GALACTOSTATIN, A NEW β-GALACTOSIDASE INHIBITOR FROM *STREPTOMYCES LYDICUS*

Sir:

β-Galactosidase (EC 3.2.1.23),[1] widely distributed in animals, plants and microorganisms, is considered to play an important role in biological regulations of carbohydrate metabolism.

Thus, several β-galactosidase inhibitors have recently been isolated from the cultured filtrates of microorganisms.[2~4] Such work showed that the strain PA-5726 of *Streptomyces* sp. produces a very potent inhibitor named galactostatin. This communication describes the isolation and characterization of galactostatin.

Strain PA-5726 was isolated from a soil sample collected in Nagasaki Prefecture, Japan, and identified as a strain of *Streptomyces lydicus*.

The inhibitory activity of galactostatin for β-galactosidase was assayed. A mixture of 0.25 ml of β-galactosidase dissolved in 0.05 M acetate buffer, pH 5.0 (0.5 mg of a crude β-galactosidase preparation obtained from Takadiastase, purchased from Sankyo Co., Ltd., Japan), 0.25 ml of inhibitor solution and 0.5 ml of *O*-nitrophenyl-β-D-galactopyranoside (ONPG) (Sigma Chemical Co., U.S.A.) in the same buffer was incubated at 30°C for 15 minutes. The reaction was stopped by adding 1 ml of 1 M Na_2CO_3 and 8 ml of water, then the optical density of the liberated *O*-nitrophenol was measured at 420 nm. Control samples, without the inhibitor solution, were treated similarly. One inhibitory unit (IU) was defined as the amount inhibiting 50% of the original activity by this method. The inhibitor was produced by shaking culture or tank fermentation of strain PA-5726.

The production medium contained glycerol 4.0%, meat extract 1.5%, Polypeptone 1.5%, KCl 0.076%, $MgCl_2 \cdot 6H_2O$ 0.042%, $FeCl_3 \cdot 6H_2O$ 0.025%, $ZnSO_4 \cdot 7H_2O$ 0.0022%, $MnCl_2 \cdot 4H_2O$ 0.0018% and the pH was adjusted to 7.0 with 5 N NaOH. Maximum production of the inhibitor was attained 5~6 days at 28°C after inoculation in shaking culture, and the inhibitory activity reached *ca.* 3,200 IU/ml in the time of harvest.

To obtain galactostatin, the culture filtrate was adjusted to pH 4.5 by adding HCl and then treated with 1.5% active carbon. The clear filtrate obtained was passed through a column of Dowex-1X8 (OH^-) and washed with distilled water. The active fraction adjusted to pH 7.0 was adsorbed on a column of Dowex 50WX8 (H^+) and eluted with 0.5 N HCl. The active fraction was applied to a column of Amberlite IRA-47 (OH^-) and desorbed with distilled water. Eluated solution was concentrated *in vacuo* and then 6% sulfurous acid solution and 2-fold volume of ethanol were added at 4°C. When this solution was kept at 4°C overnight, crystals of galactostatin bisulfite adduct formed. They were recrystallized from hot water with subsequent addition of ethanol. Galactostatin bisulfite adduct could be converted to the free compound by application to a column of Dowex-2X8 (OH^-) and desorption with distilled water. The active fraction was concentrated *in vacuo* and precipitated with ethanol. The overall yield was about 46% and 2,030 mg galactostatin was obtained from 8 liters of culture filtrate.

Galactostatin is obtained as a white amorphous powder, mp 94~98°C; $[\alpha]_D^{23}$ +85.6° (*c* 1.0, H_2O). It has the molecular formula $C_6H_{13}NO_5 \cdot \frac{1}{2}H_2O$. Calcd: C 38.30, H 7.50, N 7.44. Found: C 38.26, H 7.47, N 7.51.

Galactostatin is soluble in water, methanol, acetic acid, pyridine and dimethyl sulfoxide, slightly soluble in ethanol and 2-propanol, and insoluble in most other organic solvents. It gives positive color reactions with NH_4-silver nitrate and ninhydrin (weakly positive) test. Its Rf values on silica gel thin-layer chromatography are: 0.39 in CH_3CN - AcOH - H_2O (5:1:2), 0.29 in $CHCl_3$ - MeOH - 30% NH_4OH (1:2:1) and 0.28 in BuOH - AcOH - H_2O (3:1:1).

The mass spectrum of galactostatin presented the parent ion peak at *m/z* 161 (M−18), indicating the molecular formula $C_6H_{13}NO_5$ (MW 179.17). The IR spectrum disclosed hydroxyl and imino group (3360, 2900, 1650 cm^{-1}). ^1H NMR spectrum in 0.5 N DCl at 200 MHz revealed the configuration of galactostatin to be a D-galactose one. Anomeric protons of α and β appeared at δ 4.77 and δ 4.00. From these results it was concluded that galactostatin is 5-amino-5-deoxy-D-galactopyranose as shown in Fig. 1 and exists as a mixture of α and β anomer forms. The structure study will be reported elsewhere.[5]

Fig. 1. Structure of galactostatin.

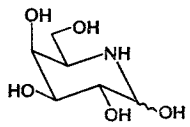

Table 1. Inhibitory activity of galactostatin on various β-galactosidases.

Origin	pH	Substrate*	IC_{50} (μg/ml)
Bovine liver	7.2	ONPG	2,450
Mouse liver	4.5	ONPG	0.33
Rat liver	4.5	ONPG	4.68
Charonia lampas	3.5	ONPG	6.68
Jack beans	3.5	ONPG	1.97
	3.5	Lactose	0.22
Escherichia coli	7.2	ONPG	6.77
	7.2	Lactose	0.38
Saccharomyces	7.2	ONPG	0.66
fragilis	7.2	Lactose	0.07
Aspergillus oryzae	4.5	ONPG	0.22
	4.5	Lactose	0.05

* Lactose concentration was 0.75% and galactose liberated was determined enzymatically with galactose dehydrogenase.

The inhibitory activities of purified galactostatin are shown in Table 1 against several β-galactosidases in acidic and neutral media. The results indicate that galactostatin is a strong inhibitor of β-galactosidases from several different sources over a wide pH range.

Galactostatin belongs to the piperidinose sugar group as can be seen from its microorganism metabolites.[6~9] For the present time many β-galactosidase inhibitors have been found as followed, pyridindolol (1-[1(*R*),2-dihydroxyethyl]-3-hydroxymethyl-9-*H*-pyrido (3,4-*b*) indole),[2] isoflavone rhamunosides,[3] HPAAO (*p*-hydroxyphenylacetaldoxime)[4] and D-galactal (1,2-dideoxy-D-*lyxo*-hex-1-enopyranose).[10] However, since galactostatin isolated here apparently differs from them in chemical nature, this inhibitor is a novel β-galactosidase inhibitor.

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References

1) WALLENFELS, K. & R. WEIL: β-Galactosidase. *In* The Enzymes. 3th Ed., Vol. 7. *Ed.*, P. D. BOYER, pp. 617~663, Academic Press, New York, 1972
2) AOYAGI, T.; M. KUMAGAI, T. HAZATO, M. HAMADA, T. TAKEUCHI & H. UMEZAWA: Pyridindolol, a new β-galactosidase inhibitor produced by actinomycetes. J. Antibiotics 28: 555~557, 1975
3) AOYAGI, T.; T. HAZATO, M. KUMAGAI, M. HAMADA, T. TAKEUCHI & H. UMEZAWA: Isoflavone rhamunosides, inhibitor of β-galactosidase produced by actinomycetes. J. Antibiotics 28: 1006~1008, 1975
4) HAZATO, T.; T. AOYAGI & H. UMEZAWA: Studies on relationships between the structure and the glycosidase-inhibiting activity of HPAAO and its analogs. J. Antibiotics 32: 212~222, 1979
5) MIYAKE, Y. & M. EBATA: Studies of β-galactosidase inhibitor galactostatin and its derivatives. Agric. Biol. Chem., in preparation
6) INOUYE, S.; T. TSURUOKA & T. NIIDA: The structure of nojirimycin, a piperidinose sugar antibiotic. J. Antibiotics, Ser. A 19: 288~292, 1966
7) MURAO, S. & S. MIYATA: Isolation and characterization of a new treharase inhibitor, S-GI. Agric. Biol. Chem. 44: 219~221, 1980
8) NIWA, T.; T. TSURUOKA, H. GOI, Y. KODAMA, J. ITOH, S. INOUYE, Y. YAMADA, T. NIIDA, M. NOBE & Y. OGAWA: Novel glycosidase inhibitors, nojirimycin B and D-mannoic-δ-lactam. Isolation, structure determination and biological property. J. Antibiotics 37: 1579~1586, 1984
9) EZURE, Y.; S. MURAO, K. MIYAZAKI & M. KAWAMATA: Molanolin (1-deoxynojirimycin) fermentation and its improvement. Agric. Biol. Chem. 49: 1119~1125, 1985
10) LEE, Y. C.: Inhibition of β-D-galactosidase by D-galactal. Biochem. Biophys. Res. Commun. 35: 161~167, 1969