STUDIES ON THE α-GLUCOSIDE HYDROLASE INHIBITOR, ADIPOSIN

III. α-GLUCOSIDE HYDROLASE INHIBITORY ACTIVITY AND ANTIBACTERIAL ACTIVITY *IN VITRO*

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Adiposin-1 and -2 exhibited potent inhibitory activities against α-amylase, human salivary α-amylase and disaccharidases isolated from porcine small intestine. The effect of adiposin-1 and -2 on hydrolysis of glucoamylase was non-competitive. Adiposin showed antimicrobial activities against some Gram-positive bacteria, Gram-negative bacteria belonging to *Enterobacteriaceae*, some anaerobic bacteria and some phytopathogenic fungi, and showed a synergistic effect on the antibacterial activity with some maltooligosaccharides. However, these antibacterial activities were suppressed by addition of various other saccharides.

α-Glucoside hydrolase inhibitors, adiposin-1 and -2* were isolated from adiposin-complex by high performance liquid chromatography using Hitachi gel, #3013N. The purification procedure and their physico-chemical properties were described in the previous paper[1].

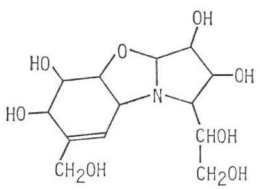
Adiposin-1 and -2 are very similar to each other in their physico-chemical properties and both are weakly basic oligosaccharides containing glucose and an unidentified amino sugar. This paper deals with the α-glucoside hydrolase inhibitory activity, the effect on hydrolysis of glucoamylase, and with the antibacterial and anti-phytopathogenic fungi activity of adiposin complex. Furthermore, the effect of various saccharides on the antibacterial activity of adiposin is also discussed.

Materials and Methods

Materials

Adiposin-1 and -2 were purified from a *Streptomyces calvus* TM-521 broth culture as described in the previous paper[1]. Adiposin-D was prepared by the hydrolysis of adiposin-complex with 0.6% H_2SO_4 at 100°C for one hour, and it was purified by SP Sephadex C-25 (H^+ form) column chromatography and high performance liquid chromatography using Hitachi gel #3013N[2]. The chemical structure of adiposin-D is shown in Fig. 1.

Fig. 1. Chemical structure of adiposin-D.



Bacterial liquefying α-amylase (BLA) and saccharifying α-amylase (BSA) of *Bacillus subtilis*, glucoamylase of *Rhizopus niveus* and invertase of *Candida utilis* were purchased from Seikagaku Kogyo Co., Ltd. α-Amylase of porcine pancreas, β-amylase of sweet potato and α-glucosidase of yeast were purchased from Boehringer Mannheim-Yamanouchi Co., Ltd. Taka-amylase and β-glucosidase of almond were purchased from Sankyo Co., Ltd. and Sigma Chemical Co., Ltd. U.S.A., respectively. α-Amylase of human saliva was prepared by the method of MEYER *et al.*[3] Pullulanase (bacterial iso-

* Adiposin-1 and -2 are identical to two of the amylase inhibitors described by OTANI *et al.*[13]

amylase) of *Enterobacter aerogenes* was a gift from Dr. Y. SAKANO, Tokyo Noko University. The sucrase, maltase and isomaltase were solubilized from porcine small intestine according to the methods of DAHLQVIST[4,5,6]. Maltooligosaccharide was provided by Dr. S. KOBAYASHI, National Institute of Food Research. Maltose, maltotriose and maltotetraose were isolated from the maltooligosaccharide by paper chromatography performed on Whatmann 3MM paper with the solvent system of *n*-butanol - pyridine - water (6: 4: 3, in volume) or 65% *n*-propanol. In addition, maltose and maltotriose were purchased from Hayashibara Biochemical Laboratories Inc.

Assay of Glucoside Hydrolase Inhibitory Activity

The assay system for the glucoside hydrolase inhibition is as follow: A mixture consisting of 50 μl of 0.007% glucoamylase dissolved in 1/20 M acetate buffer (pH 5.0), and 50 μl of test solution or water was incubated at 40°C for 10 minutes, and to this solution 400 μl of 1% soluble starch dissolved in the same buffer was added. This was incubated at 40°C for 10 minutes, and then an aliquot was withdrawn for the determination of glucose by the Somogyi-Nelson method. The inhibitory activity is expressed by the percent of inhibitor (IR) as follows:

$$IR(\%)=\frac{A-B}{A}\times 100$$

Here, A is the activity in glucoside hydrolase unit in the absence of inhibitor, B is that in the presence of inhibitor. One unit of enzyme is defined as the activity equivalent to 0.1 mg of reducing sugar liberated per minute at 40°C.

The following definition is used for the inhibitor unit; one unit of inhibitor (I.U) is equivalent to decrease of 1 enzyme unit under the above conditions. Inhibitory activities against BSA, Taka-amylase and β-amylase were determined by the same method as mentioned above. For human salivary α-amylase and pancreatic α-amylase, 1/15 M phosphate buffer containing 0.1 M sodium chloride (pH 7.0) was used instead of 1/20 M acetate buffer.

The inhibitory activities against BLA and pullulanase were determined by the modified methods of TSUZISAKA[7] and KOBAYASHI[8], respectively. For α-glucosidase, β-glucosidase, small intestinal disaccharidases and invertase, the methods of KHAN *et al.*[9], NIWA *et al.*[10], FROMMER[11] and WAHEED *et al.*[12] were used, respectively.

Antimicrobial Activity

Stock cultures maintained at our laboratory were used for the antimicrobial activity tests.

Antibacterial and antiphytopathogenic fungi activities of adiposin-1 and -2 were assayed by the disc method using heart infusion agar (HIA) for Gram-positive bacteria, 1% peptone agar containing 0.5% NaCl for Gram-negative bacteria, HIA containing 0.5% Casamino acids for anaerobic bacteria and 1% peptone - starch agar for phytopathogenic fungi. Anaerobic cultivation was carried out in a Te-Her Anaero Box Az-125 (Hirasawa Works, Tokyo).

The synergistic action of adiposin-2 and various saccharides was examined by the paper disc method using the above media, as indicated by the influence of the saccharides on the diameters of the adiposin-2 mediated inhibition zones.

Results

Glucoside Hydrolase Inhibitory Activity

The effect of adiposin-1 and -2 on various glucoside hydrolases was examined, and the result is presented in Table 1. Adiposin-1 and -2 showed potent inhibitory activities against α-amylases such as BSA, pancreatic α-amylase and salivary α-amylase, against disaccharidases such as sucrase, maltase and isomaltase from porcine small intestine and against glucoamylase. BLA and Taka-amylase were weakly inhibited, but α-glucosidase and β-glucosidase were not affected. The inhibitory activities of adiposin-1, adiposin-2, adiposin-complex and adiposin-D against glucoamylase, pancreatic α-amylase and sucrase were compared and the results are shown in Table 2. These activities of inhibition are expressed as inhibitory untits per mg of inhibitor.

Table 1. Effect of adiposin-1 and adiposin-2 on various glucoside hydrolases.
(Inhibitor concentration: 50 μg/ml)

Enzyme	Origin	Adiposin-1	Adiposin-2
BLA	*Bacillus subtilis*	−	−
BSA	*Bacillus subtilis*	+	+
Taka-amylase	*Aspergillus niger*	−	−
Salivary α-amylase	Human saliva	+	+
Pancreatic α-amylase	Porcine pancreas	+	+
Sucrase	Porcine small intestine	+	+
Maltase	Porcine small intestine	+	+
Isomaltase	Porcine small intestine	+	+
Glucoamylase	*Rhizopus niveus*	+	+
β-Amylase	Soy bean	−	−
α-Glucosidase	Yeast	−	−
β-Glucosidase	Almond	−	−
Pullulanase	*Enterobacter aerogenes*	−	−
Invertase	*Candida utilis*	−	−

Abbreviation: +, ≧50% inhibition of enzyme
−, <50% inhibition of enzyme

Table 2. Inhibitory activity of adiposin-1, adiposin-2, adiposin-complex (adiposin-C) and adiposin-D against various glucoside hydrolases.

Enzyme	Adiposin-1 (Unit/mg)	Adiposin-2 (Unit/mg)	Adiposin-C (Unit/mg)	Adiposin-D (Unit/mg)
Glucoamylase	123	817	660	0.61
Pancreatic α-amylase (Porcine pancreas)	265	334	2,662	—
Sucrase (Porcine small intestine)	2.0	4.1	3.3	0.09

Adiposin-2 was found to be most effective on glucoamylase and sucrase, while adiposin-complex showed a high inhibitory activity against pancreatic α-amylase. Adiposin-D was slightly active against all of these enzymes.

The kinetic studies of adiposin-1 and -2 showed that their inhibitory effects on starch hydrolysis were non-competitive (Fig. 2). Their *Ki* values obtained from LINEWEAVER-BURK plots were 2.52×10^{-3} mg/ml and 3.75×10^{-4} mg/ml, respectively. These results indicate that adiposin-2 is more active against exo-type amylase such as glucoamylase than against endo-type amylases such as BLA and pancreatic α-amylase, and this character is more outstanding in adiposin-2 than adiposin-1. The fact that the inhibitory activities of both adiposin-1 and -2 against the enzymes have no proportionality may be ascribed to the difference of affinity

Fig. 2. The effect of adiposin-1 and adiposin-2 on hydrolysis of glucoamylase.

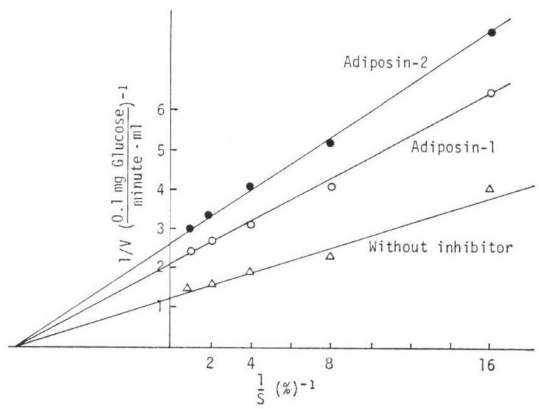


Table 3. Antibacterial activity of adiposin-1 or adiposin-2 and its combination effect with maltooligosaccharide.

Test organisms	Inhibition zone (mm)					
	Adiposin-1	Adiposin-1 $+G_2$	Adiposin-1 $+G_3$	Adiposin-2	Adiposin-2 $+G_2$	Adiposin-2 $+G_3$
Escherichia coli CSH-2 R64-11	—	13.8	13.3	—	18.6	17.3
〃 NIHJ	—	12.6	11.2	11.3	19.4	17.0
〃 SC 8600	10.1	15.8	14.9	14.5	22.4	19.4
〃 CSH-2 R1	10.3	16.4	15.2	14.6	21.8	21.1
〃 K-12	9.6	15.6	13.4	10.5	19.2	17.6
〃 ML-1410	12.4	18.2	16.2	13.6	21.8	19.4
Shigella sonnei E33 KB25	11.7	14.8	15.4	11.8	19.8	21.5
Shigella flexneri 2a	—	13.2	12.8	11.0	18.6	17.3
Enterobacter aerogenes IFO 12010	—	—	—	8.8	10.2	12.7
Salmonella paratyphi A KB 18	11.8	14.1	14.2	14.2	23.2	22.4
Bacillus licheniformis	9.8	14.6	14.8	11.7	14.2	19.0
Staphylococcus aureus 209P	11.5	11.3	11.8	12.7	12.3	12.4

(37°C, 18 hours)

Adiposin-1, adiposin-2: 50 μg/disc
Maltose (G_2), maltotriose (G_3): 100 μg/disc
Inoculum size: 10^6 cells/ml
Medium: Gram-positive, HIA; Gram-negative, 1%-peptone, 0.5%-NaCl containing agar.

defined by the length of saccharide chain.

Antimicrobial Activity

Adiposins exhibited inhibitory activities against the growth of microorganisms as well as against α-glucoside hydrolase.

As shown in Tables 3 and 4, adiposins have growth inhibitory activities against some Gram-positive bacteria such as *Bacillus licheniformis* and *Staphylococcus aureus* and some *Enterobacteriaceae*. These antibacterial activities were found to be enhanced by the addition of maltooligosaccharides such as maltose and maltotriose. The combination effect of adiposin-2 with various saccharides was examined on *Escherichia coli* CSH-2 R1 strain. None of saccharides except maltooligosaccharides exhibited the synergistic effect as shown in Table 5. On the contrary, most of monosaccharides and trehalose diminished the antibacterial activity of adiposin-2. Next, the antimicrobial activities of adiposin complex were examined against some anaerobic bacteria and phytopathogenic fungi. It in-

Table 4. Antibacterial activity of adiposin-D and combination effect of adiposin-D and maltotriose.

Test organisms	Inhibition zone (mm)	
	Adiposin-D	Adiposin-D $+G_3$
Escherichia coli CSH-2 R64-11	9.7	12.2
〃 NIHJ	13.2	12.8
〃 KP	13.8	15.2
〃 SC 8600	14.6	16.0
〃 CSH-2 R1	14.7	15.7
〃 K-12	12.3	12.4
〃 ML-1410	12.4	12.4
Shigella sonnei E33 KB25	13.4	13.9
Shigella flexneri 2a	12.1	12.6
Enterobacter aerogenes IFO 12010	—	—
Salmonella paratyphi A KB 18	12.4	13.8
Bacillus licheniformis	9.0	15.6
Staphylococcus aureus 209P	—	—

(37°C, 18 hours)

Adiposin-D: 100 μg/disc
Maltotriose (G_3): 100 μg/disc
Inoculum size: 10^6 cells/ml
Medium: Gram-positive, HIA; Gram-negative, 1%-peptone, 0.5%-NaCl containing agar

hibited the growth of a few species of anaerobic bacteria and phytopathogenic fungi as shown in Tables 6 and 7. The synergistic effect between adiposin complex and maltotriose was recognized in anaerobic

Table 5. Effect of various saccharides on antimicrobial activity of adiposin-2.

Saccharide	Inhibition zone (mm)	Combination effect*	Saccharide	Inhibition zone (mm)	Combination effect
Glucose	—	I	Sucrose	13.8	
Fructose	—	I	Lactose	14.2	
Galactose	—	I	Trehalose	—	I
D-Mannose	—	I	Cellobiose	14.9	
D-Arabinose	—	I	Isomaltose	14.8	
D-Xylose	14.4		Salicin	14.5	
D-Ribose	14.8		D-Raffinose	14.3	
L-Rhamnose	—	I	Panose	14.7	
Deoxyribose	14.3		Isopanose	14.3	
Sorbitol	—	I	Maltose	21.8	S
Mannitol	—	I	Maltotriose	21.1	S
Inositol	14.4		Maltotetraose	20.8	S
Glucosamine	—	I	Control	14.6	

(37°C, 18 hours)

Adiposin-2: 50 μg/disc Saccharide: 100 μg/disc
Test organism: *Escherichia coli* CSH-2 R1
* S: Synergistic effect I: Inhibitory effect

Table 6. Antibacterial activity against anaerobic bacteria of adiposin-complex (adiposin-C) and combination effect of adiposin-C and maltotriose.

Test organisms	Inhibition zone (mm)						
	Adiposin-C			G_3	Adiposin-C (1 mg) $+G_3$	Control*	
	1,000 μg	100 μg	10 μg			Cp	Tc
Peptococcus asaccharolyticus	—	—	—	—	—	46.4	43.8
Pc. prevotii	—	—	—	—	—	34.6	29.5
Peptostreptococcus anaerobius	—	—	—	—	—	38.0	25.1
Propionibacterium acnes	—	—	—	—	—	41.7	28.8
Prob. granulosum	—	—	—	—	—	40.3	27.8
Eubacterium lentum	—	—	—	—	—	31.8	31.3
Bacteroides ovatus	—	—	—	—	—	—	12.8
B. thetaiotaomicron	—	—	—	—	—	28.6	28.3
B. distasonis	—	—	—	—	—	23.3	16.2
B. praeacutus	—	—	—	—	—	40.3	47.6
Fusobacterium varium	—	—	—	—	—	25.5	16.2
F. nucleatum	—	—	—	—	—	41.0	38.1
Clostridium botulinum Type B	25.7	19.7	15.2	—	28.2	38.5	35.6
Cl. tetani	—	—	—	—	—	28.4	36.3
Cl. bifermentans	—	—	—	—	—	12.2	13.4
Cl. perfringens	20.9	17.8	14.6	—	23.3	18.6	19.8

(37°C, 48 hours)

Adiposin-C: 10~1,000 μg/disc Maltotriose (G_3): 100 μg/disc
* Chloramphenicol (Cp): 100 μg/disc Tetracycline (Tc): 200 μg/disc
Medium: HIA+0.5% Casamino acids

Table 7. Anti-phytopathogenic fungi activity of adiposin-1 or adiposin-2 and its combination effect with maltotriose.

Test organisms	Inhibition zone (mm)			
	Adiposin-1	Adiposin-1 $+G_3$	Adiposin-2	Adiposin-2 $+G_3$
Glomerella cingulata NKDG 16	—	—	—	—
Glomerella glycines	—	—	—	—
Alternaria kikuchiana	—	—	—	—
Gloeosporium kaki NKDG 13	—	—	—	—
Ophiobolus miyabeanus	26.2	26.5	26.8	26.4
Fusarium roseum	—	—	—	—
Fusarium lycopersici	—	—	—	—
Fusarium oxysporum	24.2	24.6	18.8	20.0
Helminthosporium sativum	—	—	—	—
Helminthosporium sigmoideum	—	—	—	—
Pyricularia sasaki	—	—	—	—
Pyricularia oryzae IFO 8775	—	—	—	—
Gibberella fujikuroi IAM 8048	28.2	28.6	21.2	20.8
Gibberella fujikuroi IAM 8058	27.8	26.8	20.8	21.4
Botrytis cinerea	—	—	—	—
Colletotrichum lagenarium	—	—	—	—

(30°C, 48 hours)

Adiposin-1, adiposin-2: 100 μg/disc Maltotriose (G_3): 50 μg/disc.
Medium: 4%-starch, 1%-peptone containing agar

bacteria, but not in fungi.

There has been no report on the antimicrobial activity of α-glucoside hydrolase inhibitors, hitherto. Accordingly, it is interesting to know whether or not α-glucoside hydrolase inhibitory activity in itself is concerned with the antimicrobial activity. Further investigations will be published elsewhere.

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