はじめに

Adenylate Kinase (AK) (EC 2.7.4.3, hAK1)

(1) アデニル酸キナーゼ (AK) は、細胞質内に普遍的に存在する酵素で、Mg^{2+}の存在下で以下の可逆的反応を触媒する。

$$MgATP^{2-} + AMP^{2-} \Leftrightarrow MgADP^{-} + ADP^{3-}$$

(2)AK は、２つのヌクレオチド基質結合部位が存在する。一つは、MgATP あるいは MgADP 部位、もう一つは AMP または ADP 部位である。この酵素は、Mg^{2+}の金属イオンの存在下、リン酸転移反応を可逆的に触媒する。

(3) ヒト AK(hAK1) は、194 のアミノ酸から構成される分子量 21,400 の球状蛋白質で、細菌から、哺乳動物まで広く存在し、３つのアイソザイムが存在する。AK1 は細胞質に存在し、骨格筋、脳、赤血球にみられる。AK2 は、ミトコンドリア内膜に、AK3 は、ミトコンドリアマトリックスに存在し、肝臓、腎臓にみられる。

(4) ３ÅX 線回折を用いたブタ骨格筋 AK の構造解析により、１０のａヘリックスと５つの b-鎖構造を有する (Schulz et al., 1974)。

(5) ヌクレオチド結合タンパク質 (ras, p21, c-has/bas protein, the b-
subunit of F1-ATPase, G-protein) と AK において、アミノ酸一次
構造の相同性の高い領域が存在し、ATP 結合蛋白 のモデルとして応用研究されている。

(6) 部位特異的変異導入による標的アミノ酸残基の変異体の作製

は、hAK1 では、R44, R97, R132, R138, R149, D119 残基において、ニワトリ AK では、P17, K21, C25, H36, T39, R44, D93,
R97, R128, R132, R138, R149, L190、大腸菌 AK では、P9, G10, K13, Q28, F86, P87, R88, L107, Y133 に関して行われ、AK の X 線回折や NMR を用いた構造機能解析研究に用いられている。

(7)X 線回折により想定された AK 立体構造モデルで AMP 結合部位が想定された。hAK1 の Arg 残基 (R44, R132, R138) は、酵素
活性に不可欠な残基であると示唆された。(Kim et al, 1990).

本研究の目的

a)hAK1 の酵素活性に最低必要と思われる重要なアミノ酸残基を決定していくために、N 末端と C 末端のリジン残基 (Lys9, Lys21, Lys27, Lys31, Lys194) に対して部位特異的変異導入を行う。

b) 野生型 AK と変異型 AK の生理学的属性を定常状態でのキネテックス解析により比較し、リジン残基側鎖に存在する陽性荷電の e-アミノ基の欠如が酵素活性に与える影響を考察する。

Introduction

Adenylate Kinase (AK) (EC 2.7.4.3, hAK_1)

1. AK is a ubiquitous enzyme catalyzing the following reversible reaction in the presence of Mg^{2+}:

$$MgATP^{2-} + AMP^{2-} \Leftrightarrow MgADP^{-} + ADP^{3-}$$

2. There are two distinct nucleotide-binding sites in AK, one for metal-chelated ATP or ADP, and the other for unchelated AMP or ADP.

3. The AK family consists of three isoenzymes: AK_1, AK_2, and AK_3. AK_1 is localized in mammalian cytosol, AK_2 in mitochondria intermembrane space, and AK_3 in mammalian mitochondria matrix.

4. The molecular weight of human AK_1 (hAK_1) is 21.7 kDa known to be one of the smallest phosphotransferases. AK has ten a-helices and five b-strands (Schulz et al., 1974).

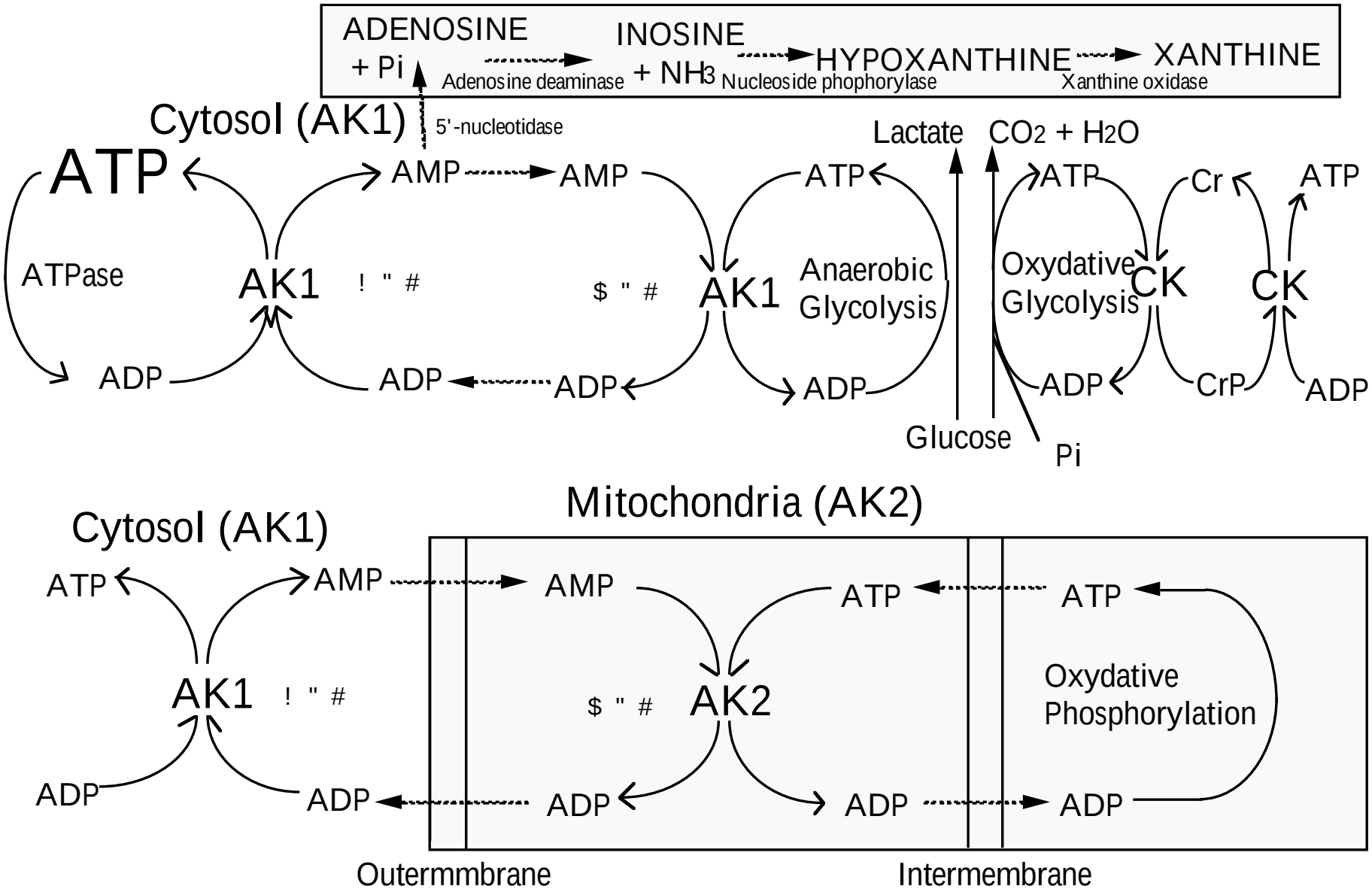
5. AK is often cited as an example of a typical ATP binding protein when structural topological comparisons or amino acid homology comparisons are made of the nucleotide-binding proteins such as ras, p21, c-has/bas protein, the b-subunit of F_1-ATPase, and G-protein.

7. Site-directed mutagenesis was carried out in hAK1 (R44, R97, R132, and R138), chicken AK (P17, K21,C25, H36, T39, R44, D93, R97, R128, R132, R138, R149, and L190), and E. coli AK (P9, G10, K13, Q28, F86, P87, R88, L107, and Y133).

8. The ATP site in the original X-ray model was reassigned to the AMP site; arginine residues in hAK1 were determined to be essential for catalytic activity (Kim et al, 1990).

In this study, to elucidate the minimum requirement of amino acid residues at the active center in hAK1 and to clarify the physiological properties of normal and abnormal AK enzymes, we substituted key lysine residues in the N- and C-terminal domains of the proposed

X-ray model (Kim et al., 1990). To elucidate the function of the positive e-amino groups of the lysine residues, we mutated Lys9, Lys21, Lys27, and Lys31 in the head domain, and Lys194 in the tail domain of hAK1. These mutants were analyzed by steady-state kinetics.


ADENOSINE
+ Pi
INOSINE
+ NH3
HYPOXANTHINE
XANTHINE
Adenosine deaminase
Nucleoside phophorylase
Xanthine oxidase
5'-nucleotidase
Cytosol (AK1)
ATP
ATPase
ADP
AK1
AMP
AMP
ADP
ADP
AK1
ATP
ADP
Anaerobic
Glycolysis
Lactate
CO2 + H2O
Glucose
Pi
ATP
Oxydative
Glycolysis
CK
ADP
Cr
CrP
CK
ATP
ADP
Mitochondria (AK2)
Cytosol (AK1)
ATP
AMP
AK1
ADP
AMP
ADP
AK2
ATP
ADP
ATP
ADP
Oxydative
Phosphorylation
Outermmbrane
Intermembrane

Materials and Methods

A) Materials.

1. The plasmid pMEX8-hAK1 (Ayabe et al., 1996, *Biochem. Mol. Biol. Int. 38, 373-381.*) was used for site-directed random mutagenesis with a Sculptor™ in vitro Mutagenesis Kit (Amersham).

B) Purification of single strand pMEX8-hAK1 DNA.

1. A single colony of TG1/pMEX8-hAK1 was cultured in 10 ml of a TYP medium containing 50 mg/ml of ampicillin overnight until the A_{600} was approximately 0.5. Helper phage (VCS-M13, Stratagene) was added to the culture medium at a multiplicity of infection of between 10 and 20 (a phage : cell ratio of between 10 : 1 and 20 : 1) with 25 mg/ml of kanamycin.
2. The single strand template DNA was precipitated with a solution of polyethylene glycol 6000 and 2.5M NaCl and extracted by phenol and chloroform.

C) Random site-directed mutagenesis of hAK1.

1. The antisense primers were 5'-CGAAGATGATYXXAGTCTTCTTAAGC-3' for Lys9 residue, 5'-GCACTGGGTACCYXXGCCAGAACC-3' for Lys21, 5'-GCA CGATYXXCTCGCACTGGG -3' for Lys27, 5'GTGTAGCCGTAYXXCTGCAC GATTTTC-3' for Lys31, 5'-CCAGCTGACCYXXTTCCATGATTTC-3' for Lys63, 5'-GTTTCGCCGCGYXXCAGCAGGCG-3' for Lys131, and 5'-CGAAGATGAT YXXAGTCTTCTTAAGC-3' for Lys194. The underlined three letters, YXX in the primer, represent a codon for each target residue (Y=G or C; X=A, G, C, or T). The phosphorylation of the primers was performed with T_4 Polynucleotide Kinase.

2. Random site-directed mutagenesis was carried out using the Sculptor™ in vitro Mutagenesis Kit and a homoduplex mutant pMEX8-hAK1 DNA was constructed with the method based on the phosphorothioate technique.

D) Purification of mutant double strand pMEX8-hAK1 plasmid.

1. The competent cells of TG1 were transformed with the above homoduplex mutant DNA, which were cultured on an LB plate containing 50 mg/ml of ampicillin. Each grown clone was transferred into 10 ml of the LB medium containing 50

mg/ml of ampicillin and purified according to the directions of the Flex Prep Purification Kit (Pharmacia).

E) Screening of mutant plasmid by DNA sequence.

1. Mutant double strand plasmid was screened by Sanger's dideoxy method with polymerase chain reaction (PCR).
2. AmpliTaq™ DNA Polymerase solution and Taq DNA Polymerase were mixed in a 9:1 ratio as a cycling mixture. Eight ml of a master mixture consisted of 2.8 mg

of double strand DNA, 2 pmol of the FITC-labeled sequence-primer, 1 ml of DMSO, which was incubated at 95^{0}C for 10 min, and immediately cooled on ice.

3. Two ml of the cycling mixture and 0.2 unit of the Perfect Match Enhancer (Stratagene) were mixed into the cooled master mixture.
4. PCR conditions were initial denaturation at 95^{0}C for 5 min, 20 cycles of 95^{0}C for 30 s, 53^{0}C for 30 s, 72^{0}C for 60 s, 20 cycles of 95^{0}C for 30 s and 72^{0}C for 60 s. PCR products were denatured by 95% formamide. The DNA sequences of both ends of the mutant plasmid were reconfirmed to avoid undesirable mutations in any other regions of the hAK1 gene.

F) Expression and purification of wild-type AK (WTAK) and mutant enzymes.

1. TG1/pMEX8-hAK1 (WTAK) and TG1/mutant-pMEX8-hAK1 were cultured in 10 ml of LB medium containing 50 mg/ml of ampicillin at 37^{0}C for 12 h; these were transferred into 250 ml of LB medium with ampicillin. The culture medium was incubated for 1 h at 37^{0}C, and isopropyl-b-D-thio- galactopyranoside (IPTG) was added at a final concentration of 1 mM and continued to culture for 16 h.
2. The harvested E. coli cells were resuspended in 10 ml of standard buffer [1 mM EDTA, 0.1 mM dithiothreitol (DTT), 20 mM Tris-HCl, pH 7.4], which was disrupted by sonication at 20 kHz, 20 W for 3 min at 4^{0}C using a sonicator. The cell debris was removed by centrifugation at 12,000 x g for 20 min.
3. The supernatant (10 ml) was loaded onto a Blue Sepharose CL-6B affinity column (ϕ 1 X 5 cm), which had been pre-equilibrated with the standard buffer, and eluted by a NaCl gradient (0 to 1 M NaCl) at a velocity of 0.5 ml/min at 4^{0}C. Each fraction was examined by a 12.5% polyacrylamide gel electrophoresis in the presence of 1% sodium dodecylsulfate (SDS- PAGE) to confirm the presence of an AK protein of 21.7 kDa. The AK fraction was concentrated by centrifuging with Centriplus-10 (Amicon).

4. The AK solution (1 ml) was then loaded onto a Superose 12 column (ϕ 1 x 30 cm) which had been equilibrated with an imidazole buffer (5 mM imidazole-HCl, 1 mM EDTA, 0.1 mM DTT, pH 6.9) and eluted at a velocity of 0.5 ml/min at 4^0C. Each fraction was also examined by a 12.5% SDS-PAGE to confirm the presence of a single AK solution as determined by the method of Lowry et al.

G) Steady-state kinetics of forward reaction of AK.

1. The forward reaction mixture in a total of 1 ml contained 75 mM triethanolamine hydrochloride (pH 7.4), 120 mM KCl, 0.2 mM NADH, 0.3 mM phosphoenolpyruvate, 0.3 mg/ml bovine serum albumin, 10 units of lactate dehydrogenase (LDH), 5 units of pyruvate kinase (PK), and 1.0 mM $MgSO_4$, and the various concentrations of $MgATP^{2-}$ and AMP^{2-} were as follows: five combinations selected from 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 3.0, 5.0 mM $MgATP^{2-}$ and at a fixed concentration of 2 mM AMP^{2-} were used in the determination of the apparent Michaelis constant (K_m) for $MgATP^{2-}$. Five combinations selected from 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 3.0, 5.0 mM AMP^{2-} and a fixed concentration of 2 mM $MgATP^{2-}$ were used in the determination of the apparent K_m for AMP^{2-}.
2. The reaction was initiated by addition of 10 ml of the recombinant hAK1 solution

diluted to desired concentrations. The initial velocity of the forward reaction was measured by observing the absorbance change at 340 nm with a Cary 2290 spectrophotometer [for NADH, in a coupled-enzyme assay in the presence of PK and LDH to monitor the ADP formation at 25 ^{0}C as previously described].

3. K_m and V_{max} were estimated using a double-reciprocal plot , and k_{cat} was calculated by dividing V_{max} by the total amount of enzyme (Et) present in the reaction mixture. One unit of enzyme activity is defined as the amount of enzyme that produces 1 mmol of ADP per minute.

Experimental Procedures

1. Culture of TG1/pMEX8-hAK1 (TG1 transformed with pMEX8-hAK1) in LB medium.
2. Addition of helper phage (VCS-M13) at a multiplicity of infection of between 10 and 20.
3. Purification of single strand pMEX8-hAK1 DNA with a solution of polyethylene glycol 6000 and 2.5M NaCl and extraction with phenol and chloroform.
4. Annealing of antisense primers to the single strand pMEX8-hAK1 DNA.
   5'-CGAAGATGATYXXAGTCTTCTTAAGC-3' for Lys9,
   5'-GCACTGGGTACCYXXGCCAGAACC-3' for Lys21,
   5'-GCACGATYXXCTCGCACTGGG -3' for Lys27,
   5'-GTGTAGCCGTAYXXCTGCACGATTTTC-3' for Lys31,

5'-CCAGCTGACCYXXTTCCATGATTTC-3' for Lys63,

5'-GTTTCGCCGCGYXXCAGCAGGCG-3' for Lys131,

5'-CGAAGATGATYXXAGTCTTCTTAAGC-3' for Lys194.

YXX represents a codon for each target residue (Y=G or C; X=A, G, C, or T)

5. Construction of a homoduplex mutant pMEX8-hAK1 DNA by site-directed mutagenesis with the method based on the phosphorothioate technique.

6. Transformation of the homoduplex mutant pMEX8-hAK1 with competent TG1 cells.

7. Culture of TG1 transformed with the mutant homoduplex DNA.

8. Purification of double strand pMEX8-hAK1 DNA.

9. Screening of mutant pMEX8-hAK1 by DNA sequence with PCR.

10. Culture and expression of the confirmed mutant pMEX8-hAK1 plasmid in LB

medium.

12. Sonication of the harvested E. coli.

13. Purification with a Blue Sepharose CL-6B chromatography and elution by a NaCl gradient (0 to 1 M NaCl).

14. Confirmation of the presence of an AK protein of 21.7 kDa with 12.5% SDS- PAGE.

15. Concentration of the AK fraction by centrifuging with Centriplus-10 (Amicon).

16. Purification of AK sample with Superose 12 chroamtography.

17. Confirmation of the presence of an AK protein with 12.5% SDS-PAGE.

18. Measurement of the concentration of AK protein.

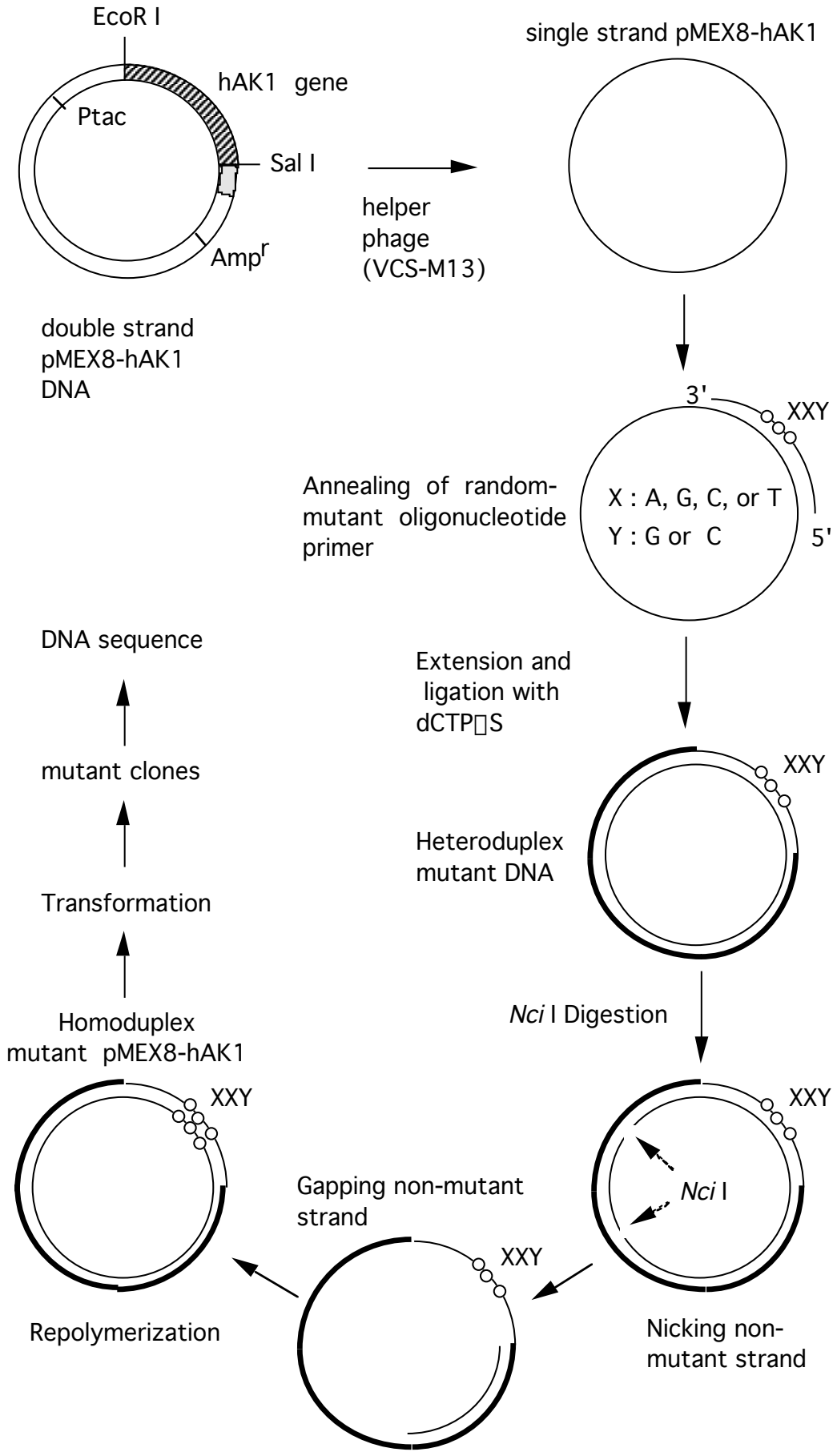
19. Assay of wild type AK and the recombinant mutant AK.

Steady-state kinetics of forward reaction of AK.

Five combinations selected from 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 3.0, 5.0 mM $MgATP^{2-}$ and at a fixed concentration of 2 mM AMP^{2-} were used in the determination of the apparent Michaelis constant (K_m) for $MgATP^{2-}$.

Five combinations selected from 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 3.0, 5.0 mM AMP^{2-} and a fixed concentration of 2 mM $MgATP^{2-}$ were used in the determination of the apparent K_m for AMP^{2-}.

K_m and V_{max} were estimated using a double-reciprocal plot , and *kcat* was calculated by dividing V_{max} by the total amount of enzyme (Et) present in the reaction mixture. One unit of enzyme activity is defined as the amount of enzyme that produces 1 μmol of ADP per minute.


EcoR I
hAK1 gene
Ptac
Sal I
Amp^r
double strand
pMEX8-hAK1
DNA
helper
phage
(VCS-M13)
single strand pMEX8-hAK1
3'
XXY
X : A, G, C, or T
Y : G or C
5'
Annealing of random-
mutant oligonucleotide
primer
Extension and
ligation with
dCTP□S
XXY
Heteroduplex
mutant DNA
Nci I Digestion
XXY
Nci I
Nicking non-
mutant strand
XXY
Gapping non-mutant
strand
XXY
Repolymerization
Homoduplex
mutant pMEX8-hAK1
Transformation
mutant clones
DNA sequence

Forward Reaction

<Reaction Mixture (1ml) of the ADP formation reaction>

75 mM Triethanolamine HCl (pH 7.5)
120 mM KCl
0.2 mM NADH
0.3 mM Phosphoenolpyruvate
1 mM $MgSO_4$
1 mM ATP
1.2 mM AMP
0.3 mg/ml Bovine serum albumine (BSA)
15 U Lactate dehydrogenase
6 U Pyruvate kinase
AK sample

$$\text{ATP} + \text{AMP} \xrightarrow{\text{AK, } Mg^{2+}} 2\ \text{ADP}$$

$$2\ \text{ADP} + 2\ \text{Phosphoenolpyruvate (PEP)} \xrightarrow{\text{Pyruvate Kinase (PK)}} 2\ \text{ATP} + 2\ \text{Pyruvate}$$

$$2\ \text{Pyruvate} + 2\ \text{NADH} + 2H^{+} \xrightarrow{\text{Lactate Dehydrogenase (LDH)}} 2\ \text{L-Lactate} + 2\ NAD^{+}$$

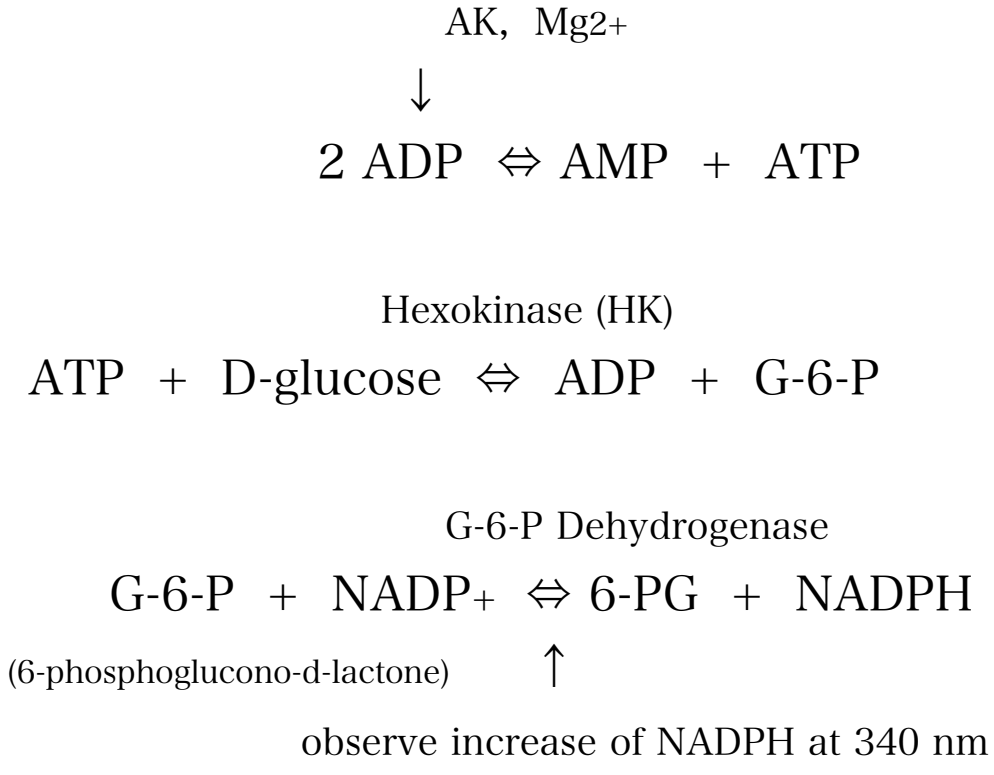
↑

observe reduction of NADH at 340 nm

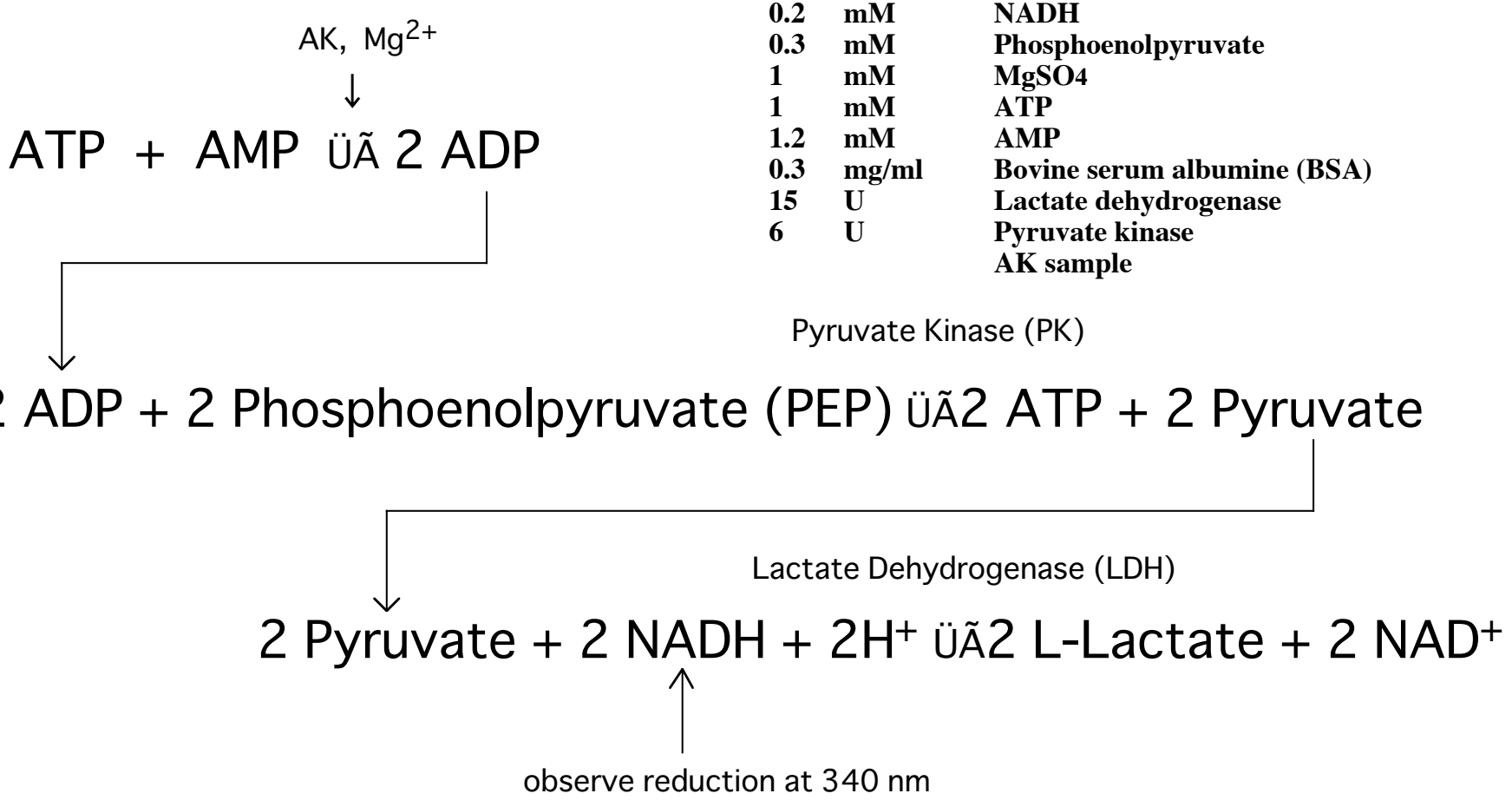
Reverse Reaction

<Reaction mixture (1ml) of the ATP formation reaction>

50 mM Tris HCl (pH 7.4)
100 mM KCl
2 mM $MgCl_2$
7 mM D-glucose
2 mM ADP
0.6 mM NADP
0.1 mM EDTA
0.5 mg/ml Bovine serum albumine (BSA)
10 U Hexokinase
5 U Glucose-6-phosphate dehydrogenase
AK sample

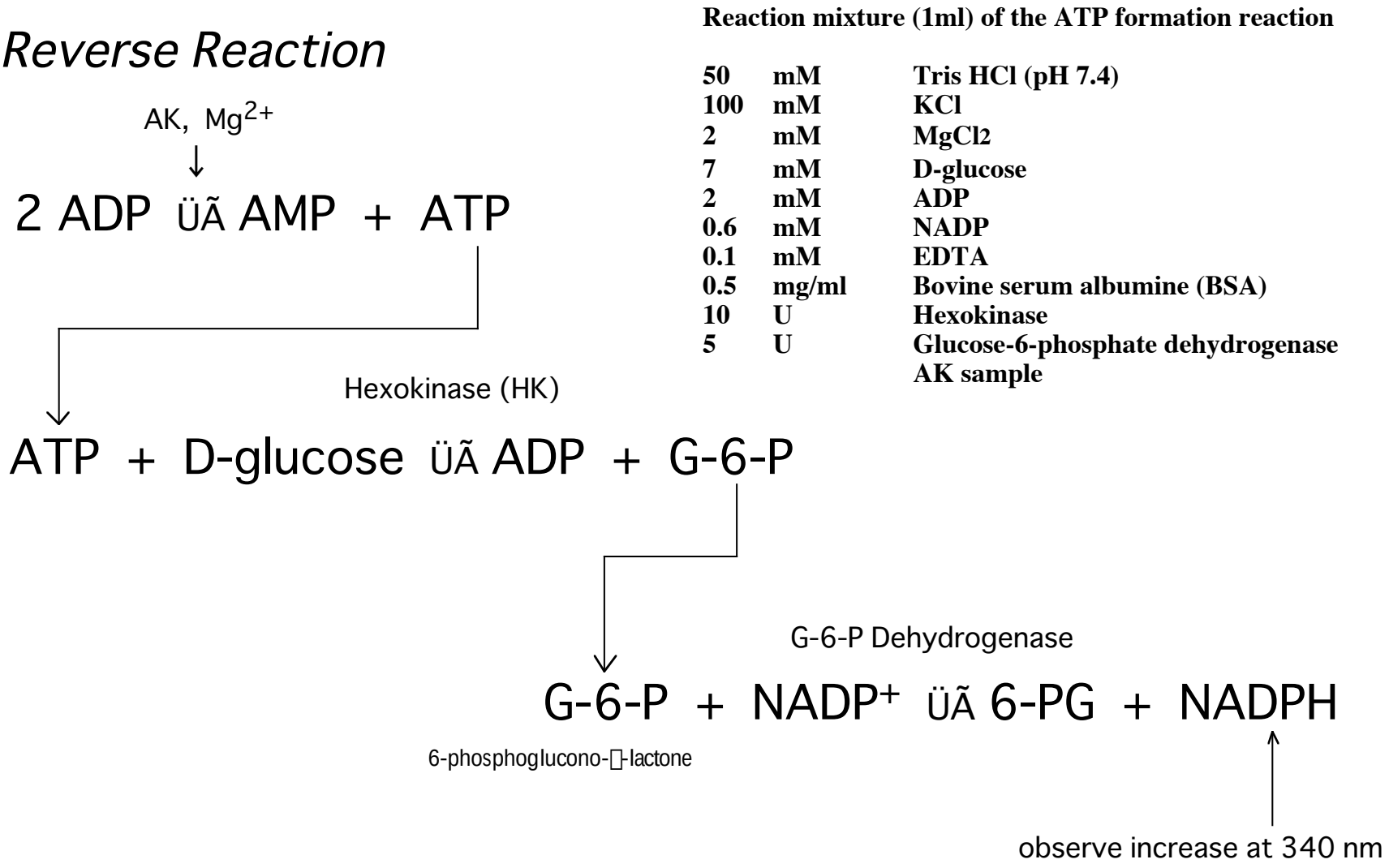


Forward Reaction



Reaction Mixture (1ml) of the ADP formation reaction

75	**mM**	**Triethanolamine HCl (pH 7.5)**
120	**mM**	**KCl**
0.2	**mM**	**NADH**
0.3	**mM**	**Phosphoenolpyruvate**
1	**mM**	**MgSO4**
1	**mM**	**ATP**
1.2	**mM**	**AMP**
0.3	**mg/ml**	**Bovine serum albumine (BSA)**
15	**U**	**Lactate dehydrogenase**
6	**U**	**Pyruvate kinase**
		AK sample



Reaction mixture (1ml) of the ATP formation reaction

50	**mM**	**Tris HCl (pH 7.4)**
100	**mM**	**KCl**
2	**mM**	**MgCl2**
7	**mM**	**D-glucose**
2	**mM**	**ADP**
0.6	**mM**	**NADP**
0.1	**mM**	**EDTA**
0.5	**mg/ml**	**Bovine serum albumine (BSA)**
10	**U**	**Hexokinase**
5	**U**	**Glucose-6-phosphate dehydrogenase**
		AK sample

One unit of activity = 1 μmol ATP/min at pH8.0 and 30 ^{0}C.

Δ O.D./min X 0.1607 = units/cuvette /aliquot X dilution factor
(10 μl) (X 100)

Extinction coefficient for reduced $NADP_{+}$ = 6.22 X 10^{6} cm^{2}/mole.

O.D. =E*c*b

E=Extinction coefficient
c= concentration (in moles/liter)
b=light path (in cm)

O. D. =6.22 X 10^{6} cm^{2} / mmol* (c)* (1 cm)
=6.22 X 10^{6} cm^{3}/mmole *(c)
=6.22 X 10^{6} liters/mole* (c).

or

O.D. = 6.22 X 10^3 ml/mmole* (c)

c=(O.D.*mmoles/6.22 X 10^3 ml) X 3 ml /cuvette

= (O.D.*□mol/cuvette)X 3ml/6.22 ml

=O.D. *□moles/cuvette X 0.1607

Δ O.D./min X 0.1607 □moles/cuvette

= units/cuvette X dilution factor/aliquote

=units/ml

Activity (U/ml) = Δ O. D. /min X 0.1607 mmoles/cuvette*dilution factor/aliquote

! " # " $ " %

One unit of activity = 1 μmol ATP/min at pH7.4 and 25 ^{0}C.

Δ O.D./min X 0.1607 = units/cuvette /aliquot X dilution factor
(1/6.22 X 10 ^{6} cm^{2}/mole) (10 μl) (X 100)

Extinction coefficient for reduced NADH
= 6.22 X 10^{6} cm^{2}/mole.

O.D. =E X c X b	E=Extinction coefficient c= concentration (in moles/liter) b=light path (in cm)

or

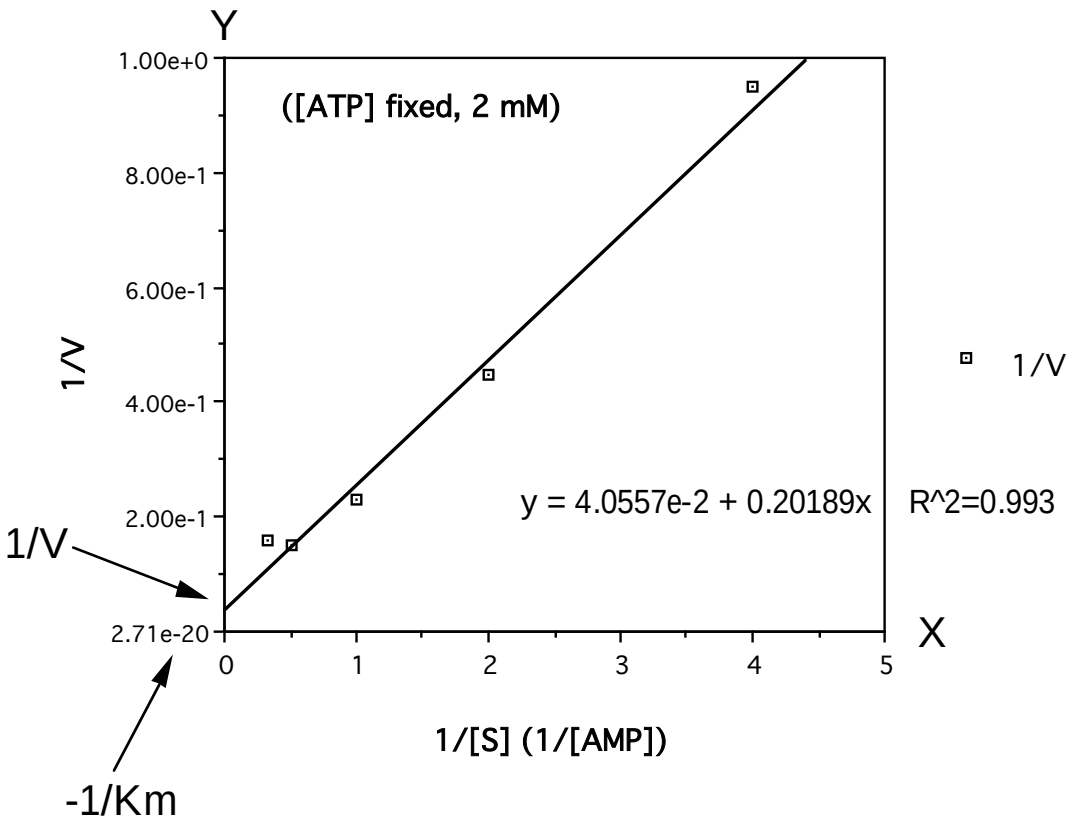
O.D. = 6.22 X 10^{3} ml/mmole X (c)

c=(O.D. X mmoles/6.22 X 10^{3} ml) X 3 ml /cuvette
= (O.D. X μmol/cuvette) X 3 ml/6.22 ml
=O.D. X μmoles/cuvette X 0.1607

ΔO.D./min X 0.1607 μmoles/cuvette
= units/cuvette X dilution factor/aliquote
=units/ml

Activity (U/ml)
= ΔO. D. /min X 0.1607 μmoles/cuvette X dilution factor/aliquote

K194P (Lineweaver-Burk Plot)



1/V=4.0557e-2 + 0.20189 ' 1/[S]

X ! " # $ % & -1/Km
Y ! " # $ % & 1/V

$$v = \frac{V[S]}{Km + [S]}$$

$$Km = \frac{k\text{-}1 + k2}{k+1}$$

$$E + S \xrightarrow{k+1} ES$$

$$E + S \xleftarrow{k-1} ES$$

$$ES \xrightarrow{k2} E + P$$

$$\frac{d[ES]}{dt} = k+1\,[E]\,[S] - (k-1 + k2)\,[ES]$$

! " # $ % & ' ( ) * + , - . / 0 1 2 3 4 5 6 7 8 9 : ; < 4 1

$$v = \frac{d[P]}{dt} = k2\,[ES],\ [E]_0 = [E] + [ES],\ [S]_0 = [S] + [ES] + [P]$$

= >) $[E]_0 << [S]$ & , ?) $[ES] << [S]_0$

@ A B C 1 4 D 1 7 E F $[P] << [S]_0$

G H :) [S] I) $[S]_0$ 1 J K L , M ! L 7 N > O 4 P , 1 % 4 Q

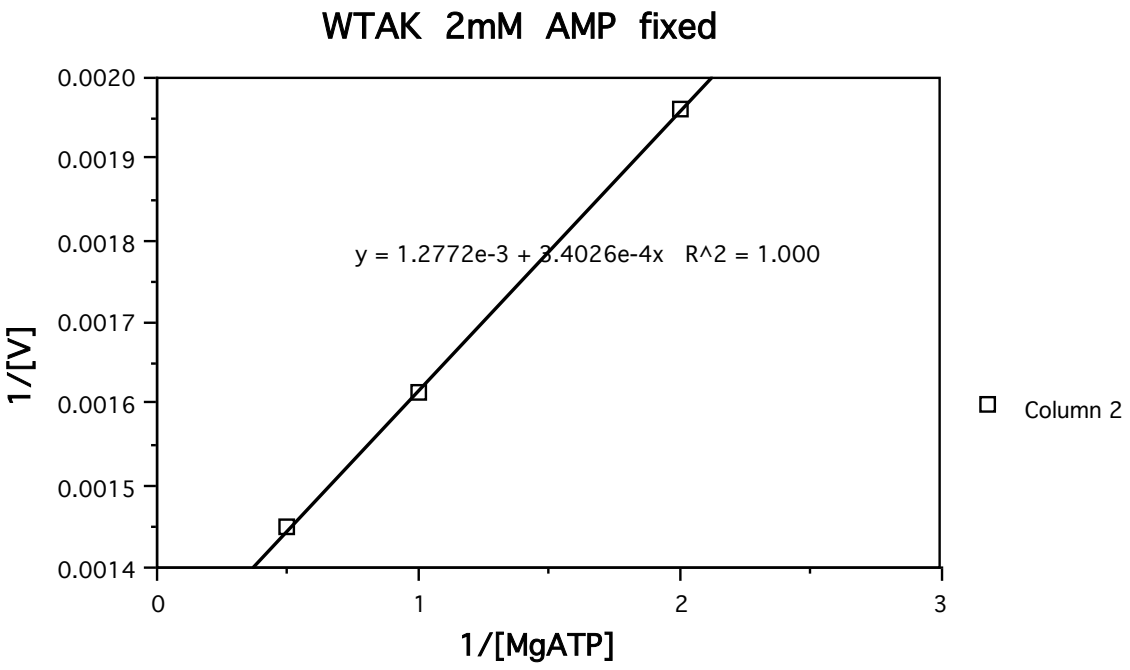
$$\frac{d\,[ES]}{dt} = k+1\,[S]\,([E]0 - [ES]) - (k-1 + k2)\,[ES]$$

D O C R S % O T) Km = (k-1 + k2)/k+1 E F

$$[ES] = \frac{[E]0[S]}{Km + [S]}\,(1 - \exp(-k+1(Km + [S])t))$$

D O /) U V W X Y Z , [ES] , [\ ] ^ _ C ` % + ? a 4 Q

$$v = \frac{d[P]}{dt} = k\,\frac{[E]_0[S]}{Km + [S]} = \frac{V\,[S]}{Km = [S]}$$


WTAK 2mM AMP fixed
0.0020
0.0019
0.0018
0.0017
0.0016
0.0015
0.0014
1/[V]
y = 1.2772e-3 + 3.4026e-4x R^2 = 1.000
0
1
2
3
1/[MgATP]
Column 2

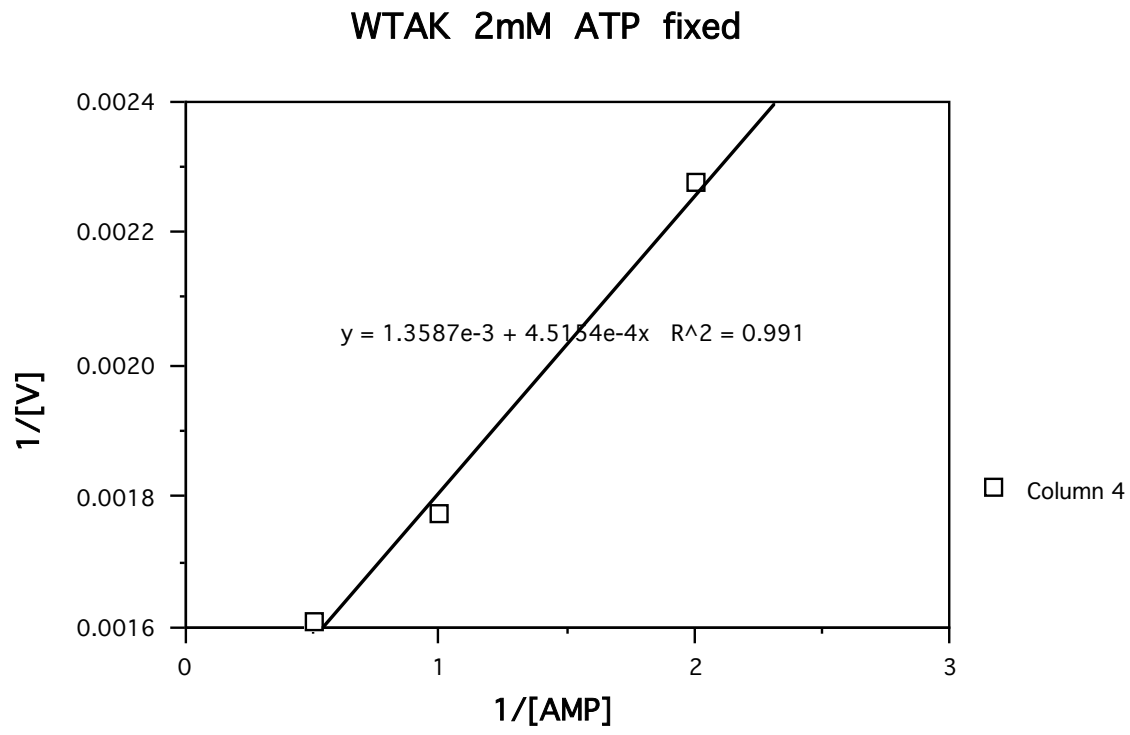

WTAK 2mM ATP fixed
0.0024
0.0022
0.0020
0.0018
0.0016
1/[V]
y = 1.3587e-3 + 4.5154e-4x R^2 = 0.991
0
1
2
3
1/[AMP]
Column 4

Table 2. Summary of kinetic parameters of wild-type hAK1 (WTAK) and muant hAK1

Enzyme	K_m(MgATP^{2-}) (mM)	(fold)	K_m(AMP^{2-}) (mM)	(fold)	k_{cat} (s^{-1})	ralative value (%)	k_{cat}/K_m(MgATP^{2-})	(%)	k_{cat}/K_m(AMP^{2-})	(%)
WTAK	0.27	(1.0) a	0.33	(1.0) a	571	(100.0) b	2.1×10^6	(100.0) b	1.7×10^6	(100.0) b
K194S	3.92	(14.5)	1.11	(3.4)	213	(37.3)	5.4×10^4	(2.6)	1.9×10^5	(11.2)
K194I	0.05	(0.2)	0.20	(0.6)	13.5	(2.4)	2.7×10^5	(12.9)	6.8×10^4	(4.0)
K194L	2.59	(9.6)	0.02	(0.1)	78	(13.7)	3.0×10^4	(1.4)	3.9×10^6	(229.4)
K194P	0.78	(2.9)	0.05	(0.2)	4.5	(0.8)	5.8×10^3	(0.3)	9.0×10^4	(5.3)
K194N	0.80	(3.0)	0.54	(1.6)	21	(3.7)	2.6×10^4	(1.2)	3.9×10^4	(2.3)
K194V	0.25	(0.9)	6.86	(20.8)	8	(1.4)	3.2×10^4	(1.5)	1.2×10^3	(7.1×10^{-2})

a Numbers in parentheses for K_m values indicate the relative change compared to the wild-type AK (the mutant/WTAK ratio).

b For calculation of k_{cat} value, molecular weight of 21,700 was employed and numbers in parentheses for k_{cat} and k_{cat}/K_m values represent % of WTAK.