5′-NUCLEOTIDASE INHIBITORY ACTIVITY OF NUCLEOTICIDIN, MELANOCIDIN A AND MELANOCIDIN B

STRUCTURE-ACTIVITY RELATIONSHIPS

KEIJIRO UCHINO, HIROSHI OGAWARA*, TETSU AKIYAMA and AKIRA FUKUCHI†

Department of Biochemistry, Meiji College of Pharmacy, 1 Nozawa, Setagaya-ku, Tokyo 154, Japan
†Central Laboratory, Nippon Flour Mills Co., Ltd., Nurumizu, Atsugi, Kanagawa 243, Japan

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Nucleoticidin and melanocidins A and B exhibited potent inhibitory activity against 5′-nucleotidases from rat liver membrane and snake venom. These inhibitors are polysaccharides with highly branched side chains having at least disaccharide units. This conclusion was supported by the results with polysaccharides of known chemical structures. The inhibitors showed non-competitive inhibition with respect to AMP, and urea-treatment caused a marked decrease or a disappearance of the 5′-nucleotidase inhibitory activity. Therefore, it is concluded that steric factors also play an important role in their inhibitory activity.

Inhibitors of membrane-bound enzymes are known to show antitumor and immunopotentiator activities[1]. Therefore, inhibitors of 5′-nucleotidase, a membrane-bound enzyme, are expected to show these activities. By testing the inhibitory activity against 5′-nucleotidase of cultured filtrates, we obtained new inhibitors, named nucleoticidin[2,3], melanocidin A and melanocidin B[4,5]. These substances had related polysaccharide structures, although nucleoticidin was produced by *Pseudomonas* sp. while melanocidins A and B were isolated from culture filtrates of *Nocardioides* sp. In addition, they showed antitumor activity. Here, we report the structure-activity relationships of these polysaccharides and related compounds.

Materials and Methods

Materials

Bleomycin, elastatinal, antipain and chymostatin were kindly provided by Prof. H. UMEZAWA, Institute of Microbial Chemistry. Pachyman, curdlan and psutulan were kindly provided by Prof. S. SHIBATA, Meiji College of Pharmacy. Carrageenan, pectic acid, amylose A (average MW: 2,900), amylose B (average MW: 16,000), amylopectin, pullulan, dextran (MW: 50,000~70,000), dextran (MW: 170,000~200,000) and mannan (yeast) were purchased from Nakarai Chemicals, Ltd. (Kyoto, Japan). All other chemicals were obtained commercially.

Assay Method of 5′-Nucleotidase Inhibitors

5′-Nucleotidase and its inhibitory activities were determined by the method described previously[2].

Urea-treatment of Polysaccharides[6]

Polysaccharides were reacted in 8 M urea solution at 70°C for 6 hours. The urea was removed

Table 1. Inhibition of 5′-nucleotidase activity.

Inhibitor	*Ki* (μg/ml)
Nucleoticidin	3.64
Melanocidin A	29.9
Melanocidin B	33.5
Mannan	26.5

Table 2. 5′-Nucleotidase inhibitory activity of polysaccharides.

Compound	Structure	IC_{50} (μg/ml)
Nucleoticidin	*	40
Melanocidin A	*	56
Melanocidin B	*	59
Mannan (yeast)	*	44
Pustulan	(1→6)-β-D-Glucan	354
Dextran (MW: 50,000～70,000)	(1→6)-α-D-Glucan	>1,000
Dextran (MW: 170,000～200,000)	(1→6)-α-D-Glucan	>1,000
Amylose A	(1→4)-α-D-Glucan	>1,000
Amylose B	(1→4)-α-D-Glucan	>1,000
Pachyman	(1→3)-β-D-Glucan	>1,000
Curdlan	(1→3)-β-D-Glucan	>1,000
Pectic acid	(1→4)-α-D-Polygalacturonic acid	>1,000
Carrageenan	→3)-α-D-Gal-(1→4)-β-D-Gal-(1→	>1,000
Agarose	→3)-α-D-Gal-(1→4)-α-D-Gal-(1→	>1,000
Amylopectin	→4)-α-D-Glc 1 ↑ 6 →4)-α-D-Glc-(1→4)-α-D-Glc-(1→4)-α-D-Glc-(1→	>1,000
Pulluran	→6)-α-D-Glc-(1→4)-α-D-Glc-(1→4)-α-D-Glc 1 ↑ 6 α-D-Glc-(1→4)-α-D-Glc-(1→4)-α-D-Glc-(1→	>1,000
Lentinan	β-D-Glc β-D-Glc 1 1 ↑ ↑ 6 6 →3)-β-D-Glc-(1→3)-β-D-Glc-(1→3)-β-D-Glc-(1→3)-β-D-Glc-(1→3)-β-D-Glc-(1→	>1,000
Permethylated-nucleoticidin		>1,000
Permethylated-melanocidin A		>1,000
Permethylated-melanocidin B		>1,000
Peracetylated-nucleoticidin		>1,000
Peracetylated-melanocidin A		>1,000
Peracetylated-melanocidin B		>1,000

* Structures are shown in Fig. 1. (Structures of nucleoticidin and melanocidins A and B were reported in previous papers[3,5]. The structure of mannan was proposed in previous papers[11,12].)

by dialysis and the products (*U*-polysaccharides) in the inner solution of dialysis bag were obtained as powders after lyophilization.

Results and Discussion

Microorganisms are known to produce various organic compounds with varied chemical structures and biological, pharmacological and medicinal activities. Then, known antibiotics and enzyme inhibitors such as benzylpenicillin, streptomycin, erythromycin, mitomycin C, tetracycline, rifampicin, daunorubicin, bleomycin, viomycin, actinomycin D, neocarzinostatin, elastatinal, antipain and chymostatin were assayed for inhibitory activity against 5′-nucleotidase from snake venom (*Crotalus atrox*) and rat liver membrane. However, these compounds did not show any inhibitory activity at 20 μg/ml. Therefore, the inhibitors we reported previously, nucleoticidin, melanocidins A and B, are useful reagents for 5′-nucleotidase study as well as for study of immunological system. These inhibitors are polysaccharides and seem to have no structural resemblance to the substrate, AMP. Kinetic analysis of inhibition of 5′-nucleotidase by nucleoticidin, melanocidins A and B and mannan with partially purified enzyme from snake venom (Table 1) supports this concept, showing that these inhibitors showed non-competitive inhibition with respect to AMP.

To clarify the relationships between structure and inhibitory activity, known polysaccharides and chemically modified inhibitors were tested for activity at 200 μg/ml with the results summarized in Table 2. Of the compounds, nucleoticidin was the most effective, showing 50% inhibition at 40 μg/ml in our assay system. Fig. 1 shows the chemical structures of active compounds. From the present experiment, clear-cut relationships of structure and inhibitory activity cannot be deduced because of the limited availability of suitable polysaccharides. However, our results suggest that the inhibitory polysaccharides are highly branched and have side-chains with at least disaccharide units. That highly branched lentinan did not show the inhibitory activity supports this suggestion.

Concerning the relationships between the conformational structure and the inhibitory activity, we noted that urea-treatment caused a marked decrease or disappearance of the activity (Table 3).

Fig. 1. Structures of 5′-nucleotidase inhibitory polysaccharides.

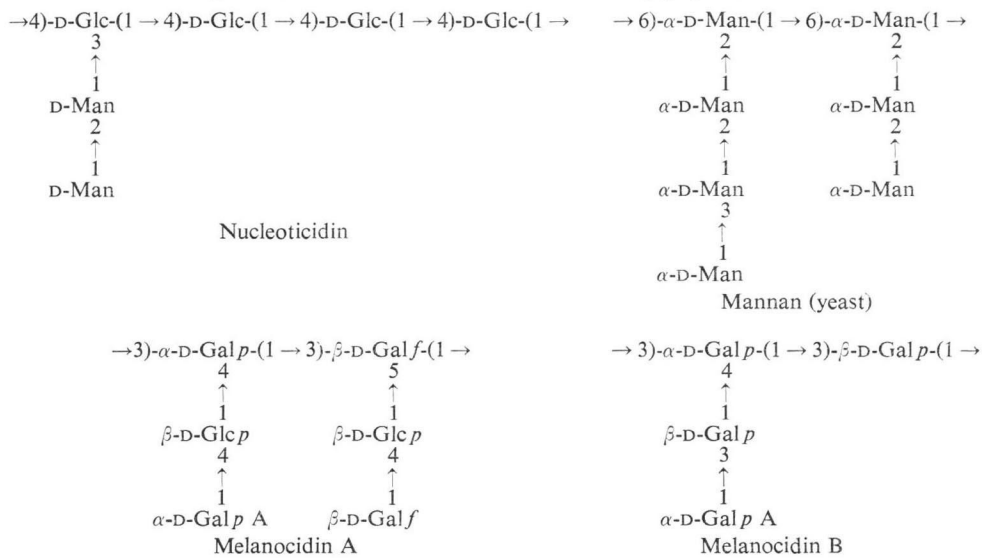

Table 3. Inhibition of 5′-nucleotidase activity by urea-treated polysaccharides.

Compound	IC_{50} (μg/ml)	*Ki* (μg/ml)
Nucleoticidin	40	3.64
U-Nucleoticidin	84	39.3
U-Mannan	148	N.D.
U-Melanocidin A	>1,000	—
U-Melanocidin B	>1,000	—
U-Pustulan	>1,000	—

N.D.: Not determined.

CHIHARA *et al.*[6~9] reported that urea changed the higher order structure of polysaccharides, and SHIBATA *et al.*[10] observed that 1,1,3,3-tetramethylurea accelerated *O*-methylation by HAKOMORI method by causing relaxation of the hydrogen bonds of polysaccharides. Hence, these results emphasize the importance of the higher order structure of polysaccharides for biological activity.

The above discussion clearly suggests that the relationships between the structure and the biological activity of polysaccharides is a complicated matter. From our experimental results, it becomes obvious that 5′-nucleotidase inhibitory activity is influenced by a number of factors such as the branching frequency, the length of branched chain and the molecular conformation. Furthermore, it is noteworthy that all 5′-nucleotidase inhibitors show antitumor activity. Therefore, our screening system for 5′-nucleotidase inhibitors is useful for finding new biologically active polysaccharides and antitumor substances in culture broths of microorganisms.

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