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SINEFUNGIN, A POTENT INHIBITOR OF S-ADENOSYLMETHIONINE: PROTEIN O-METHYLTRANSFERASE

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SUMMARY: Sinefungin and a related metabolite, A9145C, which are antifungal antibiotics isolated from Streptomyces griseolus, were found to be potent inhibitors of both calf thymus and bovine adrenal S-adenosylmethionine-dependent protein O-methyltransferase (EC 2.1.1.24). Sinefungin and A9145C exhibit inhibitory activity which are equal to or greater than S-adenosylhomocysteine, the endogenous inhibitor of this methyltransferase.

Protein carboxymethylase (S-adenosyl-L-methionine: protein-O-methyltransferase, EC 2.1.1.24) catalyzes the transfer of a methyl group from S-adenosylmethionine (AdoMet) to the carboxyl group of a protein [1]. This type of protein methylation has been implicated in both leucocyte [2] and bacterial [3] chemotaxis, as well as neurosecretory events in the parotid gland [4], the posterior pituitary gland [5], the adrenal gland [6] and hypothalamic synaptosomes [7].

Like other AdoMet-dependent methyltransferases [8], protein carboxymethylase is inhibited by the demethylated product S-adenosyl-L-homocysteine (AdoHcy) [9]. This observation suggests that analogs of AdoHcy, which are active as inhibitors of protein carboxymethylase, might be useful chemical probes to further elucidate the physiological role of this enzymatic reaction. Sinefungin, an antifungal antibiotic isolated from Streptomyces griseolus [10], is a naturally occurring analog of AdoHcy, which has exhibited interesting differential inhibitory activity toward other AdoMet-dependent methyltransferases in vitro [11-14]. In

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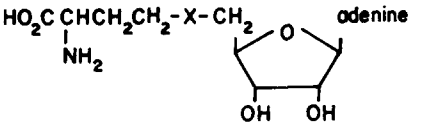
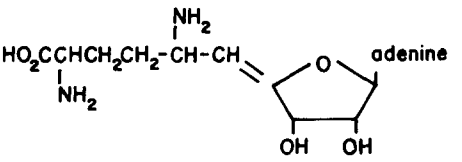
order to determine the effects of Sinefungin and a related metabolite, A9145C, on protein carboxymethylation, we have studied their inhibitor properties toward purified calf thymus and bovine adrenal protein carboxymethylases. In this communication, we report that Sinefungin and A9145C are more potent inhibitors of protein carboxymethylation than AdoHcy itself.



Compd	X
AdoHcy	-S-
Sinefungin	$-CH(NH_2)-$



A9145C

Materials and Methods

S-Adenosyl-L-[methyl-^{14}C] methionine ([methyl-^{14}C]-AdoMet, 58 mCi/mmol) was obtained from New England Nuclear. Ovalbumin and AdoHcy were purchased from Sigma Chemical Company. Sinefungin and A9145C were a generous gift from Dr. R. Nagarajan (Lilly Research Laboratories).

Protein carboxymethylase was purified from calf thymus (Pel-Freez Biologicals) or fresh bovine adrenal medulla by DEAE-Sephadex chromatography according to the procedure of Kim [15]. Protein carboxymethylase activity was assayed using [methyl-^{14}C]-AdoMet and ovalbumin as substrates. The assay mixture (0.25 ml.) contained 62 mM sodium phosphate-18.8 mM sodium citrate buffer pH 6.0, 6 mM dithiothreitol, 2 mM EDTA, 750 µg ovalbumin and 0.5-4.0 µM [methyl-^{14}C]-AdoMet. The reaction mixture was incubated at 37° for 20 minutes. The reaction was terminated by adding 0.5 ml of 0.5 M sodium borate (pH 10.0) containing 1% methanol and 3 ml of toluene-isoamyl alcohol (3:2). Each assay tube was capped and allowed to stand at room temperature for 30 min. to complete hydrolysis of the protein methyl esters. Radioactivity in an aliquot (1 ml) of the organic supernatant was determined directly by liquid scintillation spectrometry. Another 1 ml aliquot of the organic supernatant was

evaporated to dryness in vacuo at 90° and residual radioactivity determined. The difference between nonevaporated and evaporated samples provided an estimate of the [methyl-^{14}C]-methanol which was formed from AdoMet-dependent protein carboxymethylation.

Results and Discussion

Jamaluddin, et al. [9] had previously shown that AdoHcy was a competitive inhibitor of calf thymus protein carboxymethylase. As shown in Table 1, AdoHcy was also found to be an inhibitor of bovine adrenal protein carboxylmethylase, exhibiting an inhibition constant somewhat lower than that observed from the calf thymus enzyme.

When Sinefungin and A9145C were evaluated as inhibitors of protein carboxymethylase, both compounds were found to be potent reversible inhibitors. As shown in Figure 1, when AdoMet was the variable substrate for bovine adrenal protein carboxymethylase, A9145C exhibited competitive inhibitory kinetics. Similar results were also observed for Sinefungin and the calf thymus and bovine adrenal enzymes. The resulting inhibition constants for Sinefungin and A9145C are shown in Table 1.

Table 1

Effect of AdoHcy, Sinefungin and A9145C on a Purified Calf Thymus and Bovine Adrenal Protein Carboxymethylases.[a]

	Inhibition Constants, K_i ± S.E., μM[b]	
Compound	Calf Thymus	Bovine Adrenal
AdoHcy	1.03 ± 0.01	0.305 ± 0.028
Sinefungin	0.22 ± 0.03	0.529 ± 0.066
A9145C	0.024 ± 0.013	0.040 ± 0.002

a) Protein carboxymethylases were purified from calf thymus and fresh bovine adrenal medulla by DEAE - Sephadex chromatography according to the procedure of Kim [15]. Protein carboxymethylase activity was assayed using [methyl - ^{14}C] - AdoMet and ovalbumin as substrates.
AdoMet was the variable substrate (0.5 - 4.0 μM).

b) Each inhibitor showed linear competitive kinetics when plots of reciprocal velocity versus reciprocal AdoMet concentrations were made. The inhibition constants were calculated as described earlier [16].

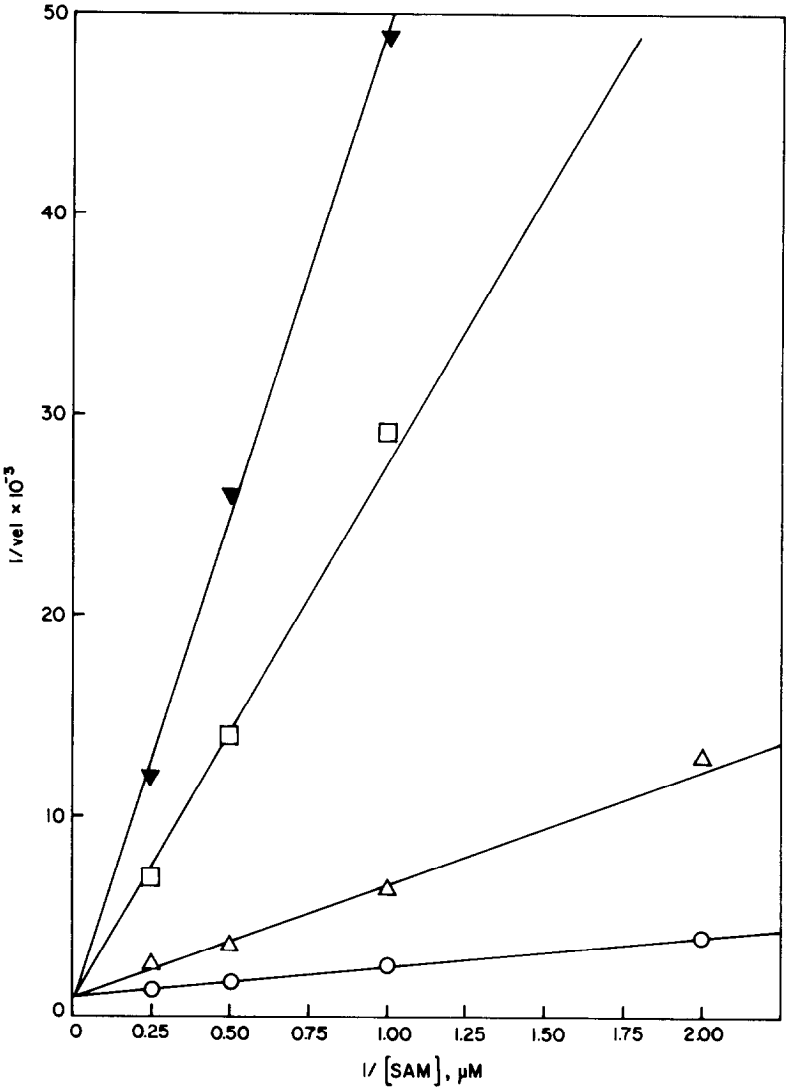


Fig. 1. A9145C inhibition of bovine adrenal protein carboxymethylase. Plots of reciprocal velocity vs. reciprocal AdoMet concentration in the presence of various concentrations of A9145C: o———o, [A9145C] = 0, Δ———Δ, [A9145C] = 0.1 μM; □———□, [A9145C] = 0.5 μM; ▲———▲, [A9145C] = 1.0 μM. Ovalbumin concentration = 3 mg/ml; vel = volatile cpm/20 min. Experimental details are described in the Materials and Methods Section.

The competitive kinetic patterns observed for AdoHcy, A9145C and Sinefungin suggest that these ligands compete for the AdoMet binding site on protein carboxymethylase, a phenomenon consistent with other AdoMet-dependent methyltransferases [8]. From the

inhibition constants shown in Table 1, it is apparent that both Sinefungin and A9145C have substantially higher affinities for the AdoMet binding site on the calf thymus protein carboxymethylase than the natural ligand AdoHcy. For example, the inhibition constant for A9145C (0.024 ∓ 0.013 μM) toward calf thymus protein carboxymethylase was approximately 1/40 of that of AdoHcy (1.03 ∓ 0.01 μM). Similar differences were also observed when the bovine adrenal enzyme was used, except that Sinefungin and AdoHcy have similar activities.

The inhibitory potencies of Sinefungin toward protein carboxymethylases (K_i = 0.25-0.50 μM) are substantially greater than those reported for Sinefungin toward protein (arginine) methyltransferase (E.C.2.1.1.23) (K_i = 3.5 μM, [12]) and protein (lysine) methyltransferase (E.C.2.1.1.25) (K_i = 4.9 μM, [12]). The inhibitory potencies of A9145C toward the arginine and lysine protein methyltransferases have not been reported.

The only AdoMet dependent systems which exhibit a degree of sensitivity to Sinefungin and A9145C comparable to protein carboxymethylase are virion mRNA (guanine-7) - methyltransferase and mRNA (nucleoside-2') - methyltransferase [11, 14]. Various small molecule methyltransferases, which were examined by Fuller and Nagaragin [13] and Borchardt and Wu [17], showed differing sensitivity to Sinefungin and A9145. For example, Borchardt and Wu [17] observed that A9145C was inactive as an inhibitor of catechol-O-methyltransferase, and moderately active as an inhibitor of phenylethenolamine N-methyltransferase (K_i = 12.4 ∓ 0.15 μM) and histamine N-methyltransferase (K_i = 1.35 ∓ 16 μM).

The results of our studies suggest that Sinefungin and A9145C are potent and fairly selective inhibitors of protein carboxymethylase. These agents might, therefore, be useful probes

for studying the role of this enzyme in chemotaxic and neurosecretory events.

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