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THE MECHANISM OF INACTIVATION OF S-ADENOSYLHOMOCYSTEINASE BY 2'-DEOXYADENOSINE

by

R.H. Abeles, A.H. Tashjian, Jr.*, and S. Fish

from the

Graduate Department of Biochemistry

Brandeis University

Waltham, Mass. 02254

and

*Laboratory of Toxicology, Harvard School of Public Health

and

Department of Pharmacology, Harvard Medical School

Boston, Mass. 02115

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Summary

S-Adenosylhomocysteinase (SAHase) is irreversibly inactivated by 2'-deoxyadenosine (Hirshfield, M.S. (1979) J. Biol Chem. 254, 22-25). In the course of this inactivation, 2'dAd becomes tightly bound to the enzyme, i.e., cannot be removed by gel filtration or dialysis. Inactivation is accompanied by reduction of the enzyme bound NAD. When the inactivated enzyme is denatured, no 2'dAd is recovered. Adenine equivalent to about 80% of the bound 2'dAd is isolated. It is proposed that 2'-deoxyadenosine is first oxidized to 3'-keto-2-deoxyadenosine by enzyme bound NAD. The 3'keto group activates the hydrogen at C-2' and facilitates elimination of adenine.

Introduction

S-adenosylhomocysteinase (SAHase) is irreversibly inactivated by 2'-deoxyadenosine and ara-adenosine. It was also shown that 2'-deoxyadenosine is associated with a protein fraction which contains SAHase after Sephadex chromatography. It was, therefore, suggested that 2'-deoxyadenosine binds tightly to SAHase (1). The mechanism of action of SAHase (2) suggested to us the following mechanism for the inactivation of SAHase by 2'-deoxyadenosine:

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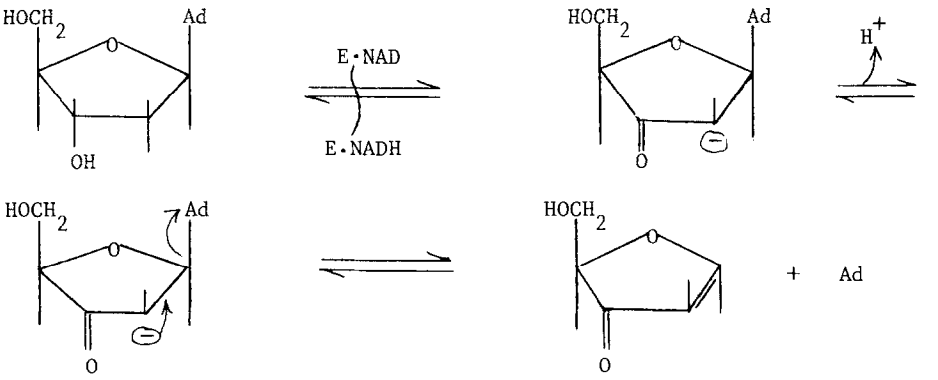

SAHase contains extremely tightly bound NAD. This NAD is utilized to oxidize the 3'position of the substrate, adenosine or SAH, during the normal catalytic process. The oxidation of the 3'position facilitates the elimination of homocysteine from SAH or the addition of homocysteine to adenosine. At the end of the catalytic cycle, the 3'position is reduced and NADH reoxidized to NAD (2). The mechanism of inactivation, shown above, involves the oxidation of the 3'position of deoxyadenosine by enzyme bound NAD. This oxidation would also occur in the normal catalytic process. Subsequent reactions differ from the normal cataltyic reaction. The presence of the keto group at C-3' activates the hydrogen at C-2' and elimination of adenine occurs. As a consequence of this elimination, the enzyme bound NADH cannot be reoxidized to NAD. This alone would lead to inactivation since NAD is required to initiate catalysis. In addition, it is also possible that the keto sugar product formed after elimination of adenine could react through a Michael-type addition with a nucleophile on the enzyme. The fact that a trans-elimination can occur with 2'-deoxyadenosine and ara-adenosine, but not with adenosine, probably accounts, in part, for the elimination of adenine from 2'-deoxyadenosine and ara-adenosine but not from adenosine. The instability of 3-keto-nucleotides is known (3). Also, an elimination reaction analogous to the one proposed here has been established for the inactivation of ribonucleotide reductase by 2'-halo-ADP (4). It has been reported that upon denaturation of an SAHase-adenosine complex, adenine and ribose are isolated. However, the identification of ribose was not convincing (5). We now report evidence in support of the proposed inactivation mechanism.

Results and Discussion

Tight Binding of 2'-Deoxyadenosine to SAHase. SAHase (1.1 I.U., sp. act. 0.66), 5 mM (2-^{3}H]-2'dAd (sp. act. 6.6 x 10^6 cpm/μmole), 1.25 mg EHNA[1], 1 mM K-EDTA , 1 mM DTT, 25 mM potassium phosphate buffer pH 7.0, were incubated in a total volume of 0.8 mL at 25°C. At 0, 10, 30 and 90 min, 2 μL aliquots were removed for enzyme activity assays and 200 μL aliquots were passed through an 0.8 x 26 cm Biogel P-6 column at 5°C. The column was eluted with 25 mM potassium phosphate buffer pH 7.0 containing 1 mM K-EDTA and 1 mM DTT. Radioactivity emerged with the large molecule fractions. At 90 min, the enzyme was essentially completely inactivated. At this point, radioactivity corresponding to 0.72 μmoles of 2'-deoxyadenosine was associated with one μmole of enzyme protein (subunit, m.w., 48,000 daltons). Since the enzyme had lost some activity subsequent to isolation (sp. act. decreased to 0.66 from 0.9), it can be concluded that one mole of 2'-deoxyadenosine binds per mole of enzyme. The data in Figure 1 show that the amount of radioactivity incorporated closely paralled the loss of enzyme activity. When the protein fraction isolated from the Biogel column was dialyzed against 25 mM potassium phosphate buffer for 24 hr, no radioactivity was lost from the protein.

Adenine Formation. Enzyme inactivated with 2'-deoxyadenosine and filtered through a Biogel column as described above was denatured by addition of $HClO_4$ (final concentration 0.33 M). The denatured protein was removed and the solution neutralized with KOH. Carrier adenine, 2-deoxyadenosine and inosine were added. The solution was then concentrated and after removal of salts, was examined by HPLC. No radioactive 2'-deoxyadenosine was found, but 77% of the radioactivity co-chromatographed with adenine, 4% with hypoxanthine, and 19% in an as yet unidentified fraction.

To determine whether adenine formation was the result of the acidic conditions used to denature the enzyme, an analogous experiment was carried out in

[1] EHNA - erythro-9-(2-hydroxy-3nonyl)adenine

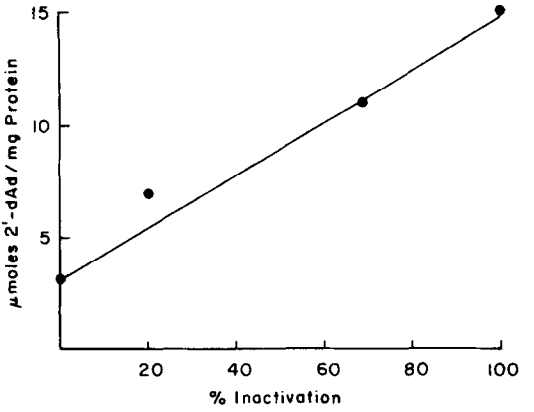

Figure 1. Inactivation fo SAHase and incorporation of [2-^{3}H]-2'-deoxyadenosine into SAHase. Experimental conditions are described in text.

which ethanol (final ethanol concentration 50%) was used to denature the enzyme. Again, no 2'-deoxyadenosine was found and the major radioactive product was adenine. Therefore, adenine was not formed through acid hydrolysis.

Formation of Enzyme-Bound NADH. 2'-Deoxyadenosine was added to SAHase and the spectrum was determined at several time intervals. The results and experimental conditions used are shown in Figure 2. A time-dependent spectral change occurred which corresponds to the formation of enzyme bound NADH (2). In the experiment presented in Figure 2, aliquots (5-10 µL) were removed periodically and assayed for enzyme activity. The data in Table 1 show that the loss of enzyme activity closely paralleled the spectral change, i.e., NADH formation.

The essential features of the inactivation mechanism which we have proposed are: formation of NADH concomitant with inactivation; and 2., formation of adenine from 2'-deoxyadenosine. These features have now been verified and thus, experimental support has been furnished for the proposed mechanism of inactivation. Obviously, further work needs to be done to define fully the mechanism. A particularly important question is the nature of the sugar moiety found after loss of adenine and whether the sugar becomes covalently bound to the enzyme. The possibility that hydrolysis of 3'-keto-2'-deoxyadenosine does not occur until after denaturation of the enzyme must also be considered. These and other aspects of the inactivation mechanism are now under investigation.

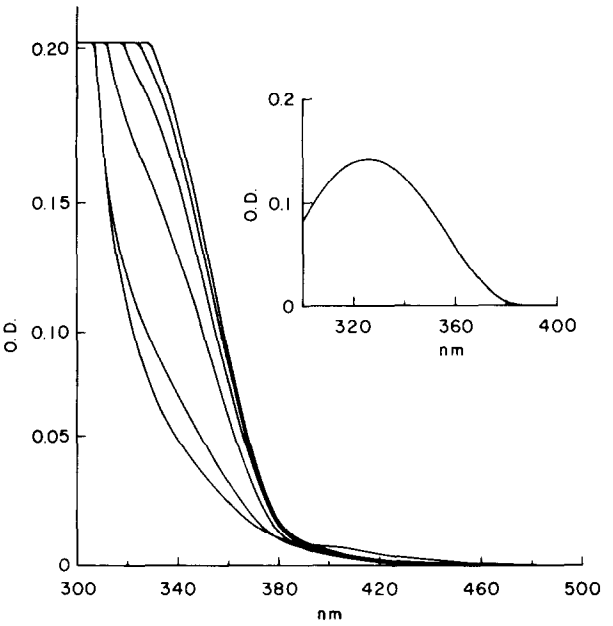

Figure 2. Spectral changes occurring during inactivation of SAHase by 2'-deoxyadenosine.

Enzyme, 1.3 I.U. sp. act. 0.68, potassium phosphate buffer 10 mM, pH 7.4, K-EDTA 1 mM, 2'-deoxyadenosine 4.6 mM. Total volume 1 mL, 25°C. Spectra taken before addition of 2'-deoxyadenosine and at 1, 7, 14, 21, min after addition of 2'-deoxyadenosine.

Insert: Difference spectrum of 32 min point. Reference cell contained all components except 2'-deoxyadenosine.

Methods

SAHase was purified from calf liver (6). The crystallization step was omitted. The enzyme was more than 95% pure as judged by SDS gel electrophoresis. The specific activity of the isolated enzyme was 0.9 I.U., but decreased somewhat on storage. Enzyme activity was assayed by the ^{3}H-release method or spectro-

Table 1
Spectral Changes and Inhibition of SAHase After Addition of 2'dAd

Time after addition 2-dAd	Inhibition %	ΔO.D. 330 nm *	% of total ΔO.D.
1	15	.018	14
7	58	.079	60
14	77	.109	83
21	90	.123	93
24	95	.132	100
32	98	—	—

*Spectral data taken from Figure 2

photometrically (2). Nucleotides were separated by HPLC with a Waters μBondapak C_{18} column 7.8 x 300 mm. The column was developed with 13% methanol in H_2O at 3 mL/min. Elution time: 2'-dAd 19 min, Ad 11.2 min, inosine 7.5 min. [2'-^{3}H]-2'-deoxyadenosine was obtained from New England Nuclear and purified by TLC (1). The compound gave a single radioactive peak in the HPLC system.

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