Kinetics Studies of a Wheat-Derived Lipase Inhibitor

Keywords: *Lipase inhibitor; wheat flour; noncompetitive inhibition*

INTRODUCTION

Reports on proteinaceous lipase inhibitor are limited to those of Satouchi *et al.* (1974), Satouchi and Matsushita (1976), and Gargouri *et al.* (1984). Satouchi *et al.* (1974) and Satouchi and Matsushita (1976) purified a proteinaceous lipase inhibitor from soybean and reported a competitive inhibitory mechanism. This inhibitor does not act directly on the enzyme molecule but acts indirectly by modifying the emulsion of the lipid substrate, blocking the enzyme–substrate contact. Supporting the finding of Satouchi and Matsushita (1976), Gargouri *et al.* (1984) demonstrated an interaction between a soybean-derived lipase inhibitor and triglyceride and suggested that the inhibitory action of the lipase inhibitor is through alteration of the surface property of the substrate. These classical lipase inhibitors may have a mechanism of action completely different from that of the wheat flour-derived proteinaceous lipase ihibitor isolated by us (Tani *et al.*, 1994), which forms a complex with the enzyme. In this study, we further examined the properties of our lipase inhibitors.

MATERIALS AND METHODS

Chemicals. Lipases derived from porcine pancreatic juice (type VI-s), *Candida cylindracea*, *Rhizopus arrhizus* (type XI), *Chromobacterium viscosum* (type XII), and *Pseudomonas* sp. (type XII) were purchased from Sigma Chemical Co. (St. Louis, MO). Other chemicals were of special reagent grade. The lipase inhibitor derived from wheat flour of commercial origin [8% (w/w) protein on a moisture-free basis] (WFLI) was purified according to the methods of Tani *et al.* (1994). Human lipase was extracted from a pancreas according to the methods of Berner and Hammond (1970) and purified using a heparin–Sepharose affinity column (Pharmacia Co., Uppsala, Sweden).

Inhibitory Activity. The inhibitory activity of WFLI to various lipases was measured according to the method of Satouchi *et al.* (1974) using linolenic acid as a standard. One unit of lipase inhibitor activity is defined as the amount that results in 50% inhibition of 1 unit of lipase.

Kinetics Study. The kinetics of WFLI on the lipases used in this study were studied using a BIAcore (Pharmacia; Panayoto *et al.*, 1993). WFLI was immobilized on a Sensor Chip CM5 (Pharmacia) and reacted with lipase. The affinity constant (K_a, M^{-1}) was determined using BIAlogue kinetics evaluation software (Pharmacia).

RESULTS AND DISCUSSION

The inhibitory activity of WFLI to various lipases is shown in Table 1. WFLI demonstrated a strong inhibitory activity against the porcine pancreatic lipase and an equally strong activity against human lipase. The activity against the *C. cylindracea*-derived lipase was approximately 11–12% the activity to porcine or human lipase. No activities were demonstrated with the other microbial lipases.

The kinetics of WFLI to human and porcine pancreatic lipases and *C. cylindracea*-derived lipase were examined (Table 2). Both the inhibitory activity and the affinity constant of WFLI to porcine and human lipases were high, indicating a high affinity of WFLI to mammalian lipases. The difference in affinity constant between porcine and human lipases is probably due to the difference in dissociation rate constant.

Table 1. Inhibitory Effect of WFLI on Several Lipase Activities

origin of lipase	WFLI[a]
human pancreas	827
porcine pancreas	905
C. cylindracea	102
R. arrhizus	ND[b]
C. viscosum	ND
Pseudomonas sp.	ND

[a] Activity of lipase inhibitor is expressed as units/mg of protein. [b] Not detected.

Table 2. Kinetic Constants of Interactions between Several Lipases and WFLI

origin of lipase	K_{assoc} (M^{-1} s^{-1})	K_{dissoc} (s^{-1})	K_a (M^{-1})
human pancreas	1.78×10^6	2.21×10^{-3}	8.05×10^8
porcine pancreas	1.48×10^6	1.62×10^{-3}	9.14×10^8
C. cylindracea	1.96×10^5	3.77×10^{-2}	5.21×10^6

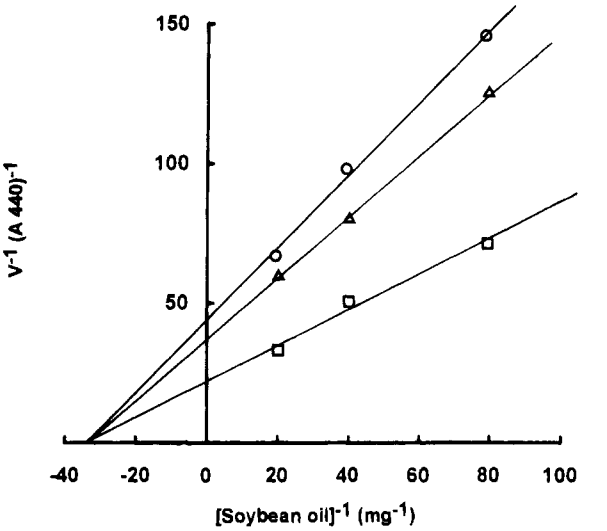

Figure 1. Competitive inhibition of porcine lipase activity by lipase inhibitor from wheat flour: (○) 5 mg/mL of WFLI; (△) 1 mg/mL of WFLI; (□) 0.5 mg/mL of WFLI. Velocities are absorbance at 440 nm.

The type of inhibition of porcine lipase effected by WFLI was studied by Lineweaver–Burk plot and was determined to be noncompetitive (Figure 1). Satouchi and Matsushita (1976) reported competitive inhibition for a soybean-derived proteinaceous lipase inhibitor. The properties of the wheat-derived proteinaceous lipase inhibitor that we purified, such as the inhibitory activity (Tani *et al.*, 1994), enzyme kinetics, and inhibition type, indicate that it may have a mechanism of action different from that of the reported soybean-derived lipase inhibitors. The complete primary structure and the lipase binding site of WFLI are now being investigated.

LITERATURE CITED

Berner, D. L.; Hammond, E. G. Phylogeny of lipase specificity. *Lipid* **1970**, *5*, 558–562.

0021-8561/95/1443-2796$09.00/0

Gargouri, Y.; Julien, R.; Pieroni, G.; Verger, R.; Sarda, L. Studies on the inhibition of pancreatic and microbial lipases by soybean proteins. *J. Lipid Res.* **1984**, *25*, 1214–1221.

Panayotou, G.; Gish, G.; End, P.; Truong, O.; Gout, L.; Dhand, R.; Fry, M. J.; Hiles, I.; Powson, T.; Waterfield, M. D. Interactions between SH2 domains and tyrosine phosphorylated PDGF β-receptor sequences. *Mol. Cell. Biol.* **1993**, *13*, 3567–3576.

Satouchi, K.; Matsushita, S. Purification and properties of a lipase inhibiting protein from soybean cotyledonas. *Agric. Biol. Chem.* **1976**, *40* (5), 889–897.

Satouchi, K.; Mori, T.; Matsushita, S. Characterization of inhibitor protein for lipase in soybean seeds. *Agric. Biol. Chem.* **1974**, *38* (1), 97–101.

Tani, H.; Ohishi, H.; Watanabe, K. Purification and characterization of proteinous inhibitor of lipase from wheat flour. *J. Agric. Food Chem.* **1994**, *42*, 2382–2385.

Received for review February 21, 1995. Revised manuscript received May 30, 1995. Accepted September 29, 1995.

Hisanori Tani,*,†,‡ Hifumi Ohishi,‡,§ and Kenji Watanabe†

The United Graduate School of Agricultural Science, Gifu University, Gifu 501-11, Japan, Department of Pathology, Institute of Gerontology, Nippon Medical School, 1-396 Kosugi-cho, Nakahara-ku, Kawasaki 211, Japan, and Fundamental Research Laboratory, Kyodo Milk Industry Company, Ltd., 180 Hinode-cho Hirai, Nishitama-gun, Tokyo 190-01, Japan

JF950116S

* Author to whom correspondence should be addressed.

† Gifu University.

‡ Kyodo Milk Industry Co. Ltd.

§ Nippon Medical School.