Communications to the editor

RIBOCITRIN, A NEW INHIBITOR OF DEXTRANSUCRASE

Sir:

Homocitric acid tririboside which inhibited dextransucrase was isolated from a culture broth of *Streptomyces* sp. MF980-CF1.

It has been reported that *Streptococcus mutans* plays an important role in the initiation of dental caries[1], and the cariogenicity depends on its dextransucrase (EC 2.4.1.5) that produces from sucrose insoluble polysaccharides, which mediates bacterial adhesion to the tooth surface[2~4].

We devised a screening method of inhibitors of dextransucrase (Fig. 1), and found a strong inhibitor in culture filtrates of the strain MF980-CF1. This is the first finding of a specific inhibitor of *S. mutans* dextransucrase.

The strain MF980-CF1 was isolated from a soil sample collected at Kawaguchiko Town, Yamanashi Prefecture. One of the media suitable for the production of the inhibitor contained maltose 2%, pharma media 1%, corn steep liquor 2%, and $CaCO_3$ 0.32%. The pH was adjusted to 6.8 with 2 M NaOH before autoclaving. The maximum production was attained on the 4~5th day in shaken culture (reciprocal shaking, 130 strokes/minute, 8 cm, 27°C).

Purification procedures are shown in Fig. 2. After 96 hours culture, the whole broth was heated in a boiling water-bath for 20 minutes, cooled and filtered. The filtrate (4.5 liters) was passed through a column of Diaion PA316 (OH-form, 5.5×85 cm). After the column was washed with five liters of water the adsorbed inhibitor was eluted with 1 M ammonium bicarbonate. The active eluate (600 ml) was concentrated to 100 ml under reduced pressure and subjected to Sephadex G-15 (4×80 cm) gel permeation chromatography using deionized water

Fig. 1. Assay method of dextransucrase inhibitors.

Reaction mixture
2.7 ml of 11 mM sucrose in 50 mM potassium phosphate buffer (pH 6.9) containing 0.04% sodium azide.
+0.3 ml of test solution[1)]
+50 μl of dextransucrase[2)]
Incubated for 14 hours at 37°C
Turbidity (600 nm) is read after agitation

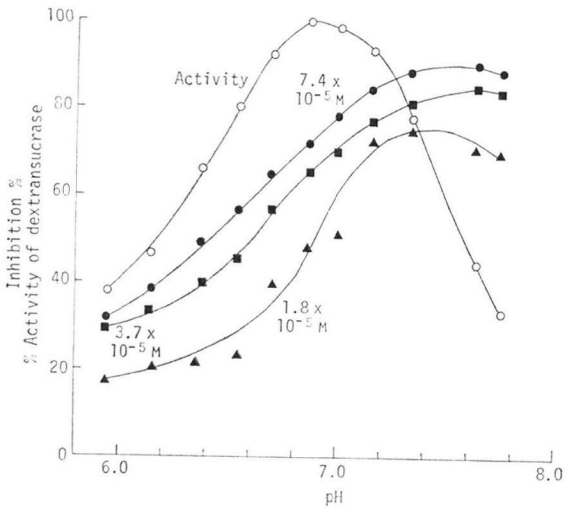
Percent inhibition = C−T/C×100
where C = turbidity of control (0.3 ml of water instead of the test sample)
where T = turbidity of test

1) Cultured broths of microorganisms were heat-treated in a boiling water-bath for 5 minutes, after centrifugation, 0.3 ml of the supernatant was used for the test.
2) Dextransucrase which was obtained by ammonium sulfate precipitation (50% saturation) from culture filtrates of *Streptococcus mutans* E49 (Brain-Heart infusion bouillon, Eiken Chem.) was used. The amount of the enzyme was adjusted to give 0.25 of turbidity after the reaction.

Fig. 2. Extraction and purification of ribocitrin.

Streptomyces sp. MF980-CF1
Cultured for 96 hours at 27°C
↓
Heat-treated in a boiling water-bath for 20 minutes
↓
Culture filtrate | Mycelium
(4.5 liters, IC_{50} 0.5 ml/ml)
↓
Diaion PA316 (OH-form, 5.5×85 cm)
eluted with 1 M ammonium bicarbonate
(600 ml, IC_{50} 80 μl/ml, yield 83%)
↓
Sephadex G-15 (4×80 cm)
eluted with water
(200 ml, IC_{50} 30 μl/ml, yield 72%)
↓
DEAE-Sephadex A-25 (HCO_3-form, 3×45 cm)
eluted with a linear gradient of ammonium bicarbonate (0.05~1.4 M)
(100 ml, IC_{50} 17 μl/ml, yield 64%)
↓
DEAE-Sephadex A-25 (Cl-form, 2.5×45 cm)
eluted with a linear gradient of sodium chloride (0.05~1.4 M)
(36 ml, IC_{50} 7 μl/ml, yield 59%)
↓
Sephadex G-15 (2.1×90 cm)
eluted with water
(30 ml, IC_{50} 6 μl/ml, yield 58%)
↓
SilicAR CC-7 (3×50 cm)
eluted with *n*-PrOH - 3 N NH_4OH (7: 3)
(124 ml, IC_{50} 24 μl/ml, yield 57%)
↓
Sephadex G-15 (2×45 cm)
eluted with water
(36 ml, IC_{50} 7 μl/ml, yield 56%)
↓
Lyophilized; white amorphous powder
(90 mg, IC_{50} 18 μl/ml, yield 56%)

Fig. 3. Effect of pH on inhibitory activity of ribocitrin and activity of dextransucrase.



as the eluting solvent. The active eluate (200 ml) was applied directly to a column of DEAE-Sephadex A-25 (HCO_3-form, 3×45 cm), and eluted with a linear gradient of ammonium bicarbonate (0.05~1.4 M). The active fractions (100 ml) were combined and concentrated under reduced pressure below 60°C. The concentrated active fraction which was diluted with deionized water was subjected to DEAE-Sephadex A-25 (Cl-form, 2.5×45 cm) column chromatography and eluted with a linear gradient of sodium chloride (0.05~1.4 M). The active fractions (36 ml) were concentrated to a syrup and applied to a column of Sephadex G-15 (2.1×90 cm). The salt free active fraction (30 ml) thus obtained was lyophilized and dissolved with a small amount of mixed solvent composed of *n*-PrOH - 3N NH_4OH (7: 3), and subjected to SilicAR CC-7 (3×50 cm) column chromatography developed by the same solvent mixture. The active fraction (124 ml) thus eluted was concentrated under reduced pressure below 45°C (pH was maintained above 7.0 by addition of 0.05 M sodium hydroxide). The resulting opaque solution was applied to a column of Sephadex G-15 (2×45 cm) using deionized water as the eluting solvent. Lyophilization of the combined active fractions gave sodium salt of the purified inhibitor as an amorphous white powder. We named this inhibitor ribocitrin, pertinent to its structure.

Properties of the sodium salt of ribocitrin are; m.p. 202~203°C (dec.); no maximum at 210~370 nm. The elemental analysis: calcd. for $C_{22}H_{31}O_{19}Na_3 \cdot H_2O$: C 38.49, H 4.85, Na 10.05; found C 38.78, H 5.09, Na 8.70. It is soluble in water, and insoluble in organic solvents. It produces a spot of Rf value 0.29 on silica gel thin-layer chromatography (*n*-PrOH - H_2O, 7: 3). It gives positive color reaction with phenol-sulfuric acid and orcinol-hydrochloride, but negative with NELSON-SOMOGYI, diphenylamine-sulfuric acid and ninhydrin. As reported in the other paper[5], ribocitrin has a unique structure consisting of three moles of ribose and one mole of homocitric acid.

The type of inhibition by ribocitrin was non-competitive with sucrose; Ki was 2.7×10^{-5} M (pH 6.9). As shown in Fig. 3, the inhibitory activity was pH-dependent. At pH 7.2~7.6, it showed the strongest inhibition. Moreover, ribocitrin at 8.9×10^{-5} M exhibited 50% inhibition against the adherence of *S. mutans* E49 on a glass surface. Ribocitrin (100 μg/ml) showed no inhibition of the growth of microorganisms including *S. mutans* E49. Intraperitoneal administration of 170 mg/kg caused no death of mice.

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