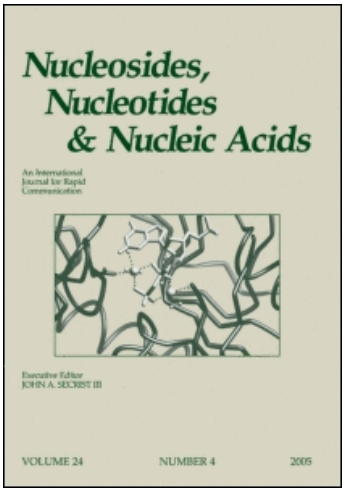

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A KINETIC STUDY OF THE RAT LIVER ADENOSINE KINASE REVERSE REACTION

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□ *Adenosine kinase is an enzyme catalyzing the reaction: adenosine + ATP → AMP + ADP. We studied some biochemical properties not hitherto investigated and demonstrated that the reaction can be easily reversed when coupled with adenosine deaminase, which transforms adenosine into inosine and ammonia. The overall reaction is: AMP + ADP → ATP + inosine + NH_3. The exoergonic ADA reaction shifts the equilibrium and fills the energy gap necessary for synthesis of ATP. This reaction could be used by cells under particular conditions of energy deficiency and, together with myokinase activity, may help to restore physiological ATP levels.*

Keywords Adenosine kinase; rat liver; adenosine; purine metabolism

INTRODUCTION

Many reports indicate that adenosine plays an important role in regulating a variety of metabolic processes. Adenosine may act as a "local hormone" in mammalian tissues showing a diversity of effects, including modulation of blood flow, inflammation, neurotransmission, pain and the endogenous response to ischemia.[1] Most basal adenosine production during normoxia is derived from the action of *S*-adenosylhomocysteine hydrolase.[2] However, during enhanced oxygen demand or metabolic load, increased amounts of adenosine are formed almost exclusively from AMP by 5′-nucleotidase. In cells, the adenosine generated may be deaminated to inosine by adenosine deaminase (ADA), phosphorylated to AMP by adenosine kinase (AdK), or transported into extracellular fluid where it acts by coupling to A1, A2, or A3 receptors.[3] Comparison of the kinetic properties of AdK and ADA suggests that under normal conditions most intracellular adenosine is metabolized by AdK.

The latter enzyme has been studied from several points of view: it has been purified to homogeneity from various mammalian tissues, has a

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monomeric structure and a MW estimated around 40,000;[4] its kinetic properties, gene expression, and structure have been described.[25,6]

In this study we analyzed the reversibility of the AdK reaction and demonstrated that it can be detected only if coupled with the ADA reaction. This series of reactions transforms adenosine into inosine + NH_3 according to Scheme 1

MATERIALS AND METHODS

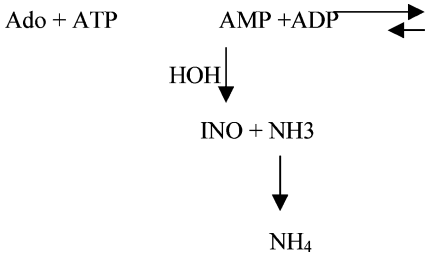
AdK was purified to homogeneity from rat liver according to the method of Yamada et al.[7] with slight modifications. The purified enzyme showed specific activity, Vmax and Km for substrates of the same order of magnitude as reported in the literature.[7] The assay mixture used to test AdK reverse activity contained: 25 mM Bis-Tris (pH 6.5), 1 mM $MgCl_2$, 1.2 mM AMP, 1.2 mM ADP, 0.9 U commercial ADA (from Sigma), 1.4 μg purified rat liver AdK and H_2O to a final volume of 0.125 ml. The mixture was incubated at 37°C for 20 minutes, deproteinized by adding concentrate perchloric acid (2.5μl), and neutralized with 5N KOH. The reaction products were analyzed by HPLC. :

An adenosine peak was not detectable in chromatograms, demonstrating the complete conversion into inosine by the ADA coupled reaction. Activity of the reverse reaction was calculated by the combined areas under the ATP and inosine peaks, compared to a calibration curve processed under the same conditions.

Time course, linearity with protein concentrations, effect of alternative substrates and salts, and Km for ADP and other alternative substrates of the reaction were also determined.

RESULTS

The reaction was linear with respect to time (up to 60 minutes) and protein concentration. Activity peaked at pH 6.5 and fell away sharply below pH 5 and above pH 8. The rate of the reverse reaction, estimated in the optimal



SCHEME 1

TABLE 1 Specific activity (S.A.) and corresponding K_m values of Reaction 1 calculated using ADP and alternative substrates. Values are the means of five experiments ± St. Dev

Substrates	S .A. ± St Dev mmol/ (min*mg)	%Activity with respect to ADP	K_m (mM)
ADP	0.815 ± 0.045	100	0.108 ± 0.005
GDP	0.322 ± 0.018	41	0.216 ± 0.009
dGDP	0.225 ± 0.011	28	0.503 ± 0.028
dADP	0.185 ± 0.008	23	1.970 ± 0.053

conditions (described in the Materials and Methods section), resulted about 6 times lower than the forward.

Table 1 reports the enzyme specific activity assayed using 0.2 mM ADP, compared to alternative substrates; the respective K m values were calculated using substrate concentrations ranging between 0.05 to 5 mM. Activity and substrate affinity were greatest for ADP, but the reaction was also effective with GDP, dADP and dGDP. Conversely, pyrimidine compounds (CDP and UDP) were not good substrates. Table 2 shows that $MgCl_2$ was the best cofactor for the reaction.

DISCUSSION

In this paper we studied the reaction AMP + ADP →Ado + ATP, catalyzed by rat liver AdK, which is the reverse of the original AdK reaction. According to the literature, this reaction is strongly directed towards the production of adenosine and ATP for energy reasons, but we found that the energetic gap can be filled by the coupled ADA reaction which transforms Ado into inosine and ammonia. This second reaction is exergonic with a ΔG^0 of -4 to -10 kcal/mol[8], has a low K_m for substrate and high efficiency, close to diffusion-limited, and occurs in most mammalian tissues. Under our conditions, the equilibrium is shifted towards ATP synthesis.

Further experiments are needed to determine whether this reverse reaction has physiological significance and could play a role in particular situations associated with ATP deficiency or increased ATP demand,

TABLE 2 Activity of Reaction 1 assayed with alternative salts at the same concentrations as $MgCl_2$ (1.2 mM), expressed as a percentage with respect to $MgCl_2$

$MgCl_2$	100%
$CoCl_2$	95%
$MgSO_4$	90%
$MnCl_2$	77%
$ZnCl_2$	13%
$CaCl_2$	4%

as during prolonged muscle work (when ATP is dramatically reduced), fructose-induced hyperuricemia with ATP depletion, situations of severe nucleotide depletion (such as rheumatoid arthritis, anemia, and cell division) and transient ischemia-anoxia.

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