J. Enzyme Inhibition, 1991, Vol. 4, pp. 359–363
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5923-DNI, A NEW DEOXYRIBONUCLEASE INHIBITOR PRODUCED BY *STREPTOMYCES* SP. STRAIN NO. A-5923

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(Received 11 October 1990)

KEY WORDS: Deoxyribonuclease inhibitor, DNase II, *Streptomyces* sp., 5923-DNI

INTRODUCTION

Deoxyribonucleases (DNases) are known to play important roles in recombination, replication and repair systems in the living cell. However, the detailed mechanism of their action has not yet been elucidated. A specific DNase inhibitor may be useful as a tool in such studies. Up to the present time, specific inhibitors have been isolated from animal tissues such as liver,[1] thymus[2] and spleen.[3] However, little has been reported about an inhibitor for DNases produced by microorganisms.

In the search for an inhibitor for DNases, we have screened various microorganisms isolated in our laboratory and, recently, have found that *Streptomyces* sp. strain No. A-5923 produced an inhibitor (5923-DNI) in the culture filtrate, which seems to be specific for DNase II (EC3.1.4.6) from porcine spleen. In this paper, we describe the purification procedure and some properties of 5923-DNI.

MATERIALS AND METHODS

Materials

DNase II from porcine spleen, RNase A from bovine pancreas, phosphodiesterase I from *Crotalus atrox* venom, phosphodiesterase II from bovine spleen, salmon DNA, thymidine-5′-monophosphate-*p*-nitrophenyl ester and thymidine-3′-monophosphate-*p*-nitrophenyl ester were obtained from Sigma Chemicals. DNase I from bovine pancreas, nuslease S_1 from *Aspergillus oryzae*, α-glucosidase from yeast and β-glucosidase from sweet almond were obtained from Boehringer Mannheim, Yamanouchi. Salicin and *p*-nitrophenyl-α-D-glucopyranoside were obtained from Nacalai Tesque, Inc. RNA from yeast was obtained from Kohjin Co., Ltd.

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Measurement of DNase II Activity

Activity was determined by measuring absorbance at 260 nm of acid-soluble hydrolysis products liberated from DNA by the enzyme reaction. The reaction mixture consisting of sodium acetate buffer (125 μl, 0.2 M, pH 5.0), EDTA·2Na (25 μl, 0.2 M, pH 5.0), KCl (50 μl, 1 M), DNase II from porcine spleen (50 μg/50 μl), distilled water (150 μl) with or without inhibitor, and salmon DNA (250 μg/100 μl) was incubated at 30°C for 30 min. After incubation, 100 μl of perchloric acid solution containing 0.75% uranyl acetate was added to terminate the reaction. After standing for 10 min at 0°C, the precipitate was removed by centrifugation. The supernatant (0.2 ml) was diluted to 3 ml with distilled water, and the absorbance at 260 nm was measured by spectrophotometer (HITACHI U-2000). One inhibitor unit (IC_{50}) was defined as the amount which reduced the activity of DNase by 50%.

RESULTS AND DISCUSSION

Isolation of 5923-DNI

Streptomyces sp. strain No. A-5923 was cultivated for 4 to 5 days under aerobic conditions at 28°C in S-medium consisting of 2% glucose, 3% starch, 1% corn steep liquor, 1% soyabean flour, 0.5% peptone, 0.3% NaCl, and 0.5% $CaCO_3$ (pH 7.0), and then the mycelia were removed by centrifugation. To the culture filtrate was added activated carbon powder (1%, w/v), and the mixture stored for 1 h at 5°C. The carbon was removed by filtration and three volume of ethanol was added to the filtrate. After removal of inactive preciptate by centrifugation, the supernatant was

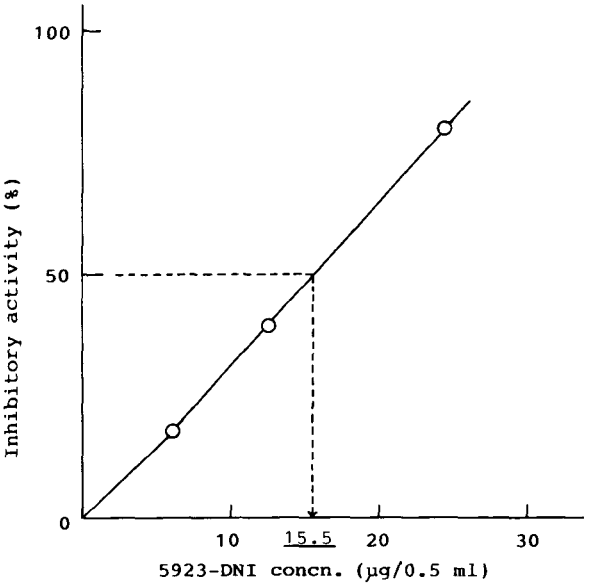

FIGURE 1 Inhibitory activity of 5923-DNI against DNase II. The 5923-DNI concentration is expressed in μg per 0.5 ml of the final reaction mixture.

concentrated *in vacuo* below 30°C, and acetone was added to the concentrate. The precipitate formed was dissolved in distilled water, and applied on Amberlite IRA-45 column (3.2 × 18 cm) and eluted with 0.5 N NH_4OH solution (pH 11). The active fractions (800 ml–950 ml) were collected and concentrated *in vacuo*. The preparation was applied on Amberlite IR-120B column (1.5 × 4 cm), eluted with distilled water, and active fractions (20 ml–60 ml) were collected. The fraction containing inhibitory activity was then applied on a Sephadex G-25 column (1.7 × 22 cm) using distilled water as eluting solvent to free from salts, and the eluate evaporate to give 5923-DNI. As shown in Figure 1, 15.5 μg of 5923 – DNI thus obtained inhibited 25 μg of DNase II.

DNase II.

Some Properties of 5923-DNI

Some properties of 5923-DNI are as follows. 5923-DNI was colorless and soluble in water, but insoluble in methanol, ethanol and acetone. 5923-DNI gave a positive reaction in the phenol-H_2SO_4 reaction,[4] but its color intensity was slight. It gave a negative reaction in the ninhydrin,[5] Fiske-SubbaRow,[6] sodium rhodizonate,[7] $AgNO_3$[8] reactions. 5923-DNI was a stable substance, as it retained 100% of its original inhibitory activity after being kept for 30 min at 60°C or 10 min at 100°C in a pH range between 7.0 and 11.0. As shown in Figure 2, the inhibitory activity of 5923-DNI was dependent on pH and temperature and was maximum at pH 6.0 and 20°C. The molecular weight of 5923-DNI was estimated to be about 2,000 to 3,000 by gel filtration on Sephadex G-25 column.[9]

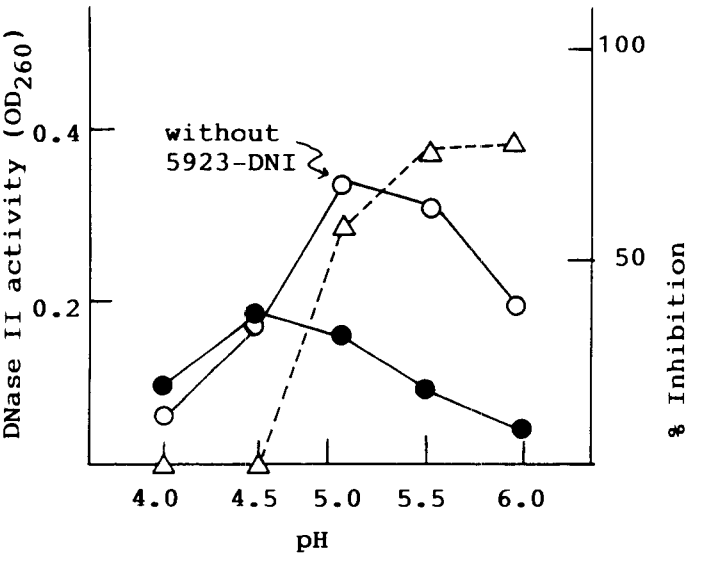

FIGURE 2 Effect of pH on the inhibitory activity of 5923-DNI on DNase II. (○) Activity without 5923-DNI; (●) activity with 5923-DNI (17 μg); (△) percentage inhibition.

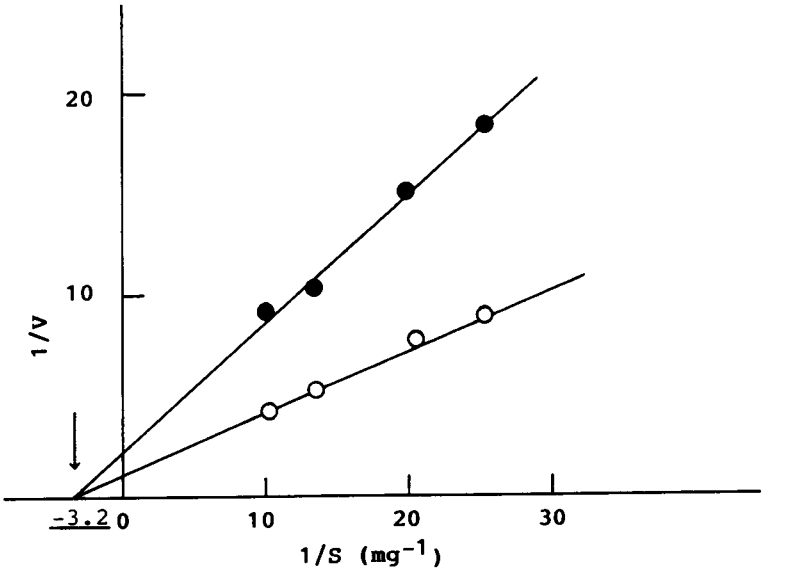

FIGURE 3 Lineweaver-Burke reciprocal plot of substrate (DNA) concentration against rate of hydrolysis by DNase II; (●) with 5923-DNI and, (○) without 5923-DNI.

TABLE I
Inhibitory spectrum of 3923-DNI

Enzyme (EC)	Substrate (conc*)	IC_{50} (μg/ml)
DNase II (3.1.4.6) from porcine spleen	DNA (0.5)	31
DNase I (3.1.4.5) from bovine pancreas	DNA (2.0)	341
Nuclease S_1 (3.1.30.1)[1] from *Aspergillus oryzae*	Denatured DNA (0.5)	101
RNase A (3.1.27.5)[2] from bovine pancreas	RNA (0.5)	> 1000
Phosphodiesterase I (3.1.4.1)[3] from *Crotalus atrox venom*	Thymidine-5′-monophosphate-*p*-nitrophenyl ester (0.09)	> 1000
Phosphodiesterase II (3.1.16.1)[4] from bovine spleen	Thymidine-3′-monophosphate-*p*-nitrophenyl ester (0.23)	183
α-Glucosidase (3.2.1.20)[5] from yeast	p-Nitrophenyl-α-D-glucopyranoside (0.8)	219
β-Glucosidase (3.2.1.21)[5] from sweet almond	Salicin (2.5)	> 1000

*Concentration was expressed as mg/ml-incubation mixture. Assay methods used were: 1. Shishido, K. and Ando, T. (1972) *Biochim. Biophys. Acta*, **287**, 477. 2. Richards, F.M. and Wyckoff, H.W. (1971) *The Enzymes*, (ed. Boyer, P.D.) Vol. 4, p. 647, New York: Academic Press. 3. Razzell, W.E. (1963) *Methods in Enzymology*, (ed. Kaplan, N.O.) Vol. 6, p. 236, New York: Academic Press. 4. Razzell, W.E. and Khorana, H. (1961) *J. Biol. Chem.*, **236**, 1144. 5. Uyeda, M., Yoshida, T., Oshima, H., Suzuki, K. and Shibata, M. (1988) *J. Enz. Inhib.*, **2**, 173.

TABLE II
Parameters for other inhibitors against DNase II.

Inhibitor (MW)	Enzyme used	IC_{50} (μg/ml-inc.mix.)	K_i (M)
Acid DNase inhibitor from beef liver (21500)[1]	Acid DNase	4.0	1.8×10^{-4}
DNase I inhibitor from calf spleen (59400)[2]	DNase I	1.2	–
	DNase II	> 100.0	–
5923-DNI from *Streptomyces* sp. (2500)	DNase II	31.0	5.8×10^{-9}

1. Lesca, P. (1976) *J. Biol. Chem.*, **251**, 116.
2. Lindberg, U. (1967) *Biochemistry*, **6**, 323.

The type of inhibition was determined by a Lineweaver-Burke reciprocal plot of substrate (salmon DNA) concentration against rate of hydrolysis by DNase II in the presence and absence of 5923-DNI (1 unit). As shown in Figure 3, the inhibition was shown to be non-competitive, and the K_i value for 5923-DNI was 5.76×10^{-9} M (1.44×10^{-5} g) and the K_m value for the enzyme was 8.21×10^{-9} M (3.12×10^{-4} g).

Inhibitory Spectrum of 5923-DNI

The effect of 5923-DNI on various enzymes were examined, and the results are summarized in Table I. 5923-DNI showed strong inhibition for DNase II from porcine spleen and weakly inhibited nuclease S_1 from *Aspergillus oryzae*, phosphodiesterase II from bovine spleen, α-glucosidase from yeast and DNase I from bovine pancreas but did not show activity against RNase A from bovine pancreas, phosphodiesterase I from venom and β-glucosidase from sweet almond. 5923-DNI is a novel potent inhibitor of DNase II and its potency is compared with that for two other known inhibitors, for which data is available, in Table II.

Thus, all data reported above suggests that 5923-DNI is a new inhibitor of microbial origin which specifically inhibits DNase II. Other properties including the structure of 5923-DNI are now under investigation and will be reported elsewhere.

References

1. Zalite, B.R. and Roth, J.S. (1964) *Arch. Biochem. Biophys.* **107,** 16.
2. Lindberg, M.U. and Skoog, L. (1970) *Eur. J. Biochem.*, **13,** 326.
3. Lindberg, M.U. (1966) *J. Biol. Chem.*, **241,** 1246.
4. Dubois, M., Gilles, K.A., Hamilton, J.K., Roberts, P.A. and Smith, F. (1956) *Anal. Chem.*, **28,** 350.
5. Lee, Y.P. and Takashashi, T. (1966) *Anal. Biochem.*, **14,** 71.
6. Fiske, C.H. and SubbaRow, Y. (1925) *J. Biol. Chem.*, **66,** 375.
7. Terho, T.T. and Hartiala, K. (1971) *Anal. Biochem.*, **41,** 471.
8. Trevelyan, W.E., Procter, D.P. and Harrison, J.S. (1950) *Nature (Lond.)*, **166,** 444.
9. Andrews, P. (1955) *Biochem. J.*, **96,** 595.