Mammalian Deoxynucleoside Kinases

II. DEOXYADENOSINE KINASE: PURIFICATION AND PROPERTIES

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SUMMARY

Deoxyadenosine kinase was partially purified 140-fold from calf thymus by fractionation with streptomycin, pro- 
tamine, and ammonium sulfate and by column chromatog- 
raphy on Sephadex G-150 and DEAE-cellulose. The molec- 
ular weight was estimated to be about 63,000 by gel filtration 
chromatography. The enzyme appears to catalyze the phos- 
phorylation of deoxyadenosine, deoxyguanosine, and cytidine 
to their corresponding nucleoside 5'-monophosphates in the 
presence of a divalent cation and a nucleoside 5'-triphos- 
phate. All common ribo- and deoxynucleoside 5'-triphos- 
phates, with the exception of deoxycytidine 5'-triphosphate, 
could act as phosphate donors.

Deoxyadenosine kinase has been found to catalyze the trans- 
ferral of a phosphate group from specific nucleoside 5'-triphosphate donors to the 5' position of dAdo1 (1). Interest in this enzyme was aroused by Klenow's observation (2) that the addition of 2 
mol dAdo to Ehrlich ascites tumor cells in vitro almost com- 
pletely inhibited DNA synthesis. This inhibition could be 
alleviated if dCyd and dGro were added simultaneously to the 
incubation mixture (3). Similar results were reported by Maley 
and Maley (4) who demonstrated that dAdo exerted a marked 
hibitory effect on the incorporation of Cyd and Urd into chick 
embryo DNA cytosine and thymine. When tumor cells were 
exposed to dAdo, dATP was shown to accumulate in these cells 
(5). Since dATP is known to act as a potent allosteric inhibi- 
tor of ribonucleotide reductase in mammalian cells (6), it has 
been suggested that the inhibition of DNA synthesis caused by 
dAdo could be mediated through its end product, dATP. It 
would therefore be of value to study the mechanism and con- 
trol of the phosphorylation of dAdo so as to understand the role 
of dAdo kinase in deoxynucleotide metabolism.

Previous work indicates that the activity of the enzyme, dAdo 

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1 The abbreviations used are: dAdo, deoxyadenosine; dGuo, 
deoxyguanosine; dCyd, deoxycytidine; dThd, deoxythymidine; 
Ado, adenine; Guo, guanine; Cyd, cytidine; Urd, uridine; 
Bicine, N,N-bis(2-hydroxyethyl)glycine.

kinase, is regulated by the inhibition produced by the deoxy-
nucleotides of adenine, guanine, and cytosine as well as by its 
relatively high apparent $K_m$ value for dAdo (1).

This paper describes more extensively the purification and 
some of the properties, and the accompanying paper (7), the 
kinetics of dAdo kinase.

EXPERIMENTAL PROCEDURE

Materials—The 14C- or tritium-labeled purine and pyrimidine 
nucleosides were obtained from Schwarz BioResearch. Non- 
radiosensitive nucleosides and nucleotides were purchased from 
Calbiochem and P-L Laboratories. Serva DEAE-cellulose 
powder, DEAE-cellulose paper (type 81), ammonium sulfate 
(enzyme grade), protamine sulfate (type 1), Sephadex G-150 
(10 to 40 μ), and cellulose-coated (Avicel) glass plates were 
obtained from Gallard-Schlesinger, Reeve Angel, Mann, Sigma, 
Pharmacia, and Analtech, respectively.

The labeled nucleotides were purified by descending chroma-
tography for 24 hours on Whatman No. 3M paper in 86% 1-but-
tanol-concentrated ammonium hydroxide (94:5:5.5, v/v). The 
chromatograms were air-dried at room temperature, and the 
radioactive spots corresponding to the material to be purified 
were eluted with 50% ethanol and stored at 20°C. Before use 
the ethanol was evaporated and the concentration of the nucleo-
sides was adjusted.

Assay of Enzyme—dAdo kinase activity was assayed by meas-
uring the incorporation of [14C]dAdo or [3H]dAdo into deoxy-
adenosine 5'-monophosphate by the binding of this latter com- 
pound to DEAE-cellulose discs (8). The incubation mixture 
(0.1 ml) contained 10.0 μmoles of Tris-HCl (pH 8.0), 1.0 μmole 
of MgCl₂, 0.1 μmole of ATP, 0.1 μmole of dithiothreitol (or 1.0 
μmole of 2-mercaptoethanol), 100 nmoles of [14C]dAdo (9 × 10⁶ 
cpm) or [3H]dAdo (1.9 × 10⁶ cpm), and 0.1 to 2.0 units of en- 
zyme. Following the addition of the enzyme to the previously 
heated (37°C) reaction mixture, the mixture was incubated for 5 
min at 37°C and then immediately diluted to 5 ml with water. 
The solution was permitted to flow by gravity through 2.5-cm 
diameter DEAE-cellulose discs that had been previously washed 
with 1 ml of 0.01 N HCl and 20 ml of water. The discs were 
then washed with 20 ml of water, dried, and placed in scintilla-
tion vials containing 10 ml of scintillation solution (4 g of 2,5 
diphenyloxazole and 100 μg of p-bis[2-(5-phenyloxazolyl)]ben- 
zene in 1 liter of toluene). The vials were counted in a Packard 
Tri-Carb liquid scintillation counter with a counting efficiency 
for 14C of 30% and for tritium of 2% under these conditions.
Table I

Purification of deoxyadenosine kinase

| Fraction      | Volume (ml) | Protein (mg) | Total units | Specific activity (units/mg) |
|---------------|-------------|--------------|-------------|-----------------------------|
| I. Extract    | 565         | 10,000       | 5,000       | 0.5                         |
| II. Streptomycin | 565        | 6,800        | 4,100       | 0.6                         |
| III. Protamine | 102         | 1,530        | 7,800       | 5.1                         |
| IV. Ammonium sulfate | 14.9     | 450          | 1,500       | 9.1                         |
| V. Sephadex G-150 | 30         | 42           | 1,000       | 38.2                        |
| VI. 20% glycerol | 9           | 42           | 1,000       | 38.2                        |
| VII. DEAE-cellulose | 13.5     | 9.0          | 652         | 72.5                        |
| VIII. 60% glycerol | 2.4         | 8.9          | 650         | 73.0                        |

One unit of enzyme was defined as the amount catalyzing the conversion of 1 nmol of dA to dAMP in 1 min under the described assay conditions. Protein was determined by the method of Lowry et al. (9) with crystalline albumin as a standard.

RESULTS

Purification of Enzyme

Extract—Frozen (−60°C) calf thymus (300 g) was homogenized in a Phillips blender in 600 ml of 20 mM cold Tris-HCl, pH 8.0, for 3 min and centrifuged at 23,000 × g for 30 min. The supernatant (565 ml) was passed through three layers of gauze. To the supernatant 0.81 ml of 14 M 2-mercaptoethanol was then added, yielding Fraction I (Table I). Throughout the purification procedure the temperature was maintained at about 4°C.

Streptomycin Treatment—To 565 ml of Fraction I, 28.2 ml of 10% streptomycin sulfate, pH 7.0, were added slowly with stirring. After mixing for 30 min the suspension was centrifuged as above and the supernatant was collected. Since the pH was found to have fallen to 6.5 it was raised to 8.0 with NH₄OH (Fraction II).

Protamine Sulfate Treatment—To 565 ml of Fraction II, 114 ml of 2% protamine sulfate, pH 7.0, were added with stirring over a 10-min period. After the mixture was stirred for 30 min, it was centrifuged at 23,000 × g for 30 min and the precipitate was retained. To the protamine sulfate precipitate 100 ml of 2% ammonium sulfate in 50 mM Tris-HCl, pH 8.0, and 20 mM 2-mercaptoethanol were added. An ultrasonic probe was used with continuous stirring for 17 min to disperse the precipitate. After the suspension was centrifuged at 23,000 × g for 30 min, the supernatant was collected (Fraction III).

Ammonium Sulfate Treatment—To 102 ml of Fraction III, 21.3 g of ammonium sulfate (0 to 35% saturation) were added with stirring over a 15-min period. After 20 min of additional stirring, the suspension was centrifuged for 30 min at 23,000 × g. The precipitate was discarded whereas the supernatant was retained. To 105 ml of the supernatant from the previous step 17.2 g of ammonium sulfate (35 to 60% saturation) were added with stirring over a 10-min period. After an additional stirring of 20 min, the precipitate was obtained by centrifugation for 30 min at 23,000 × g and dissolved in 15 ml of 50 mM Tris-HCl, pH 8.0, and 20 mM 2-mercaptoethanol (Fraction IV).

Chromatography on Sephadex G-150—A column of Sephadex G-150 (10 to 40 μ, 19.4 cm² × 50 cm) was prepared and equilibrated with 1200 ml of 50 mM Tris-HCl (pH 8.0) and 20 mM 2-mercaptoethanol (Buffer A). Through the column were passed by upward flow 14.5 ml of Fraction IV (445 mg of protein). The protein was eluted from the column with Buffer A at a flow rate of 12.5 ml per hour. Fractions (19 ml) were collected at

![FIG. 1. Sephadex G-150 (10 to 40 μ) chromatography of Fraction IV. Fraction IV (14.5 ml) containing 445 mg of protein and 4100 units of enzyme activity was eluted from a column of Sephadex G-150. 10 to 40 μ (19.4 cm² × 50 cm) with 50 mM Tris-HCl (pH 8.0) and 20 mM 2-mercaptoethanol. Fractions containing 19 ml were collected at 90-min intervals, and the deoxyadenosine kinase assay was performed as described under Experimental Procedure.]

![FIG. 2. DEAE-cellulose chromatography of Fraction VI. Fraction VI (9 ml) containing 42 mg of protein and 1600 units of enzyme activity was eluted from a column of DEAE-cellulose (0.63 cm² × 16 cm) with a linear gradient of 0 to 0.3 M KCl in 20% glycerol, 20 mM Tris-HCl (pH 8.0), and 20 mM 2-mercaptoethanol. Fractions containing 3 ml were collected at 8-min intervals, and the deoxyadenosine kinase assay was performed as described under Experimental Procedure.]

Sephadex G-150
90-min intervals, and protein concentration and enzyme activity were determined (Fig. 1). The two fractions (Nos. 15 and 16) of maximal specific activity and containing 45% of the activity applied to the column were pooled and concentrated 4.1-fold by dialyzing them (in 1-inch diameter tubing) against 500 ml of 70% glycerol in Buffer A for 4 hours. Then in preparation for the next column they were dialyzed against 500 ml of 20% glycerol in 20 mM Tris-HCl, pH 8.0, and 20 mM 2-mercaptoethanol (Buffer B) to yield Fraction V.

**Chromatography on DEAE-cellulose**—A column of DEAE-cellulose (0.63 cm² x 16 cm) was prepared and equilibrated with Buffer B. About 9 ml of Fraction V (44 mg of protein) were placed on the column and eluted with a linear KCl gradient (Fig. 2). The mixing chamber contained 43 ml of Buffer B and the reservoir contained 43 ml of 0.3 M KCl in Buffer B. Fractions of 3 ml were collected every 8 min and assayed for protein and enzyme activity. The fractions containing the highest specific activities, 16, 17, 18, 19, and 20, were combined and concentrated by dialysis against 250 ml of three different concentrations of glycerol in Buffer A: an 18-hour dialysis against 70% glycerol, followed by a 5% glycerol dialysis for 4 hours, and a final dialysis against 60% glycerol for another 4 hours. A 140-fold purification was achieved with 13% recovery. The enzyme can be stored in 60% glycerol at -20° for at least 3 months with slight loss of activity. A summary of the purification is given in Table I.

**Properties of Partially Purified Enzyme**

**Properties of Reaction**—The presence of a divalent cation and ATP in the reaction mixture was essential for activity (Table II). Of the three cations tried, Mg⁺⁺ (10 mM) produced the highest activity, with Mn⁺⁺ and Ca⁺⁺ producing a lower rate of reaction. ADP was not able to replace ATP as the phosphate donor. The sensitivity of the enzyme to mercuric ions in the absence of the thiol compound, dithiothreitol, was shown by the presence of 0.01 and 0.1 mM heavy metal, causing 25% and almost complete inhibition, respectively.

The rate of formation of dAMP from deoxyadenosine with ATP as the phosphate donor was linear with respect to time up to 5 min and enzyme concentration up to 7 μl or 33 units per ml (Fig. 3).

![Fig. 3](http://www.jbc.org/)

*Fig. 3. Effect of time and enzyme concentration on the amount of dAMP formed. The incubation mixture contained, in 0.1 ml, 10 μmoles of Tris-HCl (pH 8.0), 1.0 μmole of MgCl₂, 0.1 μmole of dithiothreitol, 0.1 μmole of ATP, and 100 μmoles of [¹⁴C]-deoxyadenosine (9.1 X 10⁴ cpm). In A, 1.7 units of Fraction VIII were added and the mixture was incubated at 37° for the time indicated. In B, the indicated volumes of Fraction VIII (470 units per ml) were added and the mixture was incubated at 37° for 3 min. In both A and B the amount of dAMP formed was assayed as described under “Experimental Procedure.”*
Effect of pH on Rate of Reaction—Under conditions of the routine assay, the partially purified enzyme (Fraction VIII) had a broad pH optimum range with the Tris-maleate-Bicine buffer (Fig. 4). There was hardly any difference (10%) in activity within the pH range of 6.5 to 8.5. Below pH 6.5 a sharp drop was evident.

Molecular Weight Determination—The molecular weight of dAdo kinase was determined by gel filtration according to the method of Andrews (10). The molecular weights of the proteins used to calibrate the column were: myoglobin, 17,800; ovalbumin, 45,000; bovine serum albumin, 67,000; and γ-globulin, 160,000 (10). Fig. 5 shows that a linear relationship between the logarithm of the molecular weight of a protein and the ratio of its elution volume to the void volume from a Sephadex G-150 (10 to 40 μ) column exists up to a molecular weight of at least 70,000. By applying this relationship to dAdo kinase, a molecular weight of about 63,000 can be obtained.

Table III

Stoichiometry of reaction with UTP as phosphate donor

The basic reaction mixture contained, in 0.1 ml, 10 μmoles of Tris-HCl (pH 8.0), 1.0 μmole of MgCl₂, 0.1 μmole of diethiothreitol, 370 nmoles of ³H-UTP (62 cpm per n mole), and 1 unit of Fraction VIII. The mixture was incubated at 37°C for 30 min. To Series B only, 360 nmoles of ³C-deoxyguanosine (95 cpm per n mole) were added at 0 min. The reaction was stopped by placing the tubes in ice. A 40-μl portion of each tube was spotted together with 0.1 μmole each of nonradioactive UTP, UDP, UMP, dAdo, and dAMP on two DEAE-cellulose thin layer plates (19). The plates were chromatographed in 0.10 N and 0.015 N HCl, respectively, and dried at 40°C. The separated compounds were scrapped off the plates and assayed for radioactivity. No enzyme was added to the zero time sample.

| Series | Time | UTP | UDP | UMP | dAdo | dAMP |
|--------|------|-----|-----|-----|------|------|
| A      |      |     |     |     |      |      |
| 0      | 370  | 25  | 5   | 0   | 0    |      |
| 30     | 366  | 25  | 10  | 0   | 0    |      |
| -4     | 0    | +5  |     | 0   | 0    |      |
| B      |      |     |     |     |      |      |
| 0      | 370  | 25  | 5   | 390 | 0    |      |
| 30     | 204  | 78  | 103 | 174 | 177  |      |
| -166   | +53  | +98 | -186| +177|      |      |

Table IV

Specificity for phosphate donors

| Phosphate donor | dAMP formed | dGMP formed |
|-----------------|-------------|-------------|
| Adenosine 5'-triphosphate | 4.2 | 5.8 |
| Guanosine 5'-triphosphate | 4.1 | 5.3 |
| Cytidine 5'-triphosphate | 0.8 | 1.1 |
| Uridine 5'-triphosphate | 3.8 | 4.4 |
| Deoxyadenosine 5'-triphosphate | <0.5 | 0.9 |
| Deoxyguanosine 5'-triphosphate | 1.2 | 1.3 |
| Deoxyxycytidine 5'-triphosphate | <0.5 | <0.5 |
| Deoxythymidine 5'-triphosphate | 3.2 | 4.3 |

dAdo kinase was determined by gel filtration according to the method of Andrews (10). The molecular weights of the proteins used to calibrate the column were: myoglobin, 17,800; ovalbumin, 45,000; bovine serum albumin, 67,000; and γ-globulin, 160,000 (10). Fig. 5 shows that a linear relationship between the logarithm of the molecular weight of a protein and the ratio of its elution volume to the void volume from a Sephadex G-150 (10 to 40 μ) column exists up to a molecular weight of at least 70,000. By applying this relationship to dAdo kinase, a molecular weight of about 63,000 can be obtained.

Stoichiometry—The stoichiometry of the reaction was determined as shown in Table III. Upon incubation of UTP (the phosphate donor in this experiment) and dAdo with enzyme Fraction VIII, the amounts of UTP and dAdo that disappeared were approximately equal and matched the formation of UDP.
and enzyme Fraction VIII. In another experiment the absence of Fraction VIII resulted in no ADP or AMP production, indicating the presence of phosphatases in Fraction VIII.

Results suggest the presence of trace amounts of UTP and UDP somewhat larger amount of UMP than UDP was formed. These results were detected upon routine incubation with 3H-deoxyguanosine (2.6 × 10^4 cpm), 10 nmoles of 3H-deoxycytidine (3.6 × 10^4 cpm), or 1.0 nmole of 3H-cytidine (1.0 × 10^5 cpm), and 30 μl of dialyzed Fraction IV. The mixture was incubated at 37° for 10 min and assayed as described under “Experimental Procedure.”

The mixture containing, in 0.1 ml, 10 μmoles of Tris-HCl (pH 8.0), 1.0 μmole of MgCl₂, 0.1 μmole of ATP, 0.1 μmole of diithiothreitol, 0.1 unit of Fraction VIII, and 20 nmoles (0.4 μCi) of the tritiated nucleoside (except 3H-uridine) was tested as acceptor. The mixture was incubated at 37° for 10 min and assayed as described under “Experimental Procedure.”

**TABLE V**  
**Specificity for phosphate acceptors**  
The incubation mixture contained, in 0.1 ml, 10 μmoles of Tris-HCl (pH 8.0), 1.0 μmole of MgCl₂, 0.1 μmole of ATP, 0.1 μmole of diithiothreitol, 0.1 unit of Fraction VIII, and 20 nmoles (0.4 μCi) of the tritiated nucleoside (except 3H-uridine) tested as acceptor. The mixture was incubated at 37° for 10 min and assayed as described under “Experimental Procedure.”

| Phosphate acceptor | Nucleotide formed |
|--------------------|-------------------|
| Adenosine          | <0.02             |
| Guanosine          | <0.02             |
| Cytidine           | 0.14              |
| Uridine            | <0.02             |
| Deoxyadenosine     | 0.78              |
| Deoxyguanosine     | 0.75              |
| Deoxycytidine      | 0.10              |
| Deoxythymidine     | <0.02             |

**TABLE VI**  
**Stability of kinase activity to dialysis**  
Fraction IV was divided into four parts and each part was diluted to a protein concentration of 10 mg per ml with the buffer in which it was dialyzed against for 48 hours. Buffer A: Tris-HCl, 200 mM, pH 8, plus 2-mercaptoethanol, 20 mM. Buffer B: Tris-HCl, 200 mM, pH 8. After dialysis Fraction IV was tested for kinase activity by using the indicated phosphate acceptors. The incubation mixture contained, in 0.1 ml, 10 μmoles of Tris-HCl (pH 8.0), 1.0 μmole of MgCl₂, 0.1 μmole of ATP (0.2 μmole with deoxycytidine and cytidine), 1.0 μmole of 2-mercaptopentanol, 100 nmoles of 3H-deoxyadenosine (1.8 × 10^6 cpm), 100 nmoles of 3H-deoxyguanosine (2.6 × 10^6 cpm), 10 nmoles of 3H-cytidine (1.0 × 10^5 cpm), and 30 μl of dialyzed Fraction IV. The mixture was incubated at 37° for 10 min and assayed as described under “Experimental Procedure.”

| Phosphate acceptor | Relative kinase activity % | Buffer A | Buffer B |
|--------------------|---------------------------|----------|----------|
| Deoxyadenosine     |                           | 100      | 93       |
| Deoxyguanosine     |                           | 100      | 55       |
| Deoxycytidine      |                           | 100      | 100      |
| Cytidine           |                           | 100      | 42       |

Specificity for Phosphate Donors—Various nucleotides and deoxynucleotides were tested for their ability to phosphorylate dAdo and dGuo (Table IV). ATP and GTP were the most effective phosphate donors, followed by UTP and dTTP which were 90 and 75%, respectively, as active. When either dAdo or dGuo was used as the substrate the order of activity of the donors was the same. Deoxyguanosine, cytidine, and deoxyadenosine 5'-triphosphates were much less capable of serving as phosphate donors. Deoxycytidine 5'-triphosphate was inactive as a phosphate donor.

**TABLE VII**  
**Effect of deoxyadenosine and deoxyguanosine on deoxycytidine kinase**  
The complete reaction mixture contained, in 0.1 ml, 10 μmoles of Tris-HCl (pH 8.0), 1.0 μmole of MgCl₂, 0.2 μmole of ATP, 1.0 μmole of 2-mercaptopentanol, 1.0 nmole of 3H-deoxycytidine (5.0 × 10^6 cpm), and 0.1 unit of Fraction VIII. The mixture was incubated at 37° for 10 min and assayed as described under “Experimental Procedure.”

| Addition                   | Concentration | dCMP formed |
|----------------------------|---------------|-------------|
| None                       |               | 0.19        |
| Deoxyadenosine             | 0.8           | 0.06        |
| Deoxyguanosine             | 1.0           | 0.12        |
| Deoxycytidine              | 1.0           | <0.01       |
| Uridine                    | 1.0           | 0.19        |
| Deoxyadenosine 5'-monophosphate | 0.3   | 0.18        |
| Deoxyguanosine 5'-monophosphate | 0.3   | 0.19        |
| Deoxycytidine 5'-monophosphate | 0.3   | <0.01       |

**TABLE VIII**  
**Effect of nucleosides and deoxynucleoside monophosphates on phosphorylation of cytidine**  
The complete reaction mixture contained, in 0.1 ml, 10 μmoles of Tris-HCl (pH 8.0), 1.0 μmole of MgCl₂, 0.2 μmole of ATP, 1.0 μmole of 2-mercaptopentanol, 1.0 nmole of 3H-cytidine (7.8 × 10^6 cpm), and 0.1 unit of Fraction VIII, and the indicated additions. The mixture was incubated at 37° for 10 min and assayed as described under “Experimental Procedure.”

| Addition                   | Concentration | CMP formed |
|----------------------------|---------------|------------|
| None                       |               | 0.75       |
| Deoxyadenosine             | 2.0           | 0.59       |
| Deoxyguanosine             | 2.5           | 0.58       |

Specificity for Phosphate Acceptors—Fraction VIII was able to catalyze the phosphorylation, to varying extents, of dAdo, dGuo, dCyd, and Cyd (Table V). Neither dAdo or dGuo was used as the substrate the order of activity of the donors was the same. Deoxyguanosine, cytidine, and deoxyadenosine 5'-triphosphates were much less capable of serving as phosphate donors. Deoxycytidine 5'-triphosphate was inactive as a phosphate donor.

Stability to Dialysis—Fraction IV was subjected to dialysis in buffer with and without 2-mercaptoethanol (Table VI). The dialyzed fraction was tested for kinase activity by using different nucleosides as phosphate acceptors. When dCyd was tested as the substrate the amount of product formed was not affected by dialysis under these conditions. However, when either dAdo, dGuo, or Cyd was used as the substrate, there was about a 50% decrease in product formation with the fraction dialyzed in the absence of 2-mercaptoethanol.
Effect of Nucleosides and Nucleotides on Phosphorylation of Deoxycytidine and Cytidine—In Table VII it can be seen that there was no effect on the amount of $^3$H-dCMP formed from $^3$H-dCyd upon the addition of either cold dAdo or dGuo in 200-fold excess with respect to dCyd. In contrast to this last experiment the addition of either cold dAdo, dGuo, or dCyd but not Urd decreased the conversion of $^3$H-Cyd to $^3$H-CMP (Table VIII). The monophosphorylated derivatives of these deoxynucleosides were also tested for their effect on $^3$H-CMP formation. The addition of dAMP resulted in a slight inhibition of the phosphorylation of $^3$H-Cyd, dGMP had no effect, and dCMP was completely inhibitory.

**DISCUSSION**

It has been shown that the addition of dAdo to mammalian cells results in an inhibition of DNA synthesis (2), possibly due to the accumulation of dATP (5), a potent allosteric inhibitor of ribonucleotide reductase (6). These findings stimulated an interest in the enzyme responsible for the phosphorylation of dAdo.

DAdo kinase was purified about 140-fold from calf thymus. The requirements of the enzyme for a divalent cation and a phosphate donor are similar to the other deoxynucleoside kinases (11, 12). Throughout the purification procedure the enzyme was fairly unstable, necessitating the presence of a reducing agent, 2-mercaptoethanol, and glycerol. The molecular weight of the enzyme, estimated to be about 63,000 by gel filtration, is similar to that of dCyd kinase (11), whereas the molecular weight of mammalian dThd kinase from tumor cells is about 700,000 (12). Certain enzymes involved in deoxynucleotide metabolism such as dThd kinase (13, 14) and ribonucleotide reductase (15) have been shown to form aggregates under various conditions. There was no evidence of aggregation of dAdo kinase in the presence of the inhibitor, dCTP.

As with the other deoxynucleoside kinases there is no absolute specificity for phosphate donors (11, 16). ATP and GTP serve almost equally well as phosphate donors, whereas UTP and dTTP are somewhat less effective. ATP, dATP, and dGTP are nearly inactive and dCTP is inactive as a phosphate donor.

The enzyme fraction appeared to catalyze the phosphorylation of dAdo, dGuo, Cyd, and dCyd. There are three possible explanations for this observation. These nucleosides could be phosphorylated by (a) the same enzyme, (b) different enzymes, or (c) the same enzyme but at different catalytic sites. This latter possibility cannot be excluded at the present time.

In a previous report data were presented that suggested that the phosphorylation of dCyd is catalyzed by an enzyme distinct from dAdo kinase (16). This was confirmed here by the inability of either dAdo or dGuo, added in 200-fold excess, to interfere with the phosphorylation of dCyd (Table VII). Further proof was shown by the distinct difference in the ability of the enzyme fraction to catalyze the phosphorylation of dAdo and dCyd after dialysis. dCyd kinase remained stable during dialysis in the absence of 2-mercaptoethanol whereas dAdo kinase lost about half of its activity under the same conditions (Table VI).

Cellular studies of Bernard and Brent offer additional evidence to support that dCyd kinase and dAdo kinase are distinct enzymes in mammalian cells. Kinase activity toward four different deoxynucleosides was measured during the cell cycle of 3T3 cells derived from mouse fibroblasts. Both dCyd and dThd kinases increased about 4- and 10-fold, respectively, during S phase, i.e. during the period of DNA synthesis, whereas the activity toward dAdo and dGuo remained constant at a relatively high level throughout the cell cycle.

The same enzyme, dAdo kinase, however, appears to catalyze the phosphorylation of dGuo as well as Cyd. In the case of dGuo this hypothesis is substantiated by the following observations: (a) the addition of cold dGuo resulted in a decrease in the rate of conversion of $^{14}$C-dAdo to $^{14}$C-dAMP (1), (b) a similar decrease in kinase activity toward dAdo and dGuo was observed in dialysis study (Table VI), (c) the same pattern of activity with inhibitors and phosphate donors was shown for dAdo and dGuo (Table IV), and (d) kinetic data presented in the following paper (7).

The finding that dAdo kinase could possibly catalyze the phosphorylation of Cyd was unexpected since Sköld (17) and Orego (18) have reported that Urd kinase can catalyze the phosphorylation of Urd and Cyd but not dAdo or dGuo. That Cyd could be a substrate for dAdo kinase was supported by several experiments. It was observed that the addition of either cold dAdo or dGuo decreased the conversion of $^{14}$C-Cyd to $^{14}$C-CMP (Table VIII). It could be argued that the phosphorylated products of these deoxynucleosides, dAMP and dGMP, inhibit the formation of CMP. However, it was shown that the addition of either dAMP or dGMP at sufficiently high concentration (0.3 mM) had no effect on the phosphorylation of Cyd (Table VIII). The complete inhibition of CMP formation in the presence of dCyd is most likely caused by dCMP as dCyd kinase has been shown to be present in enzyme Fraction VIII. If dAdo kinase catalyzes the phosphorylation of Cyd, as it appears to do, it is understandable that dCMP would interfere with the reaction as it is a potent inhibitor of this enzyme (1). Since the addition of an excess of Urd had no effect on CMP formation it is most likely that the enzyme which catalyzes the phosphorylation of Cyd is not the same as the one found by Sköld (17) and Orego (18). Furthermore, the same pattern of inhibition with deoxynucleoside triphosphates as observed with dAdo (1) and dGuo emerged when Cyd was the substrate for the enzyme.

Kinetic evidence is presented in the following paper which also suggests that the phosphorylation of Cyd is catalyzed by dAdo kinase (7).

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