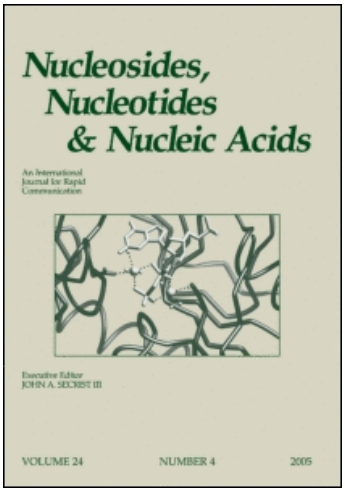

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Ability of Adenosine-2'(3')-deoxy-3'(2')-triphosphates and Related Analogues to Replace ATP as Phosphate Donor for all Human and *Drosphila melanogaster* Deoxyribonucleoside Kinases

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Ability of Adenosine-2′(3′)-deoxy-3′(2′)-triphosphates and Related Analogues to Replace ATP as Phosphate Donor for all Human and *Drosphila melanogaster* Deoxyribonucleoside Kinases

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ABSTRACT

Six non-conventional adenosine-2′- and 3′-triphosphate analogues of ATP were tested as potential phosphate donors for all four human, and *D. melanogaster*,

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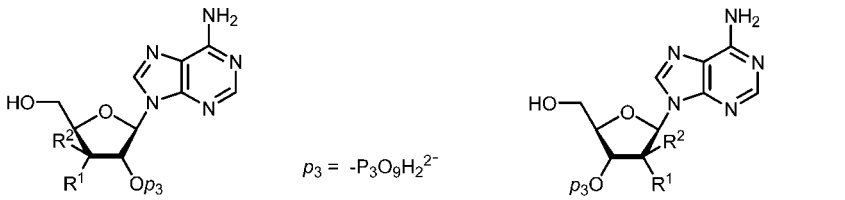
deoxyribonucleoside kinases. With dCK (only dAdo as acceptor), TK1, TK2 and dNK only 3′-deoxyadenosine-2′-triphosphate was an effective donor (5–60% that for ATP). With dCK (dCyd as acceptor) and dGK (dGuo as acceptor), sharing 45% sequence identity, donor activities ranged from 13 to 119% that for ATP. Products were 5′-phosphates. In some instances, kinetics are dependent on the nature of the acceptor, and donor and acceptors properties are mutually interdependent. Results are highly relevant to studies on the modes of interaction with the enzymes, and to interpretations of reported crystal structures of dCK and dNK with bound ligands.

Key Words: Deoxyribonucleoside kinases; Phosphate donors; Adenosine-2′(3′)-deoxy-3′(2′)-triphosphates; Donor-acceptor interdependence.

INTRODUCTION

Deoxyribonucleoside kinases are key enzymes of the salvage pathway of purine and pyrimidine deoxyribonucleosides, and catalyze the phosphorylation of deoxyribonucleosides (and analogues) to corresponding 5′-monophosphates, using nucleoside-5′-triphosphates as phosphate donors. These enzymes exhibit broad specificities for phosphate acceptors, widely profited from for development of antiviral and antitumour nucleoside analogues. Donor specificities have received much less attention. Although ATP is assumed to be the main phosphate donor, other 5′-triphosphates may also serve as donors.[1]

We have previously shown that adenosine-3′-deoxy-2′-triphosphate (ATP1, see Fig. 1) and adenosine-2′-deoxy-3′-triphosphate (ATP2) readily replace ATP as phosphate donors for dCK and, to a lesser extent, for TK1 and TK2.[2] We have now extended the foregoing by synthesis of four additional analogues with fluorinated sugar moieties (ATP3-6, see Fig. 1), and studied their donor activities with the previously reported, and two additional, kinases, human dGK and dNK from *D. melanogaster*.



adenosine-3'-deoxy-2'-triphosphate (ATP1)
R_1 = H, R_2 = H

adenosine-2'-deoxy-3'-triphosphate (ATP2)
R_1 = H, R_2 = H

adenosine-3'-deoxy-3'-fluoro-2'-triphosphate (ATP3)
R_1 = F, R_2 = H

adenosine-2'-deoxy-3'-fluoro-3'-triphosphate (ATP4)
R_1 = F, R_2 = H

adenosine-3'-deoxy-3'-fluoroxylo-2'-triphosphate (ATP5)
R_1 = H, R_2 = F

adenosine-2'-deoxy-3'-fluoroara-3'-triphosphate (ATP6)
R_1 = H, R_2 = F

Figure 1.

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RESULTS AND DISCUSSION

Phosphate donor activities, relative to that for ATP (taken as 100%), are shown in Table 1. The major substrates of TK1 (dThd) and dGK (dGuo) were used as phosphate acceptors, while two substrates were used with dCK (dCyd and dAdo), TK2 (dThd and dCyd) and dNK (dCyd and dAdo). With dCK and TK2, the second substrate (dAdo and dCyd, respectively), also effectively phosphorylated, differs significantly in kinetic parameters from the major one. For dNK all four natural 2′-deoxynucleosides are substrates,[3] leading to selection of one pyrimidine (dCyd) and one purine (dAdo) as acceptors.

With dCK (dCyd as acceptor), all analogues exhibited donor activities comparable to that of ATP, with ATP1 as active as ATP. With dAdo as acceptor, only ATP1 exhibited high activity, 60% that for ATP. With dGK, ATP1 is even more active than ATP; and ATP2, ATP4 and ATP5 exhibit activities at a level >50% that for ATP. Even ATP3 (25% activity) and ATP6 (13% activity) are reasonably good donors. It is consequently worth noting that dCK and dGK exhibit highest sequence similarity (~45% identity).[4]

Table 1. Phosphate donor activities of ATP analogues (all at 1 mM) towards deoxyribonucleoside kinases[a], expressed as initial rates relative to that of ATP (also at 1 mM, taken as 100%[b]), and with different acceptors at concentrations indicated.

	dCK			TK1	TK2		dNK		dGK
Acceptor / Donor	dCyd 2 μM	dCyd 25 μM	dAdo 50 μM	dThd 30 μM	dThd 1 μM	dCyd 50 μM	dCyd 2.5 μM	dAdo 100 μM	dGuo 10 μM
ATP	**100**	**100**	100	100	100	100	100	100	**100**
ATP1	**84**	**97**	60	5.3	7	15	10	6	**119**
ATP2	**52**	**73**	6	2.3	2.2	1.6	1	0.5	**53**
ATP3	**58**	**66**	4	0	1.7	1.7	0.2	0	**25**
ATP4	**36**	**62**	1.5	1.0	1.0	1.2	0.6	0.2	**70**
ATP5	**59**	**62**	4	0	0.9	0.7	0	0	**55**
ATP6	**38**	**59**	1	0	0.6	0.2	0	0	**13**

[a]dCK (with dAdo as acceptor), TK1, TK2 and dNK (columns in non-bold), tentatively classified as group (a); dCK (with dCyd as acceptor) and dGK (columns in bold) as group (b), as described in the text.

[b]Activities of enzymes with ATP are as follows:
dCK/2 μM dCyd – 21 nmol/min/mg
dCK/25 μM dCyd – 27 nmol/min/mg
dCK/50 μM dAdo – 540 nmol/min/mg
TK2/1 μM dThd – 130 nmol/min/mg
TK2/50 μM dCyd – 520 nmol/min/mg
TK1/30 μM dThd – 330 nmol/min/mg
dNK/2.5 μM dCyd – 3.0 μmol/min/mg
dNK/100 μM dAdo – 2.70 μmol/min/mg
dGK/10 μM dGuo – 23 nmol/min/mg.

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With the other three enzymes, only ATP1 exhibits moderate donor activity, 5–15% that for ATP. With TK2, ATP1 is 2-fold more active vs. dCyd as compared to dThd; and with dNK, it is more active vs. dCyd than dAdo. It should be noted that TK2 and dNK, with similar donor specificities, also exhibit high (40%) sequence identity.[4]

Overall, ATP1 is the most effective donor for all five kinases, whereas ATP2–ATP6 together may range from excellent to negligible activity, depending on the kinase employed. The enzymes (enzyme activities) may be tentatively classified in two groups: (a) dCK (only dAdo as acceptor), TK1, TK2, dNK with restricted donor specificity, are significantly active only with ATP1; (b) dCK (dCyd as acceptor) and dGK (relaxed donor specificity), which accept all six donor analogues at a level comparable with, or even superior to, that for ATP (Table 1).

Kinetic parameters for ATP1 and ATP2, relative to those for ATP, have also been determined. It was earlier shown that, with native dCK, both ATP1 and ATP2 were competitive with respect to ATP.[2] We have now found that, with dCK, both K_m and V_{max} values are comparable for all three donors, with 25 μM dCyd as acceptor, in line with their rates of phosphorylation (Table 1). With TK1, K_m values are also comparable, but V_{max} values are much lower for ATP1 and ATP2, in accord with their much poorer abilities as donors (Table 1). By contrast, with TK2 and dNK, K_m values for both ATP1 and ATP2 are much higher than for ATP.

Our previous,[2] and present, data add a totally new element to kinetic, and interpretation of crystallographic,[4] results, which must be taken into account in studies on the modes of binding of donors and acceptors by nucleoside kinases in general, particularly the present demonstration of interdependence of binding of donors and acceptors. Similar considerations may apply to other kinase systems.

ABBREVIATIONS

dCK deoxycytidine kinase
dGK deoxyguanosine kinase
dNK deoxyribonucleoside kinase from *Drosophila melanogaster*
TK1 cytosolic thymidine kinase
TK2 mitochondrial thymidine kinase

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