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Structural Requirements for Activity of Nucleosides as Substrates for Adenosine Kinase: Orientation of Substituents on the Pentofuranosyl Ring

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SUMMARY

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Selected nucleosides of adenine and 8-azaadenine were examined as substrates for adenosine kinase (EC 2.7.1.20), an enzyme that catalyzes the phosphorylation of many biologically active nucleosides, to evaluate the effects on substrate activity of various orientations of substituents on the pentofuranosyl ring. The enzyme used was partially purified from cultured H.Ep.2 cells. Although not all the possible isomers were studied, the results, together with other reported findings, lead to the conclusion that necessary, but not sufficient, conditions for substrate activity are the following: (a) the nucleoside should have a 2′-hydroxy group, (b) the 2′-hydroxy group should be *trans* to the purine moiety, and (c) there should exist a considerable degree of freedom of rotation about the C-1′—N-9 bond. If these conditions are met, the 3′-hydroxy group and the 4′-hydroxymethyl group may be either *cis* or *trans* to the purine ring. These results add to understanding of the mode of binding of substrates to adenosine kinase and should aid in the design of new nucleoside analogues as growth-inhibitory or antitumor agents.

INTRODUCTION

Nucleosides of purines and purine analogues possess a broad spectrum of biological activity (1, 2). Most of these compounds are active only after intracellular conversion to nucleotides, and in mammalian cells the phosphorylation of many of these nucleosides is catalyzed by adenosine kinase (3). This enzyme accepts as substrates nucleosides differing from adenosine in the nature of the 2- and 6-substituents and in the structure of the purine moiety (4–6). Although some compounds with altered sugar moieties are good substrates (4, 5, 7), there is relatively little information about the influence on substrate activity of specific changes in the orientation of the substituents of the pentofuranosyl ring. We report here the results of a study with various nucleosides of adenine and 8-azaadenine that permit conclusions regarding the importance to substrate activity of changes in the orientations of the purine moiety, the 2′- and 3′-hydroxy groups, and the 4′-hydroxymethyl group.

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MATERIALS AND METHODS

9-β-D-Arabinosyl-8-azaadenine (8), 9-α-D-arabinosyl-8-azaadenine (8), 9-β-D-xylofuranosyl-8-azaadenine (8), and "homoadenosine," 1-(aden-9-yl)-2,5-anhydro-1-deoxy-D-allitol (9), were synthesized in our laboratories by Dr. J. A. Montgomery and Ms. H. J. Thomas, who also provided a sample of [2-^{14}C]9-α-D-arabinosyl-8-azaadenine. 9-[DL-2α,3α-Dihydroxy-4-β-(hy-

droxymethyl)cyclopentyl]adenine (10), the carbocyclic analogue of adenosine, was provided by Dr. Y. F. Shealy. 9-β-D-Ribofuranosyl-8-azaadenine (8-azaadenosine) was obtained from Drug Research and Development, Division of Cancer Treatment, National Cancer Institute, through the courtesy of Dr. Harry B. Wood. 9-α-D-Ribofuranosyladenine ("α-adenosine") and 9-α-D-lyxofuranosyladenine were obtained from Terra-Marine Bioresearch, La Jolla, Calif.; [8-^{14}C]2'-deoxyadenosine [9-(β-D-2-deoxyribofuranosyl)adenine], from Schwarz/Mann; and 3'-deoxyadenosine [9-(β-D-3-deoxyribofuranosyl)adenine], from the Merck Institute for Therapeutic Research. Previously described methods (4) were used for the partial purification of adenosine kinase from H.Ep.2 cells and for the assay of nucleosides for substrate activity; further details are given in Table 1. Derivatives of both adenine and 8-azaadenine were used because of the availability of specific compounds with the desired structures and because the substitution of nitrogen for carbon at position 8 of the purine ring enhances phosphorylation by the kinase, as may be seen by comparison of the rates of phosphorylation of adenosine and 8-azaadenosine (Table 1).

RESULTS AND DISCUSSION

Table 1 contains data on the activities of various nucleosides as substrates for adenosine kinase. The values shown represent the results of 30-min assays under the standard reaction conditions described in the table; under these conditions the phosphorylation of adenosine was linear for the 30-min period. These values indicate the extent of phosphorylation of each nucleoside relative to that for adenosine under conditions in which the concentration of substrate is not limiting. For the purpose of discussion, a compound is considered to be a substrate if its activity was detectable under these conditions, and not to be a substrate if its activity was undetectable.

The compounds in Table 1 include some substrates that we studied earlier, together with data on new compounds. These particular nucleosides were chosen to yield information on the importance to substrate activity of the configuration of the pentofuranose. For a complete analysis of this problem, one would require all the isomers of the pentofuranosyladenine derivatives as well as the corresponding 2'- and 3'-deoxy derivatives. Nevertheless, one can draw from the results of Table 1 a conclusion regarding the requirements for substrate activity. It is that the 2'-hydroxy group must be present and that it must be *trans* to the purine ring. The following considerations are the basis for this conclusion.

1. 2'-Deoxyadenosine is not a substrate.

2. No substrate activity was found for α-adenosine and β-D-arabinosyl-8-azaadenine, compounds in which the 2'-hydroxy groups are *cis* to the purine ring. In α-adenosine the orientation of the purine ring differs from that in adenosine with respect to the 3'- and 4'- as well as the 2'-substituents, and hence any one or all of these three changes could be responsible for the loss of activity. However, β-D-arabinosyl-8-azaadenine differs from 8-azaadenosine only in that the 2'-hydroxy group is *cis*, rather than *trans*, to the purine ring. It might be argued that the arabinosyl moiety as a whole is responsible for the lack of activity. However, α-D-arabinosyl-8-azaadenine is a substrate and its purine moiety is *trans* to the 2'-hydroxy group, whereas the orientations of the 3'-hydroxy and the 4'-hydroxymethyl groups are opposite to those in 8-azaadenosine.

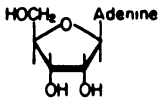
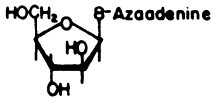
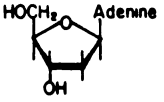
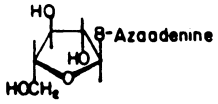
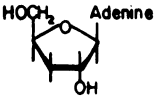
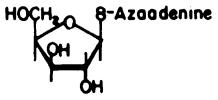
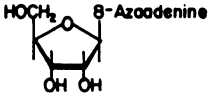
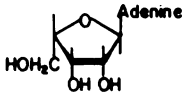
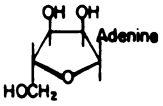
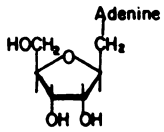
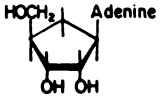
3. Neither the presence nor orientation of the 3'-hydroxy group is critical for substrate activity. Thus 3'-deoxyadenosine (cordycepin) is a substrate. Furthermore, provided that the purine moiety and the 2'-hydroxy group are in the *trans* configuration, substrate activity is evident both when the 3'-hydroxy group, with respect to the purine moiety, is *cis*, as in α-D-arabinosyl-8-azaadenine and β-D-xylosyl-8-azaadenine, and when it is *trans* as in adenosine and in α-L-lyxosyladenine.

4. The orientation of the 4'-hydroxymethyl group is not critical. Provided that the purine moiety and the 2'-hydroxy group are *trans* to one another, substrate activity is present both when the 4'-hydroxymethyl group is *cis* to the purine ring, as

TABLE 1

Activities of adenosine, 8-azaadenosine, and some related compounds as substrates for adenosine kinase

The adenosine kinase was purified either 82- or 430-fold from H.Ep.2 cells (4). Adenosine was used as a standard with each preparation and in each experiment, and the activities of the other substrates were calculated relative to that of adenosine. All assays were performed in duplicate. The rates of phosphorylation of adenosine were 69 nmoles/min/mg for the 82-fold purified preparation of kinase and 246 nmoles/min/mg for the 430-fold purified preparation. The standard reaction mixture contained, in addition to the enzyme, substrate, 1 mM; ATP, 2.5 mM; $MgCl_2$, 0.25 mM; and potassium phosphate buffer, pH 7.0, 50 mM. Incubation was carried out for 30 min at 25°, after which the reaction was stopped by immersion in boiling water and the preparation was subjected to paper chromatography in a solvent consisting of equal volumes of 93.8% 1-butanol and 44% aqueous propionic acid. Radioassays of the chromatograms were performed with a Packard chromatogram scanner. For experiments with adenosine, 2'-deoxyadenosine, and α-arabinosyl-8-azaadenine, ^{14}C-labeled substrates were used; for the other substrates, [γ-^{32}P]ATP was added to the reaction mixture. These methods have been described in detail elsewhere (4).

Compound	Relative Rates of Phosphorylation	Compound	Relative Rates of Phosphorylation
 Adenosine	100	 9-β-D-Arabinosyl-8-azaadenine	Not detectable (<0.2)
 2'-Deoxyadenosine	Not detectable (<2)	 9-α-D-Arabinosyl-8-azaadenine	16
 3'-Deoxyadenosine (Cordycepin)*	12	 9-β-D-Xylosyl-8-azaadenine	34
 8-Azaadenosine *	190	 9-α-L-Lyxosyladenine	39
 α-Adenosine	Not detectable (< 3)	 Homoadenosine	Not detectable (<3)
 Carbocyclic Analog of Adenosine *	42		

* Data for these compounds are from earlier publications (4, 7).

in adenosine and β-D-xylosyl-8-azaadenine, and when it is *trans*, as in α-D-arabinosyl-8-azaadenine and α-L-lyxosyladenine.

5. The orientation of the 2′-hydroxy group with respect to the 3′- and 4′-groups would appear to be unimportant; however, it should again be noted that not all of the possible isomers were available for evaluation. Thus substrate activity is evident when the 2′-, 3′-, and 4′-substituents are in all possible positions relative to one another, namely: all on the same side of the ring, as in α-L-lyxosyladenine; 2′-group *trans* to the 3′- and 4′-groups, as in β-D-xylosyl-8-azaadenine; 3′-group *trans* to the 2′- and 4′-groups, as in α-D-arabinosyl-8-azaadenine; and 4′-group *trans* to the 2′- and 3′-groups, as in adenosine.

6. The oxygen atom of the pentofuranosyl ring is not essential, as is evident from the activity of the carbocyclic analogue of adenosine.

Although these data show that the *trans* configuration of the 2′-hydroxy group and the purine ring is a necessary condition for effective activity as a substrate, it is not a sufficient condition, since certain alterations in the purine moiety result in compounds that are devoid of activity (4, 5). The necessity for the *trans* configuration of these moieties indicates that they probably are bound to the enzyme and that the conformation of the nucleoside required for binding to the enzyme can be attained only if these groups are *trans* to one another. Additional evidence for the importance of the spatial relationship between the 2′-hydroxy group and the purine ring is the inactivity of homoadenosine as a substrate. This compound has the groups on the pentofuranosyl ring unaltered in their orientations from those in adenosine, and differs from adenosine only in that a methylene group is inserted between the purine and pentofuranosyl rings. This compound obviously is not an *N*-glycoside, but this fact alone is not responsible for its inactivity, as is evident from the activity of the carbocyclic analogue of adenosine, which also contains no glycosidic bond.

Since "natural" nucleosides are β-D-pentofuranosides, it is the substrate activity of compounds with "unnatural" configurations, such as 9-α-D-arabinosyl-8-azaadenine, that perhaps provides the best evidence that the spatial relationship between the 2′-hydroxy group and the purine ring is a critical factor for substrate activity. The spatial relationships involved cannot be appreciated in the conventionally drawn structures of Table 1, but are apparent in the space-filling models shown in Fig. 1. The figure shows models for adenosine (A) and 9-α-D-arabinosyl-8-azaadenine (B), and for two nucleosides inactive as substrates, α-adenosine (C) and 9-β-D-arabinosyl-8-azaadenine (D). In the models for adenosine and α-arabinosyl-8-azaadenine, the 3-nitrogen atom has been placed as close to the 2′-carbon atom as possible; when this is done and the purine (or 8-azapurine) rings of the two compounds are made parallel, the molecules look remarkably similar with respect to the relative positions of the three groups that presumably are the most critical for substrate activity: the 2′-hydroxy group and purine moiety, which are suspected to bind to the enzyme, and the 5′-hydroxy group, at which the enzymatically catalyzed reaction occurs. The spatial relationship shown is selected arbitrarily for the purpose of illustration; in both compounds there is considerable freedom of rotation around the bond from C-1′ to N-9. Rotation about this bond is primarily responsible for defining the conformational state of the nucleoside (11). Nucleosides with the 2′-hydroxy group *trans* to the purine ring (and without other groups that might impede rotation; see discussion of psicofuranine below) have freedom of rotation about this bond similar to that for adenosine and can therefore assume the same conformations as adenosine. On the other hand, nucleosides with the 2′-hydroxy group *trans* to the purine ring have much less freedom of rotation about this bond and apparently cannot attain the conformation required for effective binding to the enzyme. These requirements for substrate activity can be illustrated further by considering two other nucleosides that showed no substrate activity: 2′-deoxyadenosine (Table 1) and psicofuranine (re-

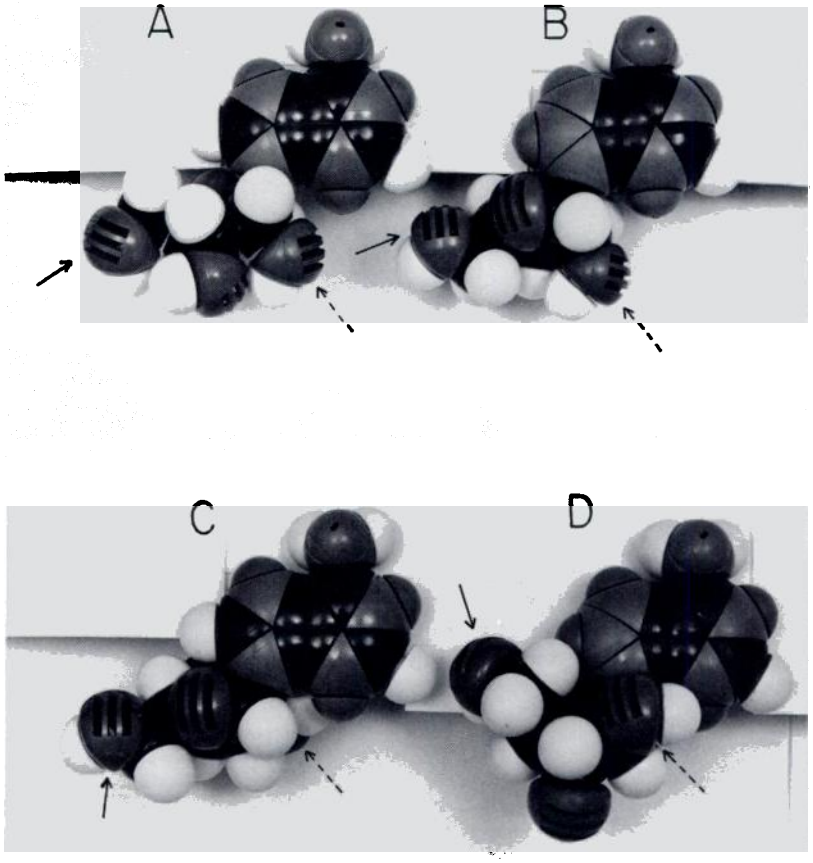


FIG. 1. *Corey-Pauling-Koltun models for adenosine (A), 9-α-D-arabinosyl-8-azaadenine (B), α-adenosine (C), and 9-β-D-arabinosyl-8-azaadenine (D)*

The solid arrows point to the 5′-hydroxy groups, and the broken arrows, to the 2′-hydroxy groups. Note that in the two α-nucleosides the oxygen atom of the pentofuranosyl ring is at the front and visible, whereas in the β-nucleosides it is to the rear and invisible. Note also, in C and D, the hindrance of rotation of the purine ring produced by the 2′-hydroxy groups.

sults not shown), which was also not a substrate for adenosine kinase from rabbit liver (5). 2′-Deoxyadenosine has as much freedom of rotation at the C-1′—N-9 bond as does adenosine; it lacks activity as a substrate presumably because it is not bound to the enzyme as a result of absence of the required 2′-hydroxy group. Psicofuranine has the constituents on the pentofuranosyl ring in the same configuration as in adenosine; its lack of activity probably is due to the fact that the severe restriction of rotation produced by the 1′-hydroxymethyl group prevents the formation of the conformational state required for binding to the enzyme.

Some other points deserve comment. The first concerns the importance to substrate activity of the 3′- and 4′-substituents. The finding that β-D-xylosyl-8-azaadenine and α-AL-lyxosyladenine both were substrates shows that it is not essential

that the 3'- and 4'-substituents have the same orientations as in adenosine. However, the observation that both these nucleosides were poorer substrates than adenosine suggests that the orientation of these groups may modify substrate activity. From the limited data available, it would appear that any alteration in the sugar moiety results in a compound that is a poorer substrate than adenosine. A second point relates to the observed inactivity of 2'-deoxyadenosine and β-D-arabinosyl-8-azaadenine. Lindberg *et al.* (5) found both 2'-deoxyadenosine and β-D-arabinosyladenine to be substrates, but rather poor ones, for adenosine kinase from rabbit liver. We found 2'-deoxyadenosine to be phosphorylated by a kinase preparation (of low degree of purification) from H.Ep.2 cells (4), but when this compound was assayed with the more highly purified enzyme, such as that used in the present study, no substrate activity could be detected. Since deoxyadenosine kinase is an enzyme distinct from adenosine kinase (12), it is likely that these reported activities of adenosine kinase preparations for 2'-deoxyadenosine and β-D-arabinosyladenine result from contamination with small amounts of deoxyadenosine kinase activity. However, it should be emphasized that our results were obtained with adenosine kinase from H.Ep.2 cells and that the enzymes from other sources might differ in their substrate preferences.

Overall, these results demonstrate that the 2'-hydroxy group and the purine moiety (or some particular portion thereof) are involved critically in the binding of nucleosides to adenosine kinase, and that the conformation required for effective binding can be achieved only when these groups are in the *trans* configuration and when there is considerable freedom of rotation around the C-1'—N-9 bond. In addition to contributing to an understanding of the mode of binding of substrates to adenosine kinase, these findings should aid in the design of new nucleoside analogues as growth-inhibitory or antitumor agents.

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REFERENCES

1. Bloch, A. (1974) in *Drug Design* (Ariëns, E. J., ed.), Vol. 4, pp. 286–378, Academic Press, New York.
2. Suhadolnik, R. J. (1970) *Nucleoside Antibiotics*, Wiley-Interscience, New York.
3. Anderson, E. P. (1973) in *The Enzymes* (Boyer, P., ed.), Vol. 9, pp. 46–96, Academic Press, New York.
4. Schnebli, H. P., Hill, D. L. & Bennett, L. L., Jr. (1967) *J. Biol. Chem.*, 242, 1997–2004.
5. Lindberg, B., Klenow, H. & Hansen, K. (1967) *J. Biol. Chem.*, 242, 350–356.
6. Divekar, A. Y. & Hakala, M. T. (1971) *Mol. Pharmacol.*, 7, 663–673.
7. Bennett, L. L., Jr., Allan, P. W. & Hill, D. L. (1968) *Mol. Pharmacol.*, 4, 208–217.
8. Montgomery, J. A. & Thomas, H. J. (1972) *J. Med. Chem.*, 15, 305–307.
9. Montgomery, J. A. & Hewson, K. (1970) *J. Heterocycl. Chem.*, 7, 443–445.
10. Shealy, Y. F. & Clayton, J. D. (1966) *J. Am. Chem. Soc.*, 88, 3885–3887.
11. Ts'o, P. O. P. (1974) in *Basic Principles in Nucleic Acid Chemistry* (Ts'o, P. O. P., ed.), Vol. 1, pp. 453–584, Academic Press, New York.
12. Krygier, V. & Momparler, R. L. (1971) *J. Biol. Chem.*, 246, 2745–2751.