INHIBITION OF S-ADENOSYL-L-HOMOCYSTEINE HYDROLASE BY THE ALIPHATIC NUCLEOSIDE ANALOGUE — 9-(*S*)-(2,3-DIHYDROXYPROPYL)ADENINE

Ivan VOTRUBA and Antonín HOLÝ

Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6

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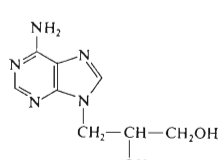
9-(*S*)-(2,3-Dihydroxypropyl)adenine, a nucleoside analogue with antiviral activity, inhibits the hydrolysis of *S*-adenosyl-L-homocysteine catalyzed by rat liver *S*-adenosyl-L-homocysteine hydrolase ($K_i = 3{\cdot}5$ μM; $K_i/K_m = 5{\cdot}6 \cdot 10^{-2}$). The effect upon the synthesis of the substrate is much less pronounced.

9-(*S*)-(2,3-Dihydroxypropyl)adenine ((*S*)-DHPA) exhibits antiviral activity against both DNA and RNA viruses[1]. The drug does not seem to affect significantly the synthesis of nucleic acids and proteins of the uninfected host cells even at high doses. Also, the structure-activity study[2] revealed that its antiviral effect is invariably lost by any change occurring either at the heterocyclic or at the aliphatic part of the molecule. This structural specificity together with the observation that the drug is hardly anabolized *in vivo* point to its effect upon enzyme(s) specific for adenine derivatives. Adenosine aminohydrolases are inhibited by (*S*)-DHPA and its racemate[1,3]; however, this inhibition is not very effective.

Another system highly specific for adenosine anabolism is S-adenosyl-L-homocysteine hydrolase (SAH-hydrolase, EC 3.3.1.1) which exhibits the regulatory role in *S*-adenosyl-L-methionine-dependent transmethylations[4–7]. Recently, it was observed that the viral mRNA methylation is inhibited by SAH and its analogues[8–13]. In the present paper we report on the inhibitory activity of (*S*)-DHPA upon the reactions catalyzed by the SAH-hydrolase isolated from rat liver.



(*S*)-DHPA

EXPERIMENTAL

Materials. Female Wistar A rats at the age of 3 months were used. The livers were removed from the rats directly after decapitation. (*S*)-9-(2,3-Dihydroxypropyl) derivatives of adenine[14], hypoxanthine[14] and xanthine[15] were prepared by reported procedures. [Adenine-U-^{14}C]-adenosine (spec. activity 5 mC$_i$/mmol, 185 MBq/mmol) was purchased from the Institute for Research, Production and Application of Radioisotopes, Prague, Czechoslovakia. L-Homocysteine stock solution was prepared by 40 h incubation (20°C) of a solution containing 10 mg of L-homocystine and 100 μl 2-mercaptoethanol in 5 ml water, pH being adjusted by conc. aqueous ammonia to 8·8. *S*-Adenosyl-L-homocysteine was prepared according to[16] and also purchased from Calbiochem-Behring (Los Angeles, U.S.A.). [^{14}C]-*S*-Adenosyl-L-homocysteine was prepared enzymatically under the conditions described below. Adenosine aminohydrolase (EC 3.5.4.4) from calf intestine was purchased from Boehringer (Mannheim, F.G.R.). Dithiothreitol was a product of Koch-Light Laboratories, England.

Enzyme preparation. SAH-Hydrolase from rat liver was purified according to the method of de la Haba and Cantoni[17] up to the point of ammonium sulphate precipitation (0—80% saturation). The precipitate was gel filtrated through a Sephadex G—25 (coarse) column (3 × 50 cm) in 0·01M potassium phosphate buffer, pH 7·6. The enzyme fraction was then purified by DEAE cellulose (Cellex D) column (2·2 × 5 cm) chromatography with potassium chloride concentration gradient (0—0·4M) in the same buffer. SAH-Hydrolase activity was eluted at 0·1M concentration. The enzyme preparation did not degrade adenosine; its specific activity was 0·27 I.U./mg protein.

Enzyme assays. The synthetic activity of SAH-hydrolase was determined in the incubation mixture (total volume, 0·25 ml) consisting of 80 mM potassium phosphate, pH 7·37, 2·4 mM dithiothreitol, 3 mM L-homocysteine, 80—400 μM [^{14}C]-adenosine, 40 μg/ml enzyme protein, and 50—500 μM (S)-DHPA. The hydrolytic activity was estimated in the assay mixture (total volume, 0·25 ml) consisting of 60 mM potassium phosphate, pH 7·37, 0·1 mM-EDTA, 10—80 μM [^{14}C]-*S*-adenosyl-L-homocysteine, 5—40 μg/ml enzyme protein, and 1·6 I.U./ml adenosine aminohydrolase. The reactions were started by the addition of the enzyme and the mixtures incubated at 37°C for 5 min. The aliquots (50 μl) were spotted on paper Whatman No 3 and chromatographed in the solvent system 2-propanol–conc. aqueous ammonia–water (7 : 1 : 2). The spots corresponding to *S*-adenosyl-L-homocysteine, adenosine and inosine markers were cut out and the radioactivity determined by liquid scintillation after the addition of toluene based scintillation mixture.

RESULTS AND DISCUSSION

The effect of (*S*)-DHPA upon the SAH-hydrolysis: Since adenosine is known to counteract the action of SAH-hydrolase[16], it is advisable to perform the assays of the SAH-hydrolase catalyzed hydrolytic reaction in the presence of adenosine aminohydrolase in order to transform adenosine formed by the reaction to inosine. In the above assay mixture containing the enzyme in excess, (*S*)-DHPA does not prevent the deamination of adenosine (the rate of adenosine deamination at $2 \cdot 10^{-4}$M adenosine is not markedly affected by the presence of $2 \cdot 10^{-3}$M-(*S*)-DHPA). However, the hydrolysis of SAH ($5 \cdot 10^{-5}$M) in the presence of adenosine aminohydrolase is significantly diminished by $5 \cdot 10^{-4}$M-(*S*)-DHPA (Fig. 1). The kinetics of this hydrolytic reaction (Fig. 2, Table I) suggests a competitive character of (*S*)-DHPA

TABLE I

Kinetic Parameters for SAH-Hydrolase Catalyzed Reactions in the Presence of (*S*)-DHPA

Values	Hydrolysis	Synthesis
K_m	$6{\cdot}25 \cdot 10^{-5}$ M (SAH)	$2{\cdot}7 \cdot 10^{-5}$ M (Ado)
K_i	$3{\cdot}5 \cdot 10^{-6}$ M	—
K_i/K_m	$5{\cdot}6 \cdot 10^{-2}$	—
$\left(\frac{\text{Substrate}}{(S)\text{-DHPA}}\right)_{50}^{a}$	$3{\cdot}2$ ($2 \cdot 10^{-5}$ M SAH)[b] $4{\cdot}0$ ($4 \cdot 10^{-5}$ M SAH)[b]	$0{\cdot}50$ ($2 \cdot 10^{-4}$ M Ado)[b] $0{\cdot}53$ ($4 \cdot 10^{-4}$ M Ado)[b]

[a] $\left(\frac{\text{Substrate}}{(S)\text{-DHPA}}\right)_{50}$ represents substrate/inhibitor ratio resulting in 50% inhibition of the reaction; [b] in parentheses, actual substrate concentrations.

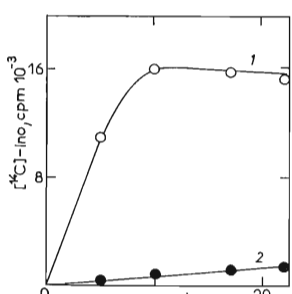


FIG. 1

Effect of (*S*)-DHPA on the Time-Course of SAH Hydrolysis in the Presence of Adenosine Aminohydrolase

1 $5 \cdot 10^{-5}$M-SAH, *2* $5 \cdot 10^{-5}$M-SAH and $5 \cdot 10^{-4}$M-(*S*)-DHPA.

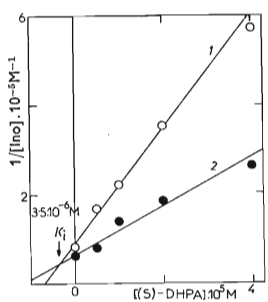


FIG. 2

Inhibition of the Hydrolysis of SAH by (*S*)-DHPA

1 $2 \cdot 10^{-5}$M-SAH, *2* $4 \cdot 10^{-5}$M-SAH. $K_i = 3{\cdot}5 \cdot 10^{-6}$M for (*S*)-DHPA.

inhibition. The K_i/K_m value ($5{\cdot}6 \cdot 10^{-2}$) reflects a comparatively high affinity of (*S*)-DHPA for the rat liver SAH-hydrolase.

A recent study demonstrated that in mice a small amount of (*S*)-DHPA is transformed in liver to (*S*)-9-(2,3-dihydroxypropyl) derivatives of hypoxanthine and xanthine[15]. None of these compounds examined in the above assay system revealed any inhibitory activity upon the SAH hydrolysis. Similarly, (*S*)-DHPA 3'-phosphate[14] which may be hypothetically formed by an *in vivo* phosphorylation of (*S*)-DHPA does not affect the SAH-hydrolase under the conditions used.

The effect of (*S*)-DHPA upon the synthesis of *S*-adenosyl-L-homocysteine. The course of (*S*)-DHPA inhibition of the SAH synthesis (Fig. 3) demonstrates that in this reaction the analogue exhibits much lower affinity for the enzyme than adenosine. In accordance with the previous reports[17-20] we have found that the synthesis of SAH from adenosine and L-homocysteine is strongly substrate-inhibited. Therefore, the data are presented as 50% inhibition of the synthesis in the presence of (*S*)-DHPA: For the concentration $2 \cdot 10^{-4}$M and $4 \cdot 10^{-4}$M of adenosine under the assay conditions, the Ado/(*S*)-DHPA ratio resulting in 50% inhibition of SAH synthesis amounts to 0·50–0·53 (Table I).

(*S*)-DHPA itself is not a substrate for SAH-hydrolase reaction with L-homocysteine. This has been demonstrated by a direct experiment with the above enzyme which shows that under the condtions of SAH synthesis [^{14}C]-labelled (*S*)-DHPA does not undergo any changes. Nevertheless, the compound is capable of inhibiting the synthesis of SAH from adenosine. This inhibition is much smaller than in the hydrolytic reaction; the different affinities of the same compound towards the same

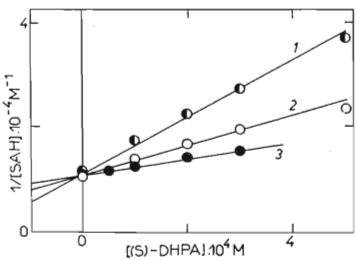


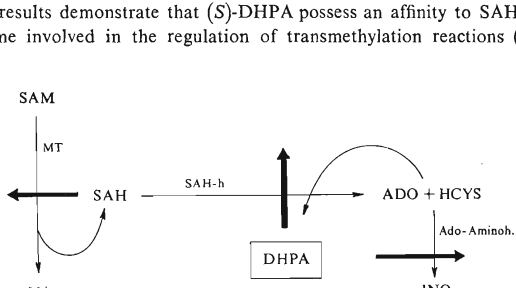
FIG. 3

Kinetics of SAH Synthesis in the Presence of (*S*)-DHPA

1 $1{\cdot}2 \cdot 10^{-4}$M Ado, *2* $2 \cdot 10^{-4}$M Ado, *3* $4 \cdot 10^{-4}$M Ado, (interception at $1{\cdot}2 \cdot 10^{-5}$M).

enzyme can be interpreted as follows: During the synthesis of SAH, (*S*)-DHPA can interfere with the enzyme by forming a complex without involvement of the catalytic site of the enzyme. Thus, (*S*)-DHPA would not differ in this respect from other adenine derivatives (except adenosine) which were also shown to exhibit mild inhibition of the synthesis. However, on the hydrolytic reaction, the analogue can occupy the position of adenosine in the enzyme–product complex, being in fact a product (adenosine) analogue. In this respect, (*S*)-DHPA differs from SIBA and other 5'-*S*-alkyl adenosine derivatives which are supposed to be SAH analogues[8,9].

These results demonstrate that (*S*)-DHPA possess an affinity to SAH-hydrolase, an enzyme involved in the regulation of transmethylation reactions (Scheme 1).



SCHEME 1

MT — Methyl Transferases
SAH-h — SAH-Hydrolase
Ado-Aminoh. — Ado-Aminohydrolases
NA — Nucleic Acids
P — Proteins
S — Steroids
C — Creatine

S-Adenosyl-L-homocysteine which is invariably formed during these reactions from *S*-adenosyl-L-methionine is a strong competitive inhibitor of methyl transferases[4–7]. Its accumulation *in vivo* by the inhibition of catabolic processes initiated by SAH-hydrolase(s) might easily be one of the reasons of the antiviral effect of (*S*)-DHPA. We suppose that the drug might exert a direct effect upon the SAH-hydrolase. This hypothesis is supported by the specific inhibition of virus growth or cellular transformation caused by viral infection, by SAH and its analogues[8–13] and, also, by a pronounced synergism of (*S*)-DHPA and adenine arabinoside[1] which is a powerful "suicide inhibitor" of SAH-hydrolase in human lymphoblasts[21,22]. Further investigation of interactions of (*S*)-DHPA with SAH-hydrolases is the subject of our present effort.

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