Note

K-251 Compounds, Inhibitors of Ca^{2+} and Calmodulin-dependent Cyclic Nucleotide Phosphodiesterase from *Streptovorticillium album*

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As reported previously, we have isolated several inhibitors of Ca^{2+} /calmodulin-dependent cyclic nucleotide phosphodiesterase (PDE) in culture filtrates of microorganisms: these compounds include indole carbazol compounds,^{1,2)} anthraquinone compounds,^{3,4)} and an isoflavonoid compound.⁵⁾ In this study, in addition, we have isolated four calmodulin antagonists, named K-251-I, T, b, and sb, from the culture broth of *Streptovorticillium album* K-251.

All experimental procedures were done by the methods described previously.⁵⁾ K-251 compounds were isolated from the culture broth of *Streptovorticillium album* K-251 by Diaion HP-10 column chromatography, ethyl acetate

extraction, repeated silica gel column chromatography, and Sephadex LH-20 column chromatography. The yield of pure K-251-I, T, b, and sb were 300, 80, 12.5, and 1.9 mg, respectively, from 36 l of culture broth. The structures of K-251-I, T, b, and sb were identical with reticulol (6,8-dihydroxy-7-methoxy-3-methylisocoumarin), tectorigenin (4',5,7-trihydroxy-6-methoxyisoflavone), daidzein (4',7-dihydroxyisoflavone), and pyridindolol (1-1-(R), 2-dihydroxyethyl-3-hydroxymethyl-9H-pyrido(3,4-b)indole), respectively, on the basis of various physicochemical properties and spectral data.

K-251 compounds inhibited the Ca^{2+} /calmodulin-dependent activity of PDE from bovine brain in a concentration-related manner. The IC_{50} values of K-251-I, T, b, and sb were 89.6, 39.6, 119, and 320 μM , respectively, at a calmodulin concentration of 4.0 U/ml. Since K-251-T and b are isoflavonoid compounds and are structurally related to K-254-I (genistein, 4',5,7-trihydroxyisoflavone),⁵⁾ we compared the inhibitory potency of K-251 compounds with that of genistein (Table I). K-251-T showed a somewhat higher degree of inhibition than K-254-I, but K-251-I, b, and sb inhibited the enzyme to a similar degree or less. The inhibitory activity of K-251 compounds was antagonized by a higher concentration of calmodulin, 320 U/ml. The kinetic analyses of K-251 compound-induced inhibition of Ca^{2+} /calmodulin-activated PDE were done by Dixon plots. The data indicates that K-251 compounds inhibited Ca^{2+} /calmodulin-activated PDE in a competitive manner with respect to calmodulin. K_i values of K-251-I, T, b, and sb were 30, 15, 65, and 170.3 μM , respectively. However, increasing the concentration of Ca^{2+} in the assay mixture did not affect the inhibitory potency of K-251 compounds.

K-251 compounds also inhibited Ca^{2+} /calmodulin-dependent PDE from bovine heart with comparable pot-

TABLE I. EFFECTS OF K-251 COMPOUNDS ON CYCLIC NUCLEOTIDE PHOSPHODIESTERASES

Enzymes	CaM* (U/ml)	IC_{50} (μM)				
		K-251-I	K-251-T	K-251-b	K-251-sb	K-254-I ⁵⁾
Bovine brain CaM-PDE**	0 ^a	1279	284	3576	> 774***	3580
	4.0 ^b	89.6	39.6	119	320	220
Bovine heart CaM-PDE	2.5 ^c	44.6	26.2	143	251	171
Bovine heart CaM-independent PDE	0 ^d	> 900	> 332	> 893	> 705	

^a The activity in the presence of 3 mM ethylene bis(oxyethylenitrilo) tetraacetic acid (EGTA), without CaCl_2 and calmodulin. The enzyme concentration was 18.6 mU/ml.

^b The activity in the presence of 4 U/ml calmodulin, and 50 μM CaCl_2 . The enzyme concentration was 26 mU/ml.

^c The activity in the presence of 2.5 U/ml calmodulin, and 50 μM CaCl_2 . The enzyme concentration was 40 mU/ml.

^d The activity in the presence of 3 mM EGTA, without CaCl_2 and calmodulin. The enzyme concentration was 25 mU/ml.

* Calmodulin.

** Ca^{2+} /calmodulin-dependent phosphodiesterase.

*** The figures expressed with > signifies considerable inhibition by the drug was not observed at this final concentration.

FIG. 1. Effect of K-251-1 and T on the Fluorescence of NPN (A and B) and TNS (C and D) Bound to Calmodulin.

Excitation was at 360 nm for both probes, and emission intensity was measured from 360~600 nm.

ency to that from brain. On the other hand, the basal activity of the brain enzyme was less influenced by K-251 compounds. K-251 compounds did not inhibit calmodulin-independent PDE from bovine heart at the concentrations indicated (Table I). Among the K-251 compounds tested, K-251-T and b inhibited protein kinase C with IC_{50} values of 252 and 262 μM , respectively; these values are about 10-fold higher than that of K-254-I (Genistein). K-251-b also inhibited cAMP-dependent protein kinase with an IC_{50} value of 374 μM . However, K-251-1 and sb inhibited neither protein kinase C nor cAMP-dependent protein kinase.

To obtain information regarding binding properties of K-251 compounds to calmodulin, we used hydrophobic fluorescent probes, such as NPN (*N*-phenyl-1-naphthylamine) and TNS (2-*p*-toluidinyl naphthalene-6-sulfonate). Fluorescence measurement were done at room temperature using a Hitachi 650-10S fluorescence spectrophotometer with a Hitachi 561 recorder based on the method of Epstein *et al.*⁶⁾ In the presence of Ca^{2+} , the fluorescent intensity of the probes was greatly increased by complexing with calmodulin (Fig. 1). Addition of 1.0 mM EGTA reversed the enhancement of the fluorescence. K-251-T inhibited the fluorescence of both NPN and TNS (Figs. 1B and 1D). K-251-1 also inhibited NPN fluorescence (Fig. 1A). In contrast, K-251-1 slightly increased further TNS-fluorescence (Fig. 1C). K-251-b and sb could

not be studied because of their intrinsic fluorescence. These results indicate K-251-1 and T can interact directly with calmodulin. Taken together, the results obtained in this study show that K-251 compounds are calmodulin antagonists.

K-251-1 (reticulol) was previously isolated from *Streptomyces mobaraensis*,⁷⁾ and its inhibitory activity on cyclic nucleotide phosphodiesterases has been also noted.^{8,9)} An isomer of tectorigenin (K-251-T) was previously isolated from fungi and streptomycetes as an inhibitor of dopa decarboxylase.¹⁰⁾ K-251-b (daidzein) was previously isolated from *Streptomyces xanthophaeus*,¹¹⁾ and its inhibitory activity on cAMP phosphodiesterase has been shown by other investigators.¹²⁾ K-251-sb (pyridindolol) was previously isolated from *Streptomyces alboverticillus* as an inhibitor of β -galactosidase.¹³⁾ However, the calmodulin antagonistic properties of these compounds had not been reported.

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