STUDIES ON THE α -GLUCOSIDE HYDROLASE INHIBITOR, ADIPOSIN

I. ISOLATION AND PHYSICOCHEMICAL PROPERTIES

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In the case of metabolic disorders such as diabetes, hyperglycemia, or hyperlipemia, it is well known that the blood levels of glucose, insulin, and lipoprotein, especially after food intake, differ from the normal levels. One of the reasonable countermeasures to these carbohydrate dependent diseases will be to limit intestinal carbohydrate digestion by the administration of α -glucoside hydrolase inhibitors. From this point of view, several oligosaccharide group amylase inhibitors have recently been isolated from the cultured broth of microorganisms¹⁻⁶.

In the course of our screening program searching for α -glucoside hydrolase inhibitors, the strain TM-521, identified as a strain of *Streptomyces calvus*⁷⁾, was found to produce such an inhibitor-complex named adiposin. This paper deals with the production, isolation and physicochemical properties of adiposin.

For the production of adiposins, 250 liters of the medium containing 3% oat meal, 1% soy bean meal and 3% starch were prepared in a 300-liter fermentor, and the pH was adjusted to 7.0, followed by autoclaving. After inoculation with 2% in volume of seed culture which had been incubated in the medium of the same composition at 30°C for 72 hours under shaking, fermentation was carried out under aeration of 200 liters/minute, 300 r.p.m. agitation and at 30°C $\pm$ 1°C. As shown in Fig. 1, the maximum production of adiposin was observed at 90~100 hours, and the inhibitory activity reached *ca.* 700 GIU/ml (one GIU is equivalent to decrease of 1 unit of glucoamylase) on average

Fig. 1. Culture process in adiposin production.

Fig. 2. Purification procedure of adiposin.

at the time of harvest, the pH of the broth being 6.0~6.4 at this time point. The purification procedures of adiposin-1 and -2 are shown in Fig. 2.

Adiposin-1 and -2 are white, amorphous solids that exhibited poorly defined infrared spectra characteristic of oligosaccharides. Properties of

adiposin-1 and -2 are presented in Table 1, acidic hydrolysis (1 N HCl, 2 hours at 100°C) data are listed in Table 2, and 400 MHz ^1H NMR spectra are shown in Figs. 3 and 4.

From these results, adiposins were found to be oligosaccharide group amylase inhibitors and contain glucose residues as their constituents.

The known amylase inhibitors possessing glucose in their structures such as BAY series amylase inhibitors¹⁾, trestatins A, B and C²⁾, S-AI³⁾, NCGAI⁴⁾ and oligostatin⁵⁾ are reported to have methyl groups in their structures, respectively. Accordingly, they can be differentiated from adiposins. In the course of our work we

Table 1. Physicochemical properties of adiposin-1 and adiposin-2.

Properties	Adiposin-1		Adiposin-2	
Appearance	White powder		White powder	
Decomposition point	138 ~ 143°C		157 ~ 162°C	
Elemental analysis	Found	Calcd.	Found	Calcd.
	C 43.09	C 42.62	C 42.79	C 43.04
	H 6.70	H 6.92	H 6.40	H 6.74
	N 2.61	N 2.62	N 1.96	N 2.01
Molecular weight (FD-MS)	522 (499 + Na)		684 (661 + Na)	
Molecular formula	$\text{C}_{19}\text{H}_{38}\text{NO}_{14} \cdot 2\text{H}_2\text{O}$		$\text{C}_{25}\text{H}_{43}\text{NO}_{19} \cdot 2\text{H}_2\text{O}$	
Optical rotation	$[\alpha]_D^{25} + 139^\circ$ (<i>c</i> 1.0, H_2O)		$[\alpha]_D^{25} + 163^\circ$ (<i>c</i> 1.0, H_2O)	
<i>pKa</i>	5.1		5.0	
UVmax	End absorption		End absorption	
Solubility Soluble in	Water, DMSO, MeOH, pyridine		Water, DMSO	
Color reaction Positive:	Anthrone, molisch, nitroprusside		Anthrone, molisch, nitroprusside	

Table 2. Rf Values of acid hydrolyzates of adiposin-1 and adiposin-2 on thin-layer chromatogram and paper chromatogram.

	Adiposin-1	Adiposin-2	Glucose
Paper chromatogram*			
65% <i>n</i> -PrOH	0.43, 0.52	0.43, 0.52	0.51
Pyridine - <i>n</i> -PrOH - AcOH - H_2O (10: 15: 3: 2)	0.52, 0.55	0.53, 0.56	0.56
Thin-layer chromatogram**			
<i>n</i> -BuOH - Pyridine - H_2O (12: 8: 5)	0.62, 0.71	0.61, 0.71	0.62
<i>n</i> -BuOH - EtOH - H_2O (3: 2: 2)	0.54, 0.56	0.54, 0.57	0.54

Detection: * Colorization with AgNO_3 .

** Colorization with concentrated sulfuric acid at 100°C for 5 minutes.

Fig. 3. ^1H NMR spectrum of adiposin-1. Fig. 4. ^1H NMR spectrum of adiposin-2.

found that adiposin-1 and -2 are identical to two of the amylase inhibitors very recently described by OTANI *et al.*⁶⁾

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References

- 1) SCHMIDT, D. D.; W. FROMMER, B. JUNGE, L. MULLER, W. WINGENDER, E. TRUSCHEIT & D. SCHAFER: α -Glucosidase inhibitors. New complex oligosaccharides of microbial origin. *Naturwissenschaften* 64: 535~536, 1977
- 2) YOKOSE, K.; S. OGAWA, K. WATANABE, T. SANO & K. SUHARA: Isolation and physicochemical and biological properties of new α -amylase inhibitors, trestatin A, B and C. Presented at the 218th Sci. Meet. Japan Antibiot. Res. Assoc., Tokyo, May 23, 1980
- 3) OHYAMA, K. & S. MURAO: Purification and some properties of amylase inhibitor (S-AI). *Agr. Biol. Chem.* 41: 2221~2228, 1977
- 4) UEDA, K. & S. GOCHO: Amylase inhibitor "NCGAI" and method for producing same. Japan Kokai 51-54,990, 1976
- 5) OMOTO, S.; S. ITOH, Y. IWAMATSU, H. HIDAKA & S. INOUE: Isolation and structures of new antibiotic, oligostatins. Presented at the 219th Sci. Meet. Japan Antibiot. Res. Assoc., Tokyo, July 25, 1980
- 6) OTANI, M.; T. SAITO, S. SATOI, J. MIZOGUCHI & N. MUTO: Novel amino sugar compound and method for producing same. Japan Kokai 54-92,909, 1979; Amino sugar compound: US patent 4,254,256, Mar. 3, 1981
- 7) BACKUS, E. J.; H. D. TRESNER & T. H. CHAMPBELL: The nucleocidin and alazopeptin producing organisms. *Antibiot. & Chemother.* 8: 532~541, 1957