conversion of ATP (1 mM) to ADP in the presence of increasing concentrations of phosphite dianion. The slope of this correlation, $(k_{HPi})_{obs}$ = (9.9 ± 0.3) x 10^{-5} M^{-1} s^{-1}, is the sum of the rate constants for *Hs*AdK1-catalyzed phosphoryl transfer from ATP to phosphite to form isohypophosphate (HPP_i), $(k_{HPi})_E$, and for activation of the *Hs*AdK1-catalyzed hydrolysis reaction by a specific salt effect, $(k_{HPi})_{salt}$. The formation of ADP by the reaction of phosphite with ATP (Scheme 3) would result in a low yield of HPP_i, undetectable by the ^{31}P analytical methods described below. Therefore, the value of $(k_{HPi})_{obs}$ = (9.9 ± 0.3) x 10^{-5} M^{-1} s^{-1} (Figure S3B) sets an upper limit of $(k_{HPi})_E \leq 1 \times 10^{-4}$ M^{-1} s^{-1} for unactivated *Hs*AdK1-catalyzed phosphoryl transfer from ATP to phosphite (Table 1).

The *Hs*AdK1-catalyzed conversion of ATP to ADP in the presence of phosphite dianion is strongly activated by EA. Figure 1A shows the effect of increasing [EA] on $(v_{obs} - v_o)$ for *Hs*AdK1-catalyzed (2 μM) reactions of saturating ATP (1 mM), where v_{obs} is the total reaction velocity, and v_o is a ≤1.1% correction for the velocity at [EA] = 0 M. The increase in $(v_{obs} - v_o)$ is consistent with EA-activation of *Hs*AdK1 for catalysis of phosphoryl transfer from ATP to HPO_3^{2-} to form HPP_i (Scheme 3). We confirm this by determining the reaction products using ^{31}P NMR.

The *Hs*AdK1-catalyzed conversion of ATP to ADP in the presence of phosphite dianion was monitored in a 1.0 mL solution in D_2O that contains 50 mM TEA (pD 7.5), 10 mM EA, 3 mM $MgCl_2$, 30 mM KCl, 25 mM phosphite (93% dianion),[1] 1 mM ATP, 30 mM phosphoenolpyruvate (PEP), 10 mM phosphonoacetate (^{31}P-standard), 1 U pyruvate kinase, and 67 μM *Hs*AK1. This reaction was monitored continuously for 16 h at 25 °C by ^{31}P NMR spectroscopy on a Varian Inova 500 MHz spectrometer, as described in the SI. Figure 2 shows the relevant changes in the ^{31}P NMR spectra during this time. The integrated areas for the singlet for PEP (−0.62 ppm) and doublet for phosphite (3.05 ppm, d, $_1J_{PH}$ = 568 Hz) decrease, as peaks for HPP_i appear (−4.50 ppm, dd, $_1J_{PH}$ = 646 Hz, $^2J_{PP}$ = 17 Hz; −5.44 ppm, d, $^2J_{PP}$ = 17 Hz).[11] No inorganic phosphate from adenylate-kinase catalyzed hydrolysis of ATP, or from hydrolysis of HPP_i was detected. This is consistent with the published 12 h halftime for hydrolysis of HPP_i at 60 °C and pH 7.4.[11]

The sum of the normalized ^{31}P peaks areas for the reactants PEP and phosphite (A_R) and the HPP_i product (A_P) were determined using a constant peak area (A_S) for the phosphonoacetate standard. There is no significant change in the sum of normalized areas of peaks for reactants and products during the reaction. Figure 3 compares the time courses for the decrease in the fraction of PEP and phosphite reactants remaining ($A_i = A_R$) with

the increase in the fraction of reactants converted to HPPi ($A_i = A_P$), with normalization of ($A_R + A_P$). The solid lines in Figure 3 show the fit of these data to the rate equation for the apparent first-order conversion of (30 mM PEP + 25 mM phosphite) to HPP_i, using a rate constant of $k_{obs} = 0.16\ h^{-1}$ and reaction end-points of 22 mM HPP_i and 11 mM (PEP + phosphite). These fits show that the reactants are converted to HPP_i as essentially the exclusive product.

Figure 3 shows that *Hs*AdK1-catalyzed phosphoryl transfer from ATP to phosphite dianion to form HPP_i can account for essentially the entire increase in ($v_{obs} - v_o$) from Figure 1A. The solid lines for Figures 1A show the nonlinear least squares fits of the kinetic data to eq 1, derived for Scheme 4, assuming $K_{HPi} >> [HP_i]$ and using the values of $(k_{cat}/K_{EA})_{obs}$ determined for reactions at different $[HPO_3^{2-}]$. Figure 1B shows the linear plot (eq 2) of values of $(k_{cat}/K_{EA})_{obs}$ from Figure 1A, against $[HPO_3^{2-}]$. The slope of this correlation is $(k_{cat})_{HPi\cdot EA}/K_{HPi}K_{EA} = (2.58 \pm 0.05) \times 10^2\ M^{-2}\ s^{-1}$ (Table 1).

Table 1 summarizes data that defines the role of the adenosyl group of AMP in activating *Hs*AdK1 for catalysis of phosphoryl transfer from enzyme saturated with ATP (1 mM). The values of $k_{cat}/K_m = 7.0 \times 10^6\ M^{-1}\ s^{-1}$ and $(k_{HPi})_E \leq 1 \times 10^{-4}\ M^{-1}\ s^{-1}$, respectively for the catalyzed reactions of AMP and phosphite dianion show that the AMP adenosyl group is responsible for a $\geq 7.0 \times 10^{10}$-fold rate acceleration for phosphoryl group transfer. This is a lower limit, because the full AMP binding interactions are probably not expressed at the transition state for the enzyme conformational change, which limits the rate of reaction of AMP.[7] This corresponds to a ≥14.7 kcal/mol stabilization of the transition state for phosphoryl transfer by the AMP adenosyl group. The ratio of the rate constants for *Hs*AdK1-catalyzed phosphoryl transfer from ATP to HPO_3^{2-} in the presence and absence of the EA activator, $\geq 2.6 \times 10^6$ M, shows that ≥8.7 kcal/mol of the adenosyl binding energy is recovered as stabilization of the transition state by the activator. This binding energy is utilized to hold AdK in an active closed conformation.[6, 12–15] By comparison, the transition state for OMPDC-catalyzed decarboxylation is stabilized 12 kcal/mol by interactions with the OMP phosphodianion and 19 kcal/mol by interactions with the OMP ribosyl and orotate moieties.[8]

Inorganic tripolyphosphate binds weakly to chicken muscle adenylate kinase (K_d = 1 mM) and undergoes phosphoryl transfer to AMP with k_{cat} that is *ca* (10^4–10^5)-fold smaller than for the reaction of ATP.[16] This shows that the adenosyl group of ATP is essential for optimal phosphoryl donor activity. *Hs*AdK1 shows a higher specificity for the phosphoryl acceptor AMP compared to the donor ATP,[17] which suggests a larger activating adenosyl binding energy for the acceptor compared with the donor nucleotide.

$$\frac{v_{obs} - v_o}{[E]} = \frac{(k_{cat})_{HPi\cdot EA}[EA]\left[HPO_3^{2-}\right]}{K_{HPi}K_{EA} + K_{HPi}[EA]} \quad (1)$$

$$(k_{cat}/K_{EA})_{obs} = \frac{(k_{cat})_{HPi\cdot EA}\left[HPO_3^{2-}\right]}{K_{HPi}K_{EA}} \quad (2)$$

Adenylate kinase functions in muscle tissues to maintain an equilibrium concentration of ATP, ADP, and AMP in order to optimize [ATP] under conditions of energy stress, created by the rapid myosin-catalyzed hydrolysis of ATP. This important function creates pressure to optimize enzyme activity, similar to that for the glycolytic enzyme TIM.[19, 20] In these and other cases, the pressure to optimize catalytic activity has driven evolution of protein motifs, where substrate binding energy is utilized to drive large, enzyme-activating, conformation changes.[2, 6]

X-ray crystal structures for AdK show that the adenosyl group of AMP sits distant from the reacting phosphates, and is held at the enzyme by hydrogen bonds with protein side chains and backbone amides.[21, 22] The ligand phosphates interact with many cationic amino acid side chains and backbone amides, so that the activator-driven conformational changes move disordered polar groups into positions that provide for optimal stabilizing electrostatic interactions with the phosphate oxygens at the transition state for *Hs*AdK1-catalyzed phosphoryl transfer.[23] We propose that the ≥8.7 kcal/mol transition state stabilization from binding of the EA activator is due to the incremental tightening of many stabilizing polar interactions that result from the ligand-driven enzyme conformational change.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Funding Sources

The authors acknowledge the National Institutes of Health Grant GM134881 for support of this work.

ABBREVIATIONS

AdK	adenylate kinase
AMP	adenosine 5’-monophosphate
ADP	adenosine 5’-diphosphate
ATP	adenosine 5’-triphosphate
***Hs*AdK1**	*Homo sapiens* adenylate kinase 1
EA	1-(β-D-erythrofuranosyl)adenine
HPP_i	isohypophosphate
OMP	orotidine 5’-monophosphate
OMPDC	orotidine 5’-monophosphate decarboxylase
SI	Supporting Information
TEA	triethanolamine
TEV	tobacco etch virus

TIM	triosephosphate isomerase

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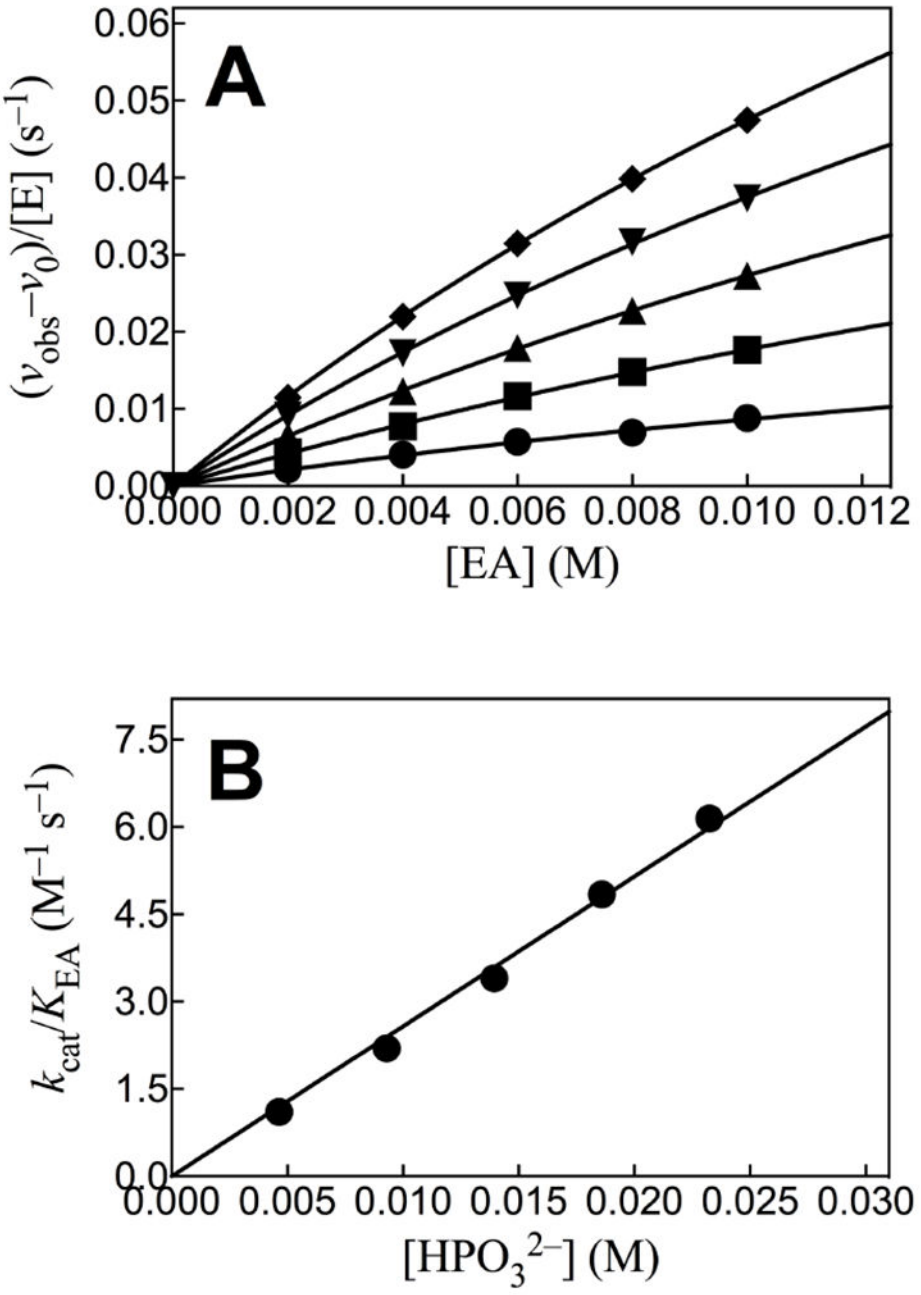


Figure 1.

(A) The effect of increasing [EA] on the velocity for *Hs*AdK1-catalyzed reactions of ATP (1 mM) at 25 °C. Key: ♦, 25 mM $[HP_i]_T$, (93% dianion); ▼, 20 mM $[HP_i]_T$; ▲, 15 mM $[HP_i]_T$; ■, 10 mM $[HP_i]_T$; ●, 5 mM $[HP_i]_T$. (B) The effect of increasing $[HPO_3^{2-}]$ on the values of $(k_{cat}/K_{EA})_{obs}$ determined for Figure 1A.

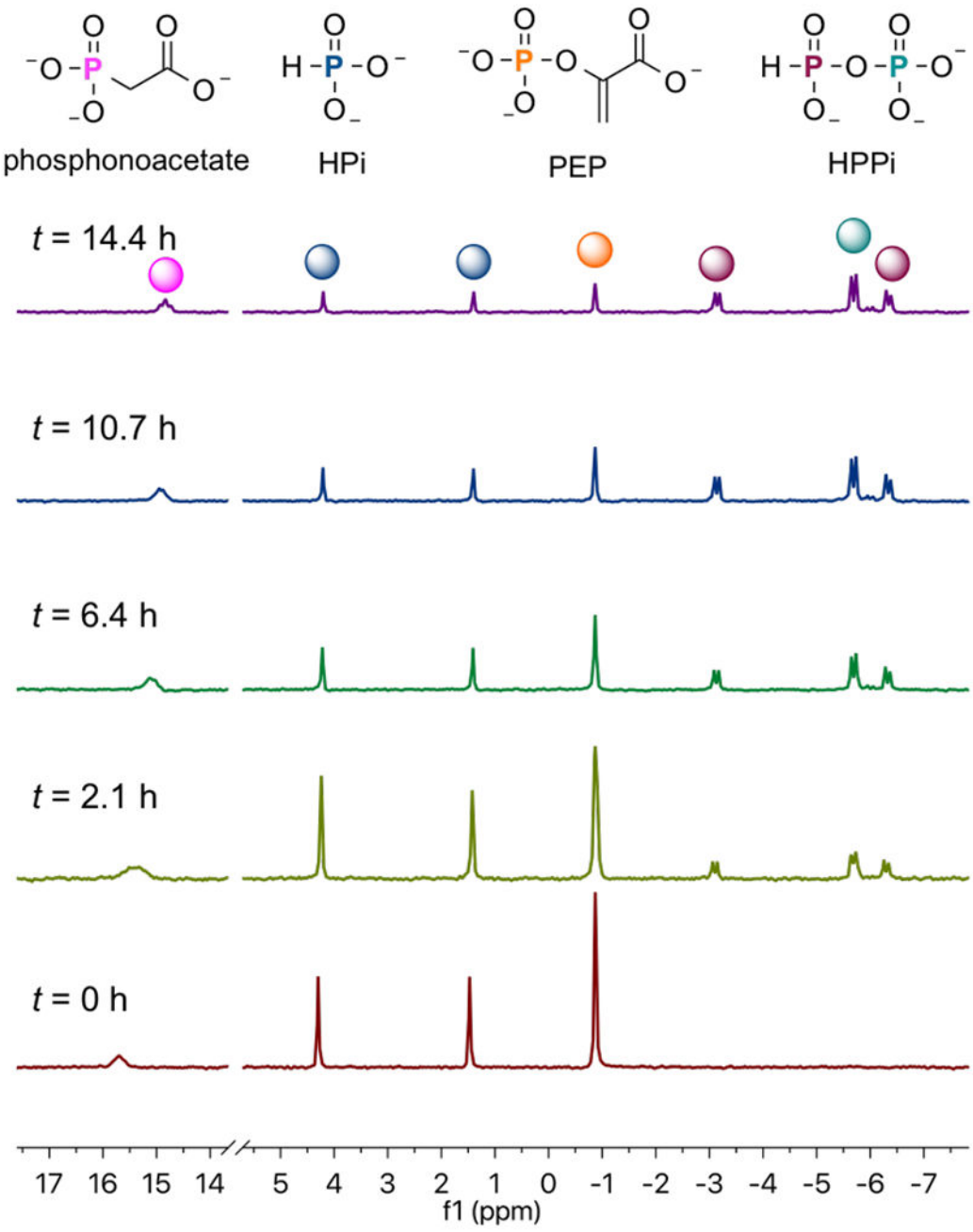


Figure 2.

^{31}P NMR spectra that show the change with time in the areas of the peaks A_R at −0.62 and 3.05 ppm for PEP and phosphite reactants, and A_p at −4.50 and 5.44 ppm for HPP_i product of EA-activated *Hs*AdK1-catalyzed reactions (Scheme 3).

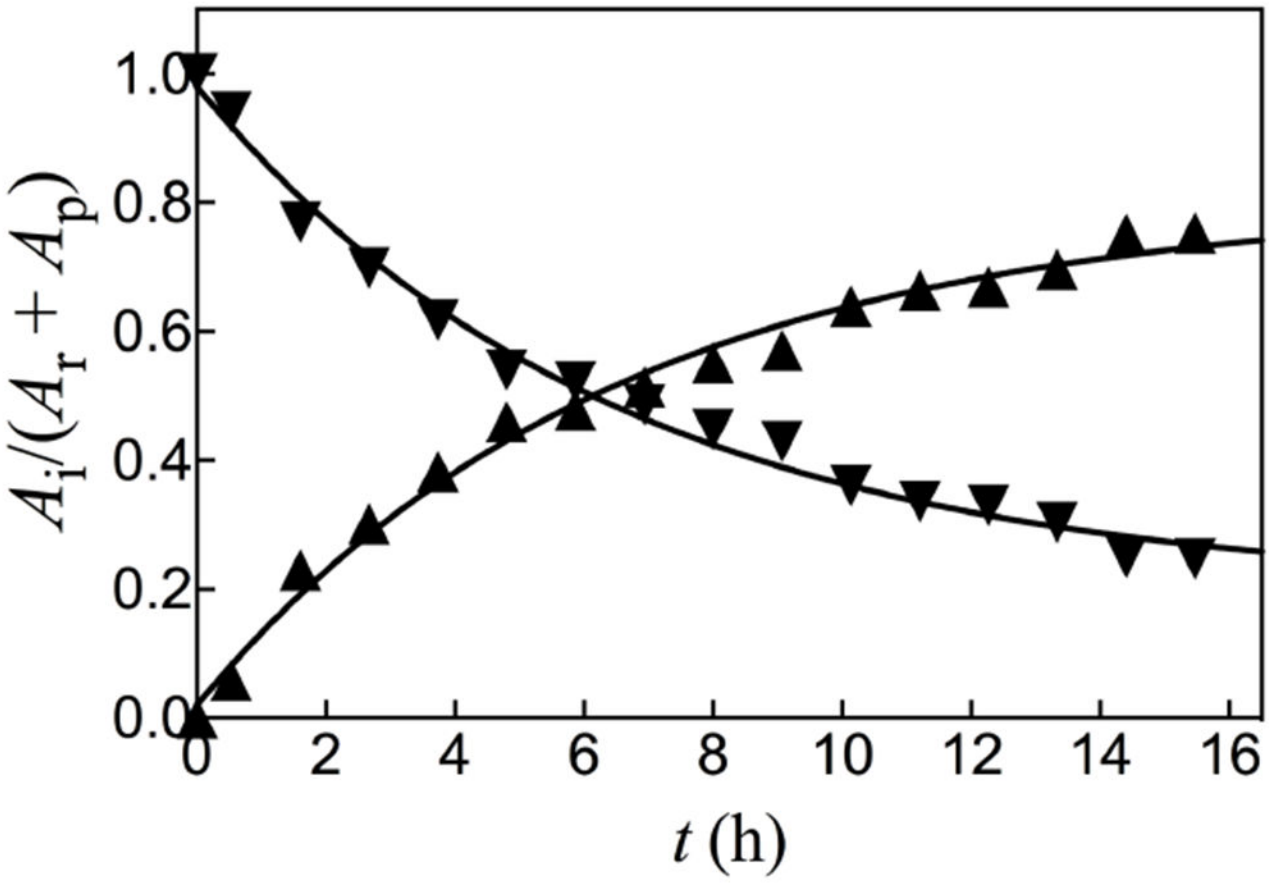
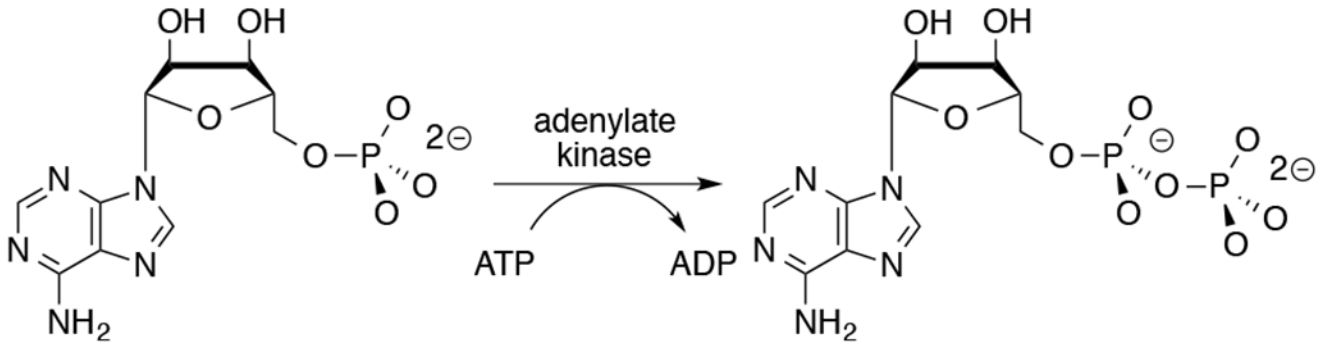
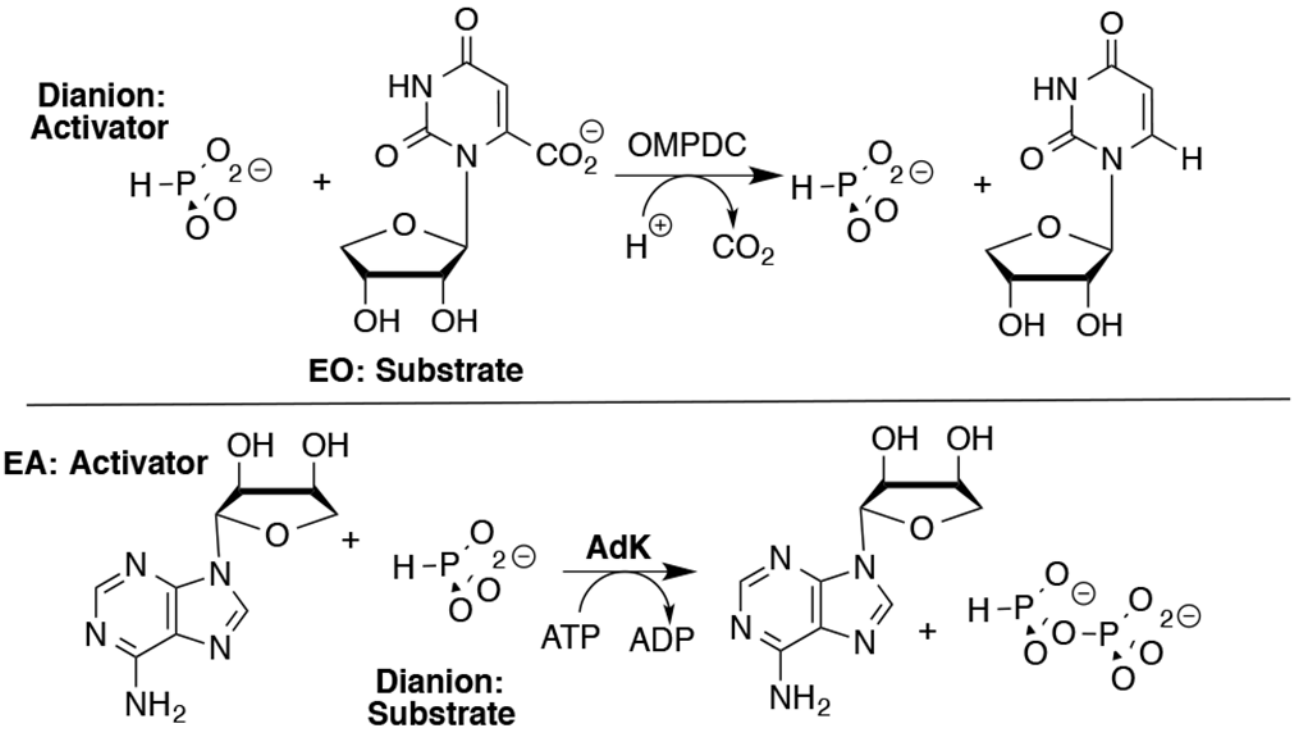


Figure 3.

The fractions of reactants PEP and phosphite remaining (▼, $A_i = A_R$) and product HPP_i formed (▲, $A_i = A_P$ at time t, determined by integration of the peaks from Figure 2.

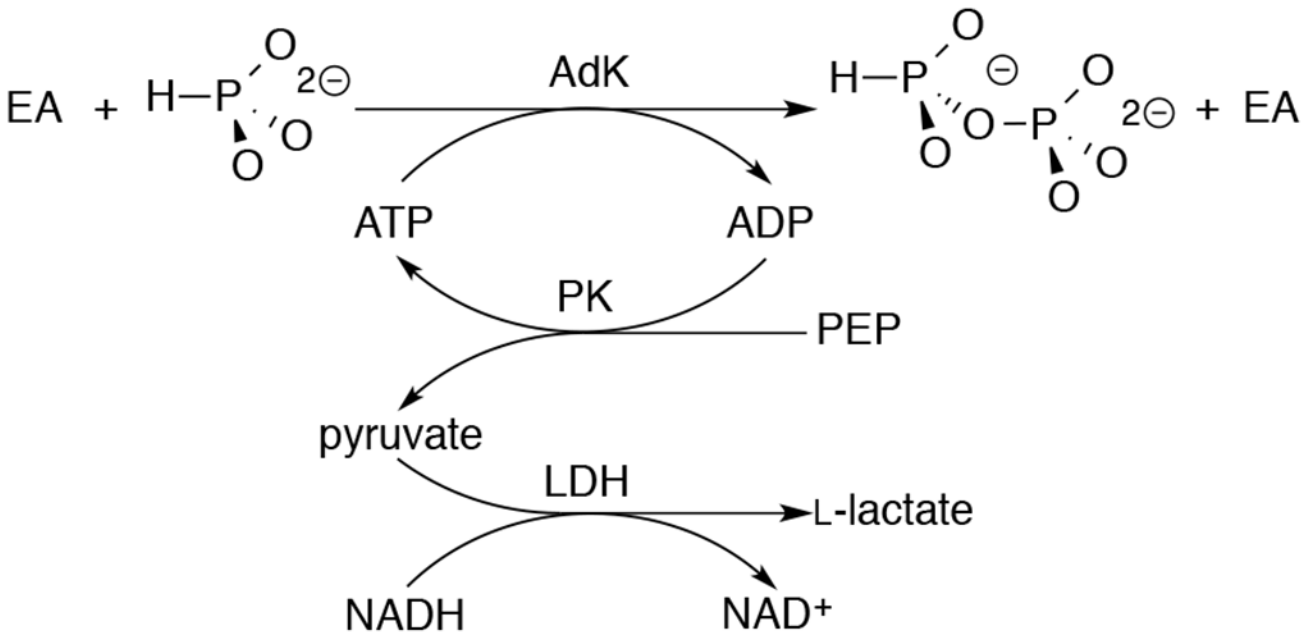


Scheme 1.
Adenylate Kinase-Catalyzed Phosphoryl Transfer from ATP to ADP.

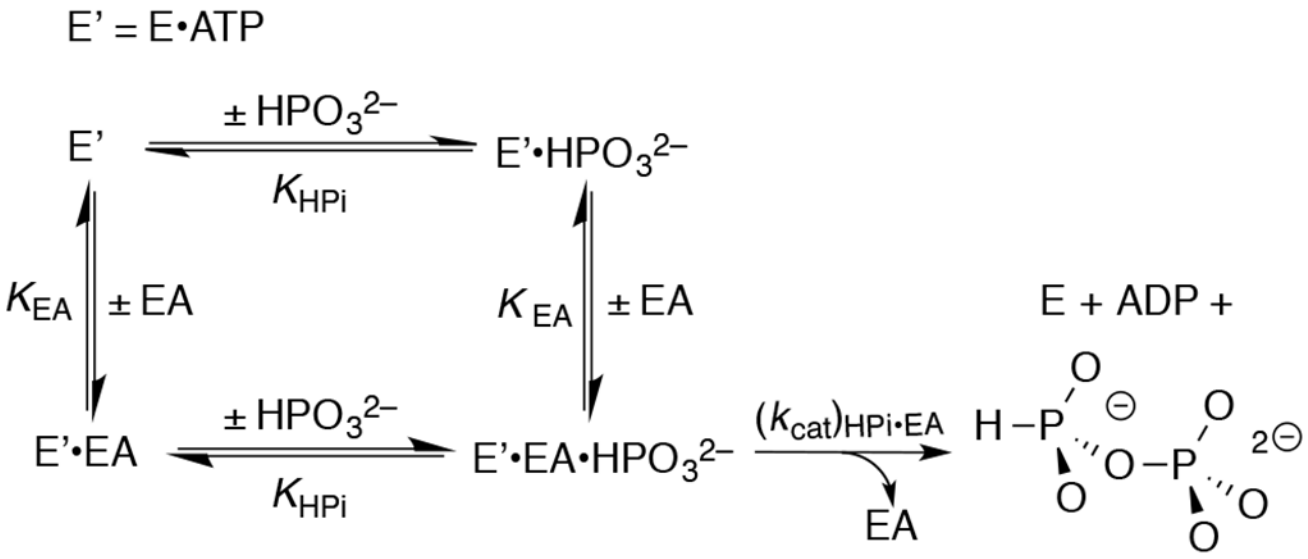


Scheme 2.

Roles of Dianion and Nucleoside (EO and EA) Substrate Pieces in Reactions Catalyzed by OMPDC and AdK.



Scheme 3.
Assay to Monitor AdK-Catalyzed Phosphoryl Transfer from ATP to Phosphite Dianion.



Scheme 4.

Kinetic Mechanism for Activation of *Hs*AdK1-Catalyzed Phosphoryl Transfer from ATP to Phosphite Dianion by the Substrate Piece EA.

Table 1.

Rate Constants and Transition-State Binding Energies $\Delta\Delta G^{\ddagger}$ for *Hs*AdK1-Catalyzed Phosphoryl Transfer from 1.0 mM ATP to AMP and to Phosphite Dianion.[a]

Phosphoryl Acceptor	Kinetic Parameter	$\Delta\Delta G^{\ddagger}$ (kcal/mol) [b]
AMP	k_{cat}/K_m (7.0 ± 0.4) x 10^6 M^{-1} s^{-1}	≥ 14.7 [c]
HPO_3^{2-} + EA	$(k_{cat})_{HPi•EA}/K_{Hpi}K_{EA}$ (2.58 ± 0.05) x 10^2 M^{-2} s^{-1} [d]	≥ 8.7 [e]
HPO_3^{2-}	$(k_{Hpi})_E$ ≤1.0 x 10^{-4} M^{-1} s^{-1} [f]	

[a] At 25 ° C and pH 7.5.

[b] Calculated from the ratio of rate constants for *Hs*AdK1-catalyzed reactions of AMP and the substrate piece (HPO_3^{2-}) or pieces (HPO_3^{2-} + EA).

[c] Assuming similar intrinsic nucleophilic reactivities for AMP and phosphite dianion.

[d] Determined from the fit of data from Figure 1B to eq 2.

[e] Calculated from the ratio of rate constant for *Hs*AdK1-catalyzed reactions of phosphite in the presence and absence of EA.[18]

[f] The slope from Figure S3B (see text).