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Metabolic Studies with an α-Nucleoside, 9-α-D-Arabinofuranosyl-8-azaadenine

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SUMMARY

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α-Arabinosyl-8-azaadenine (α-ara-8-azaA) and α-arabinosyladenine (α-araA) were toxic to H.Ep.2 cells in culture, whereas the corresponding β anomers were not toxic at the highest concentrations (375 μM) assayed. α-Ara-8-azaA was more cytotoxic than α-araA and therefore was selected for more detailed study. Cell lines deficient in adenosine kinase (EC 2.7.1.20) were resistant to inhibition by α-ara-8-azaA. Cultured H.Ep.2 cells grown in the presence of [2-^{14}C]α-ara-8-azaA contained compounds migrating on paper chromatograms like mono-, and di-, and triphosphates. Characterization of these compounds by paper chromatography and by high-pressure liquid chromatography showed them to be phosphates of α-ara-8-azaA; there were no detectable amounts of ribonucleotides and thus, presumably, no cleavage of α-ara-8-azaA to 8-azaA. There was also no detectable radioactivity in polynucleotides isolated by extraction with hot NaCl solution. α-Ara-8-azaA was a substrate for adenosine kinase partially purified from H.Ep.2 cells; the K_m was 110 μM and the V_{max} was about 15% of that of adenosine. α-Ara-8-azaA was not a substrate for adenosine deaminase; several other α-nucleosides assayed also had little or no activity as substrates for this enzyme. These results, which show that an analogue of adenosine in the "unnatural" α configuration has biological activity and differs markedly from the β anomer in biological activity and in activity as a substrate for the principal enzymes acting on adenosine, are of importance for understanding the modes of action of adenosine analogues and should also find application in the design of new nucleoside analogues with biological activity. In the course of characterizing the metabolites, a rapid and convenient method was developed for the separation, by high-pressure liquid chromatography, of the α and β anomers of ara-8-azaA; this method should also be applicable to the separation of anomers of other nucleosides.

INTRODUCTION

Although a few α-nucleosides or nucleotides have been found to occur naturally [for references, see Séquin (1)], all nucleotides found in nucleic acids and essentially all nucleosides and nucleotides occurring elsewhere are in the β configuration. For this reason α anomers have been assumed by most investigators to be biologically inert, and few have been examined for biological activity. Although growth-inhibitory activity has been reported for some α-nucleosides of adenine and adenine analogues (2, 3), the only α-nucleoside that

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has been studied extensively as a biologically active agent is α-thiodeoxyguanosine, which is phosphorylated and incorporated into DNA by terminal addition (4–6). During examination of a variety of adenosine analogues as inhibitors of growth of mammalian cells, we noted that α-araA[1] and α-ara-8-azaA were considerably more toxic than the corresponding β anomers. Since most nucleosides are toxic only after their conversion to phosphates, the cytotoxicity of these nucleosides indicated that they probably were phosphorylated intracellularly. This indication of metabolism of these "unnatural" nucleosides led to further study of α-ara-8-azaA. A preliminary report of some of these observations has been presented (7).

MATERIALS AND METHODS

Materials. Crude snake venom *(Crotalus atrox)* was obtained from Ross Allen's Reptile Institute, Silver Springs, Fla.; calf intestinal adenosine deaminase, from Sigma Chemical Company; and α-adenosine [9-α-D-ribofuranosyladenine] and 9-α-L-lyxofuranosyladenine, from Terra Marine Bioresearch, La Jolla, Cal. α-AraA and the nucleosides of 8-azaA listed in Table 4 were synthesized in our laboratories (8). [2-^{14}C]α-Ara-8-azaA (specific activity, 1.87 mCi/mmole) was synthesized, by procedures described elsewhere for the unlabeled compound (8), from [2-^{14}C]8-azaA, which was in turn prepared by ring closure of 5-amino-4-carboxamidino-*v*-triazole with H^{14} COOH (as formic acetic anhydride).

Cell cultures. The cell cultures used were the human epidermoid carcinoma line, H.Ep.2, originally established in culture by Moore *et al.* (9), and two sublines selected for resistance to certain purine analogues and deficiency in various enzymes of purine metabolism. These cell lines and their enzyme deficiencies were: H.Ep.2/MeMPR, resistant to 6-MeMPR and deficient in adenosine kinase; and H.Ep.2/FA/FAR, resistant to 2-fluoroadenine and 2-fluoroadenosine and deficient in adenine phosphoribosyltransferase and adenosine kinase. We have described elsewhere the isolation and characterization of these cell lines and their utility in studying the metabolism of purine and nucleoside analogues (10). All cell lines were grown in SRI-14 medium (11), and the effects of inhibitors were determined by cloning procedures described in Table 1.

Metabolic studies. H.Ep.2 cells grown in suspension culture were used for study of the metabolism of [2-^{14}C]α-ara-8-azaA. To a logarithmically growing culture (2.5×10^8 cells/liter) [^{14}C]α-ara-8-azaA was added for a final concentration of 2 μg/ml (0.0138 μCi/ml). Either 4 or 24 hr thereafter, cells were harvested by centrifugation and washed twice with 0.9% NaCl, after which the cells were extracted with boiling 80% aqueous ethanol. The water-soluble portion of the ethanolic extract was subjected to two-dimensional chromatography on paper, first in phenol–H_2O (72%, v/v) and then in butanol–propionic acid–H_2O (solvent A, Table 2). Radioactive metabolites were located by radioautography, and the areas of paper containing ^{14}C were cut out. The radioactive compounds were either assayed for radioactivity or eluted with H_2O and used for further characterization. From the residue remaining after ethanol extraction, sodium nucleates were isolated by extraction with hot 10% NaCl and assayed for radioactivity. These methods are described in detail elsewhere (12).

High-pressure liquid chromatography. The anomeric purity of [2-^{14}C]α-ara-8-azaA and its metabolites was determined by high-pressure reverse-phase liquid chromatography with a Waters Associates model 201 apparatus and a μBondapakC_{18} column (particle size, 10 μm). Details of the separation of anomers are given in Fig. 1.

Enzyme assays. Adenosine kinase was partially purified from cultured H.Ep.2 cells and assayed by procedures described earlier (13). The resulting preparation was purified 82-fold over the crude supernatant. The reaction conditions are given in Fig. 3. The kinetic constants were deter-

[1] The abbreviations used are: α-araA, 9-α-D-arabinofuranosyladenine; α-ara-8-azaA, 9-α-D-arabinofuranosyl-8-azaadenine; β-araA, 9-β-D-arabinofuranosyladenine; β-ara-8-azaA, 9-β-D-arabinofuranosyl-8-azaadenine; 6-MeMPR, 6-methylthiopurine ribonucleoside.

mined by Lineweaver-Burk analysis at five different substrate concentrations, which included concentrations both above and below the K_m value. Nucleosides were assayed as substrates for adenosine deaminase by measurements of changes in optical density at or near the absorption maxima for the nucleoside; prior to the assay the optimum wavelength and the change in absorption at this wavelength produced by deamination were determined for each nucleoside (Table 4). K_m and V_{max} values were obtained by Lineweaver-Burk analysis at 15 different substrate concentrations, which included concentrations above and below the K_m value.

RESULTS

Inhibition of H.Ep.2 cell lines. For the H.Ep.2/S line, α-ara-8-azaA was 6 times more toxic than α-araA; neither of the β anomers was toxic at the highest concentrations (375 μM) assayed (Table 1). The two cell lines deficient in adenosine kinase activity were markedly resistant to inhibition by α-ara-8-azaA. As shown, this pattern of response was similar to that of another adenosine analogue, 6-MeMPR, and suggested that α-ara-8-azaA was a substrate for adenosine kinase.

TABLE 1

Inhibition of H.Ep.2 cells in culture by α-ara-8-azaA and some related compounds

Approximately 100 cells were placed in 4-ounce prescription bottles containing either 10 ml of SRI-14 medium (11) or 10 ml of this medium in which the inhibitor was present at the desired concentration. After incubation for 7–10 days at 37° the medium was decanted and the cells adhering to the glass were washed with 0.85% NaCl solution (pH 7.0), fixed with Bouin's fixative, and stained with Giemsa stain. The number of macroscopic colonies present was then counted. The ED_{50} is the concentration required for 50% reduction of the number of colonies. Cloning efficiencies of control cells ranged from 40% to 60%.

Compound	ED_{50}		
	H.Ep.2/S	H.Ep.2/ MeMPR	H.Ep.2/ FA/FAR
	μM	μM	μM
α-Ara-8-azaA	2.2	>375	>375
β-Ara-8-azaA	>375		
α-AraA	14		
β-AraA	>375		
6-MeMPR	1.0	>300	>300

Radiopurity of [2-^{14}C]α-ara-8-azaA. Purity of the ^{14}C-labeled compound was determined by chromatography on paper and by high-pressure liquid chromatography. Paper chromatography revealed the presence of a labeled impurity migrating like 8-azaA in two solvents (Table 2) and amounting to about 1% of the total ^{14}C in the preparation; this may represent a true impurity or it may be an artifact of the chromatographic procedure. It was desirable also to know the anomeric purity of this sample. The paper chromatographic procedures used do not separate the anomers from one another; this separation was achieved by high-pressure reverse-phase liquid chromatography. With this procedure each anomer has a characteristic retention time, and the retention times differ enough that the anomers can be separated completely. The procedure used was essentially that described in Fig. 1; this figure actually shows an analysis of the metabolites isolated from H.Ep.2 cells, but illustrates the separation of the α and β anomers that was achieved routinely. For assay of [^{14}C]α-ara-8-azaA, a sample of 6 μg in water was placed on the column and elution was accomplished as described in Fig. 1. The labeled compound was eluted as a single peak with the retention time of the α anomer.

Metabolism of [2-^{14}C]α-ara-8-azaA. An alcohol-soluble extract of H.Ep.2 cells grown in the presence of [2-^{14}C]α-ara-8-azaA contained four radioactive compounds (Fig. 2). The pattern produced on the paper chromatogram was similar to, but less complex than, previously observed patterns for ^{14}C-adenine, and shows radioactive compounds present at positions comparable to those for AMP, ADP, ATP, and adenosine. Only one compound was present in the nucleoside areas (upper left quadrant of the radioautogram); this compound had the R_F value of the parent compound and was not further characterized. No radioactivity was detected at the position to which NAD migrates. For characterization of the metabolites, the areas of

TABLE 2

Characterization of metabolites of α-ara-8-azaA

The metabolites characterized in this table were eluted from two-dimensional chromatograms such as that shown in Fig. 2. For conversion to nucleosides, phosphates were treated with snake venom as described in the text. Paper chromatographic separations were accomplished on Whatman No. 1 paper in solvents of the following compositions: A, equal volumes of 93.8% aqueous 1-butanol and 44% aqueous propionic acid; B, 5 N ammonium acetate, saturated sodium tetraborate, ethanol, and 0.25 M EDTA, pH 9.5 (20:80:220:1, v/v). Radioactive compounds were located by radioautography. The data for metabolites in the last column were obtained by high-pressure liquid chromatography of a mixture of synthetic α and β anomers to which had been added the [^{14}C]nucleosides resulting from dephosphorylation (by snake venom) of the nucleotides; see Fig. 1 and the text for details. Abbreviations: (w), very faint spot on radioautogram; HPLC, high-pressure liquid chromatography.

Known compound or metabolic product	R_F in		^{14}C in α-ara-8-azaA by HPLC analysis
	Solvent A	Solvent B	
			%
Known compounds			
[2-^{14}C]α-Ara-8-azaA	59, 71 (w)	86, 55 (w)	>99
8-Azaadenosine	59	62	
8-Azaadenine	71	58	
β-Ara-8-azaA	56	86	
β-AraAMP	19	31	
AMP	17	13	
ADP	9		
ATP	6		
Metabolites			
Nucleotide suspected to be:			
α-Ara-8-azaAMP	26		
α-Ara-8-azaADP	16		
α-Ara-8-azaATP	9		
Nucleoside derived from metabolite suspected to be:			
α-Ara-8-azaAMP	59	83	>99
α-Ara-8-azaADP (4-hr exposure)	58	82	>99
α-Ara-8-azaATP	58	82	
α-Ara-8-azaAMP	58	82	>99
α-Ara-8-azaADP (24-hr exposure)	59	83	>99
α-Ara-8-azaATP	57	82	

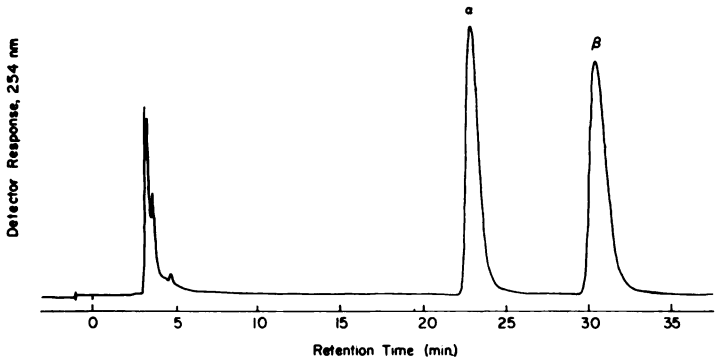

FIG. 1. *Determination of anomeric configuration of phosphates isolated from H.Ep.2 cells grown in the presence of [2-^{14}C]α-ara-8-azaA*

The conditions for growth of the cells and isolation of the phosphates are described in the text and in Table 3. The phosphates were treated with snake venom; the products were isolated by paper chromatography (solvent B, Table 2), eluted, and added to a mixture of 0.5 mg each of synthetic samples of nonradioactive α-ara-8-azaA and β-ara-8-azaA. This mixture of anomers was then subjected to high-pressure liquid chromatography; elution was accomplished with a water–acetonitrile solvent (98:2, v/v) and was monitored by measurement of optical density at 254 nm. The fractions under each peak were combined and assayed for ^{14}C. The tracing shown is for the nucleoside(s) derived from the monophosphate accumulating in the 24-hr experiment of Table 3 and is representative of the other assays. The material with a retention time of less than 5 min apparently was extraneous material eluted from the paper chromatograms, since it was not present when the α and β anomers alone were chromatographed.

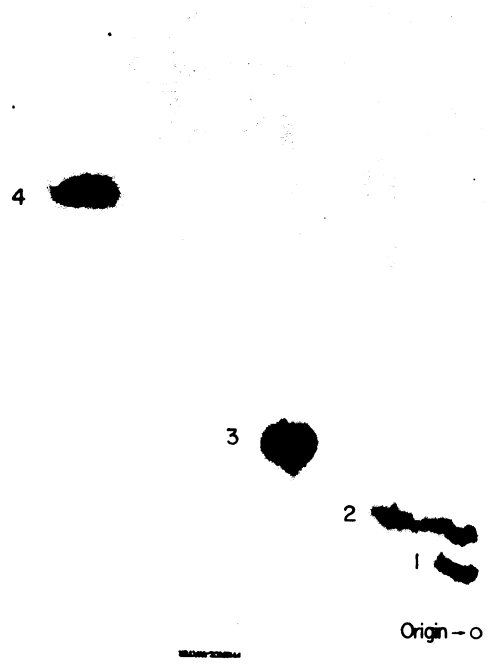

FIG. 2. *Metabolism of [2-^{14}C]α-ara-8-azaA by H.Ep.2 cells*

The figure is a reproduction of the radioautogram prepared from the chromatogram from which the data for the 4-hr experiment of Table 3 were obtained. See Table 3 and the text for details. The R_F values for the individual areas shown are as follows, where the first number is the R_F in the phenol-H_2O solvent and the second the R_F in the butanol-propionic acid solvent: area *1*, 7, 8; area *2*, 16, 15; area *3*, 36, 27; area *4*, 77, 58. The corresponding values for adenosine and its phosphates are: ATP, 8, 3; ADP, 18, 5; AMP, 38, 15; adenosine, 84, 49. On the basis of the similarity of their R_F values to those of adenosine and its phosphates, the identities of the four compounds shown were tentatively established as: *1*, α-ara-8-azaA triphosphate; *2*, α-ara-8-azaA diphosphate; *3*, α-ara-8-azaA monophosphate; *4*, α-ara-8-azaA. See Table 2 for further characterization of these compounds. The smearing of radioactivity in area *2* is commonly observed in chromatography of nucleoside polyphosphates in these solvents and may result from some hydrolysis of polyphosphates during chromatography.

paper containing the suspected phosphates were eluted, and the preparations were treated overnight at 37° with 0.3 ml of an aqueous solution of snake venom (2 mg/ml) to remove terminal phosphates. This treatment changed the migration of the suspected nucleotides to that expected for nucleosides (adenosine, 8-azaadenosine, α-ara-8-azaA, or β-ara-8-azaA) in the butanol–propionic acid solvent (Table 2). In this solvent almost all of the ^{14}C was found in the nucleoside area; there was a small amount of ^{14}C migrating to the R_F of 8-azaadenine, and only traces of ^{14}C at other positions. Chromatography in a solvent containing borate was used to distinguish arabinosyl compounds from ribosyl compounds. The nucleosides (resulting from treatment of the nucleotides with snake venom) moved in this solvent like known arabinosyl compounds (Table 2); no detectable amounts of ^{14}C were found at the positions of the ribonucleosides. For determination of the anomeric configuration of the metabolites, the phosphates were treated with snake venom as described above and the resulting nucleosides were subjected to paper chromatography in the borate solvent (Table 2, solvent B). The radioactive areas on these chromatograms were eluted, the eluates were lyophilized, and the residues were added to 0.2 ml of an aqueous solution containing 0.5 mg each of nonradioactive α-ara-8-azaA and β-ara-8-azaA. Up to 100 μl of these solutions were applied to the column, and elution was accomplished as described in Fig. 1. The fractions under the peaks corresponding to the retention times of α-ara-8-azaA and β-ara-8-azaA were pooled separately and assayed for radioactivity. For all metabolites more than 99% of the ^{14}C present in the sample was eluted with the α anomer (Table 2). Thus the metabolites in H.Ep.2 cells were phosphates of α-ara-8-azaA; the procedures employed gave no evidence of conversion to phosphates of 8-azaadenosine or β-ara-8-azaA.

Table 3 shows quantitative data on the formation of metabolites of [^{14}C]α-ara-8-azaA. As was already noted in Fig. 2, most of the ^{14}C in the cells was present as the phosphates; only 5–7% was present as the parent nucleoside. Between 4 and 24 hr the amount of ^{14}C present as the monophosphate declined moderately and that in the polyphosphates increased accordingly.

Polynucleotides, isolated by extraction

TABLE 3

Metabolism of [2-^{14}C]α-ara-8-azaA by H.Ep.2 cells

[2-^{14}C]α-Ara-8-azaA was added to logarithmically growing H.Ep.2 cells at a concentration of 2 μg/ml (0.0138 μCi/ml). Cells were harvested 4 hr and 24 hr thereafter and extracted with hot 80% ethanol, after which the soluble fraction was analyzed by paper chromatography and radioautography (see the text for details). The metabolites shown here are from the same experiment from which the data of Fig. 2 were obtained.

Compound isolated	Radioactivity		Intracellular ^{14}C	
	4 hr	24 hr	4 hr	24 hr
	dpm/10^6 cells		*% total*	
α-Ara-8-azaA	754	468	7	5
α-Ara-8-azaAMP	8319	6421	81	63
α-Ara-8-azaA di- and triphosphates	1229	3265	12	32

with 10% NaCl solution from the same cells from which the soluble nucleotides were isolated, did not contain a detectable amount of ^{14}C; the specific activity of the isolated polynucleotides (DNA plus RNA) was less than 5.4 pCi/mg.

α-Ara-8-azaA as a substrate for adenosine kinase. Figure 3 shows a Lineweaver-Burk plot for the phosphorylation of [2-^{14}C]α-ara-8-azaA by adenosine kinase 82-fold purified from H.Ep.2 cells. The calculated kinetic constants were: K_m, 110 μM, and V_{max}, 11.2 nmoles/min/mg; the corresponding values for the phosphorylation of adenosine by the same preparation were 1.8 μM and 69.3 nmoles/min/mg.

Studies with adenosine deaminase (Table 4). When α-ara-8-azaA was assayed as a substrate for adenosine deaminase, no deamination was detected, whereas deamination of the corresponding β anomer proceeded at about 17% of the rate of adenosine. Similarly, α-araA was not deaminated, but the β anomer was a substrate. To obtain additional information on the generality of the substrate preferences of the deaminase for α and β anomers, we assayed other nucleosides of 8-azapurines as well as two α-nucleosides of adenine. All the β anomers of adenine nucleosides were deaminated at easily detectable rates. α-L-Lyxosyladenine was the only one of the α anomers for which evidence of deamination was observed, and its rate of reaction was so low (0.2% of that of adenosine) that the apparent deamination may have resulted from an impurity undetected by other means.

DISCUSSION

Since the radioactive sample of α-ara-8-azaA was labeled in the 8-azapurine ring, and since it was possible that the arabinosyl group could be removed metabolically, it was necessary to determine rigorously that the metabolites were not phosphates of 8-azaadenosine. In addition it was desirable to show not only that the metabolites were arabinosyl derivatives, but that the α configuration had been retained. It was unlikely that the metabolites contained

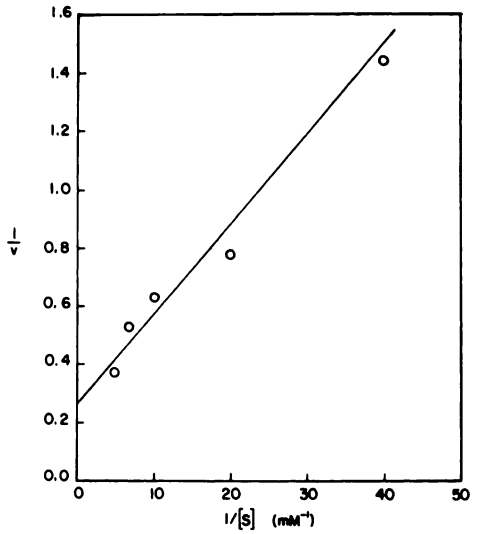

FIG. 3. *Lineweaver-Burk plot for determination of kinetic constants for phosphorylation of α-ara-8-azaA by adenosine kinase*

The incubation mixture contained, in a final volume of 0.2 ml, [2-^{14}C]α-ara-8-azaA at the indicated concentrations; enzyme 82-fold purified from H.Ep.2 cells; ATP, 2.5 mM; $MgCl_2$, 0.25 mM; and potassium phosphate buffer, 50 mM, pH 7.0. After incubation at 25° for 30 min, the reaction was stopped by immersion in a boiling water bath. The reaction mixture was subjected to chromatography on paper in solvent A (Table 2), after which the paper strips were assayed for ^{14}C in a Packard model 7201 radiochromatogram scanner. Values for the ordinates are the reciprocals of the reaction rate in nanomoles per minute.

TABLE 4

Activities of certain nucleosides of adenine and 8-azaadenine as substrates for adenosine deaminase

The rate of deamination was measured by the change in optical density at the wavelength given in the last column, which also shows the change in molar extinction resulting from the deamination. The reaction mixture contained, in a final volume of 3.0 ml, the substrate, calf intestinal adenosine deaminase (220 units/mg of protein), and potassium phosphate buffer, pH 7.5 (50 mM).

Nucleoside	K_m	V_{max}	$\Delta E/\lambda$
	μM	*μmoles/min/mg*	
Adenosine	29	435	7600/265 nm
9-α-D-Ribofuranosyladenine[a]	ND[b]		
9-β-D-Arabinofuranosyladenine[a]	120	94	7100/265 nm
9-α-D-Arabinofuranosyladenine	ND[b]		
8-Azaadenosine[a]	250	1500	5900/280 nm
9-β-D-Arabinofuranosyl-8-azaadenine	430	75	4800/280 nm
9-α-D-Arabinofuranosyl-8-azaadenine	ND[b]		
9-(2-Deoxy-β-D-ribofuranosyl)-8-azaadenine[a]	220	2000	6700/280 nm
9-(2-Deoxy-α-D-ribofuranosyl)-8-azaadenine[a]	ND[b]		
9-β-D-Xylofuranosyl-8-azaadenine	670	42	4800/280 nm
9-α-L-Lyxofuranosyladenine	400	0.9	3900/280 nm

[a] These compounds were evaluated by others as substrates for adenosine deaminase from the indicated sources: 9-α-D-ribofuranosyladenine, Taka-diastase (14); 9-β-D-arabinofuranosyladenine, human erythrocytes (15) and calf intestine (16, 17); 8-azaadenosine, Taka-diastase (14), human erythrocytes (15), and calf intestine (18); 9-(2-deoxy-β-D-ribofuranosyl)-8-azaadenine, calf intestine (19); 9-(2-deoxy-α-D-ribofuranosyl)-8-azaadenine; calf intestine (20).

[b] ND, reaction not detectable.

any derivatives of the β anomer, because there is no known enzymatic reaction for inversion at C-1′ of a nucleoside; nevertheless, it was desirable to establish this point unequivocally. The results (Table 2 and Fig. 1) show clearly that the metabolites were derivatives of α-ara-8-azaA. Thus metabolism to the phosphates occurs without cleavage of the glycosidic linkage and without alteration of configuration. The formation of the monophosphate is catalyzed by adenosine kinase, as is shown by the results of Fig. 3. The fact that anabolism proceeds to the triphosphate level in H.Ep.2 cells indicates that α-ara-8-azaAMP and α-ara-8-azaADP are substrates for nucleotide kinase(s) of these cells. No incorporation into polynucleotides was found. Internal incorporation into DNA would not be expected because, as pointed out by Séquin (1), incorporation of α-nucleotide units into a β-nucleotide chain would interfere with base pairing and base stacking. End addition of an α-nucleotide into DNA or internal incorporation into RNA is conceivable, but if either did in fact occur in these experiments it was not sufficiently great to be detected.

A point of some interest is that adenosine kinase may accept an α-nucleoside as a substrate, whereas adenosine deaminase apparently discriminates against α-nucleosides. With regard to the kinase, it should be noted that different enzymes apparently are responsible for the phosphorylation of the α and β anomers. The α anomer is clearly a substrate for adenosine kinase (Fig. 3), but we have been unable to detect phosphorylation of β-ara-8-azaA or β-araA by this enzyme. Although β-araA is phosphorylated in mammalian cells (21), the enzyme responsible has not been positively identified. Since β-arabinosylpurines and pyrimidines may be regarded as analogues of 2′-deoxynucleosides, as is evident, for example, by the activity of 1-β-D-arabinofuranosylcytosine as a substrate for deoxycytidine kinase (22), the enzyme responsible for the phosphorylation of β-araA (and presumably also of β-ara-8-azaA) would be expected to be deoxyadenosine kinase, an enzyme distinct from adenosine kinase (23). However, it has been reported recently that β-araA was not a substrate for deoxyadenosine kinase isolated from calf thymus (21).

With respect to the relative inactivity of α-nucleosides as substrates for adenosine deaminase, it should be noted that this conclusion is based on a relatively few examples. In addition to the compounds of the present study, the following α-furanosyl derivatives of adenine have been reported to have very low activity, or no activity, as substrates for the deaminase: 9-(2-deoxy-α-D-ribofuranosyl)adenine (19, 24), 9-(2-deoxy-α-L-ribofuranosyl)adenine (14, 25), 9-α-L-threofuranosyladenine (26), 9-α-L-erythrofuranosyladenine (26), and 9-α-D-ribofuranosyl-2-azaadenine (27). As noted in Table 4, several of the nucleosides studied were evaluated by others with deaminases from several sources; they have been re-evaluated here so that results with all the substrates in the table would be directly comparable.

The differences in biological activities of the α and β anomers of ara-8-azaA probably are a reflection of their activities as substrates for nucleoside kinases and adenosine deaminase. Since both anomers are phosphorylated (see above), it is probably the failure of the α anomer to be deaminated that is responsible for its greater cytotoxicity. In accord with this view are observations on the effects of inhibitors of the deaminase in increasing the antitumor effects (28), the cytotoxicity (29), and the antiviral activity (30) of β-araA, and also in increasing its conversion to β-araATP (21).

These observations with α-ara-8-azaA demonstrate that an α-nucleoside of adenine may have greater biological activity than the corresponding β anomer and may be metabolized by some of the pathways utilized by adenosine. The results raise the question whether the α and β anomers of araA and ara-8-azaA, all of which have biological activity, inhibit by the same or by different mechanisms. β-AraA has antitumor activity (31, 32) and is the most potent agent known against DNA viruses (33, 34). α-AraA, α-ara-8-azaA, and β-ara-8-azaA have not yet been studied broadly, but each has activity *in vitro* against Herpes simplex virus about equal to that of β-araA (3, 35). β-AraA is converted to the triphosphate, which inhibits DNA polymerase (36–38) and ribonucleotide reductase (36, 39). α-Ara-8-azaA is also converted to the triphosphate, and may inhibit these enzymes. Nothing yet is known about the loci of action of α-araA and α-ara-8-azaA. If they should turn out to have the same mechanism as β-araA, they might prove to be more useful agents, since they are not subject to deamination, a factor limiting the usefulness of β-araA.

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